A DESIGN FOR A RANDOMIZED CLINICAL TRIAL TO DETERMINE SAFE AND EFFECTIVE DOSES OF NIRIDAZOLE IN THE TREATMENT OF URINARY SCHISTOSOMIASIS IN SCHOOL CHILDREN

© JUMOKE MARY OMOJOLA, MB.BS.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

Mcmaster University

April 1981

URINARY SCHISTOSOMIASIS: A RANDOMIZED CLINICAL TRIAL OF DRUG THERAPY

MASTER OF SCIENCE (1981)
(Design, Measurement and Evaluation;
Clinical Epidemiology and Biostatistics)

Mcmaster University
Hamilton, Ontario

TITLE: A design for a Randomized Clinical Trial to Determine Safe and Effective Doses of Niridazole in the Treatment of Urinary Schistosomiasis in School Children

AUTHOR: Jumoke Mary Omojola, MB.BS. (University of Ibadan)

SUPERVISOR: Dr. Andrew Harper

NUMBER OF PAGES: xi + 126

ABSTRACT

Schistosomiasis is known to affect over 200 million people in tropical countries. It is well known to have variable manifestations in similar geographical regions. It is also known to have variable responses to treatments in different geographical regions.

Literature on its treatment though abundant reveals no agreement on doses of drugs to be administered.

This thesis presents a design to determine doses of a recommended drug in the treatment of haematobium schistosomiasis. Three dosage levels of niridazole (ambilhar) will be tested against a recommended dose of metrifonate. The trial will be conducted over a 12 month period and will involve infected school children in a town in Ondo State of Nigeria.

Children will be followed up during the trial period to determine the effects of treatment on their urinary egg output.

ACKNOWLEDGEMENTS

The writing of this thesis and other activities in the design, measurement and evaluation program have been accomplished as a result of input from a lot of people, faculty, staff and students. To all of them my sincere "thank you".

My appreciation goes to my thesis committee, Dr. Andrew Harper, Dr. Brian Haynes and Professor Ruth Milner for their guidance and support.

Dr. Chris Woodward deserves a special thank you for her interest and encouragement.

To my family, thank you for understanding and to my friends, thank you for caring.

TABLE OF CONTENTS

TABLES

LIST OF APPENDICES

CHAPTER I

INTRODUCTION

Schistosomiasis (synonym bilharziasis) is a chronic parasitic infection with trematode helminths occurring in mammals (31, 62, 65). The infection has been a topic of considerable interest to workers in developing countries where the infection is endemic. Several aspects of schistosomiasis that have been studied include the life cycle of the parasite, diagnosis of infection, therapy and more. Despite the extensive literature available, many issues remain unclarified. For example, there is the question of where infection ends and disease begins. Should treatment be offered to all infected individuals or only those with evidence of disease?

The schistosoma parasite is believed to be pathogenic (that is, it has the ability to produce disease). Depending on the definition of disease, pathogenicity is variable. If disease is defined as being equivalent to infection (egg detection in urine or faeces) pathogenicity is greater than if disease is defined as the presence of symptoms referable to the site of infection. For example, in haematobium infection disease is deemed to be present if an individual passes eggs in urine and has some or all of symptoms such as dysuria, haematuria and frequency of micturition (31). A distinction is usually made between infection and disease, even though in the absence of radiological, cystoscopic and other types of evidence, it

is difficult to determine what proportion of infected individuals are diseased (38, 39, 57).

On the issue of therapy there is agreement about what drugs to administer, but no agreement about the optimal dosages of these drugs. Studies in different communities report different dosages as being effective (28, 40, 75, 88). The possible reasons for this variability include the variable study subjects, differences in intensity of infection, and the strain and species of the parasite. In an infection such as schistosomiasis in which the outcome is not uniformly serious or fatal, therapeutic dosages should be determined in standardized trials.

This thesis proposes to address an aspect of therapy in children with schistosomiasis infection. Children have been selected because it is believed that they benefit more from therapy than do adults (60, 61, 63, 110). It is hoped that through a randomized clinical trial, safe and effective dosages will be identified. The treated individual is also likely to have a reduction in the risk of developing serious disease. The determination of effective and safe dosages will likely make it easier to offer chemoprophylaxis if and whenever this is decided upon.

As a prelude to the literature review and design, a brief background will be given on schistosomiasis.

1.1 The infection

Human schistosomiasis is mainly caused by three species of schistosome, *Schistosoma haematobium*, *Schistosoma japonicum* and *Schistosoma mansoni* (17, 31, 62, 73). The different species tend to occur in different geographical areas. *S. haematobium* occurs in various parts of Africa, the Middle East and on Mauritius and Madagascar (62). *S. mansoni* is widespread in parts of Africa, South America and some Caribbean Islands. *S. japonicum* occurs in Japan, China, the Philippines and other far eastern countries (62). The species of major importance in most regions of Nigeria is *S. haematobium* (12, 26, 45, 46, 80). Its therapy forms the topic of this thesis. A brief discussion of the schistosome life cycle follows. It will identify the parasitic form that is infective to man, the form identified as a measure of infection and/or disease and the form that is responsive to therapy. A less common species common in the Congo is *S. intercalatum*.

1.2 Parasite life cycle in man

The three species of schistosome commonly parasitic in man all have similar life cycles. A part of the life cycle occurs in man, who acquires the infection on coming in contact with infected water (62, 73). Infective cercarium actively penetrates the human skin from where it finds its way to the liver. In the liver maturation and mating occurs followed by migration through the portal system into the mesenteric or vesical veins where egg laying starts, depending on the species (62, 65, 73). The route by which the

schistosomulae (as the young forms are now called) reach the final destination in man is not really known. The adult worms of *S. haematobium* occur predominantly in the vesical plexus, whilst *S. japonicum* and *S. mansoni* occur predominantly in the mesenteric plexus. This state of affairs occurs in pure infections of one species. Mixed infections are quite common in the same individual. In *S. haematobium* infections eggs laid by female worms pass through the walls of the fine blood vessels and through the bladder wall into the bladder lumen, where they are voided into urine. If infected urine falls into fresh water, the eggs hatch into miracidia which invade the appropriate snail species. As long as viable eggs are excreted in urine, the individual remains a potential source of infection (27, 83).

1.3 Life cycle in the intermediate host

All schistosome parasites have a stage of asexual multiplication occurring in an appropriate fresh water snail (31, 62, 65). In *S. haematobium* infections, the miracidia penetrate the *Bulinus* species of snails within which the miracidia undergo intermediate stages of redia and sporocysts to become mature cercaria which are infective to man. When man makes contact with infected water, the cycle is repeated again.

The schistosome form representing evidence of infection is the egg. A measurable response to therapy is a reduction or cessation of egg output in urine. The literature review will discuss the magnitude of infection in Nigeria, the diagnosis and measures of infection

and disease, pathology of schistosomiasis, issues in therapy, and control methods.

CHAPTER II

LITERATURE REVIEW

2.1 Magnitude of schistosomiasis

The impact of a disease will usually be measured in terms of its effect on an individual and its general importance to the community in question.

Schistosomiasis either as the intestinal or urinary infection is a common endemic infection in tropical countries (26, 51, 56, 58, 62). An endemic parasitic infection is defined as one that is present rather uniformly in a host population. Transmission is well assured and the infection has some stability (14). A 1977 symposium on schistosomiasis estimated that nearly 200 million people in tropical countries have evidence of infection (74). In schistosomiasis the spectrum of the infection is so highly variable even within the same country that it has proven difficult to ascribe mortality to the infection with the exception of Egypt, where the infection seems to be of major public health importance (1, 2, 3, 21, 33, 71). Part of the difficulty occurs because in communities where the infection is highly prevalent, other parasitic infections are also prevalent, nutrition is inadequate, and with this multiplicity of events it is not easy to identify any single factor as causing pathology.

The magnitude of schistosomiasis will be discussed in terms

of its effect on an infected individual as a cause of morbidity and mortality and in terms of the distribution of infection in the population. Since this thesis will be concerned with haematobium or urinary schistosomiasis, the subsequent discussions pertain primarily to haematobium infections.

2.1.1 Morbidity due to urinary schistosomiasis

The significance of schistosoma infection in an endemic area is difficult to determine since multiple infections occur in the same individual. *S. haematobium* has a low pathogenicity (defined in this discussion as the proportion of infected cases who develop disease)(15, 45, 46). It has been postulated that differences in infective strains of *S. haematobium* may be partly responsible for the fact that not all infected individuals develop disease (26, 68). The course of urinary schistosomiasis has been classified into four stages (shown in Table 1). Infected individuals are usually identified during the stage of established infection. Some infected individuals are asymptomatic whilst others have dysuria, haematuria, and painful micturition. These symptoms are apparently quite compatible with normal day to day living (37, 45). Haematobium infection has been blamed for tiredness, apathy and vague ill health, although Bradley (13) found that even with the use of specially designed tests, it was not possible to demonstrate excessive lassitude in infected individuals. An alternative explanation could be that these tests were insensitive.

In Egypt bladder cancer has been extensively studied in

Table 1: A classification of the course of bilharziasis - based on parasitological, clinical and pathological aspects (62)

Stage	Parasitological	Clinical	Pathological
Stage of invasion	Migration and beginning of maturation	Incubation period, including cercarial dermatitis, if present	Slight inflammatory reactions in skin, lungs and liver
Stage of maturation	Completion of maturation and early oviposition	Toxaemic stage of the disease (or acute febrile stage) not always recognised or present	Hyperergic reactions, generalised and local, to products of eggs and/or young schistosomes
Stage of established infection	Intensive oviposition accompanied by a corresponding egg discharge	Stage of early chronic disease, characterised for instance by haematuria, or intestinal and other digestive manifestations possibly with cardio-pulmonary or other complications	Local inflammatory reactions due to ova, resulting mainly in granuloma formation. Fibrosis is not a predominant feature
Stage of irreversible effects	Prolonged infection (usually with reduced or discontinued egg extrusion)	Stage of late chronic disease, due to irreversible effects and/or sequelae or complications	Progressing formation varying with intensity of infection, and possibly other factors of fibrous tissue, with its consequences according to the organs involved

association with urinary schistosomiasis (71). The high prevalences of both urinary schistosomiasis and vesical cancer have been taken as some evidence that the association is causal. However, in West Africa and parts of the Middle East, bladder cancer is of low prevalence in the presence of a high prevalence of urinary schistosomiasis. Further studies are needed to confirm or refute a causal relationship.

2.1.2 Mortality due to urinary schistosomiasis

The importance of urinary schistosomiasis as a cause of mortality can be estimated with some certainty in autopsy studies. Cheever (20) and others in an Egyptian study of 400 autopsies considered schistosomiasis as the cause of death in 6.2% of all cases and 9.2% of all infected cases. Death is believed to be a result of renal failure following an acute infection of a hydronephrotic kidney (31). It has been hypothesized that the low death rate due to schistosomiasis may be because individuals with more severe lesions such as hydronephosis have already succumbed and their deaths (most likely at home) remain unreported (43).

2.1.3 Schistosomiasis in Nigeria

Infection with both *S. mansoni* and *S. haematobium* is widespread in Nigeria (26). Infection rates are highly variable, depending on the area being studied. Children have high prevalence rates in many areas (26, 45, 46, 84, 85). *S. haematobium* is the important infection with higher prevalence rates compared with *S. mansoni*. Cowper (26) feels

that *S. mansoni* infections are likely under-reported because of a greater difficulty of diagnosis. Studies in Epe, believed to be a highly endemic region, showed that 50% of those examined had haematobium and only 1% had mansoni infection (46, 80). The Western Region of Nigeria is believed to be endemic for both *S. haematobium* and *S. mansoni*, although *S. haematobium* is more important. *S. haematobium* infection is commonly referred to as urinary schistosomiasis. It is believed that there are two distinct species of *Bulinus* snails (*truncatus* and *globosus*), both of which cannot be infected by *S. haematobium* from the same urine specimen. These strain differences have been postulated to be partly responsible for differences in spectrum of the infection (26, 68).

2.1.4 Morbidity and mortality studies in Nigeria

Gilles and others (45) in a study of 78 primary school children aged 9-15 years noted abnormalities in their urograms. The study did not indicate the presence or absence of symptoms in relation to these abnormalities. One assumption that could be made is that since these children were apparently healthy school attenders, they were not incapacitated by their infection. Pugh and Gilles (85, 86), in a five year study, could not identify any urological abnormalities in the presence of very high urinary egg outputs. Their conclusion was that the infection did not result in any lesion. In this study of 59 males, there were no controls and no comparisons could be made with uninfected individuals or those with low egg out-

puts. It was unclear from the study what the initial urological appearance had been. There is the possibility that five years may well be too short for any noticeable change to occur.

In a study of 673 unselected autopsies *S. haematobium* eggs were recovered in 135 bladders though no death could be attributed to *S. haematobium* disease (98).

Apparently a lot of infected individual are able to tolerate their infection.

2.1.5 Measures of human infection

As a prerequisite to the determination of the importance of schistosomiasis in any community or country, certain measurements need to be taken in the population in question. Also, in order to make meaningful comparisons of different areas or in order to assess the progress of a control program, the efficacy of therapy or even the effects of secular trends, changes in these measurements need to be considered. These measures of infection will now be discussed in some detail. Several rates have been proposed as being useful in quantifying any infection or disease in groups of people (62, 67, 109). These include incidence rates, prevalence rates and, in infections, the intensity of infection. These rates are usually determined from population surveys or sample studies.

A. Incidence of schistosomiasis

The incidence of infection is defined as the number of new cases of infection detected in the population under study over a period of time (64, 67). Incidence is often expressed as a rate, the number of new cases of infection over a period of time per population at risk of developing infection. Incidence measurement involves several factors which will be mentioned briefly.

(i) The incidence rate is affected by the period of observation in relation to the risk of being infected. If the population in question is at risk for a short period of time, incidence rate can be studied during that risk period which may only last for a few days to a few weeks. The incidence rate will likely fall with removal of the risk factor. If the risk is longstanding as is exposure to schistosoma infection in an endemic area, one has to arbitrarily select the period of observation. One year will be selected in this discussion primarily because it incorporates the two seasonal periods (rainy and dry). Also, it is the more often selected period of time. Incidence of infection is presumably higher in the rainy season when the snail habitats can flourish and lower in the dry season (53). A one year period will likely even out the seasonal differences. It is still possible that during this one year period some maturing infection will remain undiagnosed (29, 54, 91, 100).

(ii) The necessity of a pretest in order to separate those who are already infected and are no longer at risk from those who are at

risk of developing infection. Only the uninfected group can be studied in order to measure incidence. Depending on the finding, if prevalence is high, it may be impossible to identify enough uninfected individuals in whom to measure incidence.

(iii) The need for a sensitive measurement tool which will not have many false negatives. The false negatives may later be identified as new cases when in fact they should have been identified during the earlier examination. Parasitological examination in schistosomiasis may prove negative when infection is light so that a false incidence rate is obtained.

(iv) The stability of the population being studied. If there is frequent migration in and out of the community some new cases identified may have contracted the infection outside the community. If the proportion of new cases who contracted infection outside the study community but moved into the community, is equal or similar to the proportion who moved out of the community after contracting infection, the incidence rate is close to the true rate. If these proportions are not similar, the incidence rate will be falsified. During the one year study period it will be assumed that this is a stable population. This is a reasonable assumption since members of the community who move usually do so at the end or beginning of a year. These numbers are not very large and more often than not children are usually left with relatives in the community (111).

Considering point (iii) above, increased sensitivity is obtained by examination of multiple urine samples in order to reduce the number of false negatives (8, 10, 16, 101). Also, if the study is executed among younger members of the population who if infected, are more likely to have higher egg excretions, the likelihood of missing new cases is reduced.

Theoretically, the incidence rate gives some indication of the effect of a manoeuvre such as chemotherapy on transmission. The reasoning is that successful therapy results in a reduction or cessation of egg excretion. This will result in decreased contamination of water and subsequently a reduction in the risk of contracting infection.

B. Prevalence

This is the proportion of the population infected (point prevalence) (64, 67). Urine specimens obtained in population surveys or selected samples are examined for schistosome eggs. Depending on the sample surveyed, prevalence data tend to be highly variable in relation to the endemicity of schistosomiasis in the population and the age structure (92, 109). Prevalence data consistently reveal a characteristic pattern of highest prevalence in individuals aged 6-20 years (1, 57, 82, 90). Prevalence rates are much lower amongst adults and even more variable amongst the under fives. In areas of high endemicity such as Egypt and Gambia, prevalence amongst the less than fives is reported to be about 50-75% approaching nearly 100% in the 6-10 year olds (2, 104). Apart from age and geographic location, sex,

proximity to water and population density are some of the factors affecting prevalence.

Sex differences in prevalence have been noted in Egypt with infection occurring predominantly in males (1, 59). Studies in Gambia reveal no sex differences (104). Pugh and Gilles (84) have suggested that this is probably because of the religious customs in Egypt which are restrictive of females who are unlikely to bathe in public areas and are therefore unlikely to contract infection. Densely populated areas close to contaminated water will likely have higher prevalence rate than populations located some distance away from water. Prevalence data may be used to assess the effect of a control program such as chemotherapy. The interpretation of results will have to be cautious since data obtained immediately following a program may reveal a decrease in prevalence rate (53, 109). This decrease is very likely a temporary effect. A 2-3 year period is necessary for a more lasting effect to be noted.

C. Intensity of infection

A third measure of human infection is the intensity of infection as determined by the egg output (14, 109). This involves quantifying the egg output. The definition of how intense an infection is seems arbitrary. With stool examination, it is usually expressed as eggs per gram of faeces, in *S. haematobium* infections as eggs per ml or per 10 ml of urine. Intensity is usually described as light, moderate or severe/heavy. The criteria for this classifi-

cation are as variable as the number of people carrying out the studies. Table 2 is an example of the varying classification.

It will be noted from Table 2 that there is no consensus of opinion regarding categorization of the intensity of schistosomiasis infection. There is, however, a widely held view that intensity of infection is relevant to development of disease, response to therapy and the potential for contamination of the environment (45, 46, 59, 66, 93, 98).

The relationship between age and intensity of infection is roughly inverse with younger individuals having higher mean egg outputs (27, 51, 59, 84). This relationship is not a simple one, as there are large individual and secular vairations (83, 93, 97). The intensity of infection bears some relationship with the prevalence of schistosomiasis. In areas with low prevalence (defined as less than 25% of population studied) over 90% of individuals have egg counts of less than 50 eggs per ml of urine whilst in areas with prevalence greater than 75%, over 65% of individuals have counts greater than 50 eggs per ml of urine and greater (50, 74).

The intensity of infection is affected by several factors, some of which are the rapidity of egg destruction in the body, host nutrition, and egg distribution among tissues in the body (100). There is some difficulty with comparison of egg counts for several reasons, some of which are the differences in counting techniques, the lack of standardization of techniques and the dependence on the expertise of the observers.

Other aspects of measuring intensity of infection involve

Table 2: Intensity of Infection

Study by	Type of Infection	Light	Moderate	Severe/Heavy
Abdel Salaam and others (1)	*S. Haematobium*	10 egg/ml of urine	15-30/ml of urine	>35 egg/ml of urine
Bassily and others (9)	*S. mansoni*	regarded 10,000 egg/24 hour stool as severe enough to require therapy		
Jordan (50)	*S. haematobium*	1-125 eggs/10 ml of urine	126-500 eggs/ml of urine	>50 eggs/ml of urine
Kloetzel (57)	*S. mansoni*			>500/gm of faeces likely to cause pathological lesions
Omer and Teesdale (75)	Both species (*S. haematobium*) (*S. mansoni*)	1-10 eggs/ml of urine	11-40 eggs/ml of urine	>41 eggs/ml of urine
Pugh and Gilles (85)	*S. haematobium*	1-32 eggs/10 ml of urine	33-256/10 ml of urine (very high)	>257-1024/10 ml of urine >1025/10 ml of urine
Warren and others (99)	*S. mansoni*	1-100 eggs/gm of faeces	101-400 eggs/gm of faeces	>400 eggs/gm of faeces

autopsy studies and are never used in living patients. Tissue egg burdens are measured and intensity is expressed per gram of tissue, bladder tissue, urinary tract tissue, rectal tissue and so on. This is hardly ethical to determine in apparently healthy individuals.

It has been suggested that schistosomiasis infection should be separated from schistosomiasis disease on the basis of the intensity of infection since the latter bears some relationship with morbidity (57). Schistosomiasis disease develops only in a minority of infected individuals (less than 10%). Even in disease, the multiplicity of factors such as malnutrition, other parasitic infection and host reactions, makes it impossible to attribute disease specifically to schistosome infection. The exception occurs in individuals with the severe manifestations of infection. These individuals form a minority (less than 3% of those infected). It is therefore difficult to decide who will defintely develop disease. However, since therapy does more good than harm, especially in children, it seems necessary to treat or at least offer therapy to heavily infected children.

D. Miracidial counts

As noted in section 1.2, a viable egg on contact with water hatches into a miracidium which invades the appropriate snail species. The potential for contamination of the environment and, subsequently, for the transmission of infection, depends on the viability of the eggs excreted (28, 84). If a large proportion of excreted eggs are dead, the individual poses minimal threat of transmitting infection.

However, an infected individual excreting large numbers of viable eggs poses a major risk of contaminating the environment. Some researchers advocate that miracidial counts should form part of the measures of infection (28, 109). This involves hatching the eggs and counting miracidia. If infected members of the community are excreting mainly dead eggs, that community has a low potential for new infections. Miracidial counts could also be used in the evaluation of anti-schistosomal drugs. If a large proportion of the eggs excreted by an individual following therapy are dead eggs, it is possible to count the individual as a therapeutic failure unless the differentiation between viable and dead eggs is made. Miracidial counting is one method of differentiating between viable and dead eggs.

2.1.6 Measures of human infections in Nigerian studies

A. Incidence

There are really no reports of incidence rates. Most studies conducted are one time cross-sectional studies yielding prevalence rates (45, 46, 80, 84, 85). Edington (30), in an autopsy study of 673 unselected cadavers, reported an "incidence rate" of 20%. This represented the proportion of cadavers in which *S. haematobium* eggs were recovered. This result strictly represents a prevalence rate since there was no way of knowing what proportion of the cadavers was initially free of infection.

B. Prevalence

Gilles and others (45) in a 1963 study of 78 school children in a southern city in Nigeria detected haematobium eggs in urine of 71 of them, a prevalence of 91%. Only 1 child had *S. mansoni* eggs in stool. The children were aged 9-15 years (within the peak prevalence period). This prevalence is likely an overestimation, considering the fact that many regions of Nigeria now have tap water supply. Pi Sunyer (80) studied 1451 individuals in Epe (a hyperendemic region for schistosomiasis). It is unclear from the study what proportion of this initial sample passed haematobium eggs in urine. However, the study reported that of the 656 individuals remaining in the study, 365 passed paematobium eggs in urine, a prevalence of 56%. No male versus female differences were noted. Prevalence rates vary from 13.5% to 18.5% in two villages studied in Northern Nigeria. The study surveyed 3352 individuals in one village and 1030 in the second village (87). Response rate to the survey varied from 76% in the first village to 93% in the second village. Only a single urine specimen was collected from each individual. Prevalence rate amongst females was about 25% of that in males. These rates differ significantly from those of Gilles and others and Pi Sunyer (45, 80). One possible explanation for the male to female differences noted in the northern study could be the religious customs of people of Northern Nigeria. Northern Nigeria is predominantly an Islamic region. This is inhibiting of female children, unlike the southern part of the country which is more heterogenous in its religious affiliations.

C. Intensity

Pugh and Gilles (86) classified infections into light, moderate, high and very high (Table 2). Higher egg outputs were noted amongst children aged 8-12 years. Children form approximately 45% of the population and they also have higher egg outputs than do adults. As such, they represent an important target group for control measures. They also benefit more from therapy (60, 61, 63, 110).

2.1.7 Conclusion

The epidemiology of *S. haematobium* infections is discussed. The morbidity and mortality associated with infection are discussed. A discussion of the diagnosis and control of schistosomiasis follows.

2.2 Diagnosis of urinary schistosomiasis

Diagnosis of infection may be clinical, immunological, radiological, parasitological or pathological. No one of these methods is completely definitive. The choice of which method to use in the identification of infected individuals depends on the aims of the study. For example, exploratory population surveys tend to use parasitological methods. Studies attempting to correlate infection with pathology often use parasitological diagnosis and radiological methods and autopsy studies are usually pathological.

2.2.1 Clinical diagnosis of infection

The clinical picture of urinary schistosomiasis is not clearly defined. Symptoms vary from nil to severe, depending on such factors as the race, nutritional status, immunity and resistance of the infected individual, the number, frequency and severity of exposures to the parasite (38, 100). When symptoms occur in urinary schistosomiasis, they are mostly referrable to the urinary tract. Lehman and Farid (59) noted that 79% (158/200) of the individuals voiding haematobium eggs in urine had symptoms referrable to the urinary system. These symptoms included dysuria, frequency of micturition and haematuria. Forsyth and Bradley (38, 39) also noted that 68% (668/976) of individuals voiding haematobium eggs in urine had urinary symptoms.

The only consistent symptoms that have been identified across studies are haematuria and dysuria. Farooq and others (33) noted clinical gradients in the symptomatology of infected individuals. Individuals with greater mean egg excretions and mixed infections were more likely to be symptomatic.

Since clinical symptoms are not always present, some individuals will be missed if diagnosis was made on the basis of clinical evidence.

2.2.2 Radiological diagnosis of infection

Whilst clinical diagnosis identified some infected individuals or groups of infected individuals, radiological examinations were

often used in the past to assess the sequelae of schistosoma infection. Lesions were noted in individuals with asymptomatic infections. There was a high frequency of hydronephosis and calcified bladders (45, 59, 110). Abnormalities were noted in 39% (38/78) of intravenous urograms of a group of healthy children (45). These abnormalities included multiple nodular filling defects and/or bladder calcifications. Presently it is hardly ethical to perform radiological examinations with no indication besides the excretion of schistosome eggs. These earlier studies have contributed information concerning the effects of therapy on some of these radiological lesions. The nodular filling defects could be reversed by treatment (32, 60, 61). They are usually found in urograms of children but not in those of adults.

The majority of urinary tract lesions apparently have no telltale symptoms or signs so that under non-study circumstances radiological diagnosis is unhelpful.

2.2.3 Parasitological diagnosis of infection

The certain diagnosis of *S. haematobium* infection involves the recovery of characteristic eggs in urine of infected individuals. Less commonly eggs may be recovered in stool. Parasitological examination may be qualitative or quantitative. Quantitative examination is usually used to establish the magnitude of infection in a community. Depending on the size, whole populations or subsamples may be examined. Quantitative examination of urine involves egg-counting and is often used to assess the severity of infection (see section 2.1.5C) and the effect of a control measure on infection.

A. Qualitative examination of infection

A specimen of urine is collected and the centrifuged deposit is examined for eggs using a light microscope. In regions where the majority of the population are infected, single urine specimens may suffice if collected during the peak period of egg output (around 12 pm - 2 pm) (83, 93, 101). The probability of finding eggs in urine increases with the number of urine specimens, per person, examined (101).

B. Quantitation of infection

Qualitative diagnosis of infection is insufficient in clinical trials attempting to study dose-response relationships. Quantitation of haematobium infection consists of egg counts on urine specimens. Egg counting is a highly subjective method, depending on the carefulness and experience of the technician counting the eggs under the microscope. Apart from the factor of observer variation, several factors affect the results of quantifying infection. These will be discussed briefly.

One factor which has engaged the interest of researchers for over three decades is the regularity of urinary egg output. Stimmel and Scott (97) were the first to attempt to find out whether there was any particular time of day during which egg excretion is maximal or minimal. This involved a long series of observations over a period of 44 days in 2 infected individuals. They noted a high variation from specimen to specimen (all urine samples were collected

separately and examined). There was a diurnal variation such that egg excretion was maximal with the noon sample when urinary volume was lowest, and minimal with the first morning sample when urinary volume was highest. This study has been the basis of all the advocation that specimens collected around noon time serve best. However, even Stimmel and Scott (97) noted that further studies with larger samples were needed. Some problems with this study include the small sample size, the fact that these two individuals were no longer residing under conditions of infection and the fact that egg output falls with age. The large variation noted in the study raises the question of whether a single urine specimen can serve as a reliable estimate of the intensity of infection since possibly that single specimen could be the one with a minimal number of eggs. Scott (93) attempted to answer some of these questions by using a larger sample (34 cases) in an endemic area (Egypt). Twenty-eight of them showed evidence of infection. He noted that over the period of the study, 7 cases at one time had no eggs detected in their urine sample. Again, there is the question of using single specimens to quantify infection. He also noted the highly variable egg counts from patient to patient from time to time. Onori (76) also noted the high variability of egg output. Two hundred boys aged 5-15 years were investigated. Timed urine specimens were collected. It was noted that the highest egg output occurred around 2 pm and the lowest egg output was noted in the 9 am specimen. Some more recent studies reached the same conclusion (83,101).

None of these studies mentioned the problem of observer errors

which potentially can contribute to the variability of egg counting. Another apparently important factor is the incubation period. In early maturing infections, egg output is likely to be absent or minimal (29, 91, 101). Any measures of egg output will likely yield negative or low results just because no eggs, or too few eggs, are being passed. The actual incubation period of schistosomiasis is yet unknown, especially when under natural conditions, it is confounded by reinfections.. In a self-infection study carried out by Barlow and Meleney (7), eggs were first noted in urine 106 and 120 days following infection with *S. haematobium*, but Forsyth and Rashid (40) noted it to be as long as 9 months in some cases. However, it is not clear from the study how they arrived at this figure.

Another factor is the duration of infection which has been stated to be as long as 5-10 years, or even longer. Warren and others (99) sutdied Yemeni immigrants to California and noted that *S. mansoni* eggs were still detected in stoool up to 5-10 years after these people had left South Yemen. In situations where superinfections occur, it is likely impossible to identify the duration of any single infection. Also, considering the variability of schistosoma infection, the duration of *S. haematobium* infection may be quite different. The study also noted that even without therapy, egg output decreased with time away from Yemen, although this could be because egg output decreases with age anyway, even in those living in endemic areas.

Egg counting, though liable to many errors, is an important variable with regard to morbidity due to and therapy of schistosomiasis. As such it must be incorporated into any therapeutic study.

Egg counting method must not only be practical, it must be reliable. This design will incorporate some checks to minimize the occurrence of false negatives and to promote the occurrence of intraobserver agreement.

A discussion of the egg counting methods is found in Appendix 1.

2.2.4 Immunodiagnosis of infection

Because of the variability and rather subjective nature of egg detection, immunodiagnostic methods were resorted to in the hope that they may prove more sensitive and accurate. Positive immunological reaction may indicate previous exposure to infection, current infection or merely the presence of antibodies to schistosomal-like antigens or other helminths (47, 54, 91, 100). There are many immunodiagnostic tests involving different antigens and measuring different antibody responses (54, 77, 91, 100). Some examples include the intradermal test (IDT), the complement fixation test (CFT), the indirect haemagglutination test (IHAT) and indirect fluroescent antibody test (IFAT). There are certainly many others. Whilst the IDT requires the individual to be present, it provides immediate results (15-20 minutes). The other tests use serum from the individual. The IDT has variable sensitivity and specificity when compared with parasitological egg detection. Table 3 shows the result of the intradermal test in comparison with parasitological egg detection (80). Cross-reactions are also common. In a developing country

Table 3: Comparison of parasitological egg testing with the intradermal test in the same individuals.

S. haematobium in urine

Intradermal test	Present	Absent	Total
Positive	266	193	459
Negative	20	69	89
Total	286	262	548

If egg detection is taken as standard:

$$\text{sensitivity} = \frac{266}{286} = 93\%$$

$$\text{specificity} = \frac{69}{262} = 26\%$$

The problem with accepting parasitological testing as standard is that the failure to detect eggs in a urine specimen is neither proof of its absence in the specimen nor the absence of infection in the individual providing the specimen.

such as Nigeria, where multiple parasitic and other infections are the rule, cross-reactions will be common and interpretation of results will likely prove difficult.

The serological tests such as the CFT, IHAT and IFAT among others, have been reviewed by Sadun (91). His conclusion was that the IFAT is the most sensitive. Whilst quantity of egg output bears some relationship to the intensity of infection, there is as yet no way of quantifying infection using immunodiagnosis. Although serodiagnostic specimens may be easier to collect and post-therapeutic sological examinations may be positive when eggs are not detected, the non-quantifiable nature of serological reaction and the occurrence of cross-reactions represent some serious disadvantages to the interpretation of results.

2.2.5 Pathological diagnosis of infection

The pathological finding in schistosomiasis is highly variable. The pathological finding has been classified into four phases as shown in Table 1. The finding depends partly on the species of the schistosome and partly on the stage of infection. Studies in living individuals are usually carried out during the stage of established infection when the effects are often irreversible.

Pathological lesions include the earlier finding of bladder congestion, oedema, and tiny ulcerations with development of nodular masses. Later stages show calcifications (believed to be calcified eggs), more extensive ulceration, fibrosis and hydronephrosis (1, 39, 59). Biopsies of similar nodular bladder masses show them to be

granulomatous reactions around schistosome eggs. These masses are observed in children but not in adults in the same geographic region (45). They are responsible for the nodular filling defects noted during radiological investigations. These studies were carried out on asymptomatic patients and it is not ethical to perform radiological examination or bladder biopsies in order to make a firm diagnosis in asymptomatic apparently healthy children.

A World Health Organization Committee on Schistosomiasis recommended autopsy studies in the hope that some correlation may be noted between tissue egg burdens and pathological findings. Tissue egg burdens (defined as eggs per gram of tissue) have been studied by several workers. Smith and others (94) studied 190 unselected Egyptian cadavers and concluded that urinary schistosomiasis disease could be separated into active and inactive disease on the basis of tissue egg burdens. Active disease, characterized by high tissue egg burdens, probably indicated that in life egg excretion was high. Activity rose with age reaching a variable peak around 6-20 years, after which the egg burdens decreased with age. Tissue egg burdens also correlated with pathological findings. Cadavers with high tissue egg burdens tended to have evidence of obstructive uropathy. As tissue egg burdens increased, so did the likelihood of finding hydroureter or hydronephrosis (20, 30, 94, 95, 98). These studies are all hospital-based and need to be interpreted cautiously. The findings may neither be generalizable to living patients nor to other regions with different endemicity. Since these autopsy studies are studies on patients dying in hospital, they are unlikely to have

been representative of the true situation since some selection bias operates to bring certain individuals to the hospital (90). There is also the possibility that a urological lesion, congenital or otherwise, may predispose to some damage in an infected individual or that the association between infection and urological lesion is just an incidental finding. These explanations are unlikely to be true in view of the repeated and consistent associations noted across studies. Despite methodological flaws of study design, there is consistent association of infection with specific recognizable clinical symptoms and pathologic lesions found by a variety of researchers in widely different areas. This lends some support to the hypothesis that schistosomiasis infection should be considered seriously as a cause of significant morbidity and perhaps some mortality and that therapy or some other method of control of transmission should be advocated. Evidence from quantitative egg studies will be discussed under measures of infection.

2.2.6 Conclusion

There are other diagnostic methods such as cystoscopy and renal function tests that have not been discussed in this thesis. They are usually reserved for detailed assessment of individual patients. Clinical diagnosis is not clearly useful. Immunodiagnosis is neither specific nor sensitive. Pathological diagnosis is not usually indicated in individuals studied. Parasitological detection of eggs in urine is a certain diagnosis of infection. However, a

negative parasitological result does not rule out the presence of infection. There is no gold standard to which parasitological findings can be compared. One could consider using two diagnostic method concurrently. As previously mentioned, contradictory results create difficulty in the ascertainment of the truth of infection (see Table 3).

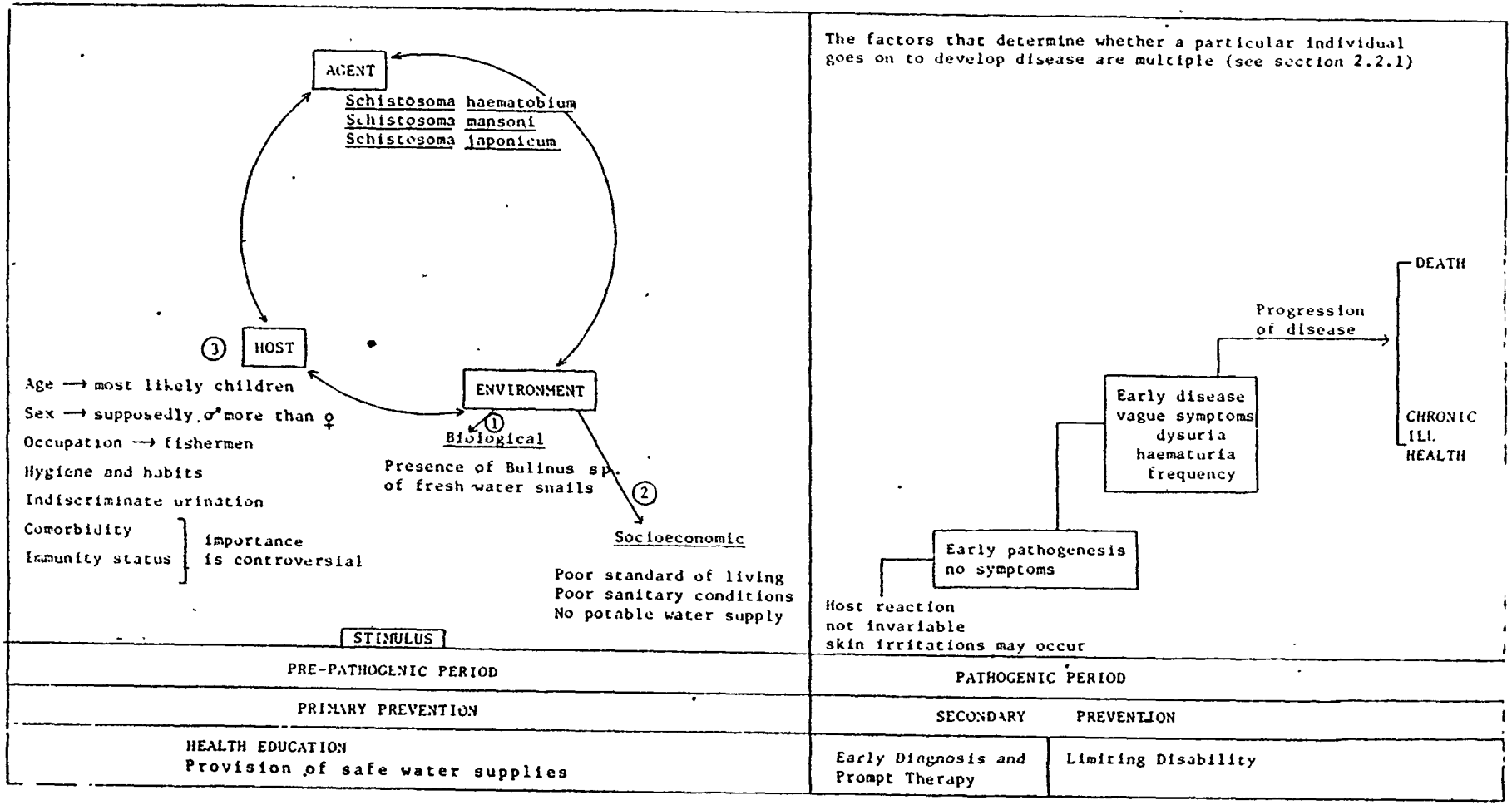
Considering the fact that parasitological diagnosis has been the most exhaustively studied and has been shown to be related to morbidity, it will be selected in this design as the method of diagnosis. Some measures will be used to minimize the variability of results.

Following the diagnosis of infection, either in a particular individual or as a member of a study population, some measure of controlling the infection is usually introduced.

2.3 Control methods

Considering the natural history of communicable diseases, the chain of transmission of infection can be broken at several points (see Fig. 1). The choice of which point to select depends on the particular communicable disease, its magnitude (prevalence) in the area under study, the availability of funds, and the intention of the researcher. In schistosomiasis the transmission of infection involves the aspects shown in Fig. 1. Control methods may be employed at points 1, 2, 3, alone, or in combination.

Figure 1 : NATURAL HISTORY OF SCHISTOSOMIASIS



2.3.1 Molluscicide or snail control

This breakage of transmission occurs at point 1 (see Fig. 1) and it involves the use of one or the other of several mulluscicides. Studies have indicated that snail control is of some effect in reducing the prevalence of infection (4, 23, 109). But the effect is not a long lasting one unless it is combined with another control method such as therapy of infected individuals or provisional safe water supplies. Snail control is complicated by the phenomenon of density dependence; the more snails that are killed, the more quickly the remaining population reproduce. The survivors are more likely to have improved survival. As such, subsequent attempts at control are both more difficult and expensive (15, 109). Snail control has been shown to be "quick and cheap" when carried out on a small scale (4). However, this study reported the immediate results and it is often necessary to wait and see for a period of 2-3 years. Even after this period, infected snails can still be detected (4, 23).

Snail control does nothing for the already infected individual. If one believes that infection can, and does, lead to disease, then snail control has to be supplemented by chemotherapy of infected individuals.

2.3.2 Environmental control

In attempting to reduce the transmission of schistosomiasis, environmental control may be used in two ways: either the provision of facilities that will likely reduce or completely eliminate

contamination of snail-infested water by modifying human activity or the alteration of the environment such that snail hosts can no longer survive since snails require certain environmental conditions for survival.

The first has been tried in Brazil, South Africa and St. Lucia (5, 52, 53, 81). The provision of domestic water supplies and, in some cases, swimming pools, resulted in a reduction in the prevalence of *S. haematobium* and *S. mansoni* infections. In the Brazil study there was a reduction in prevalence of infection over a few years in both experimental and two out of three control villages. In the South African study there was no comparison area, although the prevalence of infection decreased over a 15 year period (81). The St. Lucia study compared five experimental areas and six control areas. The former had pipeborne water supplies whilst the latter did not. Both areas were comparable in population, socio-economic status and prevalence pattern before the study. After a period of two years, it was noted that prevalence and incidence of infection had decreased in the experimental area (52). In the control areas, these measures had been unchanged or even increased in the comparison areas.

Environmental modification, such that unsuitable snail habitats are created, is expensive although it has proven effective in Japan and in the Philippines (78, 109). However, the same method in Ghana was found not to be effective alone; it had to be combined with a molluscicide (23).

The advantage of improving water supplies is that it has certain fringe benefits. Faeco-orally transmitted infections such as

ascariasis, taeniasis and sallmonellosis will likely show a downward trend. These infections have not been studied specifically in relation to schistosomiasis control. The effect of environmental control is not likely felt immediately. Prevalence will reduce rather slowly and, unless it is combined with chemotherapy, it has no effect on already infected individuals.

2.3.3 Control using antischistosomals

With the advent of effective and relatively safe antischistosomals, several attempts have been made to control transmission using therapy of infected individuals (22, 25, 36). Although therapy does take care of infected individuals and, in some cases, results in reversal of lesions observed radiologically, its effect on transmission is limited (33, 60, 61, 63, 109).

Attempts to use drugs as a control method have shown that alone it is not effective (25, 36, 109). Some of the treated individuals were passing eggs again a year later. Also, chemotherapy has a better effect in areas where prevalence of infection is high initially (defined as greater than 20%) (25, 50). In areas with low prevalence, the effect is not clearly obvious from the point of view of the community. The treated individual benefits from therapy.

In St. Lucia all three methods have been tried in the control of *S. mansoni* infections (22). A comparison of the results indicated that environmental control and molluscicide are most expensive. However, although chemotherapy proved cheapest, it required a lot of

cooperation from the individuals to be treated. As mentioned earlier, its delayed effect on transmission is not really known. However, even before large scale chemotherapy can be advocated, the effective doses need to be determined for different areas (92). This is partly because of the variable and rather complex transmission patterns of schistosomiasis and the fact that response to the same dosage of drugs has been shown to vary in different countries (25). For example, oxaminiquine in therapy of *S. mansoni* in Brazil is effective at a dose of 15 mg/kg body weight whilst up to 60 mg/kg body weight needs to be used in several parts of Africa (92). In Tanzania 7.5 mg/kg body of metrifonate in 3 doses was effective in achieving cure of *S. haematobium* infections whilst the same therapeutic regimen did not achieve cure in a study by Reddy (88) in Nigeria.

2.4 Issues in chemotherapy

This discussion will primarily involve chemotherapy of urinary schistosomiasis. The drugs that have been used for therapy of urinary schistosomiasis include niridazole (ambilhar), metrifonate (bilarcil), hycanthone, lucanthone, and oxaminiquine and antimonials. Presently, therapy of schistosomiasis utilizes niridazole and metrifonate. The assessment of dosages, side effects and effectiveness of a drug should be based on well conducted randomized clinical trials especially since the outcome of the infection is not uniformly terrible. Table 4 indicates some of the studies that have been conducted with regard to therapy of urinary schistosomiasis and that report their results in some detail. As will be noted, metrifonate has been used most frequently in recent years.

The basis for its use is the trial of Davis and Bailey (28) in Tanzania. Several issues are important to any chemotherapeutic agent, especially one to be offered to apparently healthy individuals. On the basis of the studies reported, metrifonate seems the best, followed by niridazole, although concerning the latter, fewer studies are being conducted since the discovery of metrifonate.

Niridazole has one advantage over metrifonate. It is effective against both *S. haematobium* and *S. mansoni* infections. However, several aspects of both drugs need to be mentioned briefly. A World Health Organisation committee on schistosomiasis recommended that a satisfactory antischistosomal should be easily administered for a short period of time, should be free from side effects and should produce a high rate of parasitological cure. Metrifonate fulfills these criteria fully whilst niridazole fulfills some of the criteria (109).

2.4.1 Therapeutic effects

The aim of curative therapy is the destruction of all adult worms. This should result in the cessation of egg passage in the urine. Curative therapy is not always successful and egg passage will occurs though in reduced numbers. The conclusion that cure has occurred follows the failure to detect eggs in urine following therapy. Negative urine specimens may be due to careless examination, urine collected during non-peak periods or the fact that too few eggs are being passed. A cure rate could then be calculated for a

particular regimen (expressed as the proportion of cures at a particular point in time following therapy). The differences in cure rates obtained by different workers is related to the difference in the magnitude of the egg output of the individuals in these studies.

It is also possible to identify a percentage reduction in egg output. It has been noted that at least six months following therapy the percentage reduction in egg load is constant and independent of initial egg load whilst cure rates vary across groups (see Fig. 2) (50).

Figure 2.

Relationship between egg output, cure rate and percentage reduction in egg output.

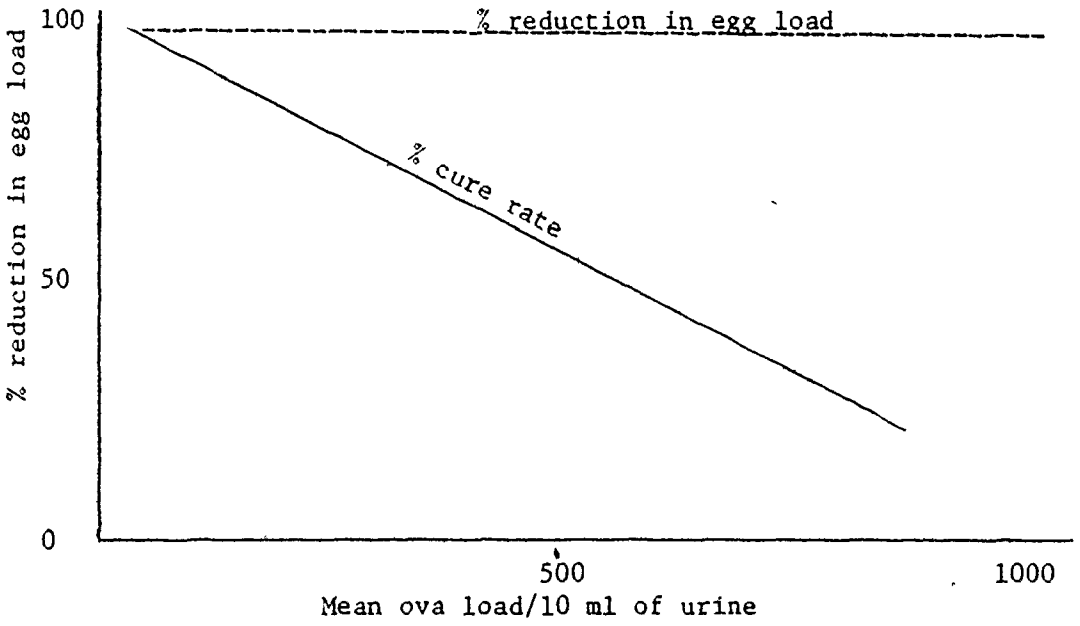


Fig. 2 indicates the dependence of cure rates on the initial egg output. It has been suggested that perhaps the percentage reduction in egg output should be used in the evaluation of drug trials instead of the cure rate. The poor cure rate noted in individuals with high egg counts does not necessarily mean that heavy infection is more difficult to treat than light infection. It may just be that cases excreting few eggs are more difficult to prove positive. Cases with large amounts of eggs in urine have a greater chance of having eggs detected in the urine. Cure rates will therefore be lower when egg output is higher. Fig. 2 was based on a single study which attempted to compare cure rates with percentage reduction in egg output. Whilst cure rates have been studied in several therapeutic trials, the percentage reduction in egg output has not been evaluated. For this reason cure rates rather than the percentage reduction in egg output will be utilized in this design.

2.4.2 Metrifonate in urinary schistosomiasis

Metrifonate is an organophosphorus cholinesterase inhibitor which was developed as an insecticide (19, 28, 88). It has been found to have some therapeutic activity against hookworm, ascaris, trichuris and intestinal schistosomiasis (21). Most studies, however, pertain to its use in urinary schistosomiasis as seen from Table 4. The trials by Davis and Bailey (28) have established that a dose of 7.5 mg/kg body weight fortnightly for 3 doses is effective in achieving an 83% cure rate. This study was a randomized trial which, although did not stratify for pretreatment variables, did examine the post-trial

Table 4: Antischistosomal Drug Trials

Reference: (Year of Study) Country of Study	Study Design and Sample Size	How Sample Generated	Drug/s	Dosage	Age of Study Group	Any Stratification Variable	Ease of Administration	Side Effects	Other Comments
9: (1979) Not indicated	Before-after study N = 17	Hospitalized patients admitted for severe bloody diarrhoea	Niridazole	12.5 mg/kg body weight	11-38 years	All males	Not indicated	Typical eosinophilic syndrome	Small sample size, highly selected group of study subjects. Non-randomized trial
27: (1969) Tanzania	1) Randomized trial into 3 groups N = 212 69 71 72 Randomization process clear No untreated controls	Pretrial screening for infected individuals (1110 screened)	Metrifonate (Trichlorophone)	5 mg/kg body weight 7.5 mg/kg body weight 10 mg/kg fortnightly maximum of 3 doses	Not indicated	Variables examined at the end of study -> sex -> weights -> miracidial counts	Oral Compliance ensured by researcher	None -> 3 doses failed to cure some high intensity infections	7.5 mg/kg seemed to achieve more cures. No miracidia in urine on 3 successive days at 2, 4, 6 months after therapy. This is the basis of most other studies advocating 7.5 mg/kg body weight as standard
	2) Randomization into 2 groups N = 69 35 34 No untreated controls	Pretrial screening	Metrifonate	10 mg/kg body weight 15 mg/kg body weight	Not indicated	-> sex -> weight -> miracidial counts	Oral	Abdominal pain, nausea, diarrhoea, vomiting. (No medication was required for these side effects)	Either at fortnightly or monthly intervals.

able 4 (continued)

eference: Year of Study) ountry of tudy	Study Design and Sample Size	How Sample Generated	Drug/s	Dosage	Age of Study Group	Any Stratification Variable	Ease of Administration	Side Effects	Other Comments
O: 1967) anzibar	Quasiexperimental study N = 76 n = 6 (community) n = 12 (college 1) n = 24 (college 2) n = 20 (college 3) n = 14 (not stated) n = 6	Grab samples from various colleges and community	Metrifonate	15 mg/kg body weight 10 mg/kg body weight 10 mg/kg body weight 7.5 mg/kg body weight 7.5 mg/kg body weight 5 mg/kg body weight 7.5 mg/kg body weight	Not indicated	All males	Not clear from article	Not stated	Mix-up of treatment doses. Decrease in plasma cholinesterase levels following therapy.

Table 4 (continued)

Reference: (Year of Study) Country of Study	Study Design and Sample Size	How Sample Generated	Drug/s	Dosage	Age of Study Group	Any Stratification Variable	Ease of Administration	Side Effects	Other Comments
48, 49: (1977) Rhodesia	Randomized placebo controlled trial using Table of Random numbers to obtain 5 groups N = 218 into 5 groups (unequal) A B C D E 66 62 56 17 17 control	Pretrial screening to separate infected from uninfected children	Metrifonate	A→ T + 7.5 mg/kg body weight; 4 weekly for 6 months B→ No therapy or prophylaxes C→ T only D, E: Non-infected children D on prophylaxes E not on prophylaxes	3->15 years	According to age, sex, home location, weight	Oral Compliance ensured No changes in packed cell volume, problems-------→	None were noticed. Plasma cholinesterase values were depressed but no cumulative effect was noted.	Trial of prophylactic activity. Attempted to blind the observers as to which individual belonged to what group. Some were lost to follow-up.
55: (1978) Nigeria	Stated as random allocation N = 57 19 19 19 3 groups But process of randomization was unclear.	Not stated	Oxaminiquine	15 mg/kg twice daily for 2 days 10 mg/kg twice daily for 3 days 10 mg/kg three times daily for 6 doses	7-14 years	Egg counts	Oral	None found	No effect on egg counts.

Table 4 (continued)

Reference: (Year of Study) Country of Study	Study Design and Sample Size	How Sample Generated	Drug/s	Dosage	Age of Study Group	Any Stratification Variable	Ease of Administration	Side Effects	Other Comments
60: (1974) Egypt	Quasiexperimental N = 200	Hospitalized patients	Niridazole to 51 patients Hycanthone to 7 patients Sodium Antimony dimercaptosuccciate to 23 patients Sodium Antimony tartrate	25 mg/kg daily for 5-7 days 3 mg/kg body weight in 1 dose 2 mg/kg body weight in 5-6 doses	5-52 years	All males	Oral	None was reported	Non-randomized trial Inconsistent therapy was offered. There was no basis for treating any patient with a particular drug. Difficult to replicat study since selection of treatment was arbitrary.
72: (1974) Rhodesia	Quasiexperimental N = 100 pts N = 100 pts N = 200 pts	Not indicated	Metrifonate	8 mg/kg for 2 doses	7-14 years 13-17 years	Intensity of infection	Oral	None stated	Follow-up less than 80%.
75: (1978) Sudan	Before-after study N = 174	Not stated	Metrifonate	12.5 mg/kg body weight for 12 days	6-14 years and men 15-50 years	None	Oral	Abdominal pain and nausea	Achieved a "high cure" rate. No eggs detected in a single urine examination.

Table 4 (continued)

Reference: (Year of Study) Country of Study	Study Design and Sample Size	How Sample Generated	Drug/s	Dosage	Age of Study Group	Any Stratification Variable	Ease of Administration	Side Effects	Other Comments
88: (1974) Nigeria	Descriptive study N = 46 at end point N = 38	Pretrial investigation	Metrifonate	7.5 mg/kg body weight in 3 doses	8-13 years	Only children with egg counts greater than 250/ml urine were selected	Oral	"Mild abdominal pain" Abdominal discomfort	Lower cure rate than that noted by Davis and Bailey (only 44%) but expressed as % reduction was about 92%. Egg count remained low up to 18 weeks after therapy.

data to determine whether important variables in the treatment groups were significantly different. There are, however, widely varying differences amongst cure rates in different study areas. Reddy and others (88), in Nigeria, noted a cure rate of only 44% using the same dosage schedule. Their study was a before-after study of 46 children who had very high urinary egg output. Jewsbury and Cooke (48), in a randomized trial, noted a cure rate of 59.3%. Also, they noted that the effect of therapy could be expected to last as long as three months, after which reinfection occurs. The dosage in this trial was 7.5 mg/kg body weight as suggested by Davis and Bailey (28). However, these differences in results were explained on the premise that urinary egg outputs were different in all these areas.

As to the argument that since morbidity and/or mortality occurs only in a small percentage of infected individuals, only disease should be treated, there is the counter argument that since metrifonate does more good than harm, then infected individuals should be treated regardless of the absence of obvious disease (57, 59, 63, 99). Considering the fact that therapy has been shown to result in reversibility of lesions and that urological lesions do occur in apparently healthy individuals, effective therapy should be offered (35, 36, 60, 61, 63, 74). If therapy is to be offered to apparently healthy individuals, then the therapy must be safe and free from side effects which may be worse than the infection. Metrifonate has been shown again and again to have no serious side effects (see Table 4). However, what has been noted is that a depression of plasma cholinesterase constantly occurs in all treated individuals (28, 40, 49).

Measurement of plasma cholinesterase in treated individuals indicates that this depression is temporary, does not affect response to therapy and normal pretreatment levels occur before the next dose in two weeks (28, 49). The significance of depressed cholinesterase in the individual is not really clear. It has been suggested that suxamethonium (a muscle relaxant used as a preliminary to general anesthesia) should be avoided when an individual is on metrifonate (49). In higher doses, nausea, vomiting, bronchospasm, abdominal discomfort, and diarrhoea may occur and it has been suggested that perhaps metrifonate should not be administered in individuals with a history of bronchospasm (28, 69).

Claims of the cheapness of metrifonate always follow the discussion of results of studies. What is apparent in all of these published studies is that metrifonate was obtained free from Bayer Drug Company for the trials. There are no formal cost studies to support or refute this claim.

Metrifonate certainly fulfills the requirements of a satisfactory antischistosomal. It can be administered orally for a period of six weeks, has relatively few side effects and causes a reduction in egg output. Dosages of metrifonate have been established more scientifically than dosages of niridazole. It will therefore be accepted as standard practice against which niridazole will be studied.

2.4.3 Niridazole in urinary schistosomiasis

It has been stated that "niridazole orally administered is the drug of choice, particularly for children and adolescents who tolerate it well" (109).

Suggested dosage has been variable, depending on the studies; 25 mg/kg body weight daily (3 doses) for 7 days or a variation of 20 mg/kg body weight (3 doses) x 10 + phenobarbitone (74). Bassily and Farid (9) noted that a dose of 12.5 mg/kg body weight for 12 days resulted in less side effects. This was a before and after study of 17 patients. The dose selection was arbitrary and the outcome of treatment was not stated.

A ranking of properties of niridazole and metrifonate by an expert committee on schistosomiasis is shown in Table 5. This comparison shows that metrifonate ranks a little higher as far as *S. haematobium* infections are concerned.

2.4.4 Conclusions

Metrifonate at 7.5 mg/kg body weight fortnightly for enough doses to achieve cure will be studied along with niridazole at varying doses. Efficacy of therapy will be related to parasitological examination.

Although therapy of schistosomiasis using metrifonate is recommended, it is often the variability of response that may be discovered. The results of administering therapy to hospitalized patients should really not be applied to non-hospitalized presumably

Table 5.

A Ranking of Properties of Niridazole and Metrifonate.

Property	Type of Schistosomiasis	Metrifonate	Niridazole
Therapeutic efficacy: Cure (a)	*S. japonicum*	unknown	++
	S. mansoni	poor	+ - ++
	S. haematobium	++ - +++	++ - +++
Estimated population coverage (b)		++ - +++	++ - +++
Estimated population tolerance (c)		+++	++ - +++
Mode of administration		++	++
Cost (d)		++ - +++	++
Overall ranking (sum of ranks for individual properties)	*S. japonicum*	not applicable	12
	S. mansoni	not applicable	12
	S. haematobium	14	13

*Throughout +, ++, +++ signify ranks 1, 2 and 3 respectively.

(a) + = 0 - 40%; ++ = 40 - 75%; +++ = >75%.

(b) Varied markedly with age: + = 0 - 33%; ++ = 34 - 66%; +++ = 66%.

(c) Varies markedly with age: + = poor; ++ = moderate; +++ = good.

(d) ++ = moderate cost; +++ = low cost.

(Taken from Ref. 109)

healthy school children. This thesis proposes to identify dosages using a randomized clinical trial. In conducting clinical trials, several issues in methodology should be considered. These will be discussed in the subsequent chapter.

CHAPTER III

RESEARCH PROPOSAL

3. Randomized Clinical Trials

Prior to the discussion of the design of the proposed study, several issues relating to randomized clinical trials will be identified. A randomized clinical trial has been defined as "an experiment as applied to clinical medicine. In it a drug, a surgical operation or other therapy is applied to patients and the outcome is compared with that observed in a suitable control group. It is essential that alternate therapies be evaluated in a well-controlled fashion using whenever possible, the techniques of random allocation and blind assignment and assessment" (42).

3.1 Methodology of clinical trials

Several issues affect the validity and interpretation of trial results. These include:

3.1.1 Study Population

Any population to be studied should be characterized properly with regard to clinical and demographic features. If a sample is to be selected, the method of generating this sample should be identified.

This enables readers to determine the relevance of the study to their own patients (89).

3.1.2 Prognostic stratification

Any factor known or suspected as having an influence on target trial outcomes should be used as a stratification variable prior to randomization. Such a factor could be demographic or could be a characteristic of the disease or condition being studied (89). This stratification also allows treatments to be evaluated within subgroups (89).

3.1.3 Random allocation of study subjects

The assignment of subjects to the different treatments must be random, not haphazard. A table of random numbers or a random number generator are some methods that could be used. This gives each member of the study population an equal chance of being assigned to a given treatment group. The possibility of a selection bias on the part of the researcher is also removed (43, 89).

3.1.4 Precise definition of the maneouvre

The administration of therapy should be described in detail. This description should permit replication of the therapy. Details should include doses administered and method of administration with the inclusion of any need for adjustment, monitoring of side effects

and treatments or other medications avoided (89, 90).

3.1.5 Contamination

The inadvertent administration of a therapeutic maneouvre designed for one group to another group should be carefully avoided (89).

3.1.6 Co-intervention

In order to assess the effects of treatments, all groups must be given the same care and attention. Additional screening, diagnostic or other maneouvres must be avoided unless they are carried out equally in all groups (44, 89).

3.1.7 Comorbidity

Any factor, such as coexistent disease or condition, known to affect trial outcomes must be considered in the prognostic stratification prior to randomization (89).

3.1.8 Compliance

Trial results should identify the extent to which study population follow therapeutic instructions. Trial outcomes should be reported for both compliant and non-compliant subjects (43, 89).

3.1.9 Diagnostic criteria

The criteria for the determination of trial outcomes must be so clear and detailed that they can be evaluated by others (89).

3.2 Some biases in clinical trials

Besides problems of methodology, several biases may be operative in the execution of clinical trials. Some of these which need to be avoided in this design are discussed. A bias is defined as "a process at any stage of inference tending to produce results that depart systematically from the true values" (70).

3.2.1 Measurement biases

(a) Diagnostic suspicion bias.

The intensity and outcome of the diagnostic process may be influenced by a knowledge of the subjects' prior exposure (90).

(b) Instrument bias.

Defects in the calibration or maintenance of measuring instruments may lead to systematic deviations from true values (90).

(c) Expectation bias.

Observers may alter their measurements so that results coincide with prior expectations (90).

3.2.2 Treatment biases

(a) The personal preferences of observers for particular treatment or allocations might bias results such that outcome measures are favourable towards or against preferred treatment (90). For example, if all the sick people demand the new treatment.
(b) The number of individuals administering therapy might influence trial results if there are large variations between them in adhering to protocol (90).

The foregoing discussion will help to clarify certain aspects of this research proposal.

3.3 Introduction to research proposal

This proposal is a randomized clinical trial designed to determine optimal doses of niridazole in the treatment of urinary schistosomiasis in school children. Niridazole is tested against a standard dose of metrifonate.

Metrifonate is believed to act both on the mature and immature stages of the schistosoma parasites by cholinesterase inhibition (69). One result is a reduction in egg output in the majority of treated individuals. In others no eggs can be detected in urine (28). Following treatment and a reduction in egg output, radiological appearances are known to change towards normalcy (63). Metrifonate is effective only in *S. haematobium* infections.

Niridazole acts on adult worms of all three schistosome

species. Egg production is inhibited and this shows up in the inability to detect eggs in urine of treated individuals (69). When eggs are detected, they are markedly reduced in numbers (28, 50, 69).

3.4 Justification of the trial

The spectrum of infection is highly variable in schistosomiasis. Some individuals have evidence of heavy infections and remain asymptomatic. Others have symptoms and complications (31, 62). The treatment is to be offered to apparently healthy school children with evidence of urinary schistosomiasis in an area in which there is opportunity for reinfections. The treatments to be offered, though shown to be effective, have potentially serious side effects. In some treated individuals side effects may necessitate discontinuation of the drugs (27, 28, 69).

Nigeria is a developing country with many pressing problems, some of which are related to communicable infections and diseases such as schistosomiasis. Other economic problems include poverty, inadequate water supply, poor housing, poor roads, under-nutrition, malnutrition and more.

In view of these other needs, mass screening and/or therapy for urinary schistosomiasis are unlikely in the near future.

Any treatment for urinary schistosomiasis is also likely to be repetitive since there is opportunity for reinfections. If one is to offer potent and potentially harmful drugs to apparently healthy

children (especially repetitively), one must be certain that the safest doses are identified. The same factor is applicable in sick, symptomatic individuals and should be even more important in healthy individuals. The randomized clinical trial, if properly executed, will contribute pertinent information regarding dosages and outcomes which before and after studies and case reports will not contribute conclusively.

3.5 Justification for selection of metrifonate and niridazole

In the treatment of urinary schistosomiasis several drugs may be used. These include antimony sodium tartrate, stibocaptate, lucanthone hydrochloride, tartar emetic and the two trial drugs, niridazole and metrifonate. Antimony sodium tartrate is one of a number of antimony compounds that could be used. It is difficult to administer (requiring hospitalization of the subject for an intravenous infusion). Continuous cardiovascular monitoring of the treated individual is essential (69). Stibocaptate is preferable to antimony sodium tartrate. It is administered intramuscularly and most treated subjects do not complete the course of treatment. It must be used soon after being dissolved in water for injection since it is unstable when dissolved. Tartar emetic requires hospitalization of subjects for treatment. It is administered intravenously. Niridazole and metrifonate can be orally administered. The side effects which have been noted are transient. There is no need to hospitalize the subjects. There is no problem with storage since both drugs are supplied in tablets which are easily stored and are relatively stable.

3.6 Trial objective

This trial will attempt to clarify several issues regarding therapy of schistosomiasis. This attempt will likely enable one to make generalizations concerning a safe and effective therapeutic regimen.

3.6.1 Research questions

Primary research questions.

(1) What are the parasitological responses to certain dosages of metrifonate and niridazole among a group of Nigerian primary school children aged 5-15 years?

(2) What dose or doses achieve maximal egg suppression with minimal side effects?

Secondary questions.

(1) What is the prevalence of urinary schistosomiasis in the study population?

(2) At the end of the study period, what is the incidence of infection and reinfection?

3.7 Choice of study design

Since outcome of infection is not a uniformly catastrophic one, there is no need for more case reports, before and after studies and trials with questionable design. A randomized trial is

necessary in order to determine effective doses in this group of children, especially because doses have been so variable across studies.

3.8 Study Procedure

3.8.1 Selection of study subjects

A. Sample source. The potential subjects for the study sample will be school children aged 5-15 years who have fulfilled the eligibility criteria. There are four primary schools in Usi-Ekiti (the region of proposed trial) with an overall student population of 2,000 pupils. The age range has been given to be between 6-15 years in the primary school. There are more males (55%) than females.

B. Sample selection.

(1) Pretrial activities. During a one-month period prior to the pretrial survey, the coordinator will obtain permission to carry out the trial from the king of the town. The usual process is to meet with the community leaders and let them know about the proposed trial. Consent on their part is usually representative of consent from the king. Several issues need to be identified during the meeting with the community leaders. All these points will also be written in Yoruba and distributed to the community leaders. These issues include the following:

(i) the study will involve collection and examination of urine specimens from school children for the diagnosis of an infection

caused by a worm and for the follow-up of the treatment to be offered.

(ii) Infected children will be offered one of four treatments.

(iii) These treatments have been shown to be effective when given to children with the infection. Some further information is necessary in order to determine the best treatment or dose of treatment. This study is being carried out in order to obtain this information.

(iv) The treatment is in the form of tablets to be swallowed and it will only be given to the children after informed parental or guardian consent.

(v) Non-participation in this study will neither jeopardize a child's education nor prevent his* or his* family's utilization of the available health services.

It is unusual for permission not to be granted for health-related studies in communities such as Usi-Ekiti since any interest in the health of members of the community is deemed to be beneficial. Whilst awaiting the king's response the coordinator will meet with the heads of schools and their assistants. The information communicated to the community leaders will also be communicated to them regarding the proposed study. Furthermore, they will be informed

---

*refers to male or female child.

that an attempt will be made to minimize the disruption of the school schedule.

It is hoped that this information period will be completed within one month. No formal consent form will be necessary for the initial urine collection which will be used to identify infected children. The estimated school population is 2,000 children in four schools. Initial urine collection will be carried out during an eight week period comprising a five-day week. Each school will be surveyed by two laboratory assistants during a two-week period. Urine collection will be carried out between 11 am and 2 pm since this period is regarded as the peak period of egg output (83, 93, 101). It has been estimated that it takes approximately 2 minutes to process each child by 2 individuals once the children are gathered together in one place. It is believed that the actual hours set aside for urine collection are sufficient. A third laboratory assistant will be involved with handling the specimens in preparation for transportation to the central laboratory. All daily samples will be driven over to the laboratory for examination. In the hours not allocated to urine collection, the laboratory assistants will prepare containers to be used for urine collection. With the help of class registers, prepasted labels will be prepared bearing name, class and an identification number which is unique to each individual. Children will be gathered by classes in order to facilitate urine collection. Body weights will be taken on a balance beam scale with children in usual school uniform and barefoot just before each child is asked to provide urine sample. Girls will be gathered first and urine samples

collected from them as they emerge from the washroom. One assistant ensures that each child receives a 250 ml reusable calibrated container. A second laboratory assistant records the volume, colour and identification number of urine samples. The colour will be recorded as yellow, pink or red. The identification number is important since this will be used to identify potential study subjects. An attempt will be made to obtain urine samples from all school children. Non-compliant children will be documented and where possible, the reasons given for not providing samples.

Results of the pretrial survey are expected within one week of its completion. Advance notification will be sent to parents/guardians of potential study subjects. Appendix II represents the English version of the note which will be written in English and Yoruba. Yoruba serves as the language of communication in the community. It is spoken by all adults and children, written and read by some children and most adults. Although English is the official school language, it is unlikely that children in the lower classes are able to read, write or speak English. The proportion of adults who can communicate in English is small in comparison to those who can read and write Yoruba. The actual or relative sizes of these proportions have not been documented in this community.

There are advantages and disadvantages of advance notification in a survey or study. Some reports show that the element of surprise is removed and that it serves as evidence that the interview is a legitimate one. It is believed that cooperation is enhanced (33, 96). It has also been noted that there may be a negative effect (18).

The potential respondent may decide not to participate as a result of the information received in the advance note. The advance note will be utilized in this study (see Appendix II).

A questionnaire will be administered to the parent or guardian of all potential study subjects. However, only children classified as having moderate or severe infections will be selected for drug therapy. The questionnaire will be designed to identify the presence of certain symptoms reported to be associated with schistosomiasis and also symptoms that may contradict the use of the antischistosomals. The questionnaire is shown in Appendix III. During the period of the interview, the consent form will be explained to the parent or guardian of an otherwise eligible child. The consent to administer therapy will be obtained at this time. It is expected that in the early period of the interview, certain responses would have identified ineligible children. Only those eligible will be given the informed consent information to read. Those unable to read either Yoruba or English will have the study explained to them. The consent form will be presented for signature or thumbprint if parent or guardian gives permission for child to participate in the study (see Appendix IV). All questionnaires will carry the identification number of each child.

(2) Eligibility criteria.

(i) When a positive urine sample is identified and egg counts are done at a later date on three urine samples, counts classified into moderate or severe categories of infection will be selected. Individuals with these counts are eligible for the trial. The classi-

fication selected is shown below.

Intensity of Infection

	LIGHT	MODERATE	SEVERE
Eggs per ml of urine	1-10	11-100	>101

(ii) The child must be aged between 6-15 years. This is the period when children are likely to be attending primary school. It is also the period of greatest benefit from treatment (32, 60, 61, 63). Since birth certification is not mandatory, it will likely prove difficult to correctly ascertain age of a child. Only children whose parents did not provide a birth certificate or remember some important event around their time of birth require some estimate of their age. These children will be assigned an age which is expected for his class at school. Since school begins when a child is 6 years old, a class three child should be 8 years old and will be taken as such.

(iii) The child should appear healthy. Inspection will usually reveal children with severe malnutrition (mostly the younger age groups). Diagnosis of severe malnutrition is usually one of pattern recognition of an underdeveloped child with enlarged abdomen, apathetic appearance, silky hair and pedal oedema. Examination of the mucous membranes (eyelids and oral mucosa) may reveal pallor as some evidence of anaemia. The inherent insensitivity of this method is recognized but in the absence of actual haemoglobin determination,

it is the simplest method.

Some of the children eligible to be in the trial may become sick prior to the administration of the appropriate medication. Symptoms such as fever, vomiting and diarrhoea are quite common. The usual treatment involves antimalarials, aspirin and antidiarrhoeals. This will be offered appropriately. Antischistosomal therapy will be given when the symptoms subside. However, persistence of one or more symptoms beyond a one month period will require further investigation. This will be offered and the children so identified will be dropped from the trial but will be offered the standard treatment.

The female children will be asked concerning menstrual history. Any female who has missed a menstrual cycle will be excluded till the next month. If the cycle returns, she will be included in the study. If the missed cycle continues, she will be completely excluded from the study. This is because of a possibility of an early pregnancy. Drugs which have not been known to be safe during pregnancy are best avoided.

There should be no evidence of anaemia (pallor of mucous membranes). One cause of anaemia is hookworm infection. This is more important in older children (30). Metrifonate is known to have some effect in hookworm infection (32). In a child with conicident hookworm infection and schistosomiasis, it is possible that a lack of reduction in egg output in schistosomiasis is due to the fact that the drug's action is divided between the hookworms and the schistosomes. There are certainly other causes of anaemia such as iron deficiency, folate deficiency and frequent malarial infections.

Children who are excluded on the basis of pallor will be offered standard therap but will be ineligible for the trial.

There should be no previous history of jaundice (yellowness of the eyes), epilepsy or febrile convulsions. Niridazole has been observed to have serious side effects in individuals with impaired liver function (69). Neurological side effects following niridazole treatment occur in children and it might be difficult to decide whether neurological side effects are due to therapy or to an unrelated febrile convulsion or epileptic fit.

The examination of potential study subjects will be carried out by the study coordinator during a one-month period prior to the actual trial. The community interviewer will administer questionnaires to those subjects who have been classified as eligible following the examination. The examination will take place at school in the head-master's office.

(iv) The children must reside within the geographic area of the study. This is estimated to be no more than 7-8 miles radius of the town hall. The inclusion of a geographical limitation is based on the premise that any infection detected is more likely to have been contracted in the study community. Also, should the need arise, it will be easier to contact study subjects.

(v) Informed parental or guardian consent is essential. This will be obtained at the time of administration of questionnaire (see Section 3.8.1B and Appendix IV).

3.8.2 Stratification

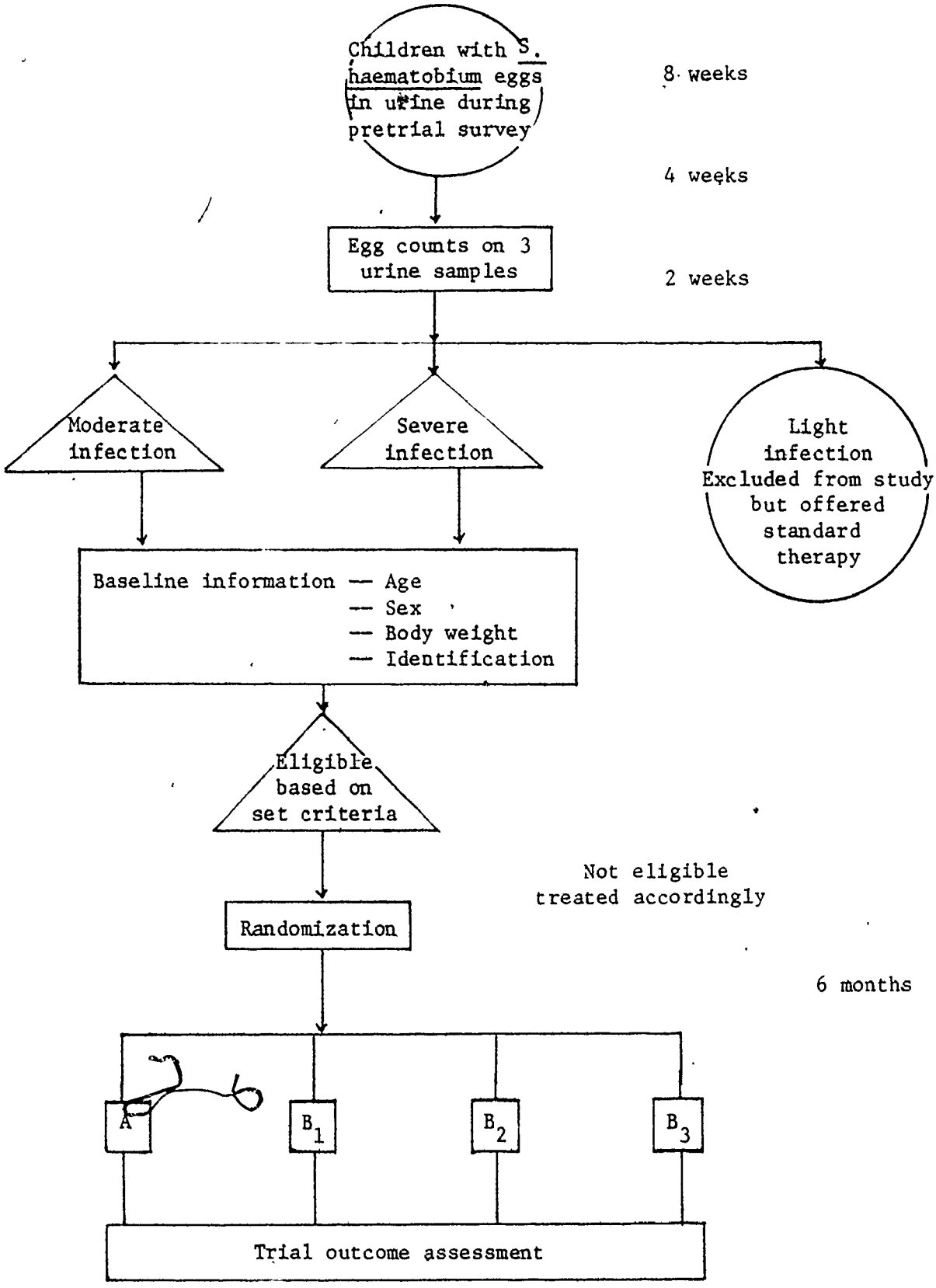
The intensity of infection as measured by the urinary egg output is a factor believed to affect response to therapy (48, 88, 106). The intensity of infection will be used as a stratification variable. According to the classification previously stated, there are three possible strata, light, moderate or severe. Children with moderate and severe infections will be included in the study. This will identify two strata.

3.8.3 Randomization

The randomization process should ensure that members of each stratum are represented in the treatment groups. The treatments are A (7.5 mg per kg body weight of metrifonate for three fortnightly doses), B_1, B_2 and B_3 which are respectively 12.5 mg/kg body weight, 17.5 mg/kg body weight and 22.5 mg/kg body weight of niridazole for five days. The randomization process should also ensure that treatment groups are of equal size and that no particular stratum is over or under represented (see Figure 3).

A table of random numbers will be used to allocate treatments. Prior to the actual administration of the drugs, the allocations will be prepared in envelopes. The treatments will be balanced after every fourth allocation. Treatments are to be administered under the supervision of a nurse. The amount of drugs depends on body weight. It is not possible to prepare the drug ahead of time of first administration. Each envelope will be drawn with a treatment group written on it. The next eligible subject will be in that treatment group.

Figure 3: A flow diagram indicating the selection of study population, stratification and randomization.



It is not possible to blind staff as to what treatment group a child belongs to once this group is initially established.

3.9 Treatment

This trial will compare three dose levels of niridazole against 7.5 mg/kg weight of metrifonate. The latter has been studied more exhaustively and in randomized trials (28, 48) than niridazole. Niridazole has one advantage over metrifonate, in that it has some effect against *S. mansoni* infection. Although the aim of this study is primarily the treatment of *S. haematobium* infections, it is unlikely that niridazole will not benefit those with multiple infections.

3.9.1 Metrifonate

A dose of 7.5 mg per kg body weight of the drug will be administered orally by study nurse. At this time the next two doses of the drug will be kept in an envelope with the child's identification number. At subsequent doses other research staff can administer this therapy without meddling with weights and dosages. The drug is packaged as tablets of Bilarcil (R) by Bayer A.G. in West Germany. A single daily dose will be administered for three doses. Each administration is separated from the other by an interval of two weeks. At time of second administration, a record of symptoms of side effects of the drug will be kept. These will be discussed with the coordinator and a decision will be reached regarding the discontinua-

tion or otherwise of treatment.

3.9.2 Niridazole

Three doses of niridazole will be tested against metrifonate. Niridazole is administered orally in tablet form. Each dose will be calculated to the nearest 25 mg. It is packaged as Ambilhar (R) by CIBA Laboratories in Switzerland. It is believed that niridazole is best administered in divided doses, either in two divided doses (27, 106) or in three divided doses (40, 61). The advantage of one over the other is not clearly apparent and two divided doses will be administered in this trial.

B_1

A dose of 12.5 mg per kg body weight of niridazole will be administered between 8-9 am and the second dose between 3-4 pm. The coordinator will supervise the administration of drugs.

B_2

A dose of 17.5 mg per kg body weight of niridazole will be administered in two divided doses for five days.

B_3

A dose of 22.5 mg per kg body weight of niridazole will be administered in divided doses for five days. Again, at the initial drug administration, the dose requirement of each individual will be

recorded on envelopes with the specific identifying number. A child's name is checked against his number and then his envelope is taken and his drug administered.

In order to minimize the possibility of contamination so that children randomized to receive niridazole do not get metrifonate and vice versa (see Section 3.1), each study subject will be required to carry a card with his identification number. The card will be left in the school premises in the subject's desk. It is to be presented when therapy is to be administered and when urine samples are provided. Misplaced or lost cards will be replaced by new ones after proper identification of the child and a crosscheck with the number and name in a master register.

All children will receive some reward for their participation in the study. The reward will include items such as erasers, pencils, pens, small notepads and other small school supplies.

3.10 Outcome measures

The most important measure is a cure. It will be divided for analysis purposes into short-term cure and a long-term cure. A short-term cure is defined as the absence of viable eggs in three consecutive urine specimens taken during the period between 11 am and 2 pm twelve weeks after therapy. A long-term cure utilizes the absence of viable eggs in three consecutive specimens taken during the period 11 am to 2 pm 24 weeks after therapy.

Failure will be regarded as any detection of viable egg twelve weeks after therapy. Failure may be due to a maturing infection or

even a reinfection. In this study a reinfection will be defined as positive urine during a follow-up period after previous urine samples at the second twelve-weekly examination had been negative.

A relapse will be defined as any positive urine within six months after a previously negative urine. The urine studies will be carried out between 11 am and 2 pm at the schools. All urine collections will be made following the administration of a 300 ml drink of Coca-cola (R) approximately 30 minutes before collection time. Whilst the three laboratory assistants gather, identify, collect and label urine, the two interviewers will ask the children regarding symptoms they have noticed in the recent weeks whilst on therapy. An extra research staff will see to the opening of the bottles of Coca-Cola and disposal of the bottles tidily so that children can return to their classrooms as soon as possible.

3.11 Compliance

Compliance problems will likely arise in three ways: the provision of urine samples, the administration of appropriate therapeutic regimen and the continued involvement in the study. The urine samples to be collected at the initial survey will not be preceded by a drink of Coca-Cola, however subsequent specimens on which egg counts will be carried out will be preceded by a drink of Coke. This will serve as an indirect incentive. Also, it is proposed that at the time of provision of a sample a reward is to be given to the child in the form of a pencil, an eraser, a small notepad or other similar items.

Therapy is to be administered under supervision of trial staff. It is quite unlikely to have problems with administering drug as long as the child is present. The issue of continual involvement in the study is important. There will be dropouts from the study, from the school due to a number of reasons. They may have side effects from treatment, they may change schools or they may move out of the community.

If a child is absent from school for two consecutive days and he is required to have therapy or provide specimens during this period, the interviewer initially assigned to administer the questionnaire to the child's parents will visit the home. During this visit urine can be collected and the reason for absenteeism will be recorded by the interviewer. If the child returns to school within the next three school days, the treatment will be administered.

There will be those subjects who complete the treatment as prescribed for the trial. There will also be those subjects who complete the trial, but not as prescribed for the trial. A comparison of those who complete the trial in accordance with the trial prescription will be carried out. Also, a comparison of those who complete the treatment, though not as prescribed, will also be carried out. There will be those who will be regarded as dropouts. This is defined as follows:

(i) Subjects on metrifonate who do not complete the treatment within ten weeks of first dose.

(ii) Subjects on niridazole who do not complete prescribed treatment within ten school days.

A record of all droupouts will be kept and where available, the reasons given for dropping out of study.

3.12 Sample size estimation

This proposed trial involves the comparison of three doses of niridazole with a standard dose of metrifonate. The outcome measure is a cure rate or the proportion of cures amongst treated subjects. Cure is defined as the absence of viable eggs in three consecutive urine specimens taken during the period between 11 am to 2 pm twelve weeks (and also twenty-four weeks) after therapy. Table 6a represents proportions that have been reported in several studies and Table 6b utilizes some of these proportions and some hypothetical proportions.

Any sample size estimations using these proportions are the basic minimum and if possible more subjects should be included in the study.

In using proportions Cohen (23) has noted the requirements for calculating a total sample size N. These are

(i) The significance criterion, a. This is the risk of mistakenly rejecting the null hypothesis and concluding that there is a difference when there is none. It will be taken as 0.01. This is because with increasing number of groups there is a chance of at least rejecting one null hypothesis even when all are true.

(ii) The value of b, where b is the risk of not rejecting the null hypothesis when in fact there is a real difference between the

Table 6a. Proportions utilized in sample size estimations.

REFERENCE	SAMPLE SELECTION	SAMPLE SIZE	DRUG & DOSAGE USED	PROPORTION OF CURES	
(9)	male hospitalized patients	17	Niridazole 12.5 mg/kg body weight	0.68	B_1 moderate infection
(26)	school children	38	Niridazole 15 mg/kg body weight	0.21	
	school children	37	Niridazole 25 mg/kg body weight	0.54	
	school children	30	Niridazole 25 mg/kg body weight	.010	
(27)	school children	23	Metrifonate 7.5 mg/kg body weight	0.58	for B_3 moderate infection
(58)	school children and adults	10	Niridazole 25 mg/kg body weight	0.90	
(86)	children	39	Metrifonate 7.5 mg/kg body weight	0.18	for A-severe infection
(100)	school children	191	Niridazole 25 mg/kg body weight	0.99	

The other proportions are not based on reported studies. Results of the trial therefore require that its power be recalculated based on the observed cure rates.

Table 6b. Sample size estimations

a = 0.01 b = 0.2

Treatment	Moderate Infection (Stratum 1) Proportion Cured	Severe Infection (Stratum 2) Proportion Cured
A	0.58*	0.18*
B_1	0.68*	0.28
B_2	0.73*	0.44
B_3	0.83*	0.60

*Indicates proportions obtained from studies reported in the literature.

groups. It will be set at 10% and 20% (that is a power of 0.9 and 0.8 respectively).

(iii) The degree of freedom u, involved. This depends on the number of treatments and the number of outcomes. In this trial there are four treatments and two outcomes (cured or not cured). The degrees of freedom equal 3((4-1)(2-1)).

(iv) The effect size index, w. This is a measure of the discrepancy between a null hypothesis and the alternate hypothesis. W measures over all the cells using the following formula.

$$W = \sqrt{\sum_{i=1}^{M} \frac{(P_{1i}-P_{oi})^2}{P_{oi}}}$$

where P_{oi} = the proportion in cell i given the null hypothesis

P_{1i} = the proportion in cell i given the alternate hypothesis and it reflects the effect of that cell

m = the number of cells.

Following the calculation of w, an appropriate table is consulted given a, b and u. Table 6 indicates the proportions that are used in the estimation of sample size. Appendix (VI) indicates the actual calculations involved.

The sample size required for all the groups from the table is 481 for stratum 1 (children with moderate infection) and 214 for

stratum 2 (children with severe infection).

If one allows for a 10% dropout rate and a 5% ineligibility rate, the sample size requirements become 602 for stratum 1 and 208 for stratum 2. These sample sizes incorporate a power of 0.9 in the study. Allowing for the same refusal and ineligibility rate and a power of 0.8, the sample sizes become 522 and 235 respectively.

Fig. 4 represents a flow diagram using the sample size calculated for stratum 1. Fig. 5 represents the expected numbers in each cell.

Two b values have been used in sample size calculations. The reason for this is related to the possibility of being unable to fulfill sample size requirements when a power of 10% is used. It is not possible to know before the trial whether the power of the study will be 0.8 or 0.9.

3.13 Criteria for success of the trial

(i) The study subjects eligible for the trial following the pretrial survey should number 757. Eligibility at this stage indicates the detection of haematobium eggs in the urine. This allows for a 10% refusal rate, a 5% ineligibility rate and a 10% dropout rate. The refusal rate is included as an estimate of the proportion who refuse to allow their children/ward to participate in the study. The ineligibility rate is an estimate of the proportion who cannot be given treatment due to comorbidity (see Section 3.8.1 Biii). The dropout rate represents those who consented to be in the study but

Figure 4: A flow diagram indicating the selection of study population, stratification, randomization and sample size for Stratum 1.

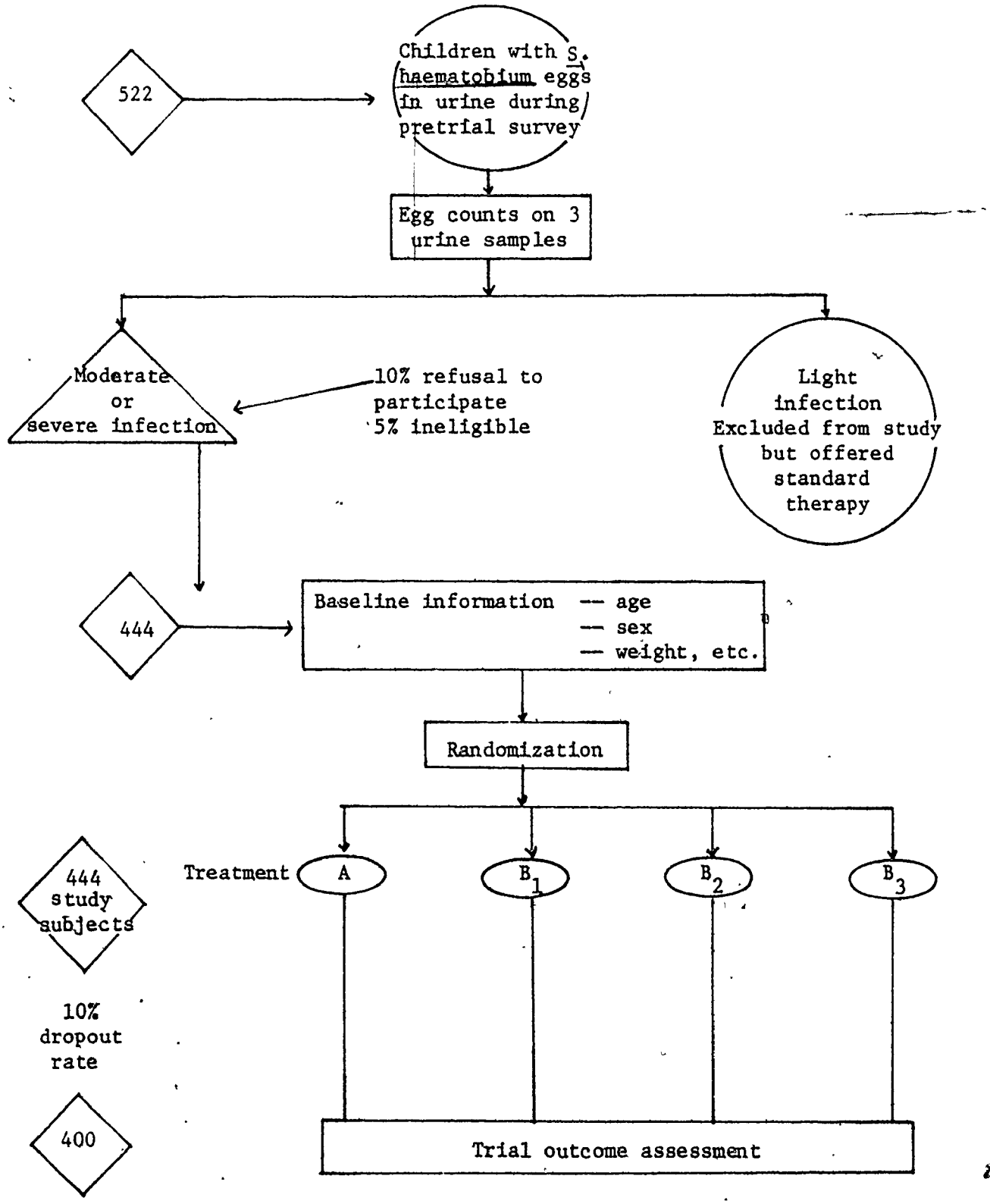


Figure 5: Urinary Schistosomiasis — Treatments and Strata and Estimated Samples Sizes

Strata	A 7.5 mg/kg body weight metrifonate	B_1 12.5 mg/kg body weight niridazole	B_2 17.5 mg/kg body weight niridazole	B_3 22.5 mg/kg body weight niridazole	
Stratum 1 Moderate infection	100 (130)(a)	100 (150)	100 (130)	100 (130)	400 (520)
Stratum 2 Severe infection	45 (59)	45 (59)	45 (59)	46 (59)	180 (216)
	145	145	145	145	180

Treatment

(a) The number in brackets incorporates a dropout rate of 10%, a refusal rate of 10%, and an ineligibility rate of 5%.

refuse final examinations or complete treatment course. These proportions may be over or under estimations. It is not possible to indicate what is expected in this community as there are no reports.

(ii) For subjects in stratum 1 (moderate infection) treatments B_1, B_2 and B_3 should be statistically significantly more effective than the standard. The same applies to individuals with severe infection. This effectiveness should not be associated with serious side effects. For example, nausea sufficient to cause actual vomiting will be regarded as a serious side effect.

3.14 Ethical considerations

When an individual actively seeks health care it is not usually unethical to offer appropriate therapy even if the therapy has some side effects (as long as the individual is well informed of these side effects). However, when treatment is being offered to individuals who did not ask for it and who presumably were healthy enough to pursue their usual activities, ethical considerations become very important. It seems insufficient to justify to a parent or guardian that involvement of their child or ward in the study, although potentially beneficial for the child, is primarily for the advancement of scientific knowledge.

As has been discussed previously, therapy has been shown to result in reversibility of some lesions, symptomatic improvement and reduction in risk of transmission of infection. On the basis of the

potential benefit to the individual and the fact that occurrence of side effects will be carefully noted and the treatment stopped if necessary, it seems ethical to carry out the study.

Considering the possible side effects that may be noted following therapy for schistosomiasis, it might be argued that niridazole should not be used. However, these side effects were noted when high doses of up to 75 mg/kg body weight of the drug were administered (41).

CHAPTER IV

MEASUREMENT

Measurements will need to be taken before actual drug trial, during the trial and after treatment. Measurements to be taken include egg counts on urine, body weights and the collection of identifying data on study subjects.

4.1 Demographic data collection

Some demographic data will be collected at the time of the pretrial survey: name, class at school and name of school of all children who provide urine specimens. Attempts will be made during this period to reach all school children or 80% or more of them (see Section 3.8.1B). This initial survey is necessary for the determination of the prevalence of urinary schistosomiasis amongst children in the community.

After the initial survey all potential study subjects will be registered. Each child will be identified by name, class at school, address, age (as given by child) and sex.

4.2 Urine studies

Urine collections will be carried out between 11 am and 2 pm. All collections will be made approximately 30 minutes following the

administration of a 300 ml drink of Coca-Cola (R) or Pepsi Cola (R) to the child. Collections will be in a 200 ml capacity disposable cup. Following the pretrial survey each child would have been given an identification number. All subsequent urine specimens provided will be marked with the identification number.

The determination of the volume and the detection of proteinuria and haematuria will be carried out. These data will be collected for purposes of information alone as they do not contribute to the execution of the trial.

Following treatment urine will be collected 28, 29 and 30 days following each dose or at a similar daily sequence following the administration of each dose such that the days fall on school days. Egg counting techniques are discussed in Appendix 1.

4.3 Quality control in microscopy

Erroneous results of egg counts may be due to fault in the instrument (the microscope) or inattention of the microscopist or inability to recognize the eggs on the slide. If it is assumed that the microscopes have been adjusted to function properly then problems of observer errors become important. Although a pretrial training is incorporated into the study, an ongoing assessment is also necessary in order to maintain some quality of the results. One method that has been used is called the "false negative rate" (8). This was used to identify laboratory technicians requiring more supervision. Slide selection involved only slides reported as negative. These were rechecked in order to identify those slides that were actually positive

(since this was a qualitative study not involving egg counts, there was no need to check positive slides). A false positive rate is not considered here because it is believed that schistosoma eggs are characteristic and are either observed under the microscope or not. Both positive and negative slides will be used in this study. The positive slides will be used to reaffirm counts and the negative slides will be used to determine a false negative rate. Slides reported on daily will be numbered 1 to n and a random 10% of these slides will be selected using a table of random numbers. Some error is expected in the classification of slides during and after treatment. A 5% error rate will be allowed each technician. This means a technician is allowed to classify as negative 1 in 20 positive slides. For this error rate only the negative slides are necessary. The calculation of this rate will form an ongoing part of the study. Technicians who have consistently high error rates will be required to utilize the training slides until better error rates are obtained.

4.4 Body weight

The dosage is dependent on the weight of the child. Weight will be measured using a balance beam scale. Children will be required to get weighed barefoot in their school clothes. All primary schools require that pupils wear school uniforms. For females this is usually a knee-length dress of simple cotton material and for males, the uniform is usually a short sleeved shirt (from same material as female dress) and a pair of shorts. It is unlikely that differences in clothing will contribute significantly to differences in body weights.

4.5 Documentation of side effects

The questionnaire administered prior to actual therapy will attempt to identify any health related problems. Parents will be asked concerning a history of nausea and vomiting in their children.

Older children at school will be questioned concerning menstrual periods. At subsequent drug administrations any side effects reported will be documented. For metrifonate side effects which have been reported include nausea, vomiting, diarrhoea and abdominal pain. These are noted more often when dosages administered are in excess of 7.5 mg/kg body weight. Niridazole has been documented as the cause of rather serious side effects such as nausea, vomiting, anorexia, headaches and occasional skin rashes. More serious side effects include mental depression, insomnia and anxiety (69). These symptoms are usually associated with high concentrations of unchanged drug in the peripheral circulation. This is often a result of some liver function impairment and failure to metabolize the drug (69). The serious side effects are also more common in treating adults with *Schistosoma mansoni* infections (106).

Following the cessation of therapy, recovery is rapid and apparently complete (29, 69, 106).

Niridazole also causes a brownish discoloration of the urine. Children on niridazole and their parents or guardians should be warned about this discoloration so that there is no alarm. The discoloration disappears with the cessation of therapy.

Although egg counts after treatments will serve as the criteria for modifying the treatment, the side effects reported will also serve

as some criteria. If vomiting is persistent enough to interfere with the child's daily life, treatment will be discontinued. A child should not be hindered from attending classes as a result of side effects of treatments. Other side effects such as diarrhoea, headache, nausea will also be carefully documented and appropriate action taken depending on the findings. This will require tracing of children who may fail to attend school because they do not want to be given these treatments. Although the cause of absenteeism may be unrelated to the trial efforts will be made to rule this out. The community interviewer initially allocated to administer the questionnaire will be responsible for follow-up of those children who are absent (see Section 3.11).

4.6 Pretrial training of personnel

Contributing to the variability in results of egg counting is the observer variation. In order to minimize this variability a pretrial training of laboratory staff will be included in the study. The technician must be able to recognize the schistosome egg in urine. *S. haematobium* egg is said to be characteristic (71). This is applicable when the observer is aware of what he is observing. The technicians will examine prepared slides of *S. haematobium* during the training period so that they will be able to make a differentiation between the morphology of dead and viable haematobium eggs and also between non-haematobium and haematobium eggs whilst looking under a light microscope. During this period sample slides with varying

degrees of infection will be used for egg counting. Intra and inter-observer variations between counts from the same slide will be noted. Twenty such slides will be used (4 with few egg counts, 6 with moderate egg counts and 10 with heavy egg counts). The use of a larger number with heavy counts is deemed necessary because it has been noted that a greater variability occurs when slides have a larger density of eggs (51). A separate set of 10 slides with a varying proportion of dead eggs will also be used during the training period. This is necessary because following treatment a greater proportion of children will be excreting dead eggs.

The acceptable level of intra and interobserver agreement will be taken as not greater than 10 eggs per ml of urine. For example, in classifying the same slide two counts by two observers should be within a 10 egg range. If one observer reports a slide as having 140 eggs per ml the same observer a second time around or a second observer should report the same slide as having a count between 130 and 150 eggs/ml of urine. It should be remembered that the actual reporting as eggs per ml is obtained through a calculation process.

There are no reports of acceptable levels of intra and inter-observer agreement with regards to egg counting. It may be found that the level that is set here is not feasible. Relevant changes will be made during the study.

The community interviewers will require some training. The guidelines given by Woodward and others (107) on the training of interviewers will be followed. The important points which the

interviewers will be expected to know and utilize are shown below.

(i) To establish rapport with the respondent.

(ii) To maintain a conversational tone and accept responses with neither approval nor disapproval.

(iii) To show consistency in asking the questions as written in the questionnaire.

(iv) To record responses as given by respondent.

(v) To be familiar with methods of neutral probing which may be used to encourage responses without "putting words into respondents' mouths".

(vi) To always check the questionnaire at the end of the interview in order to be certain that recorded responses are complete and legible.

Since the study will not employ any professional interviewers to train these community interviewers, the interviewers will be supervised and trained by the coordinator using test interviews and tape-recording sessions. The training session will last for two to three weeks during the period when the coordinator is meeting with community leaders to obtain permission.

CHAPTER V

OPERATIONAL ISSUES

The success of the proposed trial is highly dependent on the reception of the proposal in Usi-Ekiti. If the community leaders strongly disfavour the proposal then its execution is next to impossible.

The initial action is to bring the idea to the attention of the king of the township through a certain chief of the community. The king discusses issues with his advisors who are themselves community leaders. If the proposal is favoured, the community will usually hear about it via its normal sources. These include church announcements, various societies and meetings, marketplace and by word of mouth amongst friends and relatives.

Assuming that the proposal has been favoured, efforts will be made to supplement the normal routes of information. An advance note will be sent home with children who are potential study subjects (see Section 3.8.1B). It should be noted that many non-urban communities such as Usi-Ekiti welcome any interest which is perceived to be beneficial to their health.

5.1 Personnel requirements

Several types of personnel will be essential if the trial is to be properly executed. These include a coordinator, laboratory supervisor, a microbiologist, a nurse, laboratory technicians, laboratory assistants, community interviewers, a driver and a "secretary".

A coordinator will be responsible for the trial. He or she will organize all trial activities smoothly. He must be a physician, as he will carry out the initial cursory examination of potential study subjects (see Section 3.8.1B). The final writing and reporting will be the responsibility of the coordinator.

A supervisor (a microbiologist) or senior laboratory technician will be involved with supervisory activity of laboratory work. This will involve training of the technician, checking of slides and maintenance of a high quality in microscopy. Although there is no "truth" to which the results of the supervisor can be referred, his results will be accepted as final in disputed cases. The supervisor's results will also be scrutinzied for intraobserver variation. At the end of one week slides that have been reported by the supervisor will be numbered from 1 to n. A random 10% of these slides will be selected using a table of random numbers. For positive slides an acceptable level of intraobserver agreement will be set at not greater than 10 eggs per ml of urine. This means that in classifying the same slide twice, egg counts should be within a 10 egg range. If an initial egg count is stated as 130 eggs per ml the second count using

the same slide should be between 120 and 140 eggs per ml. For negative slides the supervisor is allowed to have a 5% error rate. If 20 slides reported as negative are rechecked 1 slide may be reported as positive in the recheck.

A microbiologist will supervise the supervisor. He will not be part of the study staff. He will act as impartial observer of the supervisor's results.

One laboratory technician will be trained for the trial. If it is found that he is unable to cope with the workload, one additional technician will be trained for the study.

The three laboratory assistants will be responsible for activities such as urine collection, labelling, and the provision of rewards for the children.

The nurse will give the tablets to each child with a glass of water and ensure that it is swallowed and not dropped, hidden under tongue or still in the child's hand.

The community interviewers have multiple functions in this trial. The requirements are that they be able to speak, read and write English and Yoruba. This is important, especially in the administration of the questionnaire which may require that the interviewer be familiar with various terminologies and local names for urinary schistosomiasis. Later they will be responsible for recording the side effects of treatment, reported by study subjects. They will also be utilized in tracing and follow-up of study subjects who are absent from school. They will be familiar with the geography of the area and will likely be more acceptable to members of the

community than a stranger.

A driver will be employed to transfer specimens from Usi-Ekiti to the laboratory, to offer transportation to the interviews and to perform other necessary transportational activities.

A secretary will be required to type letters, notes and reports and to perform other clerical activities.

All employees will be full time during the trial with the exception of the coordinator and microbiologist.

5.2 Budget

The study period is estimated to be 1½ years: 3-4 months of planning and pretrial survey, 8 months of trial activity and 3-4 months of data analysis and reporting. A budgetary request will be made for 1 year. Towards the end of the 1 year period a further budgetary request will be made. It is more likely that the study will be funded if budgetary requests are not overly ambitious.

5.2.1 Budget justification

Costs are involved in paying salaries of personnel employed in the study. It is expected that the preventive and social medicine department will provide space for the urine examinations. The microscopes and other equipment will have to be purchased.

A vehicle will be leased for a one-year period. This is necessary because Usi-Ekiti is 125 miles away from the laboratory. Table 7 is a detailed outline of the budget.

Table 7: Budget Details

Salaries/year	No.	Estimated % time	Cost $
i) Laboratory supervisor	1	20%	3,400
ii) Microbiologist	1	10%	2,000
iii) Laboratory technician	1	100%	10,000
iv) Laboratory assistants (@ $5,000 each)	3	100%	15,000
v) Community interviewers (@ $200/month, 2½ days/wk)	2	50%	2,400
vi) Driver	1	100%	4,000
vii) Secretary	1	100%	6,000
viii) Nurse (@ $12,000/yr. for 6 months)	1	50%	3,000
ix) Research associate	1	100%	12,000
Equipment			
Microscopes	5		7,500
Thermometers ($1.50 each)	100		150
Microbiology equipment -specimen jars -beakers -pipettes, etc.			2,000
Stationery			300
Travel			500
Miscellaneous		TOTAL:	$68,500

5.3 Timing of the study

The study involves school children and therefore allowances have to be made for school holidays. If it is timed such that pre-trial activities begin with the school year in September, pretrial urine collection, interviewer training and examination of study subjects should be complete by the time school closes for holidays. School reopens in early January till mid-April and treatment and post-therapy urine collections will be carried out during this period. It will be necessary to rotate these activities amongst the participating schools. It is intended that for children on metrifonate, first post treatment urine collection will be carried out at the end of May/June. When school reopens, urine collections will again be taken in September and October for the determination of long-term cure. If treatment falls on a school holiday it will be given on the next school day. The next three to four months will be used to analyze and report results of the study.

CHAPTER VI

ANALYSIS

6.1 Questions

1. Are the responses (cures, side effects) to the various doses of drugs administered significantly different?

2. With respect to cure rates, how do the three doses of niridazole compare with metrifonate?

3. Is there any dose-response relationship with the use of niridazole?

6.2 Comparison of responses across treatment groups

Cure rates will be determined for each treatment group and a chi-squre analysis will be used to test the null hypothesis that there is no difference in the distribution of cure rates across treatment groups within each stratum. If the hypothesis is rejected, then there is a suggestion that there is a greater than chance difference in cure rates across groups (Table 8).

Table 8. Cures across treatment groups (for each stratum).

	A	B_1	B_2	B_3
Cured				
Not Cured				

$\alpha = 0.01$

$\chi^2_3 =$

The two side effects that are often reported following treatment with niridazole and metrifonate are nausea and vomiting. A chi-square analysis will be used to test whether there is a significant difference in the distribution of these symptoms. However, only nausea severe enough to cause vomiting would be regarded as a severe side effect (see Table 9).

Table 9. Nausea report across treatment groups (for each stratum, similar one for vomiting).

	A	B_1	B_2	B_3	
Nausea					$\alpha = 0.01$
No nausea					$\chi^2_3 =$

Though the occurrence of nausea or vomiting may be significantly different across treatment groups, nausea and vomiting may or may not be different across groups. A separate analysis will be carried out to determine the occurrence of nausea and vomiting across treatment groups (see Table 10).

Table 10. Distribution of nausea and vomiting across groups (within stratum).

	A	B_1	B_2	B_3	
Nausea and vomiting					$\alpha = 0.01$
No nausea and vomiting					$\chi^2_3 =$

6.3 The nature of the dose-response of niridazole

It might be expected that cure rates will increase with the increase in dose of drug administered. The simplest method is to assign arbitrary scores to the categories and using normal regression techniques (30a). This enables the overall chi-square statistic to be partitioned into component parts. The category 'cured' will be assigned a value of 1 and the category 'not cured' will be assigned a value of 0. The three doses of niridazole will be given values -1, (B_1), 0 (B_2), 1 (B_3). These values are arbitrarily chosen and evenly spaced (see Table 12). The results of this categorization is a regression of cure (y) on doses of niridazole (2). The formula for estimating the coefficient of cure on dose is: $byx = \frac{Cyx}{Cxx}$, where Cyx and Cxx have to be calculated from frequency tables obtained by using the scores that have been chosen and the formulae to be used are

$$Cxx = \sum_{j=1}^{3} n_{.j} x_j^2 - \left(\sum_{j=1}^{3} n_{.j} x_j\right)/N$$

$$Cyx = \sum_{i=1}^{2}\sum_{j=1}^{3} n_{ij} y_i . x_{.j} - \left(\sum_{i=1}^{2} n_{i.} y_i\right)\left(\sum_{j=1}^{3} n_{.j} x_j\right)\Big|N$$

$$\text{The variance of } byx(V) = 1/N \times \frac{Cyy}{Cxx}$$

$$\text{where } Cyy = \sum_{i=1}^{2} n_{i.} y_i^2 - \left(\sum_{i=1}^{2} n_{i.} y\right)^2\Big|N$$

where x_j represents the counts of cures in the i th category.

n_i . marginal count for category i

N is the total number in the sample.

The component of the chi-square due to a linear trend can then be calculated from the formula $byx^2 | V(byx)$. The results will be tabulated as shown in Table 13.

Table 11. Cures using niridazole (assignment of scores).

Scores	B_1 -1	B_2 0	B_3 1
Cured 1			
Not cured 0			

Table 12.

Source of variation	df	χ^2	P_{Value}
Due to linear regression of cure on dose	1		
Departure from regression (obtained by subtraction)	2		

If the χ^2 is significant, one can conclude that there is a significant increase in cures with increase in dose of niridazole administered. Furthermore, one can conclude that this increase is a linear one. It is possible to find that the high but effective dose is also associated with severe side effects and poor compliance. It might be necessary to recommend a less effective dose which is not associated with such severe side effects as to make patients poor compliers.

6.4 Other results

6.4.1 Percentage reduction in egg output

The cure rates may prove not to be statistically significantly different across groups even though there is significant reduction in egg output. Clinically, reduction in egg output is important, even in those regarded as failures. If one accepts that pathological lesions are related to the egg output, any reduction in egg output is important to the infected individual. The percentage reduction in egg output is defined as follows:

$$\frac{\text{(Egg output prior to therapy) - (Egg output following therapy)}}{\text{Egg output prior to therapy}} \times 100$$

6.4.2 Compliance

Several groups of subjects can be distinguished based on compliance measures.

(i) Subjects who refuse treatment. Where possible reasons given will be documented.

(ii) Subjects who cannot tolerate treatment because of side effects. This group will be documented and analyzed (see Table 10).

(iii) Subjects who are away from school. Absenteeism from school may or may not be related to the treatment. An attempt will be made to reach this group. The community interviewer will document reasons given for absenteeism (see Section 3.11). If reasons are unrelated to side effects, this group will be regarded as dropouts.

If reasons are related to side effects then they will be regarded as belonging to (ii) above.

(iv) Subjects who have been treated but refuse final examinations. It is expected that a study subject should provide urine specimens for final examinations. Three samples of urine at specific periods of the day for three consecutive days, twelve and twenty-four weeks after last dose of treatment are to be provided by subject. Consecutiveness will be defined as a period of five school days. If within five days a subject cannot be located to provide samples, he or she will be recorded as a dropout.

6.4.3 Prevalence

The proportion of infected children amongst examined school children will be used to determine the prevalence of urinary schistosomiasis amongst school children in this community.

6.4.4 Urinary symptoms

From the results of the questionnaire, the prevalence of urinary symptoms amongst infected and uninfected children will be noted. It will also be indicated whether these symptoms are significantly different amongst infected and uninfected children.

6.5 Interpretation of chi-square

In a contingency table set up, hypothesis testing is related to independence of the variables forming the table. When a certain chi-square (χ^2) value is obtained, it is referred to a table of χ^2 distribution. The requirements for use of the table are degrees of freedom and some a priori significance level, α. If the χ^2 value obtained by data analysis is greater than the tabulated value given the degrees of freedom and α, it indicates the lack of independence or that the results are unlikely to be due to chance. A χ^2 value smaller than the tabulated value given the degrees of freedom and α leads to a conclusion that differences noted may well be a result of chance. For example, if χ^2 value obtained in analyzing table 9 is not significant, one could conclude that the incidence of nausea is not different for both drugs.

6.6 Beta analysis

Since the sample size calculations have been based on estimates from other studies, there is a possibility that the actual proportions observed in the study will be quite different. An analysis will be carried out at the end of the trial using the study sample sizes and observed proportions to determine the power of the study.

CHAPTER VII

CONCLUSIONS

A discussion of schistosoma parasitic infection has been presented. Some aspects of haematobium infection in children have been discussed. The parasite has been identified as being associated with significant morbidity and some mortality. Problems which make results of reported drug trials questionable were discussed. The need for identifying drug dosages using standardized trials were presented.

A design of a randomized clinical trial to treat 580 infected school children has been presented. The trial will take place in a single locality and will last for eighteen months.

This design will attempt to answer the question of effective dosages of niridazole in haematobium infection. Once dosages are established, it will become possible to make recommendations regarding therapy of schistosomiasis in countries where schistosomiasis is prevalent. This trial will serve as a basis for further studies regarding therapy of schistosomiasis. Consideration of a suppressive and/or prophylactic management of schistosomiasis using niridazole are possibilities for future research.

APPENDIX I

Egg counting techniques

These are of two main types: centrifugation or filtration methods.

(1) Centrifugation methods

The earliest of these methods was first described by Barlow (6) and used by Scott (93) and later in a modified form by Stimmel . and Scott (97). Mainly it consisted of using a large volume of urine, usually 50 ml. This was centrifuged and the deposit was stained with methylene blue and spread over glass slides which were allowed to dry. The dried slides could later be counted by adding a little water.

The problems with this initial technique were manifold:

(i) large number of slides needed to be counted per specimen and, especially when infection was heavy, errors were introduced;

(ii) transfer of the sediment from centrifuge tube required some washing and some dilution resulted; and

(iii) there was no allowance for the examination of part of the specimen.

However, it did have the advantage that the minimum number of precise measurements were taken since the method utilized the whole sample of urine obtained from an individual.

An adaptation of the centriguation method was used by Jordan (quoted by Bradley (14)). This involved a smaller volume (10 ml) of

urine obtained from the initial sample. Deposit from centrifuging this volume is resuspended in 0.2 ml of supernatant. A small sub-sample (60 mm^3) is transferred to a glass slide and eggs were counted (i.e., eggs in 3 ml of urine are counted).

Disadvantages of this modification include the following:

(i) requires measurement of three precise volumes;

(ii) resuspending the sediment on a tiny volume (0.2 ml) often proved difficult; and

(iii) no premanent preparations are made and recounting is impossible.

Regardless, it is less cumbersome than the first method and uses only a single slide. One possibility is the use of multiple slides per specimen. Each slide is counted and a mean of the three counts is taken as the egg count. It is quite possible that highly different results would be obtained as has been observed (104). The main disadvantage of both methods is that they need to be performed in a laboratory and both are quite time consuming.

(2) Filtration methods

This was first described by Bell (10) for quantifying *S. mansoni* eggs in stool. Several modifications of this method have been adapted for use in counting *S. haematobium* eggs in urine. Bradley's (14) modification could be used for urinary egg counting in the field. All modifications require separation of eggs onto filter paper, staining with ninhydrin and then examination under the microscope. The method requires minimum number of measurements, produces

a permanent record which can be read and re-read at leisure. Bradley (14) has used this method extensively and noted that when infections are known to be light or following therapy, a large volume up to 50 ml can be filtered whilst, prior to therapy, smaller volumes could be filtered (about 5 ml).

Filtration techniques have received more attention in recent years. Peters and others (79), Pugh (82), and Wilkins (105) have used filtration methods, or one modification of it, in several studies. Differences in these modifications include type of filter being used, inclusion or exclusion of staining, and additional normal saline. Expense often dictates the type of filters to be used and the nucleopore filters advocated by Peters and others (79) are stated to be the most expensive, though finer, and, at the same time, tended to get clogged when urine with high egg counts was examined. Wilkins (105) noted that examination of replicate aliquots from heavily infected cases was more likely to result in similar egg counts than replicate aliquots from lightly infected cases. There were consistent observer variations. Peters and others (79) noted that, although there were differences in counts between replicate aliquots, there was no statistical significance between these differences, regardless of whether the egg counts were minimal or heavy.

Secondly, some attempts should be made to minimize the inter-observer variations in reporting egg counts from the same specimen. Thirdly, the variations between replicate samples from the individual have to be noted. Fourth, the intraobserver variation in reporting the same preparation twice should be minimal.

One method that could attempt to minimize intra and inter-observer variations would be to have an initial period during which personnel are trained to use the method. Then a testing period would ensue and individuals with wide fluctuations could be retrained until insignificant differences result.

A filtration technique as modified by Peters (79) and others will be utilized in this study.

APPENDIX II

Advance Notification

Dear Parent/Guardian,

Your child/ward has been selected to participate in a study.

This study involves the treatment of a worm infection which your child/ward has. The treatment of this infection is known to be effective. The study will involve the provision of a birth certificate for the child if one is available. During the next few months your child/ward will be required to provide several urine specimens at school. Somebody will come by your house to discuss this study with you. This person will interview you only at your own convenience.

This study is not related to your taxes.

Your cooperation is essential to the success of the study and would be greatly appreciated.

APPENDIX III

Questionnaire to determine magnitude of urinary symptoms in population and to identify otherwise eligible study subjects.

Identification No.

Suggested Introduction

Hello,

I am here to discuss with you, the study mentioned in the note sent to you from your child's school.

This study is being carried out by a research group from the University of Ibadan. Please note that anything you decide to say or do will not jeopardize your child's education nor affect your family's ability to obtain health care and you can refuse to participate or withdraw at any time. If you have any questions, please do not hesitate to ask me.

(Circle appropriate number)

1. Demographic information

1.1 Name _____ Sex 1. Male 2. Female

1.2 Address _____

1.3 Respondent's relationship to child

1. Father
2. Mother
3. Other (please specify)

1.4 Age of child _____

Do you have a birth certificate for the child?

1. Yes Could a copy of it be made available to us?

2. No

If No

Probe to identify how age of child was determined.

Events occurring around time of birth such as

-a new market

-a new building opened

-festivals

-visit of an important person

-crowning of a new king

-other (please specify)

2. Health Information (If yes to 2.4-2.5, child is not eligible)

2.1 Does the child have any serious health problems?

1. Yes (please describe

2. No

3. Hesitant -don't know

-not sure

-can't recall

Probe for any of the following

2.2 Previous hospitalizations (please describe respondent's reasons for these)

1. Yes

2. No

2.3 Current medications

1. Yes (please specify)

2. No

2.4 Yellowness of the eyes

1. Yes (not eligible)

2. No

2.5 Epilepsy/convulsions

1. Yes (not eligible)

2. No

2.6 Nausea/vomiting

1. Yes

2. No

2.7 Has the child complained about his health since yesterday?

1. Yes (please describe)

2. No

2.8 Any health complaints since last week?

1. Yes (please describe)

2. No

2.9 Have you observed the child's urine in the past day or week?

Day	Week
1. Yes	1. Yes
2. No	2. No

2.10 Please state the colour

1. Yellow	1. Yellow
2. Pink	2. Pink
3. Red	3. Red

2.11 Has the child any complaints regarding urination?

1. Yes (please describe) Note 1. Pain 2. Blood in urine 3. Other

2. No

3. Don't know (probe) 1. pain 2. blood in urine.

At this stage of the interview, thank the respondent and give informed consent information to read. If unable to read, explain the content of the informed consent to the respondent. If respondent allows child to participate, present consent form for signature or thumbprint.

If respondent refuses participation, ask why.

3. Please describe reason for refusal.

APPENDIX IV

Informed Consent

A study of effective therapy of urinary schistosomiasis in school children.

Urinary schistosomiasis is an infection caused by a type of worm. Children and adults get the infection whilst swimming, playing or washing in streams or ponds around the community.

This study will try to find out the best treatments that can be offered to children. The children will be given some tablets by mouth and will be required to provide urine samples from time to time. The treatment may be accompanied by some side effects such as nausea and vomiting. Treatment will be stopped immediately if side effects are bothersome or at a request from you. The child may be withdrawn from the study at any stage. Neither you nor the child will suffer any repercussions.

Consent to Participate in Study

I, ... have read the information concerning the schistosomiasis study. It has been explained to me that the study may involve treating my child/ward with some tablets. I understand what the study involves.

I agree to participate in the study. I understand that I can withdraw from the study at any time. I hereby give my consent to participate in the study.

Date: _____ _____
Parent/Guardian signature

_____
Witness signature

In the event of inability to sign:

Thumbprint

APPENDIX V

Calculation of sample sizes

Stratum 1		Stratum 2	
Treatment	Proportion cured	Treatment	Proportion Cured
A	0.59	A	0.18
B_1	0.68	B_1	0.28
B_2	0.73	B_2	0.44
B_3	0.83	B_3	0.60

These proportions are reduced to a fraction of 1, giving the following tables:

Pi table

	Treatment (Stratum 1)					Treatment (Stratum 2)				
Proportion	A	B_1	B_2	B_3	Marginal	A	B_1	B_2	B_3	Marginal
Cured	0.15	0.17	0.18	0.21	0.71	0.05	0.07	0.11	0.15	0.38
Not cured	0.10	0.08	0.07	0.04	0.29	0.2	0.18	0.14	0.10	0.62
	0.25	0.25	0.25	0.25	1.00	0.25	0.25	0.25	0.25	1.00

Calculation of effect size, w:

$$w = \sqrt{\sum_{i=1}^{m} \frac{(P_1 i - Poi)^2}{Poi}}$$

Po table (No association) No effect due to the treatments

	Treatment (Stratum 1)					Treatment (Stratum 2)				
Portion	A	B_1	B_2	B_3		A	B_1	B_2	B_3	
Cured	0.18	0.18	0.18	0.18	0.72	0.09	0.09	0.09	0.09	0.38
Not cured	0.07	0.07	0.07	0.07	0.28	0.16	0.16	0.16	0.16	0.62
	0.25	0.25	0.25	0.25	1.00	0.25	0.25	0.25	0.25	1.00

Appendix V (contd.)

$$w = \sqrt{\frac{(0.17-0.18)^2}{0.18} + \frac{(0.18-0.18)^2}{0.18} + \frac{(0.21-0.18)^2}{0.18} + \frac{(0.15-0.18)^2}{0.18} + \frac{(0.08-0.07)^2}{0.07} + \frac{(0.07-0.07)^2}{0.07} + \frac{(0.04-0.07)^2}{0.07} + \frac{(0.10-0.07)^2}{0.07}}$$

$$w = \sqrt{\frac{(0.07-0.09)^2}{0.09} + \frac{(0.11-0.09)^2}{0.09} + \frac{(0.015-0.09)^2}{0.09} + \frac{(0.05-0.09)^2}{0.09} + \frac{(0.18-0.16)^2}{0.16} + \frac{(0.1-0.16)^2}{0.16} + \frac{(0.14-0.16)^2}{0.16} + \frac{(0.2-0.16)^2}{0.16}}$$

Stratum 1

w = 0.1942

a = 0.01

b = 0.1 (Power of 0.9) or
0.2 (Power of 0.8)

u = 3

Stratum 2

w = 0.3277

a = 0.01

b = 0.1 (Power of 0.9) or
0.2 (Power of 0.8)

u = 3

From the table

When Power = 0.9 N = 481

When Power = 0.8 N = 386

When Power = 0.9 N = 214

When Power = 0.8 N = 172

These values are conservative since the value of w in the table is not exactly as calculated above.

Appendix V (contd.)

The estimated sample sizes for a study with a power of 0.8 will be rounded off to 400 for stratum 1 and 180 for stratum 2. This will require that for each stratum the following sample sizes will be needed.

	Stratum 1	Stratum 2
After pretrial survey	N = 522	N = 235
10% refusal rate 5% ineligiblity rate	N = 444	N = 200
varying percentages for a total of 15%		
10% dropout rate during the trial	N = 400	N = 180

All these rates will likely be different in the actual trial and whatever the sample size turns out to be, a beta analysis will be necessary in order to calculate the power of the study.

BIBLIOGRAPHY

(1) Abdel-Salam, E. and Ehsan, A.: Cystoscopic picture of *Schistosoma haematobium* in Egyptian children correlated to intensity of infection and morbidity. Am. J. Trop. Med. Hyg., 27:774-778, 1978.

(2) Abdel-Salam, E. and Abdel-Fattah, M.: Prevalence and morbidity of *Schistosoma haematobium* in Egyptian children. A controlled study. Am. J. Trop. Med. Hyg., 26:463-469, 1977.

(3) Abdel-Salam, E. and Abdel-Fattah, M.: Quantitative postmortem analysis of urinary schistosomiasis in Egypt I: Pathology and pathogenesis. Am. J. Trop. Med. Hyg., 23:1054-1071, 1974.

(4) Amin, A.H. and Fenwick, A.: The control of snails on a small scale. Tropical Doctor, 8:8-12, 1978.

(5) Barbosa, F.S., Pinto, R. and Souza, O.A.: Control of schistosomiasis in small North-East Brazilian community. Trans. R. Soc. Trop. Med. Hyg., 65:206-213, 1971.

(6) Barlow, C.H.: A new method of examining urine for helminth eggs. Am. J. Hyg., 14:212-217, 1931.

(7) Barlow, C.H. and Meleney, H.E.: A voluntary infection with *Schistosoma haematobium*. Am. J. Trop. Med. Hyg., 29:78-79, 1949.

(8) Bartholomew, R.K. and Goddard, M.J.: Quality control in laboratory investigations on *S. mansoni* in St. Lucia. Bull. W.H.O., 56: 309-312, 1978.

(9) Bassily, S., Farid, Z., Higashi, G.I. and Watten, R.H.: Low-dose niridazole in the treatment of *Schistosoma mansoni*. Ann. Trop. Med. Parasitol., 73:295-296, 1979.

(10) Bell, D.R.: A new method for counting *Schistosoma mansoni* eggs in faeces with special reference to therapeutic trials. Bull. W.H.O. 29:525-530, 1963.

(11) Bell, D.R.: Clinical trials and diagnostic methods in schistosomiasis. Ann. N.Y. Acad. Sci., 160:593-601, 1969.

(12) Blair, D.M.: Bilharziasis survey in British West and East Africa, Nyasaland and the Rhodes. Bull. W.H.O., 15:203- 220 , 1960.

(13) Bradley, D.J.: The assessment of lassitude in school children with bilharziasis. Annual Report for 1961-1962 of East African Institute for Medical Research, Nairobi, 1962.

(14) Bradley, D.J.: The measurement of Bilharziasis: Prevalence and schistosomal egg output. Bull. W.H.O., 33:503-508, 1965.

(15) Bradley, D.J.: Regulation of parasite populations: A general theory of the epidemiology and control of parasitic infections. Trans. R. Soc., Trop. Med. Hyg., 66:697-708, 1972.

(16) Bradley, D.J.: A simple method of representing the distribution and abundance of helminthic infections. Ann. Trop. Med. Parasitol., 59:355-364, 1965.

(17) Brown, H.W.: Basic Clinical Parasitology. Meredith Publishing, New York, 1969.

(18) Cartwright, A. and Tucker, W.: An attempt to reduce the number of calls on an interview inquiry. Public Opinion Quarterly, 31: 299-302, 1967.

(19) Cerf, J., Lebrun, A. and Dierchx, J.: A new approach to helminthiasis control: The use of an organophosphorus compound. Am. J. Trop. Med. Hyg., 11:514-517, 1962.

(20) Cheever, A.W.: A Quantitative post-mortem study of schistosomiasis mansoni in man. Am. J. Trop. Med. Hyg., 17:58-64; 1968.

(21) Cheever, A.W., Kamel, I.A., Elwi, A.M., Mosimann, J.E., Danner, R. and Sippel, J.E.: *Schistosoma mansoni* and *Schistosoma haematobium* infections in Egypt III: Extrahepatic pathology. Am. J. Trop. Med. Hyg., 27:55-75, 1978.

(22) Christie, J.D. and Upatham, E.S.: Control of schistosomiasis mansoni transmission by chemotherapy in St. Lucia II. Biological results. Am. J. Trop. Med. Hyg., :894-898, 1977.

(23) Chu, Y.: Trials of ecological and chemical measures for the control of *Schistosoma haematobium* transmission in a Volta Lake village. Bull. W.H.O., 56:313-332, 1978.

(24) Cohen, J.: Statistical Power Analysis for the Behavioural Sciences, Ch. 7, Academic Press Inc. (London), 1977.

(25) Cook, J.A., Jordan, P. and Bartholomew, R.K.: Control of *Schistosoma mansoni* transmission by chemotherapy in St. Lucia I. Results in man. Am. J. Trop. Med. Hyg., 26:887-893, 1977.

(26) Cowper, S.G.: Schistosomiasis in Nigeria. Ann. Trop. Med. Parasitol., 57:307-322, 1963.

(27) Davis, A.: Field trials of Ambilhar in the treatment of urinary Bilharziasis in school children. Bull. W.H.O., 35:827-835, 1966.

(28) Davis, A. and Bailey, D.R.: Metrifonate in urinary schistosomiasis. Bull. W.H.O., 41:209-224, 1969.

(29) Draper, C.C.: The immunodiagnosis of schistosomiasis. Tropical Doctor, 8:19-20, 1978.

(30) Edington, G.M., von Lichtenberg, F., Nwabuebo, I., Taylor, J.R. and Smith, R.H.: Pathologic effects of schistosomiasis in Ibadan, Western State of Nigeria. I: Incidence and intensity of infection; distribution and severity of lesions. Am. J. Trop. Med. Hyg., 19:982-995, 1970.

(31) Edington, G.M. and Gilles, H.M.: Pathology in the Tropics. Baltimore, Williams and Wilkins Company, 1969.

(32) Farid, Z., Bassily, S., McConnell, E., Schulbert, A., Sabour, M. and Abdel-Wahab, M.F.: Symptomatic, radiological and functional improvement following treatment of urinary schistosomiasis. Lancet, II: 1110-1113, 1967.

(33) Farooq, M., Saaman, S.A. and Nielsen, J.: Assessment of severity of disease caused by *Schistosoma haematobium* infection and *Schistosoma mansoni* in Egypt - 49 Project area. Bull. W.H.O., 35:389-404, 1966.

(34) Ford, M.M.: The advance letter in mail surveys. Journal of Marketing Research, 4:202-204, 1967.

(35) Forsyth, D.M.: Practical difficulties in the treatment of schistosomiasis in an Arab community, Part II. Trans. R. Soc. Trop. Med. Hyg., 55:168-177, 1961.

(36) Forsyth, D.M.: Treatment of urinary schistosomiasis. Practice and Theory. Lancet, II:354- 356, 1965.

(37) Forsyth, D.M.: A longitudinal study of endemic urinary schistosomiasis in a small East African community. Bull. W.H.O., 40: 771-783, 1969.

(38) Forsyth, D.M. and Bradley, D.J.: The consequences of bilharziasis: Medical and public health implications in North-Western Tanzania. Bull. W.H.O., 34:715-735, 1966.

(39) Forsyth, D.M. and Macdonald, G.M.: Urological complications of endemic schistosomiasis in school children. Part I: Usagara School. Trans. R. Soc. Trop. Med. Hyg., 59:171-178, 1965.

(40) Forsyth, D.M. and Rashid, C.: Treatment of urinary schistosomiasis. Practice and Theory. Lancet, II:130-133, 1967.

(41) Forsyth, D.M. and Rashid, C.: Treatment of urinary schistosomiasis with trichlorophone. Lancet, II:909-912, 1967.

(42) Friedman, G.D.: Primer of Epidemiology. New York, McGraw Hill, p. 142

(43) Ibid., p. 124.

(44) Ibid., p. 126.

(45) Gilles, H.M., Lucas, A., Adeniyi-Jones, C., Lindner, R., Anand, S.V., Braband, H., Cockshott, W.P., Cowper, S.G., Muller, R.L., Hira, P.R. and Wilson, A.H.M.: *Schistosoma haematobium* infection in Nigeria II. Infection at a parimary school in Ibadan. Ann. Trop. Med. Parasitol., 59:441-450, 1965.

(46) Gilles, H.M., Lucas, A., Lindner, R., Cockshott, W.P., Anand, S.V. and Cowper, S.G.: *Schistosoma haematobium* infection in Nigeria III. Infection in boatyard workers at Epe. Ann. Trop. Med. Parasitol., 59:451-456.

(47) Hiatt, R.A., Cline, B.L. and Knight, W.B.: Limitations of the intradermal test for schistosomiasis mansoni: Experience from epidemiologic studies in a Puerto Rican community. Am. J. Trop. Med. Hyg., 27:535-541, 1978.

(48) Jewsbury, J.M. and Cooke, M.J.: Prophylaxis of schistosomiasis--Field trial of metrifonate for the prevention of human infection. Ann. Trop. Med. Parasitol., 70:361-363, 1976.

(49) Jewsbury, J.M., Cooke, M.J. and Weber, M.C.: Field trial of metrifonate in the treatment and prevention of schistosomiasis infection in man. Ann. Trop. Med. Parasitol., 71:67-83, 1977.

(50) Jordan, P.: Egg output in Bilharziasis in relation to epidemiology, pathology, treatment and control in Bilharziasis. Mostofi, F.K. (ed.), pp. 93-102, Springer-Verlag, New York, 1966.

(51) Jordan, P.: Schistosomiasis: Research to control. Am. J. Trop. Med. Hyg., 26:877-886, 1977.

(52) Jordan, P. and Unrau, G.O.: Simple water supplies to reduce schistomiasis. Tropical Doctor, 8:13-18, 1978.

(53) Jordan, P., Woodstock, L., Unrau, G.O. and Cook, J.A.: Control of *Schistosoma mansoni* transmission by provision of domestic water supplies. Bull. W.H.O., 52:9-20, 1975.

(54) Kagan, I.G. and Norman, L.: Serodiagnosis of parasitic diseases. Ch. 51 in Manual of Clinical Immunology, Bethesda, Maryland. American Society for Microbiology, 1976.

(55) Kale, O.O. and Lucas, A.O.: Oxaminiquine (UK4271) in the treatment of vesical schistosomiasis in Western Nigeria. Rev. Inst. Med. Trop. Sao Paulo, 20:55-63, 1978.

(56) Khalil, H.M., Arafa, M.S., El-Nahhal, H.S., Azab, M., Safar, E. and El-Sherif, F.A.H.: A sero-parasitological study of schistosomiasis among males from various regions of Egypt. J. Egypt. Pub. Hlth. Assoc., LII:12-24, 1977.

(57) Kloetzel, K.: "Selective" chemotherapy for schistosomiasis mansoni. Trans. R. Soc. Trop. Med. Hyg., 68:344, 1974.

(58) Kloos, H., Polderman, A.M., Desole, G. and Lemma, A.: Haematobium schistosomiasis among semi-nomadic and agricultural Afar in Ethiopia. Trop. Geogr. Med., 29:399-406, 1977.

(59) Lehman, J.S. Jr., Farid, Z., Smith, J.H., Bassily, S. and Ayad, N.: Urinary schistosomiasis in Egypt: Clinical, radiologic, bacteriologic and parasitologic correlation. Trans. R. Soc. Trop. Med. Hyg., 67:384-399, 1973.

(60) Lucas, A.O., Adeniyi-Jones, C.C., Cockshott, W.P. and Gilles, H.M.: Radiological changes after medical treatment of vesical schistosomiasis. Lancet, II:631-633, 1966.

(61) Lucas, A.O., Akpom, C.A., Cockshott, W.P. and Bohrer, S.P.: Reversibility of the urological lesions of schistosomiasis in children after specific therapy. Ann. N.Y. Acad. Sci., 160:629-644, 1969

(62) Lucas, A.O. and Gilles, H.M.: A Short Textbook of Preventive Medicine for the Tropics. English University Press Ltd., 1973.

(63) Macdonald, G., Forsyth, D.M. and Rashid, C.: Urological complications of endemic schistosomiasis in school children. Part 4. As modified by treatment. Trans. R. Soc. Trop. Med. Hyg., 62: 775-781, 1968.

(64) MacMahon, B. and Pugh, T.F.: Epidemiology: Principles and Methods. Little, Brown and Company, Boston, 1970.

(65) Maegraith, B.G.: Adam and Maegraith Clinical Tropical Disease. Blackwell Scientific Publications, Oxford and Edinburgh, 1971.

(66) Matovi, D.G.: Prospects of schistosomiasis at the Kidatu Dam Project in Tanzania. Trop. Geogr. Med., 29:266-270, 1977.

(67) Mausner, J.S. and Bahn, A.K.: Epidemiology: An Introductory Text. W.B. Saunder and Co., 1974.

(68) McCullough, F.S.: The susceptibility and resistance of *Bulinus (Physopsis) globosus* and *Bulinus truncetus rohlfsi* to two strains of *Schistosoma haematobium* in Ghana. Bull. W.H.O., 20:75-85, 1959.

(69) Meyes, F.H., Jawetz, E. and Goldfein, A.: Review of Medical Pharmacology. Lange Medical Publications. Drawer L, Los Altos, California, 1980.

(70) Murphy, E.A: The Logic of Medicine, pp. 239, Baltimore: John Hopkins University Press, 1976.

(71) Mustacchi, P. and Shimkin, M.B.: Cancer of the bladder and infestation with *Schistosoma haematobium*. J. Natl. Cancer Inst., 20:825, 1958.

(72) Niemandt, S. and Murahwa, S.: Metrifonate in the treatment of urinary bilharziasis (letter to the editor). S. Afr. Med. J., 49:1860, 1975.

(73) Nnochiri, E.: Medical Parasitology in the Tropics. Oxford University Press, 1973.

(74) Omer, A.H.S.: Treatment of severe forms of schistosomiasis. Tropical Doctor, 8:3-7, 1978.

(75) Omer, A.H.S. and Teesdale, C.H.: Metrifonate trial in the treatment of various presentations of *Schistosoma haematobium* and *Schistosoma mansoni* infections in the Sudan. Ann. Trop. Med. Parasitol., 72:145-150, 1978.

(76) Onori, E.: Observations on variations in *Schistosoma haematobium* egg output, and on the relationship between the average egg output of infected persons and the prevalence of infection in a community. Ann. Trop. Med. Parasitol., 56:292-296, 1962.

(77) Pellegrino, J., Coelho, P.M.Z. and Sampio, D.B.M.: Intradermal test with histamine in schistosomiasis mansoni. Am. J. Trop. Med. Hyg., 27:363-364, 1978.

(78) Pesigan, T.P. and Nelson, G.H.: The effect of snail control on the prevalence of *Schistosoma japonicum* in the Philippines. Bull. W.H.O., 25:479-482, 1961.

(79) Peters, P.A., Mahmond, A.A., Warren, K.S., Ouma, J.H. and Arap Siongkok, T.K.: Field studies of a rapid and accurate means of quantifying *Schistosoma haematobium* eggs in urine samples. Bull. W.H.O., 54:159-162, 1976.

(80) Pi Sunyer, F.X., Gilles, H.M. and Wilson, A.M.: *Schistosoma haematobium* infection in Nigeria I. Bacteriological and immunological findings in the presence of schistosomal infection. Ann. Trop. Med. Parasitol., 59:304-311, 1965.

(81) Pitchford, R.J.: Findings in relation to schistosome transmission in the field following the introduction of various control measures. S. Afr. Med. J., 40(suppl.):3-16, 1966.

(82) Pugh, R.N.H.: A filtration method for schistosome egg quantification. Ann. Trop. Med. Parasitol., 72:387-388, 1978.

(83) Pugh, R.N.H.: Periodicity of output of *Schistosoma haematobium* eggs in urine. Ann. Trop. Med. Parasitol., 73:89-90, 1979.

(84) Pugh, R.N.H.: Malumfashi Endemic Diseases Research Project, VII. The importance of young males from the Mahumfashi area, Northern Nigeria, in the transmission of *Schistosoma haematobium* infection. Ann. Trop. Med. Parasitol., 73:189-190, 1979.

(85) Pugh, R.N.H. and Gilles, H.M.: Malumfashi Endemic Diseases Research Project III: Urinary schistosomiasis: A longitudinal study. Ann. Trop. Med. Parasitol., 73:471-482, 1978.

(86) Pugh, R.N.H. and Gilles, H.M.: Malumfashi Endemic Diseases Research Project VIII: Follow-up of intravenous urograms of boys infected with *Schistosoma haematobium* from the Malumfashi area. Ann. Trop. Med. Parasitol., 78:293-294, 1979.

(87) Pugh, R.N.H. and Gilles, H.M.: Malumfashi Endemic Diseases Research Project IX: Urinary schistosomiasis and hypertension in the Malufashi area. Ann. Trop. Med. Parasitol., 78:293-294, 1979.

(88) Reddy, S., Oomen, J.M.V. and Bell, D.R.: Metrifonate in urinary schistosomiasis: A field trial in Nigeria. Ann. Trop. Med. Parasitol., 69:73-76, 1969.

(89) Sackett, D.L.: Design, Measurement and Analysis in Clinical Trials. Platelets, Drugs and Thrombosis Symp., Hamilton, 219-225, 1972 (Basel, Karger, 1975).

(90) Sackett, D.L.: Bias in analytic research. J. Chron. Dis., 32: 51-63, 1979.

(91) Sadun, E.H.: Serodiagnosis of schistosomiasis. Ch. 10. Immunology of Parasitic Infections, (eds.) Cohen, S., and Sadun, E. Oxford, Blackwell, 1976.

(92) Scientific Working Group on Schistosomiasis: Epidemiology and control of schistosomiasis: Present situation and priorities for further research. Bull. W.H.O., 56:361-369, 1978.

(93) Scott, J.A.: Egg counts as estimates of intensity of infection with *Schistosoma haematobium*. Tex. Rep. Biol. Med., 15:425-530, 1957.

(94) Smith, J.K., Torky, H., Mansour, N. and Cheever, A.N.: Studies on egg excretion and tissue egg burden in urinary schistosomiasis. Am. J. Trop. Med. Hyg., 23:163-168, 1974.

(95) Smith, J.K., Elwi, A., Kamel, I.A. and von Lichtenberg, F.: A quantitative postmortem analysis of urinary schistosomiasis in Egypt. II. Evolution and Edpidemiology. Am. J. Trop. Med. Hyg., 24:806-822, 1975.

(96) Stafford, J.E.: Influence of preliminary contact on mail returns. Journal of Marketing Research, 3:410-411, 1966.

(97) Stimmel, C.M. and Scott, J.A.: The regularity of egg output of *Schistosoma haematobium*. Tex. Rep. Biol. Med., 14:440-458, 1976.

(98) von Lichtenberg, F., Edington, G.M., Nwabuebo, I., Taylor, J.R. and Smith, J.H.: Pathologic effects of schistosomiasis in Ibadan, Western State of Nigeria II: Pathogenesis of lesions of the bladder and ureters. Am. J. Trop. Med. Hyg., 20:244-254, 1971.

(99) Warren, K.S., Mahmoud, A.A.F., Cummings, P., Murphy, D.J. and Houser, H.B.: Schistosomiasis mansoni in Yemeni in California. Duration of infection, presence of disease and therapeutic management. Am. J. Trop. Med. Hyg., 23:902-909, 1974.

(100) Warren, K.W.: The immunopathogenesis of schistosomiasis: A multidisciplinary approach. Trans. R. Soc. Trop. Med. Hyg., 66:417-434, 1972.

(101) Warren, K.S., Arap Siongkok, T.K., Houser, H.B., Ouma, J.H. and Peters, P.A.: Quantification of infection with *Schistosoma haematobium* in relation to epidemiology and selective population therapy. 1. Minimal number of daily egg counts in urine necessary to establish intensity of infection. The Journal of Infectious Diseases, 138:849-855.

(102) Warren, K.S., Mahmoud, A.A.F., Muruka, J.F., Whittaker, L.R., Ouma, J.H. and Arap Siongkok, T.K.: Schistosomiasis haematobium in Gaet Province of Kenya. Relationship between egg output and morbidity. Am. J. Trop. Med. Hyg., 28:864-870, 1979.

(103) Wenlock, R.W.: The prevalence of hookworm and of *Schistosoma haematobium* in Rural Zambia. Trop. Geogr. Med., 29:415-421, 1977.

(104) Wilkins, H.A.: *Schistosoma haematobium* in a Gambian community. I: The intensity and prevalence of infection. Ann. Trop. Med. Parasitol., 71:53-58, 1977.

(105) Wilkins, H.A. and El-Sawy, M.: *Schistosoma haematobium* egg counts in a Nile Delta Community. Trans. R. Soc. Trop. Med. Hyg., 71:486-488, 1977.

(106) Wolfe, H.L.: Treatment of urinary schistosomiasis with niridazole (Ambilhar) in 576 African school children. Lancet, 1:350-354, 1967.

(107) Woodward, C.A., Chambers, L.W. and Smith, K.: Guide to Improved Data Collection in Health and Health Care Surveys.

(108) World Health Organisation: Scientific Group on Research in Bilharziasis (Assessment of medical and public health importance). Report to the Director-General, Geneva, 1960.

(109) World Health Organisation: Technical Report Series, No. 515: Schistosomiasis control (Report of a W.H.O. Expert Committee).

(110) Young, S.W., Farid, Z., Bassily, S. and El-Masry, N.A.: Urinary schistosomiasis: A 5-year clinical, radiological and functional evaluation. Trans. R. Soc. Trop. Med. Hyg., 67: 379-383, 1973.

(111) Personal communication.