RHEUMATOSOMIANTS: COMPARATIVE DIAGNOSTIC METHODS IN THE
ASSSESSMENT OF URINE AND FAecal RGG COUNT

By

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schistosomiasis from Gezira area. Egg count per gram in a single sample was higher when using Kato method as compared to sedimentation technique (p < 0.001). But the sensitivity was the same for both techniques (87.6%). On the other hand egg counts from 24-hr stools samples were not higher when compared to single samples using sedimentation method. However, the sensitivity of the sedimentation method for 24-hr stool collection was higher (100%) than that of single samples (87.6%).

In 15 school children from Faki Hashim village, no daily variation was observed in the excretion of S. mansoni eggs in 24-hr stools samples, collected over a week's period (p > 0.05), using both sedimentation and Kato methods. The sensitivity of the sedimentation technique (100%) was higher than that of the Kato method (33%), based on estimates from 24-hr stool samples. On the other hand the sensitivity of the Kato method was higher in the 24-hr collection (71%) as compared to a single sample (57%).

Results from study also indicated that, there was no egg excretion in 10 patients with periportal fibrosis, followed over 7 days period collection using sedimentation of 24-hr samples.
Treatment with praziquantel (Biltricide, Bayer) was proved to be very effective in curing patients with urinary or intestinal schistosomiasis as measured by sedimentation method on the 24-hr stools samples. Treatment of urinary schistosomiasis patients with 40 mg/kg body weight resulted in significant drop of total daily eggs excretion in day 14 post treatment and 100% cure rate after 6 weeks. When the same dose was administered to intestinal schistosomiasis patients, a significant drop in their egg counts occurred seven days post treatment and they were totally cured after 7 weeks (100% cure rate) as indicated by no eggs excretion.

The study indicated a positive correlation of IgG titres (using FAST ELISA) with the geometric mean of S. mansoni collected over 24-hr. This correlation was found with both sedimentation and Kato methods (p < 0.05). However, the study showed no significant difference in the IgG titres in intestinal schistosomiasis patients pre-treatment and two weeks after treatment, while there was a significant drop in arithmetic mean egg counts of 24-hr stools samples pre-treatment and two weeks post-treatment.

From this study it can be concluded that 24-hr samples collection of urine and stools, were more accurate and...
sensitive than single spot examination in detecting schistosomiasis infection. The accurate number of eggs excreted by individual per day reflects the actual worm burden. Hence, the intensity of infection, which determines the parasite population in the community, can be worked out. The later parameter will help in laying strategies for epidemiological studies and assessment of control parameters.
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CHAPTER ONE
INTRODUCTION

1.1 General Introduction

*Schistosomiasis* is a helminthic infection caused by digenean trematodes of the genus *Schistosoma* and transmitted by fresh-water pulmonate snails. It is one of the most important parasitic diseases affecting man, and is considered a major health problem, second only to malaria, in most of the tropics (Maddisen, 1986). The disease is closely linked with the socio-economic status of the country, where it affects more than 200 million people in the underdeveloped countries of the tropics and subtropics (WHO, 1987).

The three major species of parasite which are of widespread occurrence in humans are *Schistosoma haematobium* (Weinland, 1858), *S. mansoni* (Sambon, 1907) and *S. japonicum* (Katsurada, 1904). *S. haematobium* is limited to the continent of Africa and the Middle East and is transmitted by fresh water snails of the genus Bulinus. *S. mansoni* is found essentially throughout Africa, in some areas of the Middle East, in some Caribbean islands and in many parts of South America. It is transmitted by fresh water snails of the genus Biomphalaria. *S. japonicum* has been eradicated from Japan and China but still constitutes a major problem in
paired adult worms of the intestinal species migrate to the intestinal wall to deposit eggs. Those of *S. haematobium* migrate to the vesical veins and deposit their eggs on the walls of the bladder (Maddisen, 1987).

Development of disease in schistosomiasis is a dynamic process on the part of both the parasite and the host. Since the parasite does not multiply within the definitive human host and the life span of the worm is relatively short, the number of worms within the host is related to the degree of exposure to contaminated water and the period of time over which this occurs. The key parasite factor in the pathogenesis of schistosomal disease is the eggs, many of which lodge in the host tissue and for a short period of time induce an intense localized inflammatory response (Warren, 1987). A large proportion of the ova becomes entrapped in the microvasculature of the host tissues and organs where they are surrounded by cellular immuno-inflammatory lesions called granulomas. The *S. mansoni* egg granuloma has been shown to be essentially a delayed, cell-mediated immuno-logical reaction. Both *S. haematobium* and *S. japonicum* also appear to be largely cell-mediated (Kassas et al., 1978; Cheever et al., 1985b; Olds and Stavitsky, 1986).

Acute schistosomiasis (Katayama fever) occurs 4-8 weeks
1.4 Schistosomiasis in the Sudan

In the Sudan, schistosomiasis is considered as one of the most important public health problems. It was introduced into the country through political and economic contacts with Egypt centuries before the Christian era. The free movements and common agricultural practices between the northern part of the Sudan and the southern regions of Egypt, led to the spread of the disease in the Sudan (Christopherson, 1918). Also the pilgrimage route throughout the Sudan, to and from Mecca, have an important part in the vicinity of the Blue Nile. Balfur (1904) reported the first incidence of schistosomiasis in the Sudan from one of the primary schools in Khartoum. We found that 17% of the children were suffering from urinary schistosomiasis. Later, schistosomiasis was found in all provinces of the Sudan except the Red Sea area (Christopherson, 1918).

The construction of the agricultural scheme in the Gezira area in 1925 opened a new era in the history of schistosomiasis in the Sudan. The Gezira irrigation scheme lies in an area of 180,000 feddans (1 feddan = 1.04 acre). The prevalence of S. haematobium in the Gezira area
was found to be 15% in adults and 45% in children (Stephenson, 1947). Greany (1952) observed the presence of Biomphalaria and Bulinus snails among the dense weeds in all canals in the Gezira area. The disease was reported from Wadi Halfa and other parts of the northern province and later appeared in the White Nile, Nuba Mountains, and parts of Kordofan and Darfur provinces (Annual Medical Report, 1927).

Both types of the disease were reported in Khartoum with a prevalence of 15-70% among school children in the Kober area of Khartoum North (Amin and Omer, 1972). The establishment of the Aswan High Dam increased the incidence of S. haematobium in the Nuba area of the northern province (Amin and Satti, 1973). The incidence of S. haematobium among fishermen in Lake Nuba was found to be 30% (Omer, 1978). Among the Nubian settlers in Wad Halfa, S. mansoni was found to be 10-15% (Amin and Satti, 1973) and S. haematobium 4.5% (Omer, 1978). In the Western regions of Sudan (Kordofan and Darfur Provinces), the inland rainwater resources were suitable habitats for the occurrence and distribution of Bulinus snails, while Biomphalaria snails were restricted to waterfalls in Jebel Marra. In Western Sudan, S. haematobium was found among children in the Nuba Mountains with a prevalence of 9.4 -14%. The incidence of
The national control programme for schistosomiasis was formulated in 1979 as part of the Blue Nile Health Project, the aims of which are the prevention and control of water associated diseases using an integrated comprehensive strategy. The project is made up of two large schemes, Gezira and Rahad. In the Gezira irrigated area, schistosomiasis prevalence exceeds 50% (Omer et al., 1978; Amin 1972; Amin and Fenwick 1977; Fenwick et al., 1982). The prevalence of S. mansoni was 13.2% in 1985 in the study zone monitor villages in Gezira area, compared to 15.9% in 1984. In the Rahad zone the overall prevalence in 17,000 school children was 14.7% in 1996, compared to 11.3% in 1985 and 11.9% in 1984 (Blue Nile Health Project, 1986).

Schistosomiasis exerts a significant toll on the affected populations, in human misery and suffering. The
clinical manifestations of the disease have been documented in numerous case reports. There is no agreement on the frequency with which signs and symptoms are found in relation to the prevalence and intensity of infection in various populations. These difficulties led to the involvement of many diagnostic methods and techniques.

1.3 Diagnosis of Schistosomiasis:

Parasitological methods have been described mainly for testing the presence of characteristic schistosome eggs in urine or feces samples, and less frequently by finding adult worms in tissues using histological sections (Jordan, 1982). Thus, there are many qualitative and quantitative parasitological methods. Qualitative methods are those for detecting ova and are used in clinical practice. Quantitative methods are those to determine the intensity of the infection and are used mainly for research in the fields of control, clinicopathology, and drug trials.

All the methods for examining urine and stool for helminth eggs have advantages and disadvantages. Any technique to be used depends on the aim of the examination, no technique is perfect for all purposes.

The detection of ova in urine is comparatively simple, as urine is a homogeneous fluid. The greatest number of eggs
Syringe filtration for diagnosis and detection of *S. haematobium* eggs has greatly simplified the operational aspects of field work (Bradley, 1965). The syringe filtration test is simple, sensitive and reproducible under field conditions and in hospitals; it is used as a first screening method. The cost of consumable materials, such as polycarbonate filters or paper filters which require expensive stains, may be a limitation to their use in large-scale surveys or control programs (Peters et al., 1975). Monofilament polyamide (Nytrel) filters may be appropriate for a large-scale survey, as they can be washed and reused at least 200-200 times or more (Mott et al., 1982). The hatching test is useful for assessing the quantity and hatchability of *Schistosoma* eggs resulting in an easy collection of reliable and important data, even when laboratory facilities are poor (Justesen van Bloterdijk, 1977). However, fresh material is essential for this test.

Semi-quantitative urine analysis reagent strips have only been used in a few studies to screen for pathological findings in urinary schistosomiasis. These studies showed that in areas where schistosomiasis *haematobium* is endemic, haematuria and proteinuria are common findings and parallel the prevalence of infection (Briggs et al., 1971; Ezat et al.
al., 1974), Wilkins et al. (1975) and Pugh et al. (1980) suggested that reagent strips for assessment of proteinuria or haematuria may be used as a diagnostic tool to identify heavily infected subjects. They showed that the combined criterion of proteinuria and haematuria was related to intense infection. Faldmeier et al. (1982) confirmed these findings and proved that degrees of haematuria, proteinuria and leucocyturia have been correlated with the level of egg excretion.

Handling and reading of reagent strips is extremely simple and fast, and can be performed over by untrained paramedics. Under field conditions, especially when only limited manpower is available, the combined reagent strip index may be a useful tool for diagnosis of heavily infected patients and a proof of decrease in parasite load after treatment. It should also facilitate the approach to selective chemotherapy which aims only at drastic reduction of egg excretion (Faldmeier et al., 1982).

In 1954 Kato and Miura introduced a cellophane-thick smear technique for stool examination that proved very satisfactory in Japan and which was modified and improved by many workers (Martin and Beaver, 1968; Layrisse et al., 1969; Borda and Pellegrino, 1971; Katz et al., 1972).
During epidemiological work in the Sudan, a temporary
difficulty in obtaining cellophane led to its substitution by
thick glass coverslips (Teedale and Abin, 1976). A
disadvantage of the modified technique is that the slides
cannot be stored. However, drying out can be arrested for
some time by placing the slides in a humidity chamber or by
placing a small drop of 50% glycerine in water on each
coverslip before pressing the faecal sample on it. The
subsequent evaluation of this modification by comparison with
the Bell technique (Bell, 1963) and the digestion technique
(Teedale and Abin, 1976), indicated that the modified method
was sensitive and most suitable for field conditions. The
Bell Filtration method was equally sensitive in detecting
light infections and the stained filter-paper preparation is
a semipermanent record which can be stored and examined when
convenient, but it was more tedious and complicated to
perform than the thick smear technique. The simple direct
faecal smear is necessary for the detection of moderate and
heavy infections, but light infections will be overlooked.
The techniques based on sedimentation or centrifugation have
been developed for detection of light infections. The
sedimentation method requires minimum equipments and reagents
and is suitable for field studies (Hoffman et al., 1984;
Barbosa, 1959). Other concentration methods such as formol-ether (Ritchie, 1948), acid ether (Hunter et al., 1948) and merthiolate-indigo-formaldehyde concentration (Blagg et al., 1955) techniques involve removal of fat, faecal debris and mucus by centrifugation and require more equipment and chemicals. These techniques have been modified to improve sensitivity but they are generally not suitable for large-scale epidemiological studies.

The diagnosis of schistosomiasis in the acute stage of the disease can readily be made by finding eggs in urine or stool. In the chronic stage of infection, recovery of eggs is much more difficult. Other parasitological techniques, such as rectal or hepatic biopsy, generally reveal higher prevalence rates and are valuable for confirming diagnosis in individual cases (Garcia, 1976). However, for epidemiological purposes these methods and repeated stool and urine examinations are neither practicable nor economical and other methods such as immunological tests should be employed (Kagan and Pollegro, 1981).

Immunological techniques:

Many serological tests of varying complexity have been investigated. All of these are based on the indirect
evidence of the presence of antibodies formed against schistosomes. These tests include:

(i) Cercarial Hullen reaction.
(ii) Circumoval precipitin reaction.
(iii) Intradermal test.
(iv) Complement fixation test.
(v) Fluorescent antibody test.
(vi) Gel precipitation.
(vii) Radio-immunossay.
(viii) Enzyme linked immunosorbent assay (ELISA).
(ix) FANT ELISA technique.

Immunological diagnostic tests were first used by the Japanese workers Fujinami and Nakamura (1969) to demonstrate the presence of complement fixing antibodies in sera of infected subjects. Since then many immunological tests have been developed. In general, these tests are used to confirm the clinical findings, but treatment is not given merely on the basis of immunological diagnosis. The Cercarial Hullen Reaction is not species-specific but is capable of detecting infection as early as 40-47 days after experimental infection. Highest reactivity rates (36%) were found in the younger age groups. *S. mansoni* cercariae gave better results than *S. haematobium*, whether the patient suffered
from *N. mansoni* or *S. haematobium* (Jordan and Osatley, 1967).

The circuval precipitin test is sensitive and specific in *S. mansoni* infections (Hillier et al., 1979). This reaction takes place between specific antibodies and secretions which are produced by the living miracidium inside the egg and diffuse through the egg shell wall. This test was found to be more sensitive and specific when used with fresh whole eggs of *S. mansoni* than other tests with cercariae or adult worms.

The intradermal tests are of two forms: The delayed reaction and immediate reaction. The sensitivity and size of the weal in both reactions have been shown to correlate with intensity of infection as assessed by quantitative egg counts (McKay et al., 1973). Recent experiences in Puerto Rico, Uganda and St. Lucia have shown the immediate reaction test lacks sensitivity and specificity, particularly among children. The delayed skin test is specific, but lacks sensitivity (Warren et al., 1973).

The complement fixation test is a complicated and highly delicate technique and was found unsuitable for field operations but it is considered as one of the best serological tests when conducted under optimum conditions. The sensitivity of this test seems to differ from that of the
intradermal test, where adults with a prolonged exposure to infection show a greater response than do young children (Jordan, 1982).

The fluorescent antibody test is relatively simple to perform and is by far the most sensitive of the standard procedures for sero-diagnosis of schistosomiasis. Thus, it is well suited for screening and for early detection of infection. On the other hand, this procedure tends to give a higher level of non-specific or cross reactivity than the other serologic tests, and the readings must be made on a subjective basis (McCarten et al., 1975).

The radioimmunoassay (RIA) is highly sensitive and immunologically specific, but requires a radioactive antigen and sophisticated equipments.

In recent years various studies have been concerned with the applicability of the enzyme linked immunosorbent assay (ELISA) for immunodiagnosis (Folderman and Deelder, 1977). The ELISA test can be interpreted with the naked eye, while the test itself can be carried out without elaborate laboratory facilities when all reagents are adequately prepared in advance (Folderman and Deelder, 1977). ELISA is theoretically highly sensitive. Further purification of the antigens is required in order for this method to be useful in
mepcopediology. ELISA test lacks the ability to distinguish untreated from recently treated infections, and lacks the immunological specificity as well (Nillyer et al., 1979).

The Falcon essay screening test (F.A.S.T.) system is a developing system of kinetic-based enzyme-linked immunosorbent assay (E-ELISA). The FAST-ELISA is used for detecting antibodies against S. mansoni adult microsomal antigens (MAMAs). The FAST-ELISA is ideal for field studies, due to the storage stability of MAMA antigen, ability to perform the assay with minimal equipment, sensitivity, short assay time, and ease of operation (Kemnook et al., 1986). MAMA showed highly specific activity and low cross-reactivity, when compared with other reported serologic antigens. Egg antigens, when compared with MAMA under the same k-ELISA configuration, showed significantly higher amounts of cross-reactivities (Tsang et al., 1983).

1.4 Treatment of Schistosomiasis:

Chemotherapy is one of the specific measures control against schistosomiasis, and currently represent the single most effective and practical strategy to combat human schistosomiasis, both in individuals and populations.

The objective of treatment of systemic schistosomiasis
is to cure and eradicate the infection of the non-replicating trematodes and thereby the cessation of laying of eggs which would be seeded into the tissues. The complete absence of eggs would have the advantage of interrupting transmission. In practice cure has not been easy to achieve and the custom has been to define the activity of a drug in terms of reduction of egg excretion (Mansfield, 1984).

Three widely tested effective and relatively safe drugs are now available:

(a) Praziquantel (Biltricide, Bayer): This is a quinoline drug and a major advance in the treatment of trematode infections. It is active against mature infections and inhibits egg production by the female of the distinct species of schistosomes infecting man and some other trematodes. For treating schistosomiasis, the drug is administered orally as a single oral dose of 40 mg/kg body weight in S. haematobium and S. mansoni. The currently recommended total dosage for S. japonicum is 60 mg/kg body weight given in two to three doses four hours apart (Webster, 1987). The drug is selectively toxic to the parasite, as the host is essentially unaffected (Mansfield, 1984).

(b) Metrifonate (Bilarcil, Bayer): This is an organophosphorous cholinesterase inhibitor, clinically
effective against S. haematobium. The cure rate for metrifonate was found to be (90%) against urinary schistosomiasis, but it was only 19% against intestinal schistosomiasis (Umer, 1975). It is given orally in doses of 7.5-10 mg/kg body weight, in three doses, 2 weeks apart.

(1) Oxamniquine (Mansil, Mansil, Pfizer Inc.): A tetraphthoquinoline derivative, active against S. mansoni infections. The response of the infection varies in different localities. A single oral dose of 15 mg/kg body weight in adults and a total dose of 20 mg/kg in children is adequate, given in two equally divided doses, 4-6 hour apart. A higher dosage is needed in other localities. A total dose of 60 mg/kg given as 15 mg/kg twice daily, given over two days in Sudan. The drug is effective against immature worms in mice and monkeys (Foster, 1973; Dostordino et al, 1974).

1.5 Objectives of the study:

From the methods described earlier, it is clear that there is a large number of techniques which could be used for the diagnosis of schistosomiasis. Isolated attempts have been made in India to evaluate the efficacy of individual diagnostic tests against schistosomes. However, few efforts were made to compare them with each other, e.g., by Agrawal
and Schaesbude (1982) and Panesar and Agrawal (1985). Nevertheless, there is no direct correlation between the egg-output and/or level of serum antibodies detected in the various methods described and the actual worm burden (Sca, personal communication).

Most of the diagnostic surveys on egg-output were done on midday urine samples or a single faecal sample. Some reports on egg counts were done on 24-hr urine collection or three consecutive days count for stool. Thus, no sampling throughout the 24-hr period of the day has been widely attempted. The objective of the present work is to study the variability of urine and faecal egg-output by applying some of the most widely used techniques in the field.

1.5.1 The specific objectives are:

1- To elucidate the variation of counts in urine and faecal egg-output as per a single sample and 24-hr collection.

2- To study the daily variation of total faecal egg-output for a seven days period, so as to investigate whether there are any changes and differences in the two counts recorded for the same patient on the two consecutive days of examination.

3- To determine the effect of chemotherapy on total egg-output in schistosomiasis patients and to follow these
individuals over a period of time to observe any changes in egg-output.

- To define the correlation between immunological response, using one of the recent technique (FAST ELISA), and total faecal egg-output and to determine the effect of chemotherapy on these parameters.
CHAPTER TWO
MATERIALS AND METHODS

2.1 Study areas (Fig. 1):  

2.1.1 Gezira area:  

The Gezira irrigated scheme was initiated in 1925 and the Managil Extension was completed in 1963. The Gezira-Managil scheme is irrigated from the Blue Nile River. The area comprises approximately 2 million feddans (1 feddan = 1.04 acres) of irrigated land lying between the Blue and White Niles, north of Khartoum in the central part of the Sudan. The area is almost flat with a clay soil. The main crop is the cotton which is the first export crop.

The Arab population of the area is approximately 1.5 million of which 50% are estimated to be infected with Schistosoma mansoni. In addition, there is a settled labour force of Western Sudanese and immigrants from Chad and Nigeria. Their infection rate with S. mansoni is also 50% (Fenwick et al., 1982). Additionally, up to half a million seasonal labourers come from Western Sudan to pick cotton from January to May every year (Babiker, et al., 1985).
of the area. Thirty six of these were from Wad Hamli and 49
from Zakiah village. The children ages ranged from 11 to 13
years old.

Patients with schistosomiasis, of different age groups,
reporting to the Bilharzia Research Unit, Institute of
Tropical Diseases, National Health Laboratory, were included
for the follow-up treatment study.

2.2.2 Intestinal Schistosomiasis:

Four studies were conducted on intestinal
schistosomiasis patients. The first study was conducted on
16 patients, ranging from 12 to 14 years of age, to show the
daily variation of egg output and also in 10 adult patients
with periportal fibrosis, coming to the portal hypertension
clinic, Soh University Hospital.

The second group was selected after a survey of the schools
in the Giza area was undertaken. Single and 24-hr fecal
samples were collected from 80 infected school children
ranging from 10 to 14 years of age.

The third study was to observe the effect of
chemotherapy on the circadian rhythm of egg output on 10
patients. Their ages ranged from 30 - 60 years.

The final study was conducted on 49 school children from
Giza area, aged 10 to 16 years to correlate antibody titre
to the total egg output using Falcon Assay Screeching Test (F.A.S.T.) system and to see the effect of chemotherapy on those parameters.
2.3 Urine Examination and Collection:

2.3.1 Midday Urine Collection:

Subjects found to be positive were asked to give midday (11:00 a.m. - 2:00 p.m.) urine samples in labelled plastic tubes. The tubes were shaken and 10 ml of urine were pipetted into graduated tubes, centrifuged at 1,500 r.p.m. for 2 minutes and the supernatant decanted. If a pellet of red blood corpuscles (RBCs) was seen, the deposit was washed with cold water to lyse the RBCs, and re-centrifuged. If crystals were present in the pellets, they were dissolved by the addition of 5% acetic acid or dilute HCl (10%). The cleared pellet was pipetted into a Sedgwick-Rafter chamber, the eggs were counted microscopically using 10X magnification.

2.3.2 Twenty Four Hour Urine Collection:

Each patient was given a bottle (2.5 l) for the 24-hr urine collection. The collected samples were shaken manually and four 10 ml aliquots were removed at mid-depth from each sample using a pipette. The subsamples were centrifuged at moderate speed for 2 minutes and the supernatants decanted. If there was gross haematuria in the deposit, it was washed with cold water to lyse RBCs and re-centrifuged. Crystals were dissolved by the addition of 5% acetic acid or dilute
with cold water and the suspension was strained through an 0.3 mm mesh gauge to remove large fecal debris. The sediment was allowed to settle and the supernatant fluid was poured off. The sediment was washed several times with cold water until the supernatant fluid was clear. The clear supernatant was removed and 0.85% saline was added to prevent egg hatching. The sediment was diluted with saline to a final volume of 200 ml for the 24-hr stool samples and 50 ml for single stool sample. Four aliquots of 1 ml each were taken from each sediment suspension after mixing. Each aliquot was transferred to a counting chamber and the number of eggs per gram was calculated.

2.5 Description of the FAST Enzyme-Linked Immunosorbent Assay (Hancock and Tsang, 1986):

The polystyrene beads attached to the lid of the Falcon assay test plate were sensitized with MAMA and air dried. Standards for the assay were made by diluting a serum standard derived by concentrating pooled sera from individuals infected with the Puerto Rican strain of S. mansonii. The serum standard pool was diluted with 0.1 M phosphate buffered saline, pH 7.2, containing 0.3% tween 20 (PBS-T20). Patients sera were assayed in triplicate using 1 ul of serum per well plus 97 ul of PBS-T20. A positive, a
negative serum control and PBS-T20 control were included in each 96 well microtitre plate. The total volume for each well in the titre plate was 100 μl.

The antibody units of the standards ranged from 10 to 1000 units. The sensitized plates, serum standards and peroxidase labelled goat anti-human IgG, used later for the assay, were provided by Dr. Victor Tsang, Centre of Disease Control, U.S.A. The antigen was brought to the Sudan by Dr. L. Foster in 1987, to be tested in the endemic area.

The antigen beads were rinsed with PBS-T20 followed by a rinse with distilled water. The serum plate was placed on the Mini-Orbital shaker, the lid with the antigen beads was put on the top of the serum plate and the shaker was set at speed no. 2 for 5 minutes. After shaking, the antigen plate was removed from the sera and rinsed with PBS-T20 and water, the excess was shaken off. The antigen plate was placed into the trough containing the peroxidase labelled conjugate. Then the plate was again placed on the shaker and agitated at a speed number 2 for 5 minutes. The antigen plate was removed from the conjugate, and was rinsed with PBS-T20 and water, the excess was shaken off. The antigen plate was placed in the microtitre plate containing the TMB-H2O mixture, 150 μl of the mixture per well at speed no. 2 for
1-2 minutes. Then the antigen plate was removed from the substrate to stop the reaction. The bottom of the microtitre plate was wiped and the optical density was read with a 650 nm filter. The ELISA value of 20 antibody units or less was considered negative for schistosomiasis infection in this assay (L. Foster (1987) personal communication). The antibodies unit were calculated from the optical density readings by using the standard curve. The standard serum pool was prepared from the sera of 14 Puerto Rican patients with parasitologically confirmed S. mansoni infections. The stool examination of the patients was negative for all other parasitic infections. A standard curve was included with each assay for antibody activity.

2.6 Statistical Analysis:

The statistical tests used in this study were, student’s t-test (paired), analysis of variance (ANOVA) and correlation using SPSS PC+ computer programme. (SPSS Inc., Chicago, U.S.A.). The Statgraphics statistical computer program (STSC-Inc.) was also used.
CHAPTER THREE

EXPERIMENTAL STUDIES

3.1. Study 1:

Comparison of Egg Output In A Single Sample To The 24-Hr Excretion:

This study was designed to investigate the variability of urine and faecal egg output by applying two of the most widely used techniques. For urinary schistosomiasis egg counts in a 10 ml from midday urine samples were compared with the mean egg counts/10 ml from the 24-hr urine collection of the next day. For intestinal schistosomiasis the egg count per gram of a single stool sample was compared to the egg count/gm from a 24-hr stool collection on two consecutive days.

3.1.1 Urinary schistosomiasis

Surveys of urine for schistosomiasis infection in 89 schoolboys, aged 9 to 18 years, were carried out at selected schools in two villages, north of Khartoum:

a- Wad Ramli village.
b- Zakia village.

A midday urine sample was collected from each of those students who were found to be positive and 24-hr urine
In the deposit were counted as described in material and methods. The 24-hr samples were also processed as described in the previous chapter.

Results

The results obtained showed that in 85 infected school children, the mean egg counts /10 ml of the midday samples and 24-hr urine collection was not significantly different (P > 0.05) as shown in Table (1) and Fig. (2). But 24-hr urine collection was more sensitive (100%) than midday urine samples (88.4%).

However in 47 of the patients (55.3%) showed higher egg counts in the midday samples than in mean counts of an equal volume (10 ml) from 24-hr collection. However, 7 patients (8.2%) were negative for S. haematobium eggs in the samples obtained at midday urine examination, and were positive in the 24-hr urine samples collection. On the other hand, 31 patients (36.5%) had higher egg counts in the mean of the 24-hr urine collection /10 ml than in the midday counts in an equal volume. Comparison of the geometric mean of the midday and 24-hr collection are shown in Table 1.

The total volume of 24-hr urine collection ranged from
260 to 2800 ml. This would reflect the very wide variation of the total number of *S. haematobium* eggs excreted by each one of the patients.
Fig. (2) Egg Counts In Midday & 24-Hr Urine Samples Of School Children

Geom. Mean Egg Counts/10 ml

Midday

24 hrs Collect.
The present study showed that the total egg count/day from the infected males and females is not significantly different using the (ANOVA) \( F = 0.590, P = 0.445 \) i.e. no variation due to the sex of the patient. Similarly, no correlation was found between the egg output and age of the infected children aged between 9 to 16 years \( (F = 1.863, P = 1.77) \).

Discussion

The results from this study using ANOVA test, showed that the geometric mean of egg counts of *S. haematobium*, as estimate per 10 ml from midday urine samples and 24-hr urine collections, was not significantly different \( (p > 0.05) \). These findings are not in line with those reported by Stimmel and Scott (1956), Pugh (1979) and Doebring *et al.* (1983), where the higher number of eggs were excreted at midday.

However, 38 \( (44.7\%) \) of the study group indicated that the geometric mean egg counts /10 ml from 24-hr urine sample collections, were higher than those from the midday urine sample.

Dukes (1988) reported that, the storage of urine at room temperature causes a marked destruction of ova, due to the presence of large number of bacteria. However, the problem which we continuously encountered was the presence of a high
concentration of crystals mainly phosphates and oxalates. This occasionally rendered the examination of the deposit difficult. The same author (1968) examined the relationship between urine osmolarity and egg survival. He found that if the osmolarity of urine is reduced by dilution with water a proportion of the eggs contained are destroyed. So, in the use of 24-hr pooled urine specimens, urine osmolarity varies in regular diurnal rhythm and the dilution of urine of high osmolarity with additional urine of low osmolarity may be expected to cause loss of eggs from the former.

3.1.2 Intestinal Schistosomiasis:

Selected schoolboys from El-Sydera village (Gezira Area) were examined for intestinal schistosomiasis. Single and 24-hr stool samples were collected from 89 boys positive for S. mansoni, aged between 10 and 14 years. Each 24-hr stool sample was weighed and washed several times as described in material and methods. Three slides were prepared from each single stool sample, using the modified Kato technique. The single sample was then weighed and washed by the same method.

Results

The mean egg counts from single sample using the sedimentation method were higher than those obtained from the
<table>
<thead>
<tr>
<th>Technique</th>
<th>No. of cases</th>
<th>Geom. Mean ± S.D.</th>
<th>T value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedimentation of 24-hr sample</td>
<td>84</td>
<td>33.04 ± 5.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedimentation of single sample</td>
<td>84</td>
<td>34.19 ± 5.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2)

Geometric Mean Egg Counts/gm Between Single and 24-hr Samples Using The Sedimentation Technique
<table>
<thead>
<tr>
<th>Technique</th>
<th>No. of cases</th>
<th>Geom. Mean ± S.D.</th>
<th>T value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kato of single sample</td>
<td>85</td>
<td>213.79 ± 3.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedimentation using single sample</td>
<td>85</td>
<td>54.19 ± 5.85</td>
<td>10.96</td>
<td>0.00</td>
</tr>
</tbody>
</table>
The results also reflected that there is no association between daily egg output and the sex of the patient in intestinal schistosomiasis (ANOVA $F = 10.818, P = 0.001$).

Discussion:

The significant difference ($P < 0.001$) found between the geometric mean of egg counts/gm of *S. mansoni* using the Kato technique and sedimentation method is mainly attributed to the significantly higher counts of eggs/gm (using the Kato method) as compared with the lower number of eggs obtained by using the sedimentation method. Thus it could be argued that *S. mansoni* eggs are randomly distributed in the faeces surface as could be revealed by the high counts of eggs when using Kato technique. Also, the apparent higher intensity of infection by the Kato method may be due to the sieving step which serves to concentrate the stool specimen by removing fibrous debris.

On adopting the sedimentation method, the eggs can be assumed to be distributed evenly in the washed volume. Hence, a markedly lower estimation of eggs was attained. A suggestion fairly supported by the work of Martin and Weaver (1968) and Woodstock et al. (1972), who reported that eggs were randomly distributed throughout a certain volume of stools. However, Blair et al. (1959) stated: "As the
concentration of eggs on the surface of a formed stool has been found to be higher than within, examination of scrapings of a formed stool has been advocated”.

3.7. Study II:

Variation Of Faecal Egg Counts:

The purpose of this study was to investigate the pattern of egg output from day to day in intestinal schistosomiasis patients. Two methods were applied, modified kato technique and sedimentation for 24-hr stool collection over a period of 7 days. The sensitivity of each diagnostic test was calculated according to the following equation:

\[
\frac{\text{number of positive patients}}{\text{number of (positive + false negative) patients}} \times 100
\]

3.2.1 Group I: Patients with active infection:

A group of 15 schoolboys were selected from Faki Hashim village, North of Khartoum, along the eastern bank of the Nile. Their ages varied from 12-14 years. Eight of the boys proved to be positive for S. mansoni using the modified Kato technique (Teesdale and Amin, 1976) and seven were negative in the primary survey. Twenty four hour stool samples were collected from the 15 boys for seven consecutive days. Four slides were prepared by modified kato from 24-hr stool...
described in materials and methods. Four 1-ml aliquotes were taken from the deposit after agitation and the number of eggs were counted.

Results:

Statistical analysis, using analysis of variance, indicated that there was no significant variation in daily excretion of *S. mansoni* eggs in 24-hr sample (Fig. 4 a, b), using sedimentation method over 7 days period \((F = 0.38, P = 0.89)\). Similarly testing the 24-hr samples by modified kato technique for 24-hr samples did not reveal any significant difference \((F = 0.31, P = 0.91)\).

The seven patients who were stool negative for *S. mansoni* by single modified kato method, were positive using sedimentation method for 24-hr stool samples and keeps being positive over the seven days period (Fig. 5). The other ones who were positive from the begining by modified kato method, keeps being positive for the seven days period using sedimentation for 24-hr samples (Fig. 6).

In the comparison of single and 24-hr stool samples using modified kato technique, four patients were negative for the infection in 24-hr stool samples and seven were negative in
case of the single stool samples. The sensitivity of kato method in 24-hr samples was 71% and that of kato method in single samples was 57%, but higher geometric mean egg counts were obtained by using single modified kato (Fig. 7).

The 24-hr stool samples for 16 school boys collected over 7 days, were treated with both sedimentation and kato techniques (Fig. 8). The sensitivity of the sedimentation method in this study was 10% and that for kato method was 33%.

On the other hand analysis of the results for this study showed that there was a curvilinear relationship between the weight of the stool and total daily excretion of eggs (r = 0.12).

Discussion:

Daily variation of egg excretion in schistosomiasis is important if epidemiological surveys, such as prevalence and intensity of infection, are to be determined on the basis of a one sample survey. However, few workers have analyzed the daily variation of ova excretion in urinary schistosomiasis (Stimmel and Scott, 1956; Scott, 1957; Wilkins and Scott, 1978). On the other hand, Doehring et al. (1983) analyzed the daily variation of S. mansoni ova in urine. It was slightly greater than for S. haematobium. Jordan (1982)
collection. The sedimentation for 24-hr samples was found to be more sensitive than using kato for the same 24-hr samples. Kato technique for 24-hr samples was more sensitive than kato for single samples, the sensitivity may be due to the large amount collected. Hence, the sedimentation for 24-hr stool sample can be used when sensitive technique is required and to avoid repetitive stool examination. The laborious and tedious nature of the technique may restrict its use only to small sample of the population to be followed.

The statistical analysis revealed a curvilinear relationship between the weight of the stool and daily excretion of ova. It is interesting to note that a similar curve of relationship were found by many workers on prevalence and intensity of *S. mansoni* in endemic area. Anderson (1987) stated that "this finding could be of importance in the prevalence curves and one practical consequence of this relationship is the assessment of the impact of control programmes should be based on scores of the intensity of infection as opposed to prevalence".
reduced (Abdel-Wahab et al., 1987).

In the old infection the fecundity of the worms decreased, so the number of eggs decreased. However, in our study the only patient who passed few eggs could be from those trapped in the tissue.
Fig. (4a):

DAILY VARIATION OF GEOMETRIC MEAN EGG COUNTS IN INTESTINAL SCHISTOSOMIASIS PATIENTS

[Graph showing daily variation of geometric mean egg counts over 8 days]
Fig. 4b

Legends:
Box = Middle 50% of the geometric means egg counts.
Whiskers = Flanks 25% of the geometric means egg counts.
Bar = Median of the geometric means egg counts.
Notch = The 95% confidence limit of the median.
Black small box = outliers.

As there is overlap between the notches of the plot in all days, there is no significant difference between the median of the geometric means egg counts in 18 patients.
Fig. (4b):

NOTCHED BOX & WHISKER PLOT OF EGG COUNTS IN 15 INTESTINAL SCHISTOSOMIASIS PATIENTS

Days

Geom. mean egg count

2.7 3.2 3.7 4.2 4.7 5.2 5.7

0 2 4 6 8
Fig. 7  Comparison of Egg Counts/gm
in Intestinal Schistosomiasis Patients

Geometric Mean Egg Counts/gm

24-hr Kato  Single Kato
Children Followed For 7 Days
8 Macrogol EGG Counts in 15 School
Table (4)

Pre-And Post-treatment Egg Counts In Urinary Schistosomiasis Patients

<table>
<thead>
<tr>
<th>Days</th>
<th>No. of patients</th>
<th>Mean egg count/10ml</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day pre-treat. (0-time)</td>
<td>12</td>
<td>95.67</td>
<td>148.31</td>
</tr>
<tr>
<td>1 day post-treat.</td>
<td>12</td>
<td>88.46</td>
<td>122.21</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>121.31</td>
<td>215.42</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>82.16</td>
<td>158.27</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>79.77</td>
<td>114.40</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>79.71</td>
<td>144.72</td>
</tr>
<tr>
<td>14</td>
<td>12</td>
<td>137.87</td>
<td>264.85</td>
</tr>
<tr>
<td>21</td>
<td>12</td>
<td>73.90</td>
<td>169.67</td>
</tr>
<tr>
<td>28</td>
<td>11</td>
<td>2.87</td>
<td>5.72</td>
</tr>
<tr>
<td>35</td>
<td>11</td>
<td>0.86</td>
<td>2.26</td>
</tr>
<tr>
<td>42</td>
<td>11</td>
<td>1.61</td>
<td>5.35</td>
</tr>
</tbody>
</table>

58
Table (5)

Pre- and Post-treatment Egg Counts in Intestinal Schistosomiasis Patients

<table>
<thead>
<tr>
<th>Days</th>
<th>No. of patients</th>
<th>Mean egg count/g</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day pre-treat.</td>
<td>10</td>
<td>90.47</td>
<td>125.85</td>
</tr>
<tr>
<td>1 day post-treat.</td>
<td>10</td>
<td>44.34</td>
<td>95.39</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>33.98</td>
<td>55.84</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>29.72</td>
<td>39.42</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>27.78</td>
<td>42.61</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>13.82</td>
<td>12.90</td>
</tr>
<tr>
<td>16</td>
<td>10</td>
<td>16.14</td>
<td>21.59</td>
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<tr>
<td>18</td>
<td>10</td>
<td>9.75</td>
<td>14.49</td>
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<td>21</td>
<td>10</td>
<td>4.06</td>
<td>10.13</td>
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<tr>
<td>28</td>
<td>10</td>
<td>5.79</td>
<td>18.13</td>
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<tr>
<td>42</td>
<td>10</td>
<td>0.00</td>
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</tr>
</tbody>
</table>
Discussion:

The cure rate was found to be 100% after 4 weeks. Kardaman and his colleagues (1987) obtained a 83.6% cure rate with a single oral dose. Homsića (personal communication) reported a cure rate of 99% after 4 weeks. So our figure of cure rate seems to be slightly higher when compared to these studies. This may be due to the small number of patients in our study group.

3.4. Study IV:

Comparison Of S. mansoni Total Egg Output And IgG Titres Using FAST ELISA

The investigation in this experiment was based on measuring the titre of the immunoglobulin G (IgG) in response to S. mansoni adult microsomal antigens (MAMA). The 49 patients of the study group were selected from Al Syderra village in the Gezira area, aged 10 to 15 years. All of the patients were positive for S. mansoni. The 24-hr stool samples and blood samples about 25 ml from the finger tips were collected from each patient. The stool samples were washed several times with tap water and the deposit was topped to 200 ml, as described in material and methods. The sera were separated and kept in the normal refrigerator till the next day, where the test was done. (FAST ELISA).
indicated that there is a correlation between *S. mansoni* geometric mean of the total egg count in 24-hr stool sample using sedimentation and IgG titres ($r = 0.32 \ P = 0.013$). Also there is correlation between the geometric mean egg count of 24-hr using modified kato technique and IgG titre ($r = 0.34 \ P = 0.009$).

**Discussion:**

The anti-schistosome IgG level in the group of the patients studied had a positive correlation with the total egg excretion ($P < 0.05$). This is in line with the findings of Sher *et. al.* (1977) and Fieldmeier *et. al.* (1985) who stated that the IgG and IgE were positively correlated with the intensity of the infection.

Our results showed that the FAST ELISA technique has higher sensitivity (95.9%) than the sedimentation method for 24-hr stool collection (79.6%). The test has a good prospectus to be used in epidemiological surveys.
<table>
<thead>
<tr>
<th>No.</th>
<th>Total K rate conc.</th>
<th>Total °O.D. K rate conc.</th>
<th>Total K rate conc.</th>
<th>total sati-nal per body wt. stool 24-hr eggs/1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0162720</td>
<td>12000</td>
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<td>210</td>
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</table>

continued
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<thead>
<tr>
<th>No.</th>
<th>Kato Count</th>
<th>Total E. coli</th>
<th>&quot;O.D.&quot;</th>
<th>Unit Rate</th>
<th>Stool wt./ gm</th>
<th>Eggs/ 24-hr</th>
<th>Egg Anti- per body</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
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<td>373</td>
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* Optical Density
3.4.1 IgG titres before and after treatment of human schistosomiasis mansoni:

In this experiment the study group was selected from El Faki Mashim, North of Khartoum. Fifteen school children aged between 10 and 16 years were included. The blood samples and 24-hr stool samples were collected pre-treatment and 2 weeks post treatment. The IgG titres detected in response to MAMA antigen, before and after treatment.

Results:

Statistical analysis of the data using paired t-test showed that there is no significant difference in IgG titres pre-and 2 weeks post treatment (P > 0.05). (Table 7, Fig. 11). But the geometric mean egg count pre-and 2 weeks post treatment is significantly different (Table 7, Fig 12).

Discussion:

It was found that the difference in the IgG level of the study group before and at 2 weeks post treatment with praziquantel was not significant (P > 0.05). This may be due to the fact that 2 weeks is too early to detect any change in the IgG level, while the egg excretion during the same period was significantly reduced.
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Fig. (11):

IgG TITRES IN INTESTINAL SCHISTOSOMIASIS PATIENTS PRE-AND POST-TREATMENT
Fig. (12)  
Mean Egg Counts In Intestinal Schistosomiasis Patients  
Pre- & 2 Weeks Post-treatment
CHAPTER FOUR

DISCUSSION

Epidemiological studies of schistosome infections in human communities are based on two quantitative measures of parasite occurrence and abundance, namely, the prevalence and the intensity of infection. Such studies require the determination of accurate urinary and/or faecal egg output counts particularly in areas where schistosomiasis is endemic. Knowledge of egg counts is of importance for treatment and control strategies. The daily output of eggs in faeces or urine is the only direct observation available for calculating reproduction of the parasite in the human host (Hairston, 1965). Such calculation could be taken as an approach for the analysis of the parasite population in a mathematical model. The mathematical models offer a chance for a closer collaboration between biometrician and parasitologist, and a better acquaintanceship of each with the methods of the other. Thus a predictive mathematical model of the epidemiology of any disease is desirable, both from the standpoint of intellectual satisfaction and from the standpoint of the usefulness in planning measures to control the disease (Hairston, 1965). Several attempts used
mathematical models to describe the distribution or spread of schistosomiasis in a population of snails or humans. Other attempts describe the prevalence of infection in humans and models for the whole transmission cycle including the intensity of infection in man (Jordan et al. 1982).

Therefore, the present study could be of importance since it provides quantitative estimation of excreted egg counts. Furthermore, findings from this work could be used in mathematical models which study schistosomiasis in the Sudan. The methods selected for the study in the present work are primarily parasitological and can easily be undertaken under the field conditions or a research laboratory. In addition they might satisfy some persistent needs in the current research on the statement of Sec and Cross (unpublished paper): “Estimation of total egg secretion is the key to the estimation of worm burden. Therefore, the knowledge of the fraction of the total eggs excreted daily in stool or urine, represented by the sample examined, is crucial to the estimation of worm burden and intensity of infection”.

The findings of the study indicated that the midday and 24-hr counts of S. haematobium eggs, estimated per 10 ml of urine did not significantly differ within the group
(P > 0.05). These findings are not in agreement with those reported by many workers (Scott, 1966; Bradley, 1964 and Pugh, 1979), who showed that higher numbers of eggs were found in urine collected around midday. The high counts in the midday may probably be due to the volume of urine collected or other factors rather than sampling at midday. However, results from our study showed that, the 24-hr samples were more sensitive (100%) than the midday samples (89%).

The results also showed a higher geometric mean of egg counts of *S. mansoni* per gm of faeces when using the Kato method for single samples. Lower values were obtained by using sedimentation for single samples and sedimentation of 24-hr samples respectively. A high sensitivity was obtained by using sedimentation of 24-hr stool samples (100%). Thus it could be argued that *S. mansoni* eggs are randomly distributed on the faeces surface. Thus on adopting the sedimentation method, the eggs can be assumed to be distributed evenly over the washed volume. Hence, a markedly lower and accurate estimation of eggs was attained. This result is supported by the work of Martin and Beaner (1968) and Woodstock et al. (1972), who reported that eggs were randomly distributed throughout a certain volume of stool.
However Blair et al. (1969) stated: "As the concentration of eggs on the surface of a formed stool has been found to be higher than within, examination of scrapings of the surface of a formed stool sample has been advocated."

The intensity of infection is apparently higher when using the modified Kato method. This could be due to the sieving step which may serve to concentrate the stool specimen by removing fibrous debris. However, the results of our study showed that using modified Kato on the 24-hr stool samples was more sensitive than Kato for single samples. Also sedimentation for 24-hr sample was more sensitive (100%) than using modified Kato for 24-hr collection (71%). Therefore, using sedimentation and 24-hr collection would give a more sensitive diagnostic technique. In order to overcome the daily variation of egg counts using modified Kato for single samples, some workers use the mean counts obtained from three days. The daily examination recommended by some workers can probably be replaced by some 24-hr stool collection using sedimentation method for the convenience of the patient and for saving time spent on several days sample collections.

The results of the present study also showed that there was a curvilinear relationship between the weight of the
intestinal schistosomiasis patients. On the other hand in the
urinary schistosomiasis patients there was slight increase in
the excretion of eggs in the first days post-treatment. This
result is in line with the reported by Cheever et al., 1977,
who showed that in post-mortem studies there is a difference
between the development of tissue egg burden in S.
haematobium and S. mansoni. During active infection S.
haematobium ova gradually accumulate in the tissues and
become calcified in contrast to S. mansoni infection in which
there is an equilibrium between rates of acquisition and loss
(Cheever et al., 1977). The ova persist in the tissues and
subjects without an active S. haematobium infection may have
high tissue egg burden, with associated pathology, while
comparatively few S. mansoni ova remain in tissues of
subjects when the worms of infection are no longer active.

The estimation of antischistosome IgG by using FAST
ELISA technique which is fast and specific, was found to be
positively correlated (P < 0.05) with 24-hr collection of
S. mansoni eggs using sedimentation method. However IgG
titres before and two weeks post-treatment did not differ,
while the daily egg-output dropped significantly.
In conclusion from these studies, we can state that, single samples of stool using modified Kato and midday urine using the filtration technique are less sensitive than estimates from 24-hr samples collection. Hence, single samples could be used in large scale epidemiological surveys of intestinal and urinary schistosomiasis. On the other hand the 24-hr estimates can be recommended for more accurate research work in order to provide more reliable base-line data on drug trials, control programmes and for use of data in mathematical models descriptive to that area.
CHAPTER FIVE
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38
تم إجراء هذه البحث لدراسة التباينات في جودة بويج البليارسيا الكيماوية والبليارسيا النباتية، والتي من هذه الدراسات هو تأثير الألوان على جودة بويج البليارسيا. تم استخدام طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمال طريقة الكثبر لقياس نسبة السكريات في البويج البليارسيا. وتم استعمل