BILHARZIA IN SOUTH AFRICA WITH SPECIAL REFERENCE TO THE LABORATORY DIAGNOSIS

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Bilharzia is increasing in importance in South Africa for a number of reasons. It has loomed larger since malaria was brought under control by the application of long-acting insecticides and it has been possible to assess more accurately the damage done by the infection.

The control and virtual elimination of malaria has also made possible great agricultural development in the river valleys of the lowveld of Northern Natal and the North-Eastern Transvaal. This has favoured the intermediate snail hosts of the bilharzia parasite, for they have flourished in the dams and canals of the great irrigation systems, which had been built to water the extensive tropical fruit gardens. These agricultural developments have also brought many thousands of susceptible people to live in the region.

Good roads have also provided easy access to vast tracks in the endemic region of the country for campers, picnickers and other visitors. The increase in popularity of fishing and water-sports generally have also exposed a number of people to infection. Good hotels and pleasant scenery and the promise of pleasant hikes and expeditions have tempted many thousands of the dwellers in the larger cities in the highveld to visit these regions during the weekends and holidays.

Several of these large cities have also extended their suburbs into regions where rivers, streams and dams, harbour the intermediate host snail, and their inhabitants often unknowingly risk infection by paddling or swimming in them.

It is probable that the indigenous people in these areas have been exposed to bilharzial infection since time immemorial, but these recent developments have greatly increased the danger of heavy infections and so have greatly aggravated the problem, for the severity of the disease is closely related to the weight of infection.

GEOGRAPHICAL DISTRIBUTION OF THE SNAIL INTERMEDIATE HOST

It may be recalled that as a general rule in South Africa the rivers flowing into the Indian Ocean between Humansdorp in the Eastern Province in the south and the Limpopo in the north (and north from there) harbour the snail intermediate hosts of the human bilharzia parasite. These are:

Bulinus (*Physopsis*) *africanus*, intermediate host of *Schistosoma haematobium* and also the intermediate host of *Schistosoma mattheei*, a parasite of cattle, sheep and other bovidae, but one which may also parasitize man. *Biomphalaria pfeifferi*, the intermediate host of *S. mansoni*, the cause of intestinal bilharzia.

Bulinus africanus is prevalent throughout the endemic region. *Biomphalaria pfeifferi* is more restricted in its distribution and is found patchily in the tropical and sub-tropical parts of Africa. Recent surveys undertaken jointly by the State Health Department and the South African Institute for Medical Research have confirmed this generally accepted distribution of the infested waters. However, certain exceptions have become apparent. At present the Sundays and the Fish Rivers and their tributaries in the Eastern Province are free from the host snails. On the other hand, several tributaries of the Vaal River have been found to harbour these snails. *Bulinus africanus* has been found in foci in the Mooi River, the Bamboespruit, the Schoonspruit and the Hartz River and more recently in a tributary of the Klip River, south of Johannesburg.

Biomphalaria pfeifferi has been found in the pools of water in stream beds near Buxton in the North-Western Cape. It seems probable that these are newly-discovered but old-established foci of the snails. The details of this survey will be reported in a separate paper. For the present it is relevant to note that a patient who contracts bilharzia will give a history of having lived in or visited and come into contact with water in a region where the intermediate hosts are found.

LIFE CYCLE OF THE PARASITES

The problem of diagnosis, particularly in the early stages of the infection, arises with increasing frequency. Before consideration of diagnostic tests, it is necessary to recall the life cycle of the bilharzial parasite and the clinical manifestations of the disease.

The stage infective to man, the cercaria, which, characteristically, has a forked tail, is shed from the intermediate host snails. Probably more are shed in the day-time than at night and they are more active in daylight, their forked tails acting like propellers. They survive for 24 - 48 hours, during which time they must find their definitive host, man, or die. If successful in their quest, they shed their tails and actively pierce the skin, gain entrance and then travel via the lymphatics and veins to the right side of the heart and then to the lungs. In the lungs they apparently pass through the capillaries to the pulmonary veins and then to the left side of the heart and are then presumably widely disseminated. Ultimately, some reach the intrahepatic branches of the portal vein by a route which has not yet been clearly defined. Here they develop to maturity. There are separate sexes. The male is shorter and broader and encloses the female in a groove made by the ventral folding of the sides of the body, called the gynaecophoric canal. The pairs migrate against the blood stream of the portal circulation to the smaller venules of the veins of the pelvic plexuses in the case of *S. haematobium*, where the eggs are laid and then dissolve their way into the bladder and are excreted in the urine. The miracidium hatches from the egg in water and then in turn seeks out and infects the intermediate host *Bulinus* (*Physopsis*) *africanus*.

In the case of *S. mansoni*, the worms make their way to the venules of the inferior mesenteric veins and the eggs are laid in the mucous and sub-mucous membrane of the large intestine and are normally excreted in the faeces. On gaining access to water, the miracidium hatches and seeks out and infects in turn its intermediate host, *Biomphalaria pfeifferi*.

Five stages in the evolution of the disease are recognized.

1. The Stage of Infection during which the Schistosomes are Puncturing the Skin

It is clinically characterized by intense irritation. In individuals sensitized by previous contacts, marked inflam-

mation may occur, giving rise to a condition known as cercarial dermatitis. It is important to remember that this condition is most frequently caused by non-human cercariae, frequently the cercariae of the parasites of birds, which do not develop further in man.

2. The Stage of Migration

During this stage, the parasites are migrating through the blood and lymph vascular system of their host. The symptoms of this stage begin 1 - 4 weeks after infection and include fever, sometimes associated with rigors, general body pains, frequently urticaria and other allergic manifestations, irritation of the lungs resulting in cough, occasionally slight haemoptysis and some patients develop asthmatic attacks; discomfort and tenderness in the region of the liver which is the result, as Bersohn and Lurie have shown, of an acute hepatitis associated with considerable derangement of liver function.

3. The Stage of Egg-Laying

In urinary bilharzia due to *S. haematobium*, the earliest symptom of this stage is vague lower abdominal pain associated with pain or irritation on micturition followed by haematuria, which is characteristically terminal, and aggravated by exercise. The blood is bright red and the haematuria continues for several years. It is of interest to note, as Gerritsen, Walker, De Meillon and Yeo[1] showed, that the amount of blood lost in adult Bantu sufferers from urinary bilharzia is insufficient to cause hypochromic anaemia. While bladder involvement and haematuria are the characteristic features of this form of bilharzia, the adult worms and their eggs are frequently found in the tissues of the large intestine and frequently involve the appendix, sometimes causing subacute appendicitis.

In *S. mansoni* infections the commonest manifest symptom is diarrhoea. It might be so slight as not to worry the patient or may be so severe as to simulate acute dysentery. The condition often becomes chronic, simulating ulcerative colitis. In some cases the brunt of the disease falls on the viscera, particularly the spleen and liver, which are often enlarged, sometimes considerably so.

The worms of both *S. haematobium* and *S. mansoni* often go astray in their wanderings. Considering the tortuous course of their journey and the number of possible wrong turnings on their way, this is not surprising. They may reach any point in the tributaries of the portal system or of the pelvic plexuses and veins. Adult worms often also get into the systemic venous system and eggs have been found in every tissue. Their presence results in subacute and chronic inflammation. When a vital organ such as the brain or spinal cord is involved, the sequelae may be serious.

4. Stage of Cicatrization

The production of fibrous tissues laid down in the inflammatory reactions around the worms and their eggs is responsible for most of the serious late manifestations of bilharziasis.

In *S. haematobium*, stricture of the ureter mainly results from the fibrosis of the base of the bladder and may be followed by hydro-ureter, hydronephrosis, often pyelonephrosis and chronic nephritis. This progression is one of the commonest causes of death directly attributable to bilharzia. However, such deaths are rare.

The involvement of the liver and spleen in both *S. haematobium* and *S. mansoni* infections may result in a condition simulating Banti's disease with the development of anaemia and a tendency to severe haemorrhage from oesophageal varices.

5. Stage of Malignant Change

In South Africa, bilharzia is the commonest disease preceding the development of cancer of the bladder, which is a form of cancer frequently encountered in the indigenous people of the endemic regions. Cancerous changes associated with bilharzia lesions elsewhere in the body have been observed, but are relatively rare. The role of bilharzia as a predisposing condition of cancer of the liver remains a vexed question. In Southern Africa it occurs much more frequently in populations of the highly endemic zones than elsewhere, but it is probable that environmental factors other than bilharzia are responsible for the development of this condition.

LABORATORY DIAGNOSTIC TESTS

Detection of Ova

It should be emphasized at the outset that the best and most reliable laboratory test for the confirmation of the diagnosis of bilharzia is the finding of ova in the excreta or in the tissues. They have a characteristic morphology and are easily recognized. The ova of *Schistosoma haematobium* are about 150 mμ long by 60 mμ broad, have a terminal spine and are found typically in the urine. The ova of *S. mansoni* are also about 150 mμ long by 60 mμ broad, have a lateral spine and are found in the faeces. The eggs of *S. mattheei* have terminal spines but are larger than *S. haematobium* and have a definite shoulder and a lateral compression. They are most often found in the faeces but occasionally in the urine. The eggs of *S. haematobium* are rarely found in the faeces and *S. mansoni* eggs are rarely found in the urine.

For the detection of ova of *S. haematobium*, the usual procedure in the laboratory is to collect a specimen of urine and then to examine the deposit obtained by centrifugation. Alternatively, the patient should be asked to empty his bladder. This urine is discarded and he is then asked to squeeze out a few more drops, which are examined microscopically for the characteristic ova. If present, they are easily found and recognized. Vigorous exercise immediately before collecting the specimen is said to increase the chances of finding the ova.

In the field, although a centrifuge is helpful, it is not essential. Instead the urine specimen may be allowed to stand for 1 or 2 hours. The deposit which forms is then examined microscopically. If an immediate examination is not possible, a film of the deposit may be made on a microscopic slide and allowed to dry for later study when convenient.

For the detection of *S. mansoni* in the faeces, the whole stool specimen should be examined. This is best done by emulsifying it in saline and then examining the suspension in a petri dish under an entomological microscope. When this is not possible a portion of stool should be selected

for study. If the stool is formed then the flecks of mucus on its surface are most likely to contain ova and should be picked off for examination.

In addition to finding them in the excreta, the eggs of each of the 3 species are readily found in snips taken from the rectal mucosa. This is a painless but somewhat fearsome procedure. The ova also may be found in biopsy specimens taken from various sites of the body, including the liver. Indeed, bilharzia eggs have been found in almost all conceivable sites of the body including the eyelids and eyebrows. When present, the findings of eggs give a clear clue, both to the diagnosis of bilharzia as well as to the species of the worm responsible for the infection.

In patients infected with *S. mansoni*, eggs may be found in the faeces as soon as 5 weeks and often 8 weeks after exposure. In *S. haematobium* these intervals are about twice as long, varying from 10 to 15 weeks.

The mature worms survive for many years in their human hosts. In a few cases the passage of eggs in the urine has continued for as long as 30 years. In most patients with chronic long-standing infections there tends to be a marked reduction in the number of eggs excreted with the passing of time.

The direct method of diagnosis by finding the ova has its limitations, a few of which will be noted. Eggs are not excreted in the early stages of the infection in the time between exposure and the maturation of the worms.

In chronic infections the excretion of eggs is greatly diminished and often intermittent and their detection is correspondingly difficult.

In a significant proportion of patients, the worms stray from their predestined course to the veins of the bladder and intestine and their eggs are not excreted in the urine or faeces, but are lodged in other tissues and organs. Their presence may give rise to ill-defined symptoms. Occasionally, the lesions involve vital organs such as the brain, spinal cord and nerves, giving rise to serious and occasionally life-threatening disease.

Their presence in the lungs may lead to chronic pneumonitis with fibrosis, sometimes resulting in cor pulmonale.

In such cases the diagnosis is suggested by a history of exposure to water in an area where bilharzia is known to be endemic. The finding of an eosinophilia is also suggestive of a helminthic infection, the most frequent of which, in this region, is bilharzia.

In a large proportion of these cases the diagnosis can be established definitely by applying the immunological tests.

Immunological Tests

The serological tests which have been studied at this Institute for their value in the diagnosis of bilharzia include the complement-fixation, cercarial agglutination, cercarial immobilization and swelling, miracidial immobilization, circum-ovum precipitin, gel-diffusion, sensitized carbon particle agglutination, sensitized red-cell agglutination and the fluorescent antibody test. In addition, the value of several different antigens in intradermal tests have been investigated.

The findings in these investigations will not be reviewed at present; attention will be given only to the tests currently available in routine laboratory practice in South Africa. These are the complement-fixation test, the fluorescent antibody test and the intradermal skin test.

The complement-fixation test. The bilharzial complement fixation is a well-established laboratory test and has been routine practice at the Institute since 1937.

The first antigens used were prepared from miracidia hatched from eggs passed in the excreta of human patients. In the test as performed at present, the antigens are prepared from cercariae, adult worms and miracidia of *S. mansoni*. There is a close immunological relationship between *S. mansoni, S. haematobium* and *S. mattheei* and therefore antigens derived from *S. mattheei* may be used instead of the human worms. In a series of tests reported by Lurie and De Meillon,[2] the antigen prepared from *S. mattheei* gave clear results in human infections. However, as the test is now done, the antigens are prepared from cercariae, adult worms and the miracidia of *S. mansoni*.

There is general agreement that the complement-fixation test has a high degree of sensitivity and specificity. It has also been shown that in a large proportion of cases the reaction becomes positive from 3 to 5 weeks after exposure to infection, i.e. before the parasites are mature and eggs are passed. The complement-fixation test, when properly performed with a sensitive antigen, detects from 75 to 95% active infections and gives a low non-specific response with normal sera. Chaffee's modification of the preparation of antigen has largely eliminated false positive reactions with syphilitic sera which are apparent with crude saline extracts of antigen. It has been found that bilharzia antigen reacts with sera from patients with paragonimus and trichiniasis, but neither of these infections are seen, except in immigrants, in South Africa and this difficulty rarely arises. There appear to be weak cross-reactions with sera of cases of hydatid and possibly some other helminthic infections. However, it may be taken as a general rule, that a clear-cut strong reaction in the complement-fixation test is indicative of bilharzial infection. Weaker reactions have to be interpreted more cautiously, taking into consideration the other manifestations of the patient's illness.

The reaction tends to diminish with the passage of time and in infections of many years' duration the test is often negative. Following successful treatment, it also tends to become negative but as the reaction may persist for many months, and sometimes for years before doing so, it is not wise to rely on this test as a criterion of cure. The persistence of a positive reaction in the complement-fixation test, after treatment, is certainly not to be regarded as indicating that further courses of treatment are necessary.

The strength of the complement-fixation test does not measure the weight of infection nor does it distinguish in its usual form between the several species which may infect human patients.

The complement-fixation test when performed under optimal conditions is a most reliable test. However, it has several disadvantages. The blood for the test requires venepuncture and must be collected under sterile conditions and rapidly transported to the laboratory. The test itself is complicated and requires for its proper performance highly-skilled technical staff provided with special

facilities. The results, because of the large number of factors involved, tend to be variable from time to time and many sera prove to be anticomplementary, particularly those from cases of the more severe subacute forms of the disease.

The fluorescent antibody test. This test, developed particularly by Dr. Sadun, has also proved to be of great value in the diagnosis of bilharzia. Indeed, in routine use it appears to be slightly more sensitive and detects a higher proportion of positive cases. In general, the results run parallel. An added advantage of the fluorescent antibody test is that it gives clear results with anticomplementary sera. The amount of blood needed for the test is small and can be collected onto blotting paper from a finger prick. The antigen is a specially prepared suspension of cercariae, usually of *S. mansoni*. The antibodies in the patient's serum adhere to the surface of the cercariae and are detected indirectly by the addition of fluorescing anti-human globulin. The test requires a fluorescent microscope, but these are now standard equipment in most parasitological laboratories. Reading the results also requires considerable experience and skill on the part of the technologist responsible. A small number of tests can be done much more rapidly than a corresponding number with the complement-fixation test. However, when a large number of tests are required, the complement-fixation test can perhaps be more easily and rapidly read. There is a close correspondence between the results of the complement-fixation test and the results of the fluorescent antibody test but there are a few sera which give a negative reaction with one and a positive reaction with the other. Like the complement-fixation test, the fluorescent antibody test first becomes positive about 3 weeks after exposure to infection and regularly gives positive results within 5 weeks of infection. Like the complement-fixation test also, the result of the test cannot be relied upon to assess the effectiveness of treatment.

Skin tests. The intradermal test was introduced many years ago for the study of bilharzia. It has been extensively tested in South Africa in surveys sponsored by the World Health Organization to determine the incidence of infection. In these surveys 3 antigens—Kagan, Chaffee and Melcher antigens—were compared, each antigen having been prepared according to the methods described by the author. The Kagan antigen is a relatively crude saline extract, the Melcher antigen a more highly purified product. Under South African conditions all 3 antigens gave clear-cut results, the best differentiation between a positive reaction and a negative reaction being seen with Melcher's purified antigen. Equally good results were obtained with a more highly purified antigen fraction prepared by Dr. Kent. These findings will be described in detail in a separate paper. At present it suffices to note that in the groups of schoolchildren tested, the number giving positive skin tests was larger than the number excreting eggs and larger than the number giving positive serological reactions. Children in schools in non-endemic areas gave clear-cut negative results except in a few instances, and in each one of these, their history showed that they had lived in, or had been on holiday in a bilharzia endemic region. In children under 5 years old, a significant proportion of bilharzial cases as determined by the detection of ova in the excreta gave negative skin test results. It is clear from these findings that in schoolchildren and in adults the skin test detects a higher proportion of infections than the direct examination of excreta or biopsy material. False positive reactions are rare, but this aspect of the test in patients with other helminthic infections still has to be clearly defined. In patients with bilharzia the reaction does not diminish with time nor is it affected by successful treatment.

In children under 5 years old and possibly in older individuals with recently acquired infections, a significant proportion of those excreting bilharzia ova give negative results in the skin test.

The use of the skin test in the diagnosis of bilharzia in individual cases is somewhat limited. Its main value is to obtain from its use in surveys, a clear indication of the incidence of bilharzia in a region. It may also prove of value in assessing the effect of control measures in preventing infection.

Criteria of Cure

From this review it will be apparent that reliance should not be placed upon the results of the immunological tests in assessing the effectiveness of treatment.

The best criterion of cure at present available is the continued absence of viable ova from the excreta and biopsy specimens. It is recommended that specimens for examination should be collected every day for 1 week, at intervals of 3 months, for 1 year. If bilharzial ova are not detected during this time and the patient remains free of symptoms, it may be presumed that he has been cured.

In patients who were not passing ova before treatment, the effect of treatment must be judged on the clinical findings.

SUMMARY

Bilharzia is increasing in importance in South Africa because of the increasing opportunities of infection and the likelihood of heavy infections.

The value of the tests currently used in laboratory practice in South Africa is briefly reviewed and it is noted that the best method of diagnosis is the detection of the characteristic ova in the excreta, or in biopsy material.

In many cases, however, ova are not excreted and reliance for specific diagnosis must be placed on immunological tests. The complement-fixation test and the fluorescent antibody test are reliable specific tests. Positive reactions are first given 3-5 weeks after infection and persist for many years of active infection, but in long-standing cases, the reactions diminish in intensity and may become negative.

The intradermal test with currently available antigens is of great value in surveys to determine the incidence of bilharzia in a community. It detects a high proportion of cases in older children and adults.

In children under 5 years old a significant proportion of those with bilharzia give negative results in the skin tests.

In assessing the effect of treatment, reliance cannot be placed on the results of the immunological tests. The best criterion of cure is the continued absence of ova from the excreta. In other cases not excreting ova before treatment, this has to be judged from the clinical findings.

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