EXPERT COMMITTEE ON
BILHARZIASIS

First Report

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EXPERT COMMITTEE ON BILHARZIASIS
First Session
San Juan, Puerto Rico, 4-10 October 1952

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EXPERT COMMITTEE ON
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First Report

The Expert Committee on Bilharziasis met from 4 to 10 October 1952 in the School of Medicine of the University of Puerto Rico, San Juan. The committee's session followed that of the Joint OIHP/WHO Study Group on Bilharziasis in Africa, convened by the World Health Organization and the Office International d'Hygiène Publique in Cairo, Egypt, in October 1949.

Election of officers

The committee elected as Chairman, Dr. W. H. Wright; as Vice-Chairman, Dr. J. Oliver Gonzalez; and as general Rapporteurs, Dr. D. M. Blair and Dr. J. Gaud for the English and French versions respectively of the committee's report. The committee further requested some of its members to act as specialized rapporteurs for those sections of its report towards the preparation of which they had contributed basic papers: Professor E. C. Faust (section 2.1), Dr. J. Oliver Gonzalez (section 2.2), Dr. W. H. Wright (section 3.1), Dr. J. Gaud (section 4), Dr. D. B. McMullen (section 5.1), and Dr. D. M. Blair (section 5.2).

The session was also attended by two members of the WHO Expert Advisory Panel on Parasitic Diseases.

Agenda

The agenda presented by the Director-General was approved and adopted.

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1 The Executive Board, at its eleventh session, adopted the following resolution:

The Executive Board

1. Notes the first report of the Expert Committee on Bilharziasis;
2. Thanks the members of the committee for their work; and
3. Authorizes publication of the report.

(Resolution EB11.R11, Off. Rec. World Hth Org. 46, 4)

Tribute to Dr. Abdel Azim

Before dealing with its agenda, the committee paid a silent tribute to a deceased colleague, Dr. Mahmoud Abdel Azim, Director-General of the Rural Health Department, Ministry of Public Health, Egypt, who had presided over the session of the Joint OIHP/WHO Study-Group on Bilharziasis in Africa, held in Cairo in 1949, and had since carried out bilharziasis surveys for WHO in the Eastern Mediterranean Region.

1. GEOGRAPHICAL DISTRIBUTION OF BILHARZIASIS

1.1 As a result of the surveys recommended by the Joint OIHP/WHO Study-Group on Bilharziasis in Africa and approved by the Executive Board at its fifth session, the following documents were available to the committee for its consideration:

Abdel Azim, M. (1951) "Bilharziasis survey in some countries of the Eastern Mediterranean Region" (WHO Regional Office for the Eastern Mediterranean, unpublished working documents EM/BIL/1, EM/BIL/1 Corr.1, and EM/BIL/1 Add.1)

Ayad, N. (1952) "Interim report of bilharziasis survey in various countries of the WHO Eastern Mediterranean Region: Eritrea, Ethiopia, British and Italian Somaliland, the Sudan and the Yemen" (WHO Regional Office for the Eastern Mediterranean, unpublished working document EM/BIL/2)

Blair, D. M. (1952) "Bilharziasis survey of British Africa (October 1950-February 1951)" (Unpublished working document WHO/Bilharz/2)

Gaud, J. (1951) "Compte rendu d’une mission d’enquête sur les bilharzioses en Afrique occidentale et en Afrique centrale"


Gaud, J. (1952) "Compte rendu d’une mission d’enquête sur les bilharzioses à Madagascar et aux îles Mascareignes"


1.2 The committee also had the benefit of verbal information furnished by its members on their respective countries and on countries in which

4 Off. Rec. World Hlth Org. 25, 6
they and their associates had had occasion to work, such as British West Africa, China, Japan, Liberia, the Philippines, and Taiwan.

1.3 Summarizing the information available, the committee was of the opinion that, while it showed the wide distribution of actual and potential vectors of bilharziasis in tropical and subtropical areas throughout the world, and of human infection from various schistosome species, it gave no adequate measure of the true prevalence and severity of bilharziasis as a disease or as a cause of disability or of death.

1.4 The committee noted in this connexion that, since most health administrations were themselves ignorant of the true extent of *Schistosoma* infection in their own territories, one could not expect to collect accurate information on prevalence.

WHO consultants sent for surveys could do no more than demonstrate here and there methods for the detection of bilharziasis and its vectors, stimulate the interest of individual workers, and help the national health administrations to realize the medical, social, and economic importance of the disease.

1.5 For these purposes, and with a view to drawing up epidemiological conclusions of general interest and practical value, the committee recommended that the WHO-sponsored bilharziasis surveys be published, preferably in the form of a monograph.

1.6 While the present report, it was hoped, would facilitate epidemiological surveys and diagnosis of individual as well as of mass *Schistosoma* infection, the publication of comparatively simple keys for the determination of vector snail species, together with instructions for the collection of specimens, such as that issued by the Department of Health of Southern Rhodesia, could be of great value to the non-specialists who would make surveys of the intermediate hosts of *Schistosoma*.

1.7 Such conclusions as the authors of the African survey drew from their field observations are presented in section 3 of the present report.

1.8 On the basis of information provided by the surveys sponsored by WHO, of the information collected and presented by the members of the committee in a series of preparatory documents, and of the interchange of opinion which took place during its session, the committee adopted the views and recommendations contained in the present report.

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5 The committee preferred the word infection to infestation when referring to the presence of any stage of *Schistosoma* in a host, as well as in snails, the word infestation being left to designate the presence of the *Schistosoma* in its free living form in water.

6 Clarke, V. & Alves, W. (1952) *A key to the families and genera of some South Central African gastropods*, Salisbury
2. CRITICAL ANALYSIS OF METHODS OF DIAGNOSIS OF BILHARZIASIS, WITH SPECIAL REFERENCE TO THEIR APPLICABILITY

In a known endemic area of bilharziasis physicians may make a presumptive diagnosis on the basis of frank haematuria in the vesical type of infection or the tender, enlarged liver and spleen associated with fulminating dysentery in the intestinal types. Yet many errors result from unsupported clinical diagnoses, since several other disease syndromes simulate those of bilharziasis and in many lightly infected patients the characteristic symptoms are lacking. Thus, laboratory diagnosis is essential.

The laboratory methods available for diagnosis of bilharziasis are twofold: (1) demonstration of *Schistosoma* eggs in the excreta and in the tissues, and (2) immunological tests.

2.1 Available Methods for Recovery of *Schistosoma* Eggs

2.1.1 Before considering available methods for the recovery of *Schistosoma* eggs, it is necessary to understand where these eggs are laid and under what conditions they become available for diagnosis. The mature, fertilized, female worms reside in the smaller branches of the caval and portal venous systems, adjacent to, or within, the walls of the urinary bladder and other pelvic organs, or in the intestinal wall. *Schistosoma haematobium* most frequently lives in the vesical venous plexus but may oviposit in the rectal, haemorrhoidal, or pudendal veins before arriving in the vesical plexus. *S. japonicum* has a predilection for the superior mesenteric venules but frequently migrates into the venules draining the large intestine and oviposits there; *S. mansoni* is usually situated in the inferior mesenteric venules but at times wanders into the superior mesenteric venous drainage. Both of these intestinal schistosomes are commonly carried into, and become lodged in, the intrahepatic portal vein, where they lay their eggs, and all three of these blood flukes may migrate as far afield as the pulmonary arterioles, and even bypass the hepatic and pulmonary capillaries to deposit their eggs in the blood-vessels of the skin, central nervous system, and other ectopic locations.

During this biological incubation period eggs are not produced; hence their recovery can not be employed for diagnostic purposes. In the acute stage, eggs tend to filter rather rapidly into perivascular tissues, and many escape into the lumen of the urinary bladder or intestines, to be evacuated in the urine or stools. As the infection gradually progresses to the chronic state, host-cell reaction around infiltrated eggs tends to wall them off in the
tissues, so that fewer and fewer eggs are evacuated in the excreta, while those that do escape are frequently degenerate and may be encased in a coat of host cells. Thus, there is usually no lack of eggs for diagnosis during the early overt stage of bilharziasis or during acute exacerbations resulting from physical exertion, but increasing difficulty is experienced later in recovery of eggs in the urine or stool. This is particularly true for *S. mansoni*, which lays very few eggs per day; yet even in *S. japonicum* infection eggs may be difficult to find in the stools of a chronic case.

2.1.2 Recovery of Schistosoma eggs from the urine

2.1.2.1 The following description applies for the most part to *S. haematobium*, occasionally to *S. mansoni*, and very rarely to *S. japonicum*. The eggs filtering through the wall of the bladder are usually passed towards the end of the period of micturition and, together with cellular detritus and salt crystals, rapidly settle to the bottom of the container into which the patient urinates. A 250-350-ml, inverted, conical urinalysis glass serves as a satisfactory sedimentation jar, from which a sample of the sediment may be examined microscopically. This will ordinarily provide demonstration of the characteristic living or dead eggs, which are terminal-spined in the case of *S. haematobium* and lateral-spined in the case of *S. mansoni*. In chronic infection, exercise of the patient before urination frequently provides eggs in the urine sediment, which otherwise would be negative.

2.1.2.2 Hatching technique

Eggs which contain living and hatchable miracidia can be made visible with the aid solely of a hand lens by adding water to the lightly centrifuged deposit from the specimen of urine. Such miracidia hatch quickly, usually within five to ten minutes. Care must be observed to use for dilution water free from added chemical agents and freed from free-swimming infusoria which might be confused with the miracidia of *Schistosoma*. This is best done by heating the water before use to a temperature of 60°C.

The advantages of this method of diagnosis are that the equipment is very cheap and staff are quickly trained. All eggs in a deposit are given an opportunity to hatch out miracidia so that the diagnosis of very light infections is possible. The method is of particular value as a test of cure after treatment of *S. haematobium* infections.

2.1.3 Recovery of Schistosoma eggs from the stool

In early acute infections the stool is frequently dysenteric in character, either with an excess of blood and mucus and essentially no intact faeces, or with flecks of blood and mucus on the outside of elements of formed
faeces. This applies irrespective of the species of *Schistosoma*, but is particularly true in *S. japonicum* infection, since the larger number of eggs laid by the mother worms produces a comparably greater number of open lesions in the intestinal wall and hence more haemorrhage and mucus secretion. Whenever there is macroscopic evidence of blood in the stool, this component is the most likely material for demonstration of eggs. Also, during acute exacerbations of a chronic infection, blood and mucus are most likely to contain the eggs. However, during the early dysenteric stage eggs will be viable, with a mature or maturing miracidium inside each shell, whereas in reactivated chronic infection they are most likely to be degenerate, calcified, or expelled from the intestinal mucosa surrounded by a fuzzy coat of host cells. The dysenteric type of intestinal evacuation will frequently provide evidence of *Schistosoma* eggs by direct microscopic examination without concentration, whereas in chronic cases direct faecal films are more apt to be negative.

In lightly infected individuals harbouring *S. japonicum* and *S. mansoni*, there is typically a period of reduced intestinal irritation following the prodromal and acute stages. Under such circumstances, and particularly if the patient has not been physically active, there may be no gross evidence of blood and mucus in the stools—only normally formed faeces. Under these circumstances the eggs will be rather widely, although not uniformly, distributed throughout the faecal mass, so that the chances of finding eggs in the average direct microscopic faecal film are relatively poor. This situation calls for concentration techniques.

### 2.1.3.1 Methods of concentrating eggs from the faeces

These methods consist of flotation, sedimentation, and a combination of these processes. Both techniques make use of the principle of relative increase in the number of eggs as a result of loss of a large amount of the faecal material. Theoretically, there should be no loss in the number of eggs during concentration, but in practice there is almost always some loss. The practical goal is to eliminate as great a proportion of the faecal material and as small a number of eggs as possible.

#### 2.1.3.1.1 Flotation and centrifugal flotation

These methods have been employed very effectively for concentration of eggs of hookworms, *Ascaris*, and *Trichuris*. However, eggs of *Schistosoma* shrink in the hypertonic solution required for carrying out flotation, so that the egg yield in the surface film is at times less than in the direct faecal preparation, and those eggs which are recovered are of inferior diagnostic quality. Thus, flotation can not be considered to be a satisfactory procedure for concentrating *Schistosoma* eggs.
2.1.3.1.2 Sedimentation. This method is based on the principle that, following suspension of the particulate components of a stool in several parts of water, the heavier elements, including the eggs of helminths, settle to the bottom of the container. The settling may be hastened by the use of detergents, or by employing 10% ethyl alcohol in place of water, or by adding 0.5% glycerol to the water. A satisfactory procedure for simple sedimentation of *S. japonicum* and *S. mansoni* eggs is as follows: (1) Thoroughly comminute 10 g of stool, preferably without macroscopic blood and mucus, in 200 ml of 0.5% glycerinated water relatively free from colloidal particles. (2) Pour through two layers of surgical gauze into a 250-350-ml, conical, sedimentation glass. (3) Allow to sediment for 30-40 minutes, then decant the supernate. (4) Resuspend the sediment in 0.5% glycerinated water, allow to sediment for 20 minutes, and again decant the supernate. (5) Resuspend a third time in 0.5% glycerinated water, allow to stand for 10 minutes, and decant the supernate. (6) With a pipette transfer about 0.05-0.1 ml of the bottom sediment onto a microscope slide, spread out into a film about 35 mm × 20 mm, and mount with a 40 mm × 22 mm cover-glass. *Schistosoma* eggs in the film will not be damaged by the manipulation, will be easily found, and can be readily identified.

2.1.3.1.3 Macro-centrifugulation. The process of sedimenting 10-15 g of faeces may be hastened by suspending the faeces in 100 ml of tepid water, straining through two layers of gauze, and then centrifugalizing in 50-ml tubes at 1,500 revolutions per minute (r.p.m.) for 30 seconds. After decantation of the supernatant liquid, the sediment is resuspended in water and recentrifugalized. The process is repeated until the supernate is clear. After the final decantation, four drops of sediment are pipetted onto a microscope slide, mounted with a 40 mm × 22 mm cover-glass, and examined.

2.1.3.2 Hatching technique

Direct sedimentation or macro-centrifugalization also allows the large residue of unexamined clean sediment to be resuspended in water, so that mature viable eggs of *Schistosoma* will hatch overnight and be seen to swim about in the water. Exposure of *S. mansoni* eggs to direct light will hasten hatching. Water used for dilution should be heated to 60°C so that it contains no living infusoria; otherwise they may be confused macroscopically with the free-swimming miracidia.

2.1.3.3 Acid-ether technique

This method sediments eggs and protozoan cysts after clearing the faecal detritus and mucus with the acid and dissolving fatty constituents in the ether. For recovery of *Schistosoma* eggs in the stool, modifications of the Telemann technique have proved to be most satisfactory. Workers
at first used one gram of faeces, suspended in 5 ml of a 15% solution of hydrochloric acid (38-40% concentrated HCl), with specific gravity of about 1.080. The technique was then modified by adding 0.06 ml of the detergent, Triton NE, to the acid suspension of the faeces; an appreciably higher yield of diagnosable Schistosoma eggs was obtained. Then it was found that immature and degenerate eggs were frequently destroyed by the strong acid, and the method was further modified by substituting a solution of sodium sulfate (specific gravity 1.080) for half of the acid in the suspending medium. This allowed Schistosoma eggs of all types in the stool to be concentrated in the suspension in a diagnosable state. The steps in this procedure are as follows: (1) One gram of stool is suspended in 2.5 ml of HCl solution (specific gravity 1.080) + 2.5 ml of Na₂SO₄ solution (specific gravity 1.080) + 0.06 ml of concentrated Triton X-30 (= Triton NE). (2) This suspension is poured through two layers of surgical gauze into a 15-ml test-tube, 5 ml of ether are added, and the suspension is emulsified by thorough shaking. (3) The tube is then centrifugalized at 1,500 r.p.m. for one minute. (4) After the interphase between the ether and acid layers has been loosened from the inside of the tube with a wooden applicator, all the liquid contents of the tube are poured off the sediment. (5) The entire sediment (approximately 10-20 mg) is transferred to microscope slides, mounted with cover-glasses, and examined for Schistosoma eggs. Practically all eggs in the original one-gram sample of faeces are present in the concentrate in a diagnosable condition. If the sample is truly representative, diagnosis on the basis of the concentrate is dependable.

2.1.4 Biopsy techniques

Eggs of S. mansoni can be detected in scrapings of the mucosa and submucosa of the sigmoid colon and rectum much more frequently at necropsy than by examination of the faeces of the living patient. Proctoscopic biopsy in suspected cases of bilharziasis considerably increases positive diagnoses made by examination of the excreta. The site where the greatest concentration of eggs is deposited is in the vicinity of the plicae transversales recti (valves of Houston). The sample from the suspected area may be obtained by aspiration, scraping, or punch techniques. The specimen is placed on a microscope slide, mounted with a thick cover-glass or a superimposed slide, and examined under low power of the microscope. Punch specimens may also be fixed in formalin, sectioned, stained with haematoxylin and eosin, and then examined as tissue sections. Frequently, nests of eggs are found in dilated venules within the mucosa.

It is well known that terminal-spined eggs of S. haematobium and related species can often be detected in rectal biopsy snips even when none of these eggs can be found by examination of a specimen of urine.
Fresh biopsy specimens can be used to detect viability of the eggs. Occasionally punch biopsy of urethral or bladder mucosa will reveal infection with *S. haematobium* or *S. mansoni* when sedimented urine has been consistently negative.

### 2.1.5 Evaluation of methods for demonstration of Schistosoma eggs

Potentially all cases of bilharziasis may be diagnosed on the basis of microscopic demonstration of the eggs as soon as egg production begins. When eggs are numerous in the excreta, no problem in diagnosis is presented; when they are few, concentration techniques must be employed.

Concentration of eggs present in the urine is a very simple procedure. The patient, after exercise, passes the specimen directly into a conical urinalysis glass of 250-350-ml capacity, and the sedimentation of particulate material is allowed to take place for 10 minutes. After the supernate is decanted, some of the sediment is pipetted onto a microscope slide and examined. The only equipment needed consists of urinalysis jars, pipettes, microscope slides and cover-glasses, and a compound microscope. For field surveys the urine may be collected in labelled glass jars with metal screw tops and brought in batches to a dispensary laboratory for processing. This technique is a simple, practical one, readily available for mass examination.

In intestinal bilharziasis it is usually possible in active infections to recover the eggs from flecks of blood and mucus attached to the outside of the faeces or in undisguisedly dysenteric or mucoid stools. Only direct films, preferably mounted with a cover-glass, are needed for detection of these eggs.

In light infections without accompanying dysentery or diarrhoea, the eggs are incorporated in small numbers in formed faeces. Likewise, in late chronic cases eggs may be found only sporadically in any considerable numbers in the intestinal evacuations. These cases call for concentration methods. Greatest concentration is accomplished with the HC1-Na$_2$SO$_4$-Triton-ether technique; but ether is expensive and difficult to transport and keep under field conditions, the technique is untidy, particularly in hot, humid climates, and there is considerable likelihood that the small amount of stool which is processed may contain no eggs, even though they may be present in other parts of the specimen. This method is therefore not a practical one for mass diagnosis.

The most dependable technique for concentration of *S. mansoni* eggs present in small numbers in the stool is simple sedimentation, employing 0.5% glycerinated water as the diluent. Urinalysis glasses are employed as
in vesical bilharziasis and a minimum of 10 g of stool may be sedimented at one time. Stool specimens may be collected in the field in waxed pasteboard containers and transported to the laboratory for examination. The only disadvantage is the relatively long time required for adequate sedimentation of the stool. If the laboratory is provided with an electric centrifuge equipped with buckets for 50-ml tubes, centrifugal sedimentation will hasten the process.

Careful weighing of the advantages and disadvantages of the several methods available for concentration of *Schistosoma* eggs from the stool warrants the conclusion that the most practical one for mass diagnosis is sedimentation.

Biopsy techniques are very valuable for use in individual cases or small groups of cases, where proctoscopy and cystoscopy are available, or where biopsied specimens are removed for diagnosis during exploratory surgery. But on account of its very nature, biopsy is not generally adapted for mass diagnosis.

2.1.6 Summary of recommended procedures

The following practical procedures are recommended for the recovery of *Schistosoma* eggs for the diagnosis of bilharziasis:

1. Eggs passed in the urine accumulate in the sediment at the bottom of a conical urinalysis glass. This is a simple concentration technique adapted to mass diagnosis.

2. In active cases of intestinal bilharziasis eggs may be found in flecks of blood and mucus on the outside of formed faeces or in undisguisedly dysenteric stools; direct film examination will usually reveal the eggs amongst the cellular and mucus components of the exudate.

3. In formed faeces it is frequently necessary to concentrate the eggs.

   (a) Although greatest concentration is effected by the HCl-Na$_2$SO$_4$-
       Triton-ether technique, this method is complicated, expensive, untidy,
       and, since only one gram of faeces is processed, negative diagnosis may
       not be reliable.

   (b) Sedimentation in 0.5% glycerinated water is simple, produces effective
       concentration, and, since 10 g or more of the stool specimen
       are processed, it may ordinarily be depended on as an adequate
       sampling of the stool. It is well adapted for mass diagnosis.

4. Biopsy is particularly helpful in providing a check on sedimented
   specimens of stool and urine, and for examination of material removed
   surgically. It is not adapted for mass diagnosis in most areas, but con-
   stitutes a valuable potential diagnostic procedure.
(5) Hatching of miracidia from sedimented concentrates of *Schistosoma* eggs in water is valuable as a public-health measure in providing an index of the percentage of viable eggs evacuated by schistosome-infected patients.

### 2.2 Immunological Tests in the Diagnosis of Bilharziasis

Past and recent investigations have indicated that the immunological diagnosis of bilharziasis can now be accomplished with a reasonable degree of accuracy. Complement-fixation, flocculation, and intradermal tests have been devised, all of which give positive reactions in individuals infected with bilharziasis and in experimental animals. There is, therefore, a definite humoral response by the host which seems to persist for a number of years. The specificity and sensitivity of the various tests utilized, as well as antibody development during infection, present one of the most fascinating fields of study in immunity to parasitic infections.

On the second or third week after exposure to infection, easily detectable complement-fixing antibodies appear in the blood. Later, positive intradermal reactions are observed, usually beginning in the fourth to the sixth week after exposure. Apparently, skin sensitivity increases with duration of infection. Individuals coming from endemic areas, with a long-standing infection, even after adequate treatment give a positive intradermal reaction as much as four years later.

Precipitin and flocculation tests also become positive subsequent to the appearance of complement-fixing antibodies.

#### 2.2.1 Types of antigens used in immunological tests

The antigens which have been used in immunological tests for the diagnosis of bilharziasis have been prepared from the following sources:

1. Cercariae
2. Adult worms
   1. homologous
   2. heterologous
   3. distantly related species.

The cercariae used in preparing antigens have been those of the locally prevailing species: *S. haematobium* in Africa, *S. mansoni* in America, and *S. japonicum* in the Western Pacific Region. Extracts of the free cercariae and of the infected snail livers have been used with success for intradermal, complement-fixation, and flocculation reactions.
The adult forms of *S. mansoni* have been the main source of adult worm material for the preparation of antigens. This is explained by the fact that *S. mansoni* infections can be maintained in the laboratory, so that this material is recovered with relative ease. Infections of experimental animals with *S. japonicum* and *S. haematobium* are more difficult to produce in the laboratory.

Antigens have been prepared from adult schistosomes of bovine origin and other less-related trematodes, such as: the liver fluke, *Fasciola hepatica*; the frog lung-worm, *Pneumocoele medioplexus*; the ox trematode, *Eurytrema coelomaticum*; and the free-living turbellarian, *Planaria maculata*.

For the intradermal test, the antigens prepared from either the dry, well-pulverized cercarias or from adult forms of one of the species of schistosomes give satisfactory results. The skin reaction is produced about the same time (10-15 minutes after injection), and there appears to be no difference in intensity of the reaction in successive stages of the disease. Also the percentage of false-positive reactions due to hypersensitivity of the skin to non-specific substances is not significantly different. The potency of both types of antigens remains the same if properly stored at 6°C.

The cercarial antigens are more reliable for the complement-fixation test than the antigens prepared from adult worms. Adult worms have been shown to contain anticomplementary substances which lead to false reactions.

The antigens prepared from the less-related trematodes, such as *Fasciola hepatica* and *Pneumocoele medioplexus*, do not give as reliable results as the antigens prepared from the schistosome group. When large numbers of individuals with laboratory-proved schistosome infections are skin-tested, a fairly high percentage of negative reactions are obtained with these heterologous antigens. This is particularly so with extracts prepared from adult *F. hepatica*. There is also an observable loss of potency with time, which does not occur in the schistosomal antigens.

Investigations should be conducted in order to isolate the common substance present in these various trematode antigens. Stable antigens from easily available sources might be prepared, and would be useful in regions where only one trematode infection prevails.

### 2.2.2 The intradermal reaction

The skin becomes sensitized to schistosome antigens subsequent to the development of circulating antibodies. Positive skin-reactions are usually observed in human beings from the fourth week after exposure.

Duration of the skin sensitivity depends upon two factors: *(a)* the duration of infection, and *(b)* the possible role of factors associated with
sensitization of the skin which persist even after cure following adequate treatment.

2.2.2.1 Volume and dilution of antigen injected and criteria of a positive reaction

The volume of antigen which is injected intradermally has varied from 0.01 to 0.1 ml. Good results have been claimed by various investigators using small volumes, such as 0.01-0.03 ml. The tissue damage caused by the injection of 0.1 ml of a highly diluted antigen probably does not affect the nature of the reaction.

A dilution of antigen commonly used is 1 : 10,000 when prepared from adult or larval forms. In individuals coming from endemic areas, where the infection was probably acquired during childhood, there is no marked difference in the percentage of positive reactions whether 1 : 5,000 or 1 : 10,000 dilutions are used in the test. However, this may be different in recent infections; in such cases more-concentrated antigens should be used. Thus, it has been reported that, in a group of 64 patients infected with S. japonicum, the 1 : 5,000 dilution of cercarial antigen gave more satisfactory results than the 1 : 10,000.

The criteria for a positive reaction are also a matter on which authors differ. A difference of at least 3 mm between the diameter of the weal produced by the antigen and that of the control has been considered sufficient to indicate a positive reaction. Others consider an extension beyond the ink line around the original weal an indication of positivity, provided the weal formed by the control solution has remained the same. In individuals known to harbour S. mansoni, intradermal reactions have been observed which do not exceed by 1 mm the reaction formed by the control injection. This, according to the criteria mentioned above, would be classified as a negative reaction. Outlining the antigen and control injections with ink would reveal the fact that one weal has extended, while the other has not; this fact should be considered as significant as the increase in diameter of the weal.

The false-positive intradermal reactions obtained with either cercarial or adult-worm antigens occur so rarely that they are claimed to be insignificant. The fault of the intradermal test lies chiefly in the false-negative reactions which may occur, particularly in cases of recently acquired bilharziasis, when highly diluted antigens are used.

2.2.2.2 Skin reaction by age-groups

In children, the intradermal reaction is not as intense as in adults. Furthermore, a low percentage of infected children may give negative reactions. The degree of skin sensitivity thus increases with age. Negative
reactions among infected children coming from endemic areas may be due to the fact that in repeated infections an immunological condition develops in which there is an excess of antigen, which neutralizes the antibody very rapidly and does not permit detection of antibodies by the laboratory tests now available.

Among adults, the intradermal reaction gives a positive result usually in over 90% of the infected individuals. This fact is the point of greatest importance in the value of the test. The results vary, however, following treatment of patients who have harboured the infection for varying periods of time. Thus, it has been reported that the skin test using a 1 : 5,000 dilution gives a negative result in 60% of the patients within six months after treatment. It must be considered that treatment affects the adult worms, but probably has no effect on the eggs that have lodged in the tissues. In recently acquired infections submitted to effective treatment no eggs or only small numbers of them are lodged in the tissues. In long-standing infections, on the other hand, eggs may serve as a source of antigen for a period of time, which keeps the patient sensitive to the antigen after the death of the adult worms.

In persons coming from endemic areas, who have been repeatedly exposed to infection and who have harboured the infection for many years, the reaction accordingly remains positive for longer periods of time. In addition to the factors which increase sensitization of the skin resulting from duration of infection and of antigenic stimulation by the eggs in the tissues, there is the possibility that, in spite of adequate treatment, a small number of adult worms may remain alive and continue to sensitize the host. The test is thus of limited value for determining the efficacy of treatment in patients coming from endemic areas.

The intradermal test has a definite value as a means of screening schistosome-infected individuals in the field. It does not, however, give absolute evidence of persisting infection. After sorting out the positive reactors, examination of their stools, urine, and rectal biopsy-tissue should follow, in order to determine whether the infection is still active.

2.2.3 The complement-fixation test

The complement-fixation reaction is regarded as an accurate and reliable test for revealing possible infection before eggs have been demonstrated in the urine, stools, and biopsy specimens.

The complement-fixation test, unlike the intradermal test, can be used to study some of the factors involved in group reactions to the antigens prepared from the various trematode species which infect man. Thus, it has been found that when *S. mansoni* antigens were used reactions were obtained
with the sera from patients infected with *S. japonicum*, but that the titre was lower with the heterologous than with the homologous antigen.

Antigens prepared from the cercariae are superior to those from the adult worms because the latter are anticomplementary and less stable.

Although the complement-fixation test offers the advantages mentioned, it is at present not adapted to field work. It requires trained personnel and materials not easily available under field conditions.

### 2.2.4 The flocculation reaction

The ease of the technique of the flocculation reaction, and the simple equipment it requires, hold promise as to its value in the field. More work, however, is needed, and in as many regions of the world as possible, to evaluate the test. From the small number of reports available, it is clear that the test is highly specific and sensitive.

### 2.2.5 The formaldehyde or formol-gel test

The formaldehyde or formol-gel test should be discarded as a serological aid in the diagnosis of bilharziasis since a large number of individuals known to be infected give a negative reaction to it.

### 2.2.6 Recommendations

It is recommended that immunological studies should be conducted in various laboratories in order to furnish information on the following points:

1. Are the antigens which sensitize the skin the same as those responsible for the development of circulating antibodies?

2. Is a particular fraction of the antigens prepared from adult worms and cercariae the cause of the response of the patients' sera in serological tests? If this were found to be so, it might lead to the development of a more specific and/or sensitive test.

3. Can immunity to bilharziasis be acquired? If so, what are the factors involved?

Answers to these questions would constitute a great advance in knowledge of the human aspects of bilharziasis.
3. EPIDEMIOLOGY OF BILHARZIASIS

3.1 The Snail in the Epidemiology of Bilharziasis

3.1.1 Morphology and taxonomy of intermediate hosts

One of the most important problems in the epidemiology of bilharziasis concerns the morphology and taxonomy of molluscan intermediate hosts. There is continuing confusion about the status of many species of snails. This applies particularly to those concerned in the transmission of bilharziasis in Africa.

Malacological studies are also needed for other areas, such as the Eastern Mediterranean Region, the Americas, and the Western Pacific Region. Most of the identifications of African species reported in the literature have obviously been based on such tenuous and inconstant factors as shell characteristics, which frequently vary from individual to individual. Many so-called species are known under a variety of names. There is need for the development of a sound molluscan classification based on the anatomy of the soft parts of the animal.

3.1.1.1 In order to facilitate further the development of a reliable system of snail identification, it is recommended that the arrangement made by WHO since 1950 for collective action in this respect be continued and expanded.

It is suggested that three malacologists possessing a special knowledge of vectors of human schistosomes be selected to form a Subcommittee on Malacology of the Expert Committee on Bilharziasis. The members of this subcommittee should be prepared to undertake identification, based on the internal anatomy of known, suspected, or potential intermediate hosts, of the human schistosomes forwarded by health administrations and interested individuals in countries in which bilharziasis is endemic.

The regional priorities for such identification might be in the following order: Africa, the Eastern Mediterranean, the Americas, the Western Pacific.

3.1.1.2 In so far as is possible, it is desirable that health services and individuals should divide given snail collections into three parts and supply each of the three malacologists participating in the work of identification with what would appear to be identical material from the same locality.

3.1.1.3 The members of the subcommittee should provide, after consultation, suitable recommendations for the collection and preservation of the material and define the data which should accompany the specimens. It is anticipated that an agreement may be reached by the members of the sub-
committee on the identification of the majority of the species represented by the material reaching them. However, for the purpose of scientific opinion and for promoting liaison between the subcommittee and the Expert Committee on Bilharziasis, it is recommended that the members of the subcommittee be given the privilege of attending the next session of the committee.

3.1.2 Ability of certain species of molluscs to serve as vectors

The ability of certain molluscs to serve as intermediate hosts for human bilharziasis in Africa is still in need of definitive study. The claim that Bulinus tropicus is capable of serving as an intermediate host of S. haematobium has never been confirmed, although this species is said to serve as an intermediate host for S. mansoni in Kenya. Various workers have been unable to infect this species. Claims that Lymnaea natalensis is capable of transmitting S. haematobium have not been substantiated. Two investigators have not been able to infect this species. Likewise, L. natalensis undulansae in the Belgian Congo and in French Sudan proved refractory to infection.

The situation with regard to Pyrgophysa forskali, claimed to be the intermediate host of S. haematobium in Mauritius, is even more confusing. Recently, the WHO-sponsored survey in Mauritius revealed the presence of only one possible intermediate host, a species of Bulinus which contained haematobium-like cercariae, in the areas where bilharziasis was present. P. forskali in the Belgian Congo could not be infected. However, this species is suspected to be the intermediate host of S. haematobium in Portuguese Guinea, and is found to act in this capacity at Tudun Wada near Kaduna, northern Nigeria. Certainly much more needs to be known concerning the role of this species in the transmission of bilharziasis in Africa.

Studies in French Sudan indicated that Physopsis tchadiensis could be infected with difficulty by S. haematobium. Physa strigosa (= Physopsis africana) was readily infected with both S. haematobium and S. mansoni, a rather surprising result concerning the latter species. Planorbis adowensis (= Biomphalaria ruppellii) could be infected with S. mansoni. Biomphalaria alexandrina var. choanomphala was found to be an intermediate host of S. mansoni in Lake Albert. Planorbis stanleyi from Lake Kivu was demonstrated to be an intermediate host of S. mansoni in that locality. In the Lango district of Uganda, one worker claims to have demonstrated experimentally that Physopsis nasuta is the intermediate host of S. haematobium. Confirmation of this finding would be highly desirable.

Because of conflicting reports and possible errors in identification, it is desirable that additional studies be carried out on the ability of Physopsis
tehdialis, Pyrgophyusa forskali, and Physopsis nasuta to transmit S. haemato-lobum on the African continent.

3.1.3 Bionomics of molluscan intermediate hosts

Except in respect of certain species of Oncomelania, there is an almost complete lack of information on the bionomics and ecology of molluscan intermediate hosts of the human schistosomes. Such information would be of great importance in relation to the epidemiology of bilharziasis and its control.

It is recommended that WHO endeavour to stimulate, through the various means at its disposal, research on this important problem.

3.1.4 Vector-parasite relationships

During the past few years a considerable amount of information has become available concerning the relationships between the intermediate host and various strains of schistosomes.

Snail vectors of S. mansoni from five countries in the Western hemisphere and two countries in Africa were tested for their susceptibility to various strains of S. mansoni from different countries. Australorbis glabratus from the Dominican Republic, Puerto Rico, Surinam, and Venezuela were more susceptible to strains of S. mansoni from the Western hemisphere than to a strain of the parasite from Egypt. In contrast, A. glabratus from Brazil was refractory to almost all of the strains of S. mansoni employed in the study. However, while snails of this species from São Salvador (Bahia), Brazil, were refractory to all of the parasite strains employed, snails of this species from Recife (Pernambuco), Brazil, could be readily infected with a strain of S. mansoni representing a cross between Recife and Puerto Rican parasites. Biomphalaria pfeifferi from Liberia was a suitable host for the strains of S. mansoni from the Western hemisphere and for the Egyptian strain of parasite. On the other hand, Planorbis boissyi from Egypt was receptive only to the Egyptian strain and only slightly receptive to one Egyptian and Puerto Rican cross-strain.

Additional studies showed that A. glabratus from Puerto Rico demonstrated little susceptibility to S. mansoni from the Dominican Republic, although A. glabratus from the latter country was quite susceptible to the parasite from Puerto Rico. A. glabratus from São Salvador (Bahia), Brazil, continued to be refractory to infection with all strains of S. mansoni.

The resistant A. glabratus from São Salvador has been crossed with susceptible A. glabratus from Puerto Rico. It was found that susceptibility to schistosome infection was a heritable characteristic, and that the strains
were not genetically alike. The findings indicated that in the non-susceptible *A. glabratus* the parasite is destroyed and disposed of within 24-48 hours after penetration. As at least part of this response, there is a cellular reaction around the parasite. In the susceptible snails, no response to the parasite was observed. It is suggested that the non-susceptible snails possess a natural antibody not characteristic of the susceptible strain of snails.

Additional studies indicate that similar differences in susceptibility exist between known molluscan intermediate hosts and strains of *S. japonicum*.

Such data are of considerable interest from the standpoint of the epidemiology of bilharziasis and would seem to indicate that the ability on the part of any mollusc to transmit the disease must be intimately linked up with its genetic and physiological constitution. To what extent environmental factors enter into this ability has not been determined.

3.1.4.1 Research is needed on the relative susceptibility of various intermediate hosts to infection with various human schistosomes and with mammalian *haematobium*-like schistosomes in Africa. Some of the conflicting reports concerning the ability of certain molluscs to serve as intermediate hosts might be resolved through studies of this sort. It is recommended that WHO endeavour to stimulate the interest of African workers in this type of research.

3.1.4.2 Nothing is known concerning the influence of chemical, physical, and meteorological environmental factors on the susceptibility of molluscan intermediate hosts to schistosome infection. There is reason to believe that these factors may be important in the epidemiology of bilharziasis. It is recommended that WHO commend to research workers in various endemic areas the advantages of undertaking studies along this line.

3.1.5 Physiology of intermediate hosts

Studies on the anaerobic metabolism of various species of snails have shown that the anaerobic resistance of the *Planorbidae* and the operculate snails is greater than that of the *Lymnaeidae* and *Physidae*. All species consumed carbohydrate under anaerobic conditions and produced carbon dioxide and organic acids; lactic acid was identified as one of these acids. In further studies on the anaerobic metabolism, the fact was confirmed that under anaerobic conditions freshwater snails produce volatile acids, and that those formed by *Australorbis glabratus* were identifiable as propionic and acetic acids. Evidence indicated that species not resistant to anaerobiosis are killed primarily by the accumulation of lactic acid, while the resistant species are more tolerant to the lack of oxygen due to the fact
that they accumulate in their tissues the less-toxic fatty acids rather than lactic acid.

It has been found that *A. glabrat*us is able to maintain an approximately steady rate of oxygen consumption over a wide range of oxygen tensions. These findings explain the ability of certain freshwater snails to escape the action of molluscicides* by burrowing into the mud as well as their faculty for resisting desiccation. It may be expected that species with similar respiratory characteristics would not be easily killed by asphyxiation simply by being driven into surroundings poor in oxygen. It was also determined that the oxygen consumption of *A. glabrat*us increased with rising temperature in the range of 0.3°-37°C. A molluscicide, therefore, which acts via the alimentary tract may be expected to be less effective at lower temperatures and more effective at higher temperatures, a consideration which should receive attention in any effort to control intermediate hosts.

The effect of *S. mansoni* infection on *A. glabrat*us has been studied. It was determined that such infection does not interfere seriously with the storage of fat in the body of the snail and that the oxygen consumption remains normal. However, the infected snails were found to have a reduced polysaccharide-content, which may have been due either to impaired carbohydrate digestion and resorption or to toxic action by the parasite.

Experiments have been conducted with tissue homogenates and minces of *A. glabrat*us to determine the enzyme systems operating in this species. Under aerobic conditions, the presence of cytochrome oxidase, succinic and malic dehydrogenases, and fumarase was demonstrated. The findings indicate that catalysis of carbohydrate metabolism is through glycolytic degradation to the pyruvate-lactate level with a mechanism suggestive of the Krebs tricarboxylic-acid cycle for the terminal oxidation of carbohydrate intermediates. It is hoped that information of this sort may lead to a better understanding of the action of molluscicides.

The relationship of oxygen tension to the shedding of cercariae by *A. glabrat*us infected with *S. mansoni* has been studied. Under conditions of anaerobiosis, the snails ceased to shed cercariae or only shed them in very small numbers. Furthermore, infected *A. glabrat*us were less able to withstand anaerobic conditions than were uninfected snails. When cercariae of *S. mansoni* were subjected to anaerobic conditions, they became inactive and lost their tails. Maintenance under such conditions for one hour or more at 25°C significantly reduced their infectivity for mice. However, cercariae showed no reduced infectivity for mice when shed in an aerobic environment from snails previously maintained under anaerobic conditions.

* Of the three current terms "molluscacides", "molluscicides", and "molluscocides", the committee adopted the third because it is used in French and easily adaptable to English.
In other words, conditions of anaerobiosis do not affect cercariae which are still within the snail host. It was found also that the anaerobic metabolic level of both infected and uninfected *A. glabratus* from the Dominican Republic was higher than that of a Puerto Rican strain of the same species. Thus there is the suggestion that physiological differences in geographical strains of snails may account, at least in part, for differences in ability to carry species of *Schistosoma*.

3.1.5.1 It is recommended that additional studies be carried out on the physiology of molluscan intermediate hosts. While considerable progress has been made during the last few years, much additional information is needed. Such data might well provide clues to variations in susceptibility to infection and to the mode of action of molluscicides.

3.2 Economic and Social Conditions in the Epidemiology of Bilharziasis

The surveys carried out under the aegis of WHO in Africa and in the Eastern Mediterranean Region during the last two years justify the reiteration of the views expressed at Cairo by the Joint OIHP/WHO Study Group on Bilharziasis in Africa:

3.2.1 “In certain areas the impaired productivity of infected individuals is not obvious owing to the fact that they unconsciously adapt their working efforts and output to their lowered strength. But when such patients are subjected to heavier labour, ... their physiological balance breaks down, and subjective as well as objective symptoms of bilharziasis suddenly appear.”

Unfortunately no quantitative knowledge concerning the relation between infection and the loss of productive power of the individual is yet available.

3.2.2 It may, however, be stated that infection caused by *S. haematobium* is as a rule borne with fewer signs by the population affected than that caused by *S. mansoni*. This tolerance accounts for the non-recognition by the health authorities in many territories of bilharziasis as a local health problem.

The inhabitants of a bilharziasis area will not ask for treatment for a condition such as haematuria which is so frequent that they consider it normal. In consequence, bilharziasis will not figure in the statistics of in- and out-patients, in which only complaints are listed, with the result that bilharziasis morbidity and mortality are considerably underestimated.

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7 *World Hith Org. tech. Rep. Ser. 1950, 17, 6*
3.2.3 In spite of the formal cautionary notice issued by WHO to all governments and interested intergovernmental agencies, on the risk of introducing or increasing the intensity of bilharziasis as a result of irrigation schemes, it is obvious that co-operation between health administrations and the authorities responsible for irrigation has not in many areas been achieved or been as close as was necessary.

3.2.4 In many areas large-scale migrations of population are taking place and may well result, as has been the experience in the past, in an extension of the prevalence of bilharziasis, unless the initial local population is efficiently treated before new-comers are allowed to settle, or, if such a population is free from bilharziasis, unless the immigrants are carefully screened and are treated when found infected. The need for such screening of the human population and the control of the vector snails becomes even more important when the migration is into an irrigated area.

3.2.5 The present distribution of bilharziasis in the world appears to depend to a greater extent on human factors, of which migration is one, than on factors relating to the potential snail vectors.

When administrative action, or the development of industry, results in the concentration of a partially infected but previously scattered population in a dense community, a considerable increase in the infection-rate in that population usually ensues. Other human factors, such as the development of cultures requiring irrigation or of artificial fish-ponds, or intensive fishing, may also result in a serious increase in the incidence of bilharziasis.

3.2.6 It is the obvious responsibility of the local authorities to compensate for the increased risk due to the concentration of the population by providing the people with sanitary equipment and conditions as well as with adequate health education. It must be recognized that the very concentration of the population makes these measures practicable.

4. STANDARD PROCEDURES FOR EPIDEMIOLOGICAL SURVEYS OF BILHARZIASIS

A survey should provide adequate information on:

1) the true incidence and the social importance of bilharziasis (section 4.1);

2) the factors involved in its maintenance and possible spread (section 4.2);

3) the methods of control which are suggested by study of these factors (section 4.3).
If the extent of the survey is such as to prevent the surveyor from collecting all facts personally, he has to rely on a series of other workers and the value of the survey will depend very largely on the reliability of these workers. This reliability will be increased by the knowledge that investigations of field samples are likely to be made to check their statements.

The data received from various sources are naturally to be checked against each other, just as the results yielded by one method are to be checked against those furnished by another method.

4.1 Measurement of the Incidence of Bilharziasis and of its Social Importance

4.1.1 Human infection-rate

4.1.1.1 The human infection-rate would appear, a priori, to be a reliable indication of the parasite incidence. However, after excluding all possible technical errors, there are still problems of interpretation which arise. These difficulties of interpretation may be due to four causes:

1. Uncertainty as to the place where the disease was contracted. This may have been definitely outside the country or region covered by the survey. People nowadays move about a great deal. Children, however, move away from a locality to a lesser extent.

2. Variation of the rates from one place to another. Infection-rates vary greatly between one human community where conditions are favourable to infection and another where conditions are unfavourable. Generalizations from rates based on surveys of small communities may be misleading.

3. Variation of rates from one year to another. Although bilharziasis is usually an endemic disease, epidemic occurrences are seen in countries with an irregular climate. In such countries, it is advisable to be cautious in the interpretation of results; the latter should be considered valid only for the time at which they are compiled.

4. Variation of rates according to age, sex, ethnic group, and occupation. Considerable differences are sometimes found between the infection-rates of children and those of adults, of males as compared with females, or between the different ethnic groups in a given locality. Whether these differences are due to biological factors (nature of the epidermis, immunity) or to social factors (different habits and customs), they must be taken into consideration when interpreting the significance of human infection-rates.

4.1.1.2 The best method for dealing with these difficulties is to extend the survey in space and time, and to cover each of the various classes of
the population. The ideal can rarely be achieved and the question then arises of the choice of persons most worth examining.

Boys of school age generally constitute the best human material. Apart from the fact—which is of some practical importance—that they lend themselves to examination more readily than any other social category, 

(a) they are less likely to have travelled than adults;

(b) they are a category whose social life is most comparable between countries (the infection-rates for boys are the best material for comparison between countries);

(c) the difference in incidence due to occupation plays little part in the case of children;

(d) children of school age are generally the category with the highest infection-rates.

For comparison of racial groups an examination of girls, men, and women should be made, if possible. It is probable that the differential factors here are social as much as racial. Social differences are least in children and greatest in adults.

4.1.2 Morbidity- and mortality-rates

4.1.2.1 The figures for morbidity- and mortality-rates can be obtained from the following sources:

(1) returns from out-patient clinics;

(2) field surveys or systematic case-finding in supervised communities, such as schools, the Armed Forces, and groups of labourers;

(3) returns from in-patient records;

(4) returns from laboratories.

4.1.2.2 Although these figures may be easy to obtain, they are more difficult to interpret. By themselves they are of no use. They become of value only when considered in relation to:

(a) the number of inhabitants of the country, region, or province whence the statistics come; or

(b) figures showing the scope of the medical activities in the area considered. Bilharziasis cases should be related to the total number of persons seen by the health units from which the returns were obtained; hospital cases of bilharziasis to the total number of in-patients; and positive stool or urine examinations to the total number of such examinations carried out in the laboratories concerned.
In both cases the rates thus obtained depend to a large extent on factors other than the incidence which it is proposed to measure. Among them the following may be cited: the availability of the main health units to persons living in bilharziasis foci; the extent of case-finding efforts; and the health-education level of the population.

In the first case, (a), another source of error to be considered is the degree of perfection or otherwise of the medical facilities of the country concerned. In the second case, (b), the incidence of the great endemicoepidemics which may be prevalent constitutes a source of error. This is a factor which unfortunately often goes unrecognized.

In actual practice these conditions lead to two conclusions:

1. Medical statistics should be related to size of population and extent of medical activities.

2. The above-mentioned sources of error should be assessed during the survey.

4.1.3 Anatomical Localization, Clinical Gravity, and Complications

4.1.3.1 Infection with *Schistosoma* is often well tolerated and without clinical signs. However, this relative benignity appears to vary considerably from country to country without the cause of these variations being as yet thoroughly understood. One of the points which a survey should endeavour to establish is the proportion of clinical cases and serious complications to the number of persons infected. The ratio "disease incidence: infection incidence" is a very important element in assessing the social significance of bilharziasis.

4.1.3.2 The relationship between the data referred to in sections 4.1 and 4.2 gives only an indication which is quantitatively inexact and qualitatively inadequate. Other relevant factors should be sought. They may be drawn from a study of:

1. Post-mortem examinations—a source of information which is the more valuable the more systematically such examinations are carried out;

2. Statistics from pathological-anatomy laboratories;

3. Statistical comparisons between the frequency in a given human group of the incidence of bilharziasis, on the one hand, and the incidence of some disease or symptom which is a possible complication of bilharziasis, on the other hand;


The information obtained from the above-enumerated sources should not be neglected, but its interpretation is very difficult. Errors have often
been made in this regard: too much attention has been paid to the positive correlations and the negative findings have been ignored.

4.1.3.3 In any case, exact quantitative details of the frequency of each clinical sign and its severity, or of bilharziasis complications, can be obtained only by systematic investigations dealing both with the determination of the infection-rate and with the clinical aspects of the disease.

4.2 Study of Conditions Governing the Transmission of Bilharziasis

4.2.1 Geographical data

Geographical data should be recorded in every survey. Their interpretation, however, is difficult in the present state of our knowledge of bilharziasis.

It is certain that climate has an influence on the disease. Apart from the climatic factor, in which temperature appears to be an essential element but whose importance it is difficult to gauge, the influence of other physiogeographical factors on the transmission of bilharziasis has not been adequately evaluated. They may, however, be of influence in regard to both transmission and prophylaxis.

4.2.2 Helminthological data

The species of Schistosoma and, as far as possible, the frequency of each species should be established for the various possible vertebrate hosts.

4.2.2.1 Human infection-rates have been discussed in section 4.1.1 (page 25). These rates can give an indication of the ability of the disease to spread:

(a) by observation of their evolution through the years (history of the disease);

(b) by comparison with the data studied later in this report, particularly in section 4.3.3 (page 32).

4.2.2.2 The concept of animal infection and its incidence has a certain importance, either because the animal schistosomes may be adaptable to man, or because the animal infection may introduce a source of error in the interpretation of the snail infection-rates (see section 4.2.3).

4.2.3 Malacological data

The presence of snails which are suitable intermediate hosts is a sine qua non for the spread of the disease to areas which so far have been free
from it. In a country where bilharziasis is known to exist, a search for infected snails in all habitats and at all seasons is the only method of ascertaining the places where and periods when human infection may take place. This shows the importance of malacological data and the need to give them an important role in bilharziasis surveys.

Unfortunately, in the present state of knowledge, interpretation of such data is extremely difficult. The vector status of certain species of snails has been established exactly, but we are still ignorant of:

(a) the vector potentialities of a number of species;

(b) the biology and ecology of the vector species (apart from certain very special cases);

(c) the possibility of the adaptation of any local races of *Schistosoma* which may exist to other species of snail.

When to these difficulties is added the chaos still reigning in the taxonomy of the possible gastropod vectors of bilharziasis, it will be realized what importance should be placed on snail surveys. The aim should be to collect as much information as possible; analysis and interpretation can come later.

(1) Search should be made everywhere for possible snail vectors, both in zones where bilharziasis is prevalent and in those where the disease is still unknown. Such investigations are often difficult.

(2) Samples from each type of habitat should be identified in an exact manner by specialists.

(3) Systematic research should be carried out on the infection of the various snail species at different seasons and in different kinds of places. The species of *Schistosoma* causing the snail infection should be verified not only morphologically but also, whenever possible, experimentally.

(4) The experimental infection of local snails by human *Schistosoma* strains, both local and exotic, should be encouraged.

### 4.2.4 Human data

Human factors play a considerable part in the spread of bilharziasis to regions so far free from the disease, as well as in increasing the incidence of the disease in regions or countries only slightly affected.

#### 4.2.4.1 Extension to zones free from the disease

The fact that there are regions which are free from bilharziasis even though snail vectors are present should lead to the greatest attention being paid to human migrations capable of importing the disease into such regions.
Study of such movements should form part of a bilharziasis survey. The following points should be noted:

(1) Spontaneous human interchange and any tendency for it to grow or to decrease;

(2) Government projects for transfers of population (resettlement of inhabitants of regions which have become desert, attraction of labour, etc.);

(3) The extent of endemic bilharziasis in the country of origin of the immigrants, the number of the latter, their occupations, and the proportion of children in immigrant populations (all these factors should be taken into consideration in assessing the degree of danger).

4.2.4.2 Local aggravation of incidence

Among the many human factors which can upset endemic equilibrium it is felt that two are of special importance:

(1) Concentration of population near the breeding-places of infectable snail vectors increases the chances of transmission of the disease. This is a different concept from that of population density per unit area. In near-desert regions, for example, the concentration around water-sources is very high, although the population density per square mile is very low. The agricultural concept of "number of inhabitants per acre cultivated" is the one which best measures the data required.

(2) The introduction of certain methods in agriculture or stock-breeding, e.g., irrigated or flooded crops, or fish culture in ponds, is particularly important from this point of view.

4.3 Orientation of Preventive Action

The preventive methods which it is theoretically possible to employ against bilharziasis are numerous. None of them, however, is entirely effective. The simultaneous application of several of them is the ideal solution, each complementing or reinforcing the effect of the others. However, for economic reasons it is generally necessary to choose such methods as may be expected, in view of local conditions, to be the most effective and acceptable.

Assessment of the local factors governing the effectiveness of each preventive method is in practice the most important part of a bilharziasis survey. It is also the most complicated. What is required is the integration of bilharziasis-control measures in the health programme and even in the general economy of the country.
4.3.1 Study of conditions governing transmission

Study of the conditions governing the transmission of bilharziiasis is clearly the essential technical basis for the logical orientation of preventive action. The information which should be collected has been listed in section 4.2, in particular under 4.2.3 and 4.2.4 (pages 28-30).

4.3.1.1 The prospects of good results from the use of <i>moluscocides</i> will be limited, the more extensive, the more irregular in configuration, and the more inaccessible the habitats of infectable snails. It is also necessary to take into account how quickly different types of habitats become repopulated at the various seasons.

4.3.1.2 The results given by <i>protective measures against cercariae</i> will depend more particularly on the extent of those periods of the year when snail infection occurs, and on the occupations exposing human beings to infection.

4.3.1.3 <i>Prevention of snail infection</i> will depend on the sites of snail breeding-places and of dwellings, and on human occupations or habits favouring snail infection.

4.3.1.4 The results of <i>treatment of infected persons</i> will depend on the number treated, on whether clinically asymptomatic forms are common, and on human occupations or habits favouring snail infection.

4.3.2 Study of economic conditions

The best theoretical solutions can be logically derived from the data collected on local conditions of transmission of the disease. However, such solutions are not necessarily the best in practice. The applicability of preventive methods varies, in fact, according to local resources and facilities.

4.3.2.1 Health-service facilities

Health-service facilities are measured:

(a) by the concentration of medical personnel and of nursing personnel; this should be related not only to the number of inhabitants, as is usual, but also to the area of the country. A low population density, entailing the need for much travelling, limits the efforts of the health services;

(b) by the size of the health-service budgets. The proportion of these budgets devoted to bilharziiasis control should be ascertained.

These figures cannot be interpreted without bearing in mind important endemo-epidemic diseases other than bilharziiasis which are prevalent in the country. Their incidence and social repercussions may compel the
health services to reduce the material effort devoted to bilharziasis. But, conversely, some of these diseases can be prevented at the same time as bilharziasis by certain common preventive methods, which should be chosen in such cases.

4.3.2.2 General resources of the country

The health services naturally carry on bilharziasis-control activities. However, they are not the only bodies to be concerned with this problem. Their activities are necessarily influenced by some of the general economic conditions of the country. A complete survey should therefore collect information on:

1. administrations or enterprises concerned with bilharziasis and the budget which they could devote to control of this disease;
2. the state of the general economic equipment of the country (communications, ease of access to the population, etc.);
3. the availability in the territory concerned, the cost, and the opportunities for local production of drugs and chemical compounds for control measures.

4.3.3 Study of ethnological conditions

The human data connected with conditions of transmission (see section 4.2.4, page 29), must clearly be taken into consideration in laying down the lines to be followed by preventive action. This point will not be enlarged upon. However, the economic and nutritional state of the population concerned must also be borne in mind.

The standards of intelligence and education represent ethnological conditions which cannot be neglected. Whatever may be the preventive methods employed, their good effect will always depend on the health education of the population. When this is perfectly satisfactory, it is no longer necessary to impose any preventive methods. Even if the results of health education can be expected to appear only in the future, such education should nevertheless be undertaken. The methods to be employed, however, depend on certain data which the survey should establish, namely:

(a) average intelligence of the social classes affected by the disease;
(b) dialects and languages generally understood;
(c) educational system and quality of the teaching personnel;
(d) encouragement of health education, either theoretical or by demonstration by bodies other than schools.
4.3.4 Results of previous experiments

Finally, whatever may be the theoretical conclusions derived from the various data, there is one type of information of great practical value which should not be neglected—namely, the results obtained in previous experiments in prophylaxis carried out in the country with which the survey is concerned. These experiments may have given a good result and will thus be worthy of general application. More often, they will have met with failure, and analysis of the reasons for this failure will be instructive.

5. CONTROL METHODS

5.1 Molluscocides and Other Means of Snail Destruction *

The control of molluscan intermediate hosts is the most important single method of preventing bilharziasis. The control of these molluscs may be considered under two general headings: (1) the application of molluscocides, and (2) the changing of physical and biological relationships between the molluscs and their environment.

5.1.1 Molluscocides *

The testing of thousands of chemicals as molluscocides, usually on an empirical basis, has probably gone beyond present knowledge of the biology and ecology of the snails. It may be that a better understanding of these would be of great value in their control, with or without the use of molluscocides. Such an approach might assist materially in the practical solution of the problem. From present knowledge it appears that molluscocides are effective when used in sufficient quantity, but that they are expensive.

There seems to be general agreement on most of the qualities desired in a molluscocide: the chemical should at low concentration be toxic to snails and to their eggs in all stages; it should not be dangerously toxic to man, domestic animals, or crops; it should not be easily inactivated by physical or chemical means; it should be cheap; and it should be easy to handle. Its solubility seems to be the only point on which there is disagreement. The matter can be settled only when the problem is better understood; at present little is known about the metabolism and the physiology

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* Dr. D. B. McMullen acted as special rapporteur for this section of the report. At the request of the committee, additions were provided by Dr. W. H. Wright. The information is given in some detail in view of its practical importance to health administrations and because most of it is still unpublished elsewhere.

* See footnote, page 22.
of snails, and the mechanisms through which they are affected by mollusco-
cides.

In the control of aquatic vectors of schistosomes, copper sulfate has
been the most commonly used molluscicide, especially in Egypt. Its
cheapness, availability, lack of toxicity to higher animals, and the ease with
which it can be handled are in its favour. The fact that it combines readily
with organic matter and that it can be washed away quickly leaves some-
thing to be desired. Data indicate that under certain conditions 0.6-1 part
per million (p.p.m.) in standing water and 10-20 p.p.m. in flowing water
is effective against aquatic snails. It has also been used in the control of
amphibious snails, but in order to be effective it should be applied when
the snail colony is covered with water. When a temporary dam is used the
porosity of the soil soon results in the disappearance of the solution. On
the other hand, it is impractical to use it during a period of continuous
flow.

In Japan, calcium cyanamide is the molluscicide most commonly used
against amphibious snails. It changes rather quickly into non-toxic com-
pounds, but when applied properly the results are good. At the rate of
18-20 kg per 1,000 m² it will kill most of the snails, but it costs about
$7.50 for a single application to such a surface.

In recent years thousands of additional chemical compounds have been
screened for their molluscocidal activity against the intermediate hosts of
the human schistosomes. Most of these tests have been made on Australor-
bis glabratus, an aquatic snail, and on Oncomelania nosophora, an amphi-
bious snail. Only those compounds which are most active need be men-
tioned. These include a rather large number of phenolic compounds, a
halogenated hydrocarbon, and some organic phosphorus compounds.

The phenolic compounds appear to be the most important. Of the
more than 700 of these that were tested, the most active were found among
the highly halogenated phenols, the dinitroalkyl- and dinitroaryl-phenols,
the "bisphenol" type of compound, and mercurated phenols. On the
basis of availability and cost, pentachlorophenol or its salts appear to be
the molluscocides of choice against the aquatic snails. These compounds
are also effective against the amphibious snails but some of the dinitro-
alkyl compounds are even more so.

Many of the laboratory-screened halogenated phenol compounds have
been tested under field conditions in different parts of the world. In sta-
tionary waters a considerable number of these compounds have been found
effective for the destruction of aquatic molluscs in concentrations of
5-10 p.p.m. Such compounds include: pentabromophenol; sodium penta-
chlorophenate; 2:4:6-tribromophenol; 2:4:6-triiodophenol; copper
pentachlorophenate; 2:6-dichloro-4-nitrophenol; rosin amine D penta-
chlorophenate; bis (3:5:6-trichloro-2-hydroxyphenyl)-methane; 3:5-dibromo-2:4:6-trichlorophenol; bivalent copper acetylacetone; 2:2'-ethylene-bis-4-chloro-6-isopropylphenol; bis (3-bromo-5-chloro-2-hydroxyphenyl)-methane; 2:4:x-trichloro-6-phenylphenol; and certain restricted compounds. For the amphibious snails, applications of from 4 g to more than 10 g per square metre have been found necessary to achieve effective control with most of these compounds. Preliminary experiments indicate that 2:4:5-trichlorophenol, its sodium salt, and 2:2'-ethylene-bis (4:6-dichlorophenol) are highly toxic to these snails. The relative unavailability and high cost of some of these compounds preclude their practical use at this time.

A considerable number of compounds have also been tested for their ability to destroy aquatic snails in flowing water. At the present time, sodium pentachlorophenate has proved to be the most practical of the more effective compounds. The chemical has been applied in various ways. In the form of briquettes added directly to the stream, it dissolves and maintains sufficient residuals over varying lengths of stream, depending on local conditions. For instance, at Tudun Wada, near Kaduna, Nigeria, $3.00-worth of sodium pentachlorophenate eradicated all Biomphalaria pfeifferi and Physopsis africana from a stream 1.5 miles (2.4 km) in length and the stream remained free of these snails for a period of nearly 11 months. In another area at Rigachikun, northern Nigeria, these same species of snails were eradicated and remained absent for 10 months from a stream 3 miles (4.8 km) in length at a cost of less than a halfpenny (about half a cent) per person of the population in the area. In both instances, the initial dosage provided a concentration of 10 p.p.m. for 6 hours. In the State of Pernambuco, Brazil, a pilot control project covered an area of 5-6 km² (about 2 square miles). Treatment of all streams and watercourses in the area with sodium pentachlorophenate at a concentration of 10 p.p.m. provided good control of Tropicorbis centimetrulris. Three months after treatment only 46 specimens of this species could be found in the entire area whereas thousands had been present before treatment. Other field trials in Brazil have given promising results. Various other methods of application have been employed, including dusting, the incorporation of the chemical into sawdust, and its incorporation with a binding agent to prolong the rate of solution. In some areas, it has been determined that the amount of mud in the body of water influences the molluscidal activity of the compound. This factor must be taken into account when measuring the dosage applied. It is possible also that sunlight may lessen the effectiveness of the chemical. Additional studies are needed to determine the most effective means of application under all conditions.

Sodium pentachlorophenate also destroys the eggs of aquatic snails. Laboratory experiments indicate that a concentration of 10 p.p.m. for
8-10 hours, or of 5 p.p.m. for 24 hours, will destroy all or nearly all the eggs of *Australorbis glabratus*. Failure of snail populations to return for months after field-test applications of the chemical indicates a similar ovicidal effect when the chemical is employed under practical conditions.

Pharmacological studies have indicated that sodium pentachlorophenate can be employed with safety. Its cost compares favourably with that of other mollusccicides. Simple colorimetric tests have been devised for measuring the concentrations of the chemical under field conditions, a distinct advantage in determining effective dose-rates over various lengths of water-courses. It is concluded that concentrations of 5-10 p.p.m. are of practical value for the destruction of the aquatic molluscan intermediate hosts of the human schistosomes.

Experiments have shown that the introduction of sodium pentachlorophenate into stationary or flowing water is relatively ineffective against the amphibious molluscan hosts of *S. japonicum*. The best control with this compound has been attained by spraying the colonies when they are living on a moist surface. On moist soil, with a minimum of vegetation, an application of about 4 g per square metre in a 1:200 solution gave good results. When vegetation was dense or when the snails were found in loose stone walls 10 g or more were needed. In the Yamanashi and Kurume endemic areas in Japan it was found that effective control of *Oncomelania nosophora* could be obtained with an application of 4-7 g of the compound per square metre. In the Philippines, the application of 10 g per unit was used on *O. quadrast* and the results were not always good. Dense vegetation and the removal of the soluble compound from the area by frequent rains apparently reduced the efficiency of the mollusccide in this area. In Yamanashi, an average of 6.6 kg of sodium pentachlorophenate were used per 1,000 m² and the cost of a single application for this area was about $4.00. In the Kurume area, 98.14% of the snails were eliminated after the first application made in the spring. By the autumn of that same year counts showed that the snails were returning so that it was apparent that a sufficient number of snails had remained to repopulate the habitat. Over a two-year period four applications were made and there was a 99% reduction in the snail population. Further studies will be necessary to determine whether such areas become repopulated. If repopulation takes place, it may be impractical to eradicate a snail species from a large endemic area but at present nothing is known about the critical population-levels capable of maintaining the infection in the colonies.

Copper pentachlorophenate was active as a mollusccide in laboratory tests but it is insoluble in water and relatively so in other common solvents, so that its distribution under field conditions offers a problem. It was found to be effective against aquatic snails at a concentration of 10 p.p.m. It was only moderately toxic to *O. nosophora* in preliminary field tests.
In these tests, copper pentachlorophenate was obtained in small particles by mixing solutions of copper sulfate and sodium pentachlorophenate. The copper salt was more difficult to handle and was no more effective than the sodium compound. In a situation in which it was desirable to have an insoluble compound the former would be worth trying.

The two dinitrophenol compounds that have been most adequately tested are 2-cyclohexyl-4:6-dinitrophenol and its dicyclohexylamine salt. Related compounds that are highly mollusccidal, at least for the amphibious snails, include: 2-sec.-butyl-4:6-dinitrophenol; 4:6-dinitro-2-amylphenol; 2:4-dinitro-6-caprylphenol; 2:4-dinitro-3-methyl-6-t-butylphenol; 2:4-dinitro-3-methyl-6-iso-propylyphenol; 2:4-dinitrophenylphenol; and 2-iso-propyl-4:6-dinitrophenol. Some of these show greater mollusccidal activity in the laboratory than the first two compounds mentioned, but at present they either are too expensive, or are not produced commercially, or they have not been tested in the field.

The compound, 2-cyclohexyl-4:6-dinitrophenol, is the most economical of the two mentioned above, so it will be used as an example of this group of compounds. In tests on amphibious snails it was found to be among the best chemicals used on O. quadrasi and O. nosophora. In high dilution it is also lethal to the eggs of these two species. In Yamanashi, Japan, a single application of this compound in the autumn, at the rate of 1.65 g per square metre, gave an average reduction of 84.3% in the snail population in three large areas. A single application in the spring in three more areas gave an average reduction of 95.1%. In three areas where the compound was applied both in the autumn and in the spring the reduction was 97.8%. Parallel tests were made, comparing this compound with sodium pentachlorophenate. These indicate that the former is the most efficient of the known available mollusccides for the amphibious snails. In the amounts used it does not injure crops. In the Philippines, the application of 10 g per square metre stimulated rice growth. At the rate of application in Japan, the cost for 1,000 m² was $3.90, and increased use and production of this chemical will decrease this cost.

In tests on aquatic snails, 2-cyclohexyl-4,6-dinitrophenol was effective against Australorbis glabratus and Bulimus contortus, and 1-3 p.p.m. were lethal to the eggs of the former after a 24-hours' exposure. In field tests, using Bulimus contortus and Planorbis boissyi, it was found that a concentration of 3-5 p.p.m. was effective in stationary water. Copper sulfate under similar conditions, for a comparable kill, required 10-20 p.p.m. The dinitro-compound also had the advantage as an ovicide and there was a residual effect that lasted from two to four weeks. Parallel tests with this compound and with sodium pentachlorophenate have not been reported.

Of the halogenated hydrocarbons, benzene hexachloride (BHC) has shown some promise in laboratory experiments. In those with aquatic
snails, the delta-isomer was the most active. In tests on amphibious
snails, the gamma-isomer was the most toxic in the laboratory; but it
was not effective in field tests. More-effective and less-expensive compounds
are available.

In laboratory tests some organic phosphorus compounds were found
to be among the more potent molluscocides. Of these, tetra-iso-propyl
monothionopyrophosphate was the most active. Such compounds cause
marked body extension in *Oncomelania nosophora*. Mixing them with
compounds like the dinitrophenols markedly increased their molluscocidal
power, apparently because the phosphates increased the exposure to the
compounds that commonly cause withdrawal into the shells. It is doubtful
whether their usefulness offsets the dangers and expense involved.

The use of molluscocides does not necessarily ensure that a control pro-
gramme will be a short one. To obtain any degree of control would pro-
bably require at least five years. Recent work indicates that the time of
the year at which molluscocides are applied can be an important factor in
their effectiveness. The flow of water needed for rice culture makes certain
periods unsuited to molluscocide application so that control should be
carried out before or after the growing season. Chemicals are of no value
during the hibernation period. It has been shown that temperature
materially affects molluscocidal activity of chemicals. If this information
can be used without upsetting the agricultural economy of an area, it is
quite possible that it would help materially.

5.1.2 Biological methods of control

Biological methods of control that have been tried include drainage,
drying, filling, clearing of vegetation, flushing, and encouraging predators.
Here again, as in the case of the molluscocides, one runs up against eco-
nomic problems and the pressure to follow age-old patterns used to raise crops
in order to live. Even as simple a procedure as lining the irrigation ditches
with cement, or using cement to fill crevices in rock-lined ditches, would
constitute a financial burden that could not be borne by the landowners.

Ducks have been suggested as predators but their value is quite limited.
It is known that certain kinds of fish, the larvae of fire-flies, and crayfish
will feed on snails. It is doubtful if they actually have much effect on snail
populations, and when other methods of control are used many of these
predators are eliminated.

Observations in the laboratory and the field by various individuals have
indicated that snail colonies sometimes disappear but reasons for this are
not understood. The effect of bacteria, fungi, and viruses on snails and
their environment has received little attention so far and it is possible that
something of value would be discovered in investigations on the subject.
The successful attack on this disease will be accomplished by a team: the engineer, the malacologist, the parasitologist, the sanitarian, the physician, and the chemist, widely divergent in training, speaking in the beginning different technical languages, but with a common purpose in mind.

5.1.3 Recommendations for making snail control more effective

The following measures for making snail control more effective are recommended:

1. place greater emphasis on the study of bionomics, ecology, and physiology of the snail hosts;
2. investigate bacteria, viruses, etc., that might assist in snail control;
3. on the basis of known facts, stress environmental control, i.e., drainage, irrigation, vegetation clearance, agricultural practices, and sanitation;
4. where feasible, use the molluscicide best suited to the host and environment;
5. make observations on repopulation and infection of snails in control areas.

5.2 Treatment

5.2.1 Criteria of cure

A feature of therapeutic trials in bilharziasis has been the lack of agreement on what constitutes evidence of cure. At its simplest, particularly in *S. haematobium* infections, some would measure "cure" as an amelioration of symptoms, a sense of well-being, and the absence of eggs from a single specimen examined some time after treatment. Others would demand more stringent standards. Finally, there are those who have doubts as to whether complete eradication of the parasite is achieved even in *S. haematobium* infections and think that it is certainly less likely to be achieved in *S. mansoni* and *S. japonicum* infections.

The establishment of elaborate criteria of cure for the routine treatment of bilharziasis is not a practical possibility. It is rarely, if ever, possible, when treating primitive peoples, to arrange a satisfactory follow-up examination. In fact, if treatment requires that the patient return repeatedly, then it is unlikely that standard courses of treatment will be completed.

5.2.2 Standard methods of treatment

The behaviour of the same parasite in different localities shows much variation, so that it is necessary to establish a standard method of treatment
for each species of parasite for each area. In assessing the value of the various drugs and the techniques and dose-schedules to be used, it is important that the therapeutic results of treatment should be assessed by the same methods, so that results may be easily compared with data obtained in other areas. The pattern of the disease and its average intensity vary so much that it is necessary for each region to decide what standard method should be adopted for each type of infection. The selection of a standard method of treatment is, of course, influenced by other factors than therapeutically such as the cost of the drug, the simplicity of administration, and whether skilled staff are required for the purpose.

5.2.2.1 It is most important that persons with newly developed and active infections be selected for the trial, that the subjects should be proved to be passing eggs regularly, and that a high proportion of these eggs should be viable and hatchable.

In order to obviate the criticism that early relapses may be due to the maturing of young worms which had escaped the effects of the drug, subjects should, if possible, not have been subjected to infection for three months before the trial. It may, therefore, be difficult to arrange satisfactory trials in countries with bilharziasis due to irradiation, and where the disease is truly endemic.

5.2.2.2 Where the course of treatment is of short duration, the first post-treatment examinations need not be made until a week after treatment has begun. In cases where the drug must be given for periods of two weeks or more, a weekly follow-up starting two weeks after treatment will suffice.

5.2.3 Examination techniques

The value of many therapeutic trials in the past was much reduced because the viability or otherwise of the eggs observed had not been established. It is well known that dead eggs can be shed into urine or stool for months after cure. Even in active and recent infections, a proportion of the eggs passed into the excreta are non-viable and non-hatchable, presumably because they have been unduly delayed in their passage through the tissues after being extruded by the worms. From experimental evidence in *S. japonicum* infection it would appear that eggs remain viable in the tissues for only 21 days at the longest. It is, therefore, assumed that if living eggs are seen the producing worms were still alive 21 days earlier.

5.2.3.1 Specimens of excreta should be examined weekly for the first month and thereafter fortnightly for a period of six months after treatment. In view of the difficulty in protecting persons from reinfection in many areas where trials of drugs and of standard treatments should be made,
there may be difficulty in protecting trial subjects from reinfection for such a period.

Each examination should consist of the investigation of at least two separate specimens taken on consecutive days. If conditions permit, this can be extended to the examination of specimens on three consecutive days.

Additional indirect evidence of cure may be given by an increase in weight and improvement in the general physical condition, especially in young subjects, and by an increase in mental alertness and capacity for physical exertion.

5.2.3.2 S. haematobium infections

In trials of drugs used against S. haematobium the following standard procedure is suggested:

(1) Urine samples should be passed by the patient after some exercise has been taken.

(2) Samples should be examined without undue delay—if possible, within an hour of being passed.

(3) If the specimen is to be centrifugalized, this should be at a rate of not more than 1,000 r.p.m. and for not more than 2 minutes.

(4) The sedimented or centrifugalized deposit should be examined microscopically; the appearance of the eggs and the contained miracidia may give useful indications of the early effect of the drug.

(5) The specimen should also be examined by a miracidia-hatching technique. Hatched miracidia should be looked for several times during the first 20 minutes, and the specimen should not be discarded as negative until carefully examined one hour after the addition of water to the deposit.

5.2.3.3 S. mansoni infections

In therapeutic trials against S. mansoni infections, the follow-up and the establishment of satisfactory criteria of cure are much more difficult.

The standard method for the examination of stool is described in section 2.1.3 (page 7). Miracidia-hatching techniques should also be employed. An additional refinement in the test is the examination of a rectal biopsy-snipping, from which also the viability of the egg can be estimated. Rectal biopsy can also be of value in the follow-up of S. haematobium infections.

5.2.3.4 S. japonicum infections

The same methods and procedures as recommended for use in S. mansoni infections can be used in S. japonicum trials.
5.2.4 Drugs and techniques in the treatment of bilharziasis

A number of preparations which have been described in the past are no longer used to any extent. These include salts of emetine, acriflavine, and salts of copper.

The drugs now in general use have various advantages and disadvantages and these have to be given full consideration before the drugs can be accepted as standard preparations and procedures in any area, quite apart from questions of therapeutic efficiency.

5.2.4.1 Potassium antimony tartrate and sodium antimony tartrate

Sodium antimony tartrate (SAT) causes fewer toxic signs than potassium antimony tartrate (PAT) and solutions diluted ready for use are stable. The drug is cheap. It is not well tolerated by subjects affected by *S. mansoni*, and even less by those affected by *S. japonicum*, unless the salt is administered in dilute solution, preferably not stronger than 0.5%.

There is more than sufficient evidence in the literature that when full doses of these salts are given intravenously, on a regular schedule, the therapeutic efficiency is high. It is least efficient in *S. japonicum* infections, but it still remains the most effective agent available at present for this infection. These antimony salts have the following disadvantages:

1. The need to use the intravenous route requires a degree of technical skill which seriously limits the amount of treatment that can be given in less-well developed areas.

2. The length of the course of treatment results in a very high default-rate.

3. The toxicity of these salts of antimony given in doses over a long period is high and towards the end of the full course of treatment the toxic signs and symptoms are sometimes rather alarming.

Efforts have been made to mitigate some of these disadvantages by introducing more-intensive courses, whereby the total amount of antimony administered can be reduced by about one half. Accelerated schedules of treatment are now in more general use; these range from an intensive course of six "slow" injections, spaced over 48 hours, and used chiefly in *S. haematobium* cases, through a variety of schedules lasting as long as ten days. These modifications have helped to reduce the high default-rate experienced in long courses of treatment. The inherent disadvantage of having to use the intravenous route remains, and thus, in many areas, the agencies able to provide such forms of treatment are few.
5.2.4.2 *Antimony pyrocatechol sodium sulphonate*\(^9\)

A trivalent antimony preparation which can be given either intravenously or intramuscularly. It is a costly drug, especially when the dosage is increased and given more intensively in an effort to improve therapeutic efficiency. The preparation is very much less effective when given by the intramuscular route. In fact, this route is justified only in the case of the treatment of small children whose small veins prevent the use of the intravenous route. It may also be justified at remote treatment centres where staff skilled in intravenous techniques are not available.

5.2.4.3 *Lithium antimony thiomalate*\(^10\)

This preparation, which resembles in many ways antimony pyrocatechol sodium sulphonate, can be given both intramuscularly and intravenously. It is relatively costly and ranks below antimony pyrocatechin sodium sulphonate in therapeutic efficiency.

5.2.4.4 *Miracil D hydrochloride*\(^11\)

Miracil D hydrochloride is a complex thioxanthone preparation. The drug is costly, but there is some evidence that the price is being reduced as the preparation is more widely used. This is the only effective schistosomical agent which is taken by mouth, being dispensed in compressed tablets. It has an unpleasant taste, but is free from serious toxicity, although there are unpleasant side-effects, such as abdominal pain, nausea, constipation, and mental depression. The course of treatment is short—three to five days—and the side-effects pass quickly when treatment is completed.

The drug has been shown to be most effective in *S. haematobium* infections. There is some evidence that, by doubling the total dose, *S. mansoni* infections may be cured, but it is without value in *S. japonicum* infections.

It lends itself to mass treatment of populations and to treatment on an out-patient basis, particularly at schools because it is well tolerated by children. Its disadvantages are the high cost of the drug—but this is greatly offset by the fact that no skilled staff are needed to administer it—and the shortness of the course of treatment, which prevents defaulting from treatment.

\(^9\) Sodium-antimony-bispyrocatechol-3 S-sodium disulphonate, known by a variety of names, including stibophen (*Pharmacopeia Internationalis*), Fouadin, Reprodal, and Fantorin.

\(^10\) Known also as Anthiomaline.

\(^11\) Known also by a variety of names, including lucanthone hydrochloride (British approved name), Nilodin, and 3735 RP.
5.2.4.5 Present situation in the chemotherapy of bilharziasis

Standards and methods of assessing cure have altered much in recent years and it is difficult to compare recent work with trials made years ago. It would be a most useful service if the drugs now in common use could be tested again in as many endemic areas as possible, the trials being conducted to a standard pattern. A tentative classification of these drugs, showing the effect of the various administration-techniques described, is given in table I in the hope that this may stimulate the compilation of therapeutic-efficiency tables in each local area.

**TABLE I. COMPARATIVE EFFICIENCY OF DRUGS AND TECHNIQUES USED IN BILHARZIASIS TREATMENT**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treatment technique</th>
<th>Percentage efficiency in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. haematobium</em> infection</td>
</tr>
<tr>
<td>SAT</td>
<td>Intensive course</td>
<td>85-90</td>
</tr>
<tr>
<td>Miracid D</td>
<td>Oral route</td>
<td>80-85</td>
</tr>
<tr>
<td>SAT</td>
<td>Classical course</td>
<td>75-80</td>
</tr>
<tr>
<td>Fouadin</td>
<td>Intravenous route</td>
<td>60-65</td>
</tr>
<tr>
<td>Fouadin</td>
<td>Intramuscular route</td>
<td>50-55</td>
</tr>
<tr>
<td>Anthiomialine</td>
<td>Intramuscular route</td>
<td>50-55</td>
</tr>
<tr>
<td>Anthiomialine</td>
<td>Intravenous route</td>
<td>45-50</td>
</tr>
</tbody>
</table>

5.2.5 Possibilities of control by therapeutic measures

Complete control of bilharziasis by destruction of the definitive stage of the parasites in man has long been a dream. Treatment of infected human populations as a method of control has been tried in various parts of the world in past years, but nowhere with such courage and persistence as in Egypt. Such treatment had a remarkable effect on the severity of the infection and on the incidence of serious complications, but had little effect on the general incidence of the disease.

It does not seem possible to derive much benefit in bilharziasis control from the treatment of patients attending hospitals, however efficient the
treatment may be and however dutiful the patients may be in remaining under medical care. Mass treatment as a means of control must be taken to the people on a much broader front than in hospitals and must be given to infected populations in their own environment. A drug for this purpose must be easy to administer, non-toxic, require a short period of treatment, and be cheap. Miracil D hydrochloride in the treatment of *S. haemato-
bium* infections is the only preparation even approaching such an ideal. Treatment of infected human populations—particularly of the younger age-
groups, who, from their habits, are the most likely to maintain the infection of snails—is, however, a logical and effective aspect of bilharziasis control when combined with the control or elimination of vector molluscs.

The situation is not so clear where *S. japonicum* infection is concerned because other mammals probably provide suitable reservoirs for the worms. It seems, therefore, that attempts to control bilharziasis solely by the treatment of infected human beings are not likely to be effective. Budgets devoted only to mass treatment could be spent more profitably on other aspects of control.

6. BILHARZIASIS CAUSED BY *S. JAPONICUM*

The committee was aware that the present report is definitely incomplete in respect to the distribution and epidemiology, and, therefore, to a certain extent in respect to the control, of *S. japonicum* infection. The committee hoped that, after covering forms of bilharziasis which prevail in Africa, in the Americas, and in the Eastern Mediterranean Region, it would have an opportunity to consider the forms of the disease which occur in the Western Pacific Region.

The committee fully realized the importance of domestic animals, in addition to man, as reservoirs of *S. japonicum*, a situation to which the Joint WHO/FAO Expert Group on Zoonoses has drawn attention. The committee felt, however, that local studies were necessary before it was in a position to pronounce definitely on the role of the various species of domestic animals, such as dogs, cats, swine, oxen, water-buffaloes, etc., in the epidemiology and possible spread of bilharziasis caused by *S. japonicum*.

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