Bull. Org. mond. Santé / *Bull. Wld Hlth Org.* 1958, **18**, 945-961

THE INTRADERMAL TEST IN THE DIAGNOSIS OF BILHARZIASIS

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SYNOPSIS

The diagnosis of bilharziasis by detection of schistosome eggs in the urine or faeces of patients is often not possible in the later stages of the disease, when elimination of the eggs is rendered increasingly difficult. To overcome this difficulty, an immunological test has been developed, based on the cutaneous response to an injection of an antigen prepared from the cercariae, adult worms, eggs or miracidia of *Schistosoma*.

In this paper, the author reviews the various procedures for obtaining the basic material for antigen preparation, describes briefly the method of preparation, outlines the nature and properties of the specific antigens, and discusses the skin-testing procedure, with special reference to the criteria for evaluating the results. He points out that the intradermal test may prove useful in epidemiological surveys as well as in the diagnosis of individual cases.

The diagnosis of bilharziasis during the initial stage of the disease (frank haematuria, in the vesical type, and toxaemic manifestations accompanied by gastro-intestinal disorders, in the intestinal types) may be easily made by detecting schistosome eggs in the patient's urine (*S. haematobium*) or faeces (*S. mansoni* and *S. japonicum*). However, at later stages, the number of worms may decrease and the elimination of eggs becomes more and more difficult, owing not only to the gradually increasing cellular reactions that tend to wall them in, but also to the growing connective tissue which prevents them from escaping. Therefore, in endemic areas, the prevalence of bilharziasis, when evaluated by examining stool or urine samples, or both, from unselected people, always gives figures lower than expected, since most patients are in the chronic stage of the infection.

Attempts to overcome these inherent difficulties in diagnosis led to the use of immunological methods, which have proved to be very helpful for confirming the diagnosis of individual cases of schistosome infections.

When used in epidemiological surveys, such methods provide a fairly good approximation of the true prevalence of the disease.

In this report the writer will review the procedures for procuring the basic material for antigen preparation and the main aspects regarding the use of the intradermal test for the diagnosis of schistosome infections.

Procurement of Basic Material

Cercariae

The following methods for concentrating schistosome cercariae for antigen preparation have been devised:

(*a*) *Centrifugation of living cercariae* (Oliver-González & Pratt, 1944; Katzin & Most, 1946) *or of dead cercariae killed by heat* (Lurie & De Meillon, 1951). As to the first method, Bozicevich & Hoyem (1947) and Standen (1950) have pointed out that the cercariae, even when spun at 4000 revolutions per minute (r.p.m.) for 5 minutes are able to swim back actively to the top immediately after centrifugation.

(*b*) *Immobilization of cercariae by chemical or physical agents prior to sedimentation.* Bozicevich & Hoyem (1947) used ethanol and Martins (1949) a small quantity of ether to narcotize the cercariae, which then sink to the bottom. The sedimentation may also be accomplished by putting the cercarial suspension into the refrigerator (4-10°C). The latter method was employed by Bozicevich & Hoyem (1947), Mayer & Pifano (1949) and Oliver-González, Bauman & Benenson (1954).

(*c*) *Deposition of cercariae on filter-paper.* This technique, used by Alves & Blair (1946), has also been employed by Blair & Ross (1948), Rodrigues da Silva & Costa (1949), Standen (1950) and Pereira (1951). Antigens may be prepared with the filter-paper still wet or after it has been dried.

(*d*) *Accelerated vertical migration of cercariae.* Standen (1950, 1952) claims to have easily concentrated cercariae of *S. mansoni* by putting the cercarial suspension in a separating-funnel, cooling its lower part with ice-cold water and illuminating its top from the sides. After a few minutes the cercariae will have gathered together in the bright upper layer of the liquid. The contaminants are then removed by drawing off the lower layer.

(*e*) *Concentration of cercariae in crucibles and funnels with fritted-glass bottoms.* The porosity of the plates should be such as to let only water and small contaminants pass through, so that the cercariae are easily concentrated (Pellegrino & Macedo, 1955).

Appreciable amounts of *S. mansoni* cercariae practically free from undesirable matter may be obtained by the following method (Pellegrino & Nunes, 1956):

1. Infected snails, previously washed and cleaned, are dispensed in beakers containing about 300 ml of water, in groups of 20-50. They are then exposed to the sun or to electric light for 1-3 hours, in order to induce the shedding of cercariae.

2. The cercarial suspension is poured through 4 layers of surgical gauze to remove snail faeces and other debris.

3. The cercariae are concentrated in Büchner funnels, with a capacity of 350 ml or greater, fitted with plates of medium porosity (maximum pore size, about 20μ).

4. The heavy cercarial suspension is poured into conical flasks and placed in the refrigerator for sedimentation.

5. The supernatant is discarded and the cercariae in the remaining small volume of liquid are transferred to a beaker so that a thin layer is formed.

6. The material in the beaker is frozen under dry ice and then desiccated in vacuum in the presence of calcium chloride. The dried cercariae are kept in glass ampoules and sealed in vacuum.

About 100-150 mg of dried cercariae may be obtained in a day, which is a highly satisfactory yield for antigen preparation (Pellegrino—unpublished data, 1957).

Adult worms

The recovery of adult schistosomes from the mesenteric-portal system may be easily accomplished by means of simplified perfusion techniques (Brandt & Finch, 1946; Yolles et al., 1947; Pan & Hunter, 1951; Ruiz, 1952, 1953b; Pellegrino & Siqueira, 1956). The perfusion is generally performed in two stages:

1. *Perfusion of the liver*, in which the physiological salt solution runs under pressure through the hepatic veins into the portal system, reaching the exit at the portal vein.

2. *Perfusion of the mesenteric vessels*, in which the solution is allowed to flow into the aorta and run off by the portal vein.

A previous injection of heparin, which prevents blood from clotting, renders the perfusion and the collection of the worms easier.

Most schistosomes lodged in the mesenteric-portal system are carried away with the perfusion fluid. When a large number of worms is required, as for antigen preparation, the guinea-pig has proved to be a suitable animal despite being considered a poor host of *S. mansoni* (Moore, Yolles & Meleney, 1949; Stirewalt, Kuntz & Evans, 1951). Pellegrino & Siqueira (1956) were able to recover 51 702 worms after liver and mesenteric perfusions carried out on 38 highly infected guinea-pigs.

Eggs

Interest in egg antigens was aroused when Oliver-González, Bauman & Benenson (1954, 1955) reported that skin-tests with such antigens, negative in most patients before treatment, became positive after therapy in about 80% of cases. These workers used the liver and intestines of infected mice and hamsters as sources of eggs. The liver and intestines were cut up into small pieces, homogenized in a Waring blender and then strained through a finely woven sieve into conical flasks for the sedimentation of eggs. Most extraneous matter was removed after several sedimentations in saline. Griffiths & Beesley (1955) concentrated *S. mansoni* eggs from infected mouse livers homogenized in a Waring blender and then strained through several sieves of gradually decreasing meshes. The resulting suspension was poured through organdie and silk to remove small contaminants. Recently Coker & Lichtenberg (1956) outlined a simple procedure for isolating *S. mansoni* eggs from the liver of experimentally infected hamsters by a combination of comminution, filtration and sedimentation through a viscous medium (sucrose).

As the number of *S. mansoni* eggs in the small intestine of mice is remarkable 8 weeks after infection and the tendency to granuloma formation is still little pronounced at that stage (Brener, 1956), the following technique for egg concentration has been devised (Pellegrino—unpublished data, 1957):

1. Mice infected with about 150 cercariae (*S. mansoni*) are killed, preferably between 8 and 11 weeks after exposure, and their intestines removed.

2. The small intestine is cut off and thoroughly opened and washed. It is then placed in a Petri dish containing physiological salt solution and left at 37°C for 2 hours.

3. The intestinal mucosa is scraped with the aid of a microscope slide in order to remove most of the available material.

4. The suspension is homogenized in a Waring blender for 1 minute and, after being filtered through 4 layers of surgical gauze, is finally strained through a sieve (US Standard No. 100).

5. The eggs are allowed to settle in a crystallizing-dish, 6 cm high, for 15 minutes.

6. The supernatant is removed, by means of a pipette connected to a vacuum pump, so that a thin layer (about 0.5 cm) is left.

7. Physiological salt solution is poured into the crystallizing-dish up to a height of 3 cm and the sedimentation of eggs is observed through a stereoscopic microscope. When sedimentation is complete, the supernatant is pipetted off as in step 6.

8. This washing in saline is repeated as many times as are necessary to remove most of the intestinal debris. As the eggs settle rapidly, only 4-5 minutes are required for each washing.

9. The egg suspension is poured into a centrifuge tube and spun at 1500 r.p.m. for 5 minutes.

10. The sediment is frozen under dry ice and desiccated in the presence of calcium chloride.

The amount of eggs obtained by this technique is quite satisfactory for antigen preparation.

Miracidia

Blair & Ross (1948) make reference to the use of an extract prepared with *S. haematobium* miracidia, stating that this antigen, even after having been stored at room temperature for several years, provided results similar to those obtained with cercarial antigen of the same species of *Schistosoma.* However, they were forced to stop using it, owing to difficulties in securing a satisfactory yield of miracidia. Recently, Sherif (1956) described a method for collecting and washing schistosome eggs (mainly of *S. haematobium*) by means of which a plentiful supply of miracidia may be obtained. The miracidia are killed by adding 96% ethanol to the original suspension and are then centrifuged and dried under vacuum.

Simplified techniques for concentration, based on the positive phototropism and negative geotropism of miracidia, have been devised (Stunkard, 1946; Ingalls et al., 1949; Kagan, Short & Nez, 1954; Chaia, 1956).

When appreciable amounts of *S. mansoni* miracidia are required, the following procedure can give fairly good results (Pellegrino—unpublished data, 1957):

1. *S. mansoni* eggs from the intestines of infected mice are concentrated as previously described.

2. The saline egg suspension is poured into a glass crucible fitted with a plate of medium porosity.

3. Dechlorinated water is added to the suspension in the crucible so that any excess of sodium chloride is carried away by percolation.

4. The egg suspension, now in dechlorinated water, is exposed to the light of a 60-watt electric lamp. The ova will soon hatch, liberating free-swimming miracidia.

5. With a pipette, the miracidial suspension is transferred to another crucible. Immature and degenerate eggs, as well as empty shells, will remain at the bottom of the first crucible. The heavy miracidial suspension is then transferred from the second crucible to a watch-glass and, finally, is frozen and desiccated for antigen preparation.

Preparation of Antigens

As has been shown, desiccated schistosome cercariae, adult worms, eggs and miracidia can be easily obtained for antigen preparation. Therefore, the use of extracts from the digestive glands of infected snails (Fairley & Williams, 1927; Manson-Bahr, 1929; Taliaferro & Taliaferro, 1931; Vogel, 1932; Ramsay, 1934; Martins, 1949; Davies & Eliakim, 1954) or of heterologous antigens (Hassan & Betashe, 1934; Culbertson & Rose, 1942; Mayer & Pifano, 1945b, 1946; Guerra, Mayer & Di Prisco, 1945; Peres & Pena, 1945; Culbertson, Rose & Oliver-González, 1947; Martins, 1949; Lurie & De Meillon, 1951) should not be encouraged. Cercarial and adult-worm extracts have been widely used as skin-testing material. Egg antigens have been employed by Oliver-González, Bauman & Benenson (1954, 1955) and Pellegrino (unpublished data, 1957). Miracidial antigens have been used by Blair & Ross (1948) and, more recently, by Sherif (1956).

As a rule, antigens are prepared with desiccated and finely ground material. Extracts of fresh cercariae or adult worms have occasionally been employed as antigens (Bozicevich & Hoyem, 1947; Pellegrino—unpublished data, 1957). Physiological and Coca solution have been used as the extracting medium, phenol or merthiolate sometimes being added. Extraction is generally carried out at room temperature (2-3 days) or in the refrigerator at 4-8°C (2-8 days). It has also been performed at 36-37°C during short periods (Pesigan et al., 1951, 1954; Oliver-González, 1953; Oliver-González & Pratt, 1944; Davies & Eliakim, 1954). Wright et al. (1947) effect the extraction of allergenic substances from adult worms in a relatively short time: 12 hours in the refrigerator. Extracts have been preserved with phenol (0.3-0.5%) or merthiolate (1:5000 to 1:10 000). In some cases Seitz filters (Mayer & Pifano, 1945a, 1946; Rodrigues da Silva & Costa, 1949; Pesigan et al., 1951, 1954; Pessôa & Barros, 1953; Sherif, 1956) or porcelain ones (Coutinho, 1948, 1949, 1951, 1952a) have been employed.

Purified antigens (removal of lipoids, isolation of polysaccharides) have been prepared by some authors (Martins, 1949; Coutinho, 1952b; Ruiz, 1953a; Horstman, Chaffee & Bauman, 1954; Pellegrino et al., 1956).

Nature and Properties of Antigens

According to Martins (1949) and Ruiz (1953a), the active fraction of antigens that is capable of eliciting cutaneous responses in patients with bilharziasis must be a polysaccharide. In fact, substances acting chemically as polysaccharides have been extracted from cercariae and adult worms (*S. mansoni*) and successfully used for skin-testing. However, Pellegrino

et al. (1956) believe that the polysaccharides—at least those extracted from *S. mansoni* cercariae by Fuller's technique—may not be the only active fraction.

Data concerning the storage of antigens seem to indicate that they are highly stable. Pratt & Oliver-González (1947) did not detect any loss of potency in cercarial antigens which had been stored in the refrigerator for one year. According to Mayer & Pifano (1945a), Blair & Ross (1948), Martins (1949), Coutinho (1951), Lurie & De Meillon (1951) and Pellegrino (unpublished data, 1957), antigens commonly used for skin-tests in bilharziasis do not undergo any appreciable changes during storage. However, Pesigan et al. (1951) observed that an adult-worm antigen (*S. japonicum*) prepared three months previously was slightly less potent than a fresh one.

Technique of Intradermal Test

The intradermal test is generally performed on the volar surface of the forearm and, less frequently, on the dorsal surface. It must be mentioned, however, that skin-tests performed on the scapular region are significantly more intense (Mayer & Pifano, 1945a; Martins, 1949; Pellegrino—unpublished data, 1957).

The amounts of antigen injected vary from 0.01 ml to 0.1 ml. Recently, the smaller volumes (0.01-0.05 ml) have been chosen, to prevent false-positive reactions due to tissue injury. Antigen dilutions range from 1:1000 to 1:10 000, though, occasionally, stronger or weaker concentrations have been used.

Positive reactions are normally immediate and reach their maximum 15 minutes after injection of the antigen. However, since Pessôa & Barros (1953) have noticed that patients in the later stages of the disease, showing spleen enlargement and anaemia, may react slowly (30 minutes or more), observation for a longer period is sometimes required. Reading is done by observing the wheal and recording its characteristics, diameter length, etc., or by taking a tracing of it on transparent paper (Oliver-González & Pratt, 1944), or even by drawing its outline in ink and then pressing it against slightly wet absorbent paper (Martins, 1949; Pellegrino & Macedo, 1956; Pellegrino, Memória & Macedo, 1957).

Delayed reactions have occasionally been recorded (Khalil & Hassan, 1932; Boza, 1942; Martins, 1949; Pessôa & Barros, 1953).

Criteria for Evaluation of Results

A critical review of the diagnosis of bilharziasis by the intradermal test has shown that a number of criteria have been used for the interpretation of results. Such criteria may be grouped as follows:

Inaccurate or subjective criteria

Regrettably, some authors who have carried out intradermal tests using antigens prepared from *S. bovis* or other helminths—Khalil & Hassan, 1932 (*S. bovis* antigen); Hassan & Betashe, 1934 (*Fasciola gigantica* antigen); Culbertson, Rose & Oliver-González, 1946 (*Planaria maculata* antigen); Oliver-González et al., 1953 (*Fasciola hepatica* and *Pneumoneces mediopleXus* antigens, simultaneously with that of *S. mansoni* cercariae)—have used inaccurate criteria for the evaluation of the results. Lurie & De Meillon (1951), for instance, just state that " a definite extension in the size of the wheal was taken as a positive result ".

Criteria based on enlargement of initial wheal

Blair & Ross (1948) and Coutinho (1951) consider an intradermal test positive when 15 minutes after the antigen injection the wheal diameter is twice its initial size.

Criteria based on increase of antigen wheal in relation to control wheal

To several authors, a reaction in which the diameter of the antigen wheal exceeds that of the control wheal by 3-4 mm or more is considered positive (Katzin & Most, 1946; Bozicevich & Hoyem, 1947; Wright et al., 1957; Pesigan et al., 1951, 1954; Davies & Eliakim, 1954; Horstman, Chaffee & Bauman, 1954 ; Oliver-González, Bauman & Benenson, 1954).

Criteria based on diameter of antigen wheal

The size of the wheal diameter at the 15 minutes' reading is used as a basis of interpretation by several authors who, however, do not agree as to the diameter size beyond which a reaction can be considered positive: 20 mm (Fairley & Williams, 1927); 23 mm (Ramsay, 1934); 12 mm (Peres & Pena, 1945); 10 mm (Davies & Eliakim, 1955; Sherif, 1956).

Criteria based on degree of intensity of positive reactions

Most South American workers classify positive reactions into several groups according to their intensity (Mayer & Pifano, 1945a, 1949; Lopes, 1945; Coutinho, 1948, 1949, 1952a, 1952b; Martins, 1949; Rodrigues da Silva & Costa, 1949; Pereira, 1951; Pessôa & Barros, 1953; Ruiz, 1953a). Wheal enlargement, pseudopodia formation and, in some instances, the presence of erythema and pruritus are usually taken into consideration.

Criterion based on size of wheal area

Aiming at a more accurate and objective evaluation of results, Pellegrino & Macedo (1956) suggested the following procedure: 15 minutes after the

antigen injection (0.05 ml on the volar surface of the forearm) the outline of the wheal is drawn in ink and transferred, by pressure, to slightly wet absorbent paper. The wheal area is then determined with a planimeter or in the following way: a series of circles, outlining areas ranging from 0.5 to 1.2 cm^2, is drawn on thick transparent paper or celluloid; beside these circles, 3 rectangles, with areas of 1.0, 2.0 and 4.0 cm^2 and surrounded by a chequered area of which the small squares measure 0.05 cm^2, are also drawn. It is very easy to obtain a rapid and fairly good estimate of the wheal area by placing the transparent paper over the wheal transfer. The following criteria were established after preliminary tests on patients with bilharziasis as well as on healthy individuals:

(*a*) *negative reactions*: wheals with areas up to 0.9 cm^2;

(*b*) *doubtful reactions*: wheals with areas of 1.0 or 1.1 cm^2;

(*c*) *positive reactions*: wheals with areas of 1.2 cm^2 or more.

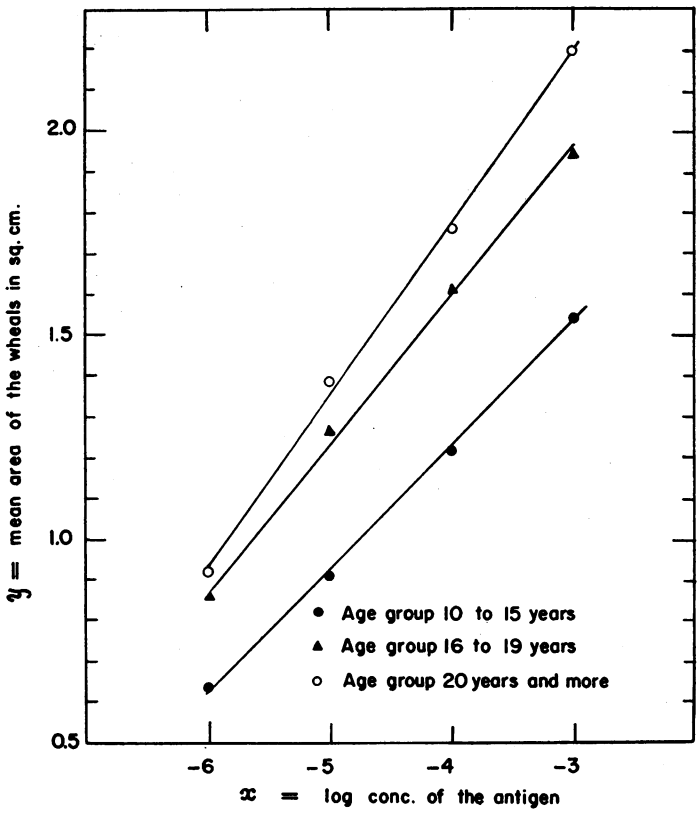
On estimating the wheal areas obtained by intradermal tests using cercarial antigen in concentrations ranging from 10^{-3} to 10^{-6}, in three age-groups of patients with bilharziasis, Pellegrino, Memória & Macedo (1957) demonstrated the existence of a linear relationship between the mean area responses and the log concentrations of the antigen (see figure below).

Sensitivity and Specificity of Test

In the course of schistosome infection the skin becomes sensitized to specific antigens subsequent to the development of circulating antibodies. Positive intradermal tests are usually observed from the fourth week after exposure (WHO Expert Committee on Bilharziasis, 1953). Wright et al. (1947), using *S. mansoni* antigens diluted 1:1000, obtained negative intradermal tests in American soldiers newly infected with *S. japonicum* during the Leyte campaign; on the other hand, tests performed with the same antigen in local patients were positive. Mayer & Pifano (1945a, 1946) observed positive reactions 35 days after *S. mansoni* exposure and Lurie, De Meillon & Stoffberg (1952) observed them during the seventh week of disease in one case (*S. haematobium*). The duration of the infection probably contributes materially to sensitization and, early in the course of the disease, such sensitization is not at a high level (Wright et al., 1947).

The data at present available on the sensitivity of the test cannot be adequately compared, either because of the diversity of antigens and criteria of interpretation or because of the lack of uniformity in the groups studied. It is known that the cutaneous reaction is stronger in adults than in children (Mayer & Pifano, 1946; Martins, 1949; Lurie et al., 1953; Pessôa & Barros, 1953; Pellegrino, Memória & Macedo, 1957). According to Martins (1949),

INTRADERMAL TEST USING CERCARIAL ANTIGEN IN PATIENTS WITH ACTIVE *S. MANSONI* INFECTION: REGRESSION OF MEAN AREA OF WHEALS ON LOG CONCENTRATION OF ANTIGEN *



10-15 years: 100 individuals
16-19 years: 50 individuals
20 or more years: 100 individuals

* Reproduced from Pellegrino, Memória & Macedo (1957) by kind permission of the editors of the *Journal of Parasitology*.

men give more pronounced responses than women and negroes more than white people.

It is generally admitted that the skin-test with proper antigens is positive in about 90% of bilharziasis patients. Excluding the results recorded in children (Tavares da Silva (1945): 45.6%; Martins (1949): 78.2% with cercarial antigen and 83.3% with adult-worm antigen; Lurie et al. (1953): 11.0% with cercarial antigen; Pessôa & Barros (1953): 87.5%; Pellegrino & Macedo (1956): 79.0%), there are few cases in which figures lower than 90% are reported (Bozicevich & Hoyem (1947): 63.0% with cercarial antigen

and 80.0% with adult-worm antigen; Rodrigues da Silva & Costa (1949): 84.0%; Pereira (1951): 88.6%; Pesigan et al. (1951): 76.5%; Pesigan et al. (1954): 84.1%.

A higher degree of sensitivity has been attained by performing the test with cercarial, adult-worm and miracidial antigens instead of with extracts derived from infected snails or heterologous antigens (Martins, 1949; Mayer & Di Prisco, 1949; Prata, 1953; Prata et al., 1956; Sherif, 1956). However, the diversity of criteria adopted for reading the test has rendered difficult any comparative study. According to Martins (1949) the proportion of false-positive reactions in non-exposed individuals is about 5%. This proportion may be reduced with the use of purified antigens. Positive intradermal tests were occasionally observed by Hsü & Ameel (1955) with antigens of *S. mansoni* (cercariae) and *S. japonicum* (adult worms) in cases of cercarial dermatitis. Chung et al. (1955) observed positive skin-tests in 6 out of 18 cases of paragonimiasis using *S. japonicum* antigen. No cross-reactions were observed in 8 patients with clonorchiasis (Chung, Weng & Hou, 1955).

Standardization of Antigens

The antigens used in the intradermal test for the diagnosis of bilharziasis have not generally been properly standardized before use. Thus, arbitrary dilutions, commonly ranging from 1: 1000 to 1: 10 000, have been made from desiccated material. In some cases, preliminary tests have been performed on bilharziasis patients as well as on healthy individuals to check the effectiveness of the antigen (Mayer & Pifano, 1946; Bozicevich & Hoyem, 1947; Coutinho, 1951, 1952b).

Some workers standardize antigens by estimating the approximate number of cercariae or adult worms in a given volume of extract (Mayer & Pifano (1945a): 500 worms per each 3-4 ml of extracting medium; Alves & Blair (1946) and Blair & Ross (1948): 1000 cercariae per ml; Rodrigues da Silva & Costa (1949): 2000 cercariae per ml; Pesigan et al. (1951, 1954): 500-1000 worms per ml; Pessôa & Barros (1953): 500 worms per each 4-5 ml of extracting medium).

Quantitative determination of nitrogen (0.001 mg of N per ml) was used by Lopes (1945) to standardize cercarial (*S. mansoni*) antigens.

Several workers have tried to standardize antigens biologically, with the aim of determining suitable doses for the test, by injecting different dilutions and volumes into patients with bilharziasis (Bozicevich & Hoyem, 1947; Katzin & Most, 1946; Martins, 1949; Most et al., 1950).

Recently, attempts have been made to base biological standardization (Pellegrino—unpublished data, 1957) on the linear relationship between the log concentration of antigen and the mean areas of the wheals (Pellegrino, Memória & Macedo, 1957).

The Intradermal Test in Epidemiological Surveys

The prevalence of bilharziasis in endemic areas is generally assessed by examination of the stools (*S. mansoni* and *S. japonicum*) and urine (*S. haematobium*)—usually a single sample of each—of unselected people. Laboratory proof of the infection, however, is often not attained, especially in light or advanced cases of *S. mansoni* or *S. japonicum* infections when few eggs are eliminated, either because they are scarce or because elimination is rendered difficult by the growing intestinal fibrosis. It is generally accepted that, even when the most efficient methods of concentration are used, stool examination does not reveal more than 70% of the patients with bilharziasis mansoni. Pesigan (quoted by Davies & Eliakim, 1955) believes that, in epidemiological surveys, the examination of a single faecal sample cannot show more than 30% of patients infected with *S. japonicum*. The use of immunological methods for the diagnosis of bilharziasis in epidemiological surveys, then, presents many advantages: saving of time, equipment and personnel; quick results; increased accuracy; etc. In mass treatment campaigns, the intradermal test provides a reliable means of screening out infected persons; stool or urine samples of the positive reactors can then be examined for schistosome eggs.

The use of intradermal tests as a means of diagnosis in epidemiological surveys has not been resorted to as often as it should have been. So far as the writer is aware, only a few authors have reported on the use of skin-testing for such a purpose (Ramsay, 1934; Pesigan et al., 1954; Horstman, Chaffee & Bauman, 1954; Davies & Eliakim, 1955).

The screening of infected individuals and the estimation of the prevalence of the disease, in a given region, are rendered easier when the wheal area is taken as the basis for evaluating the results. The data obtained in surveys may be plotted on graphs where the ordinates represent the frequency of a given wheal area and the abscissae the size of the areas. Two different frequency curves, which overlap slightly, are usually obtained: the first curve gives the proportion of individuals presenting negative reactions; the second one gives the proportion of positive reactors. It must be taken into consideration that age, sex and race may influence sensitivity to the test.

The Intradermal Test as a Criterion of Cure

The reactions to intradermal tests before and after specific treatment are still open to investigation. Authors do not agree as to the value of such tests as a criterion of cure.

Since Fairley & Williams' report in 1927, it has been shown that positive reactions may persist after treatment. This finding has been confirmed by many investigators, although some cases of negative reactions in cured patients have been observed (Manson-Bahr, 1929; Taliaferro & Taliaferro, 1931; Khalil & Hassan, 1932; Oliver-González & Pratt, 1944; Lopes, 1945; Mayer & Pifano, 1945a, 1946; Rodrigues da Silva & Costa, 1949; Oliver-González, 1953; Davies & Eliakim, 1954; Oliver-González, Bauman & Benenson, 1954; Pesigan et al., 1951).

Martins (1949) carried out intradermal tests, before and after treatment, on 214 individuals passing eggs of *S. mansoni* and did not obtain a single negative reaction after treatment, merely observing a decrease of reaction intensity in 65 patients (42 cured; 23 failures). On the contrary, he pointed out that most patients showed more intense reactions after completion of therapy. Similar observations have been reported by Mayer & Pifano (1946). According to Martins (1949) and Pellegrino (unpublished data, 1957), no value should be attached to the intradermal test in the assessment of cure.

Alves & Blair (1946), Katzin & Most (1946) and Most et al. (1950) claim that, where patients have been skin-tested with a reliable antigen and found to give a positive reaction, a reversion to a negative reaction after treatment is supporting evidence that a cure has been effected. Alves & Blair (1946) suggest that further treatment is needed in cases showing positive skin reactions six months after the end of a course of therapy. Rodrigues da Silva (1948) thinks that the intradermal test is valuable for checking active cases of bilharziasis.

Recent reports from Oliver-González and his co-workers (Oliver-González, Bauman & Benenson, 1954, 1955; Oliver-González, Ramos & Coker, 1955) have shown that only a small percentage of patients with active *S. mansoni* infection (8.3%) give positive cutaneous reactions when the test is performed with egg antigen. However, after treatment, the percentage of positive reactors gradually increases, reaching about 80% after one year. According to Oliver-González, Bauman & Benenson (1954), negative results before therapy are due to the neutralization of sensitizing antibodies by the schistosome eggs continuously laid in the tissues, and therefore cutaneous sensitization to schistosome eggs can be demonstrated by the intradermal test only after the spontaneous death of the worms or successful therapy. These findings have not been confirmed by Pellegrino (unpublished data, 1957). Oliver-González and his colleagues suggest that the intradermal reaction with egg antigen, together with the circumoval precipitation test, would provide a good criterion for evaluating the efficacy of drug therapy. On the other hand, Sherif (1956) claimed that bilharziasis patients (*S. haematobium* and *S. mansoni*) who had completed a full course of specific treatment all gave negative reactions when tested with miracidial antigen 3-6 months after the completion of treatment.

Passive Transfer of Cutaneous Allergy

Taliaferro & Taliaferro (1931) were the first to show that the sera of bilharziasis patients contain a circulating antibody capable of sensitizing the skin of normal individuals. Guerra, Mayer & Di Prisco (1945) confirmed this finding and demonstrated that it is possible, by passive transfer, to differentiate antigens prepared with *S. mansoni* adult worms from those prepared with *Fasciola hepatica.*

Recently, several passive-transfer tests have been performed with the sera of patients infected with *S. mansoni.* In most cases the Prausnitz-Küstner test was positive. By heating the sera at 56°C for 2 hours, the antibody responsible for the skin sensitization is destroyed. The reverse Prausnitz-Küstner test always gave negative results (Pellegrino—unpublished data, 1957).

Conclusions

The intradermal test is a useful method for the diagnosis of bilharziasis, especially in the chronic stage of the disease, when it is often not possible to detect schistosome eggs in the urine or faeces of the patient.

From the data at present available on this test, it would appear that further studies are required on the following aspects:

(*a*) Standardization of the technique of the test on an objective basis.

(*b*) Use of the test in epidemiological surveys.

(*c*) Preparation of purified antigens.

(*d*) Biological standardization of antigens, based on the linear relationship between the log concentration of antigen and the mean area response.

(*e*) Behaviour of the intradermal and passive-transfer tests before and after treatment.

RÉSUMÉ

Le diagnostic de la bilharziose par mise en évidence des œufs de schistosomes dans l'urine et les fèces est souvent impossible aux stades avancés de la maladie, alors que la fibrose intestinale étendue rend leur élimination difficile. Pour obvier à cet inconvénient, on a mis au point un test immunologique fondé sur la réponse cutanée à l'injection d'un antigène préparé à partir de cercaires, de vers adultes, d'œufs ou de miracidia. Ce test a donné de bons résultats dans le diagnostic individuel. Dans les enquêtes épidémiologiques, il indique avec une assez bonne approximation la prévalence de la maladie.

L'auteur décrit les méthodes d'extraction des substances antigéniques. L'antigène desséché et finement broyé est reconstitué au moment de l'injection. La fraction active semble être un polysaccharide; certains auteurs estiment que cette fraction n'est pas seule en jeu.

Le test intradermique est généralement effectué dans le bras; la quantité injectée varie de 0,01 à 0,1 ml. Les réactions sont immédiates, le plus souvent, et atteignent leur maximum en 15 minutes. Des réactions différées ont cependant été observées. On a proposé divers critères d'estimation des réactions. L'auteur et ses collaborateurs ont suggéré une méthode de relevé et de calcul de la surface de la zone réagissante et de classement des réactions selon cette surface. Ils ont démontré qu'il existait un rapport linéaire entre la moyenne des réponses et le logarithme de la concentration de l'antigène, et ont proposé une méthode de standardisation de l'antigène sur cette base. Ce sont des anticorps spécifiques qui déterminent la sensibilisation de la peau. Le test devient positif environ 4 semaines après infection. Au début, la sensibilisation peut être faible, et il est probable que la durée de l'infection contribue à la sensibilisation cutanée. On admet généralement que le test cutané pratiqué avec des antigènes convenables est positif chez 90% des malades. La proportion des réactions faussement positives (évaluée à 5% dans certains travaux) diminuera à mesure que l'on purifiera l'antigène. Dans les enquêtes épidémiologiques, il est notoire que l'examen des fèces ne permet pas de déceler plus de 70% des malades (s'il s'agit d'un examen unique, cette proportion peut s'abaisser à 30%, ainsi que l'ont montré des expériences avec *S. japonicum*). Le test cutané présente de nombreux avantages: économie de temps, d'appareils et de personnel, résultats rapides, plus grande précision. On n'a pas encore tiré de ce test tout le parti possible, dans les enquêtes épidémiologiques. La valeur du test comme critère de guérison est encore discutée. Les anticorps produits par l'infection bilharzienne peuvent être transmis par injection, d'un individu malade à un individu sain, et provoquer la réaction cutanée. L'anticorps responsable de la sensibilité cutanée est détruit par chauffage de 2 heures à 56°C.

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