

**Comparative study of Bantu children with and without bilharziasis.** Groups of children from two neighbouring Bantu schools, near Rustenburg, having access to different water supplies—one infested with *S. haematobium* and the other not—are being compared with regard to diet, anthropometry, blood chemistry, haematology, and parasitology. A record is also being kept of the clinical findings, studies are being carried out on the eyes and teeth, and various physiological and intelligence tests given. Observations up to the present suggest that the differences between child populations with and without bilharziasis are small. The general impression, therefore, is that the handicap imposed by bilharziasis may well be less than is usually believed. The work is continuing.

## Studies on Immunity to Bilharziasis Mansoni: Evaluation of the Circumoval Precipitin Test \*

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Antibodies against the eggs of *S. mansoni*<sup>a</sup> have been detected in the sera of humans infected with this parasite. Precipitins are formed around the membrane when the eggs are incubated in the serum at 37°C. The antibody activity of sera obtained from chronic cases of bilharziasis mansoni is apparently greater than that of sera from early infections. This is believed to be a factor of paramount importance in the diagnosis and prognosis of clinical bilharziasis mansoni. As the great majority of infections in Puerto Rico seem to be acquired during childhood or adolescence, the circumoval precipitin reaction might point to the presence of infection when other orthodox methods of diagnosis have failed. Experience has demonstrated that the examination of the faeces for ova and rectal biopsy yield equivocal results in many instances. In other words, the criteria now available for determining the effectiveness of a drug in the treatment of bilharziasis mansoni are inadequate. It was therefore believed that by performing circumoval precipitin tests in a large group of patients suffering from the disease—whether or not they had been treated—a dual purpose would be accomplished:

(1) It would be possible to evaluate the reliability of the circumoval precipitin reaction as a diagnostic test;

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<sup>a</sup> Oliver-González, J., Bauman, P. M. & Benenson, A. S. (1955) *Amer. J. trop. Med. Hyg.*, 4, 443

(2) The test could be employed to determine the results of treatment, along with other criteria currently in use, such as improvement in the clinical manifestations of the disease or the presence of living and dead ova in the stools and in tissue removed from one of the rectal valves.

The present report deals with the evaluation of the circumoval reaction in 46 cases of bilharziasis mansoni. Living or dead ova were observed in all cases, either in the stools or in biopsy specimens of rectal tissue, at the time when the test was performed.

The subjects were male Puerto Ricans, United States veterans irrespective of age, who were known to be infected with *S. mansoni*. Most of them had been in San Patricio United States Veterans Administration Hospital at San Juan, Puerto Rico, at some time in the past for a disease other than bilharziasis, and the diagnosis had been established as a result of routine faecal examinations; the infection with *S. mansoni* was considered as an incidental finding, not related to the primary disease responsible for the patient's admission to hospital. A small number of patients (not more than 10) presented clinical manifestations, such as abdominal pain associated with bloody stools and tenesmus. There were also several cases of visceral bilharziasis, with hepatosplenomegaly and chemical evidence of impaired liver function, associated with anaemia, leucopenia and thrombocytopenia. Some cases presented evidence of portal hypertension, such as a history of repeated haematemesis and oesophageal varices demonstrable by X-ray examination.

To obtain the sera, 8-10 ml of venous blood were withdrawn from the cubital veins and allowed to coagulate.

### Materials and apparatus

- (1) Livers from 2-4 mice or hamsters, which had been infected with *S. mansoni* 8-10 weeks previously.
- (2) Osterizer or electric blender (washed with distilled water).
- (3) 1.7% saline, at room temperature.
- (4) Sera from infected animals or human subjects inactivated for 30 minutes at 56°C. Before use, a further 10 minutes' inactivation is required.
- (5) Two sieves (mesh 50 and 100) to fit test cups (250 or 500 ml).
- (6) Five culture tubes for discarding washes.
- (7) Glass slides and cover slips.
- (8) Vaseline.
- (9) Capillary pipettes with rubber teats.

### Procedure

- (1) After crushing a piece of liver between two slides and examining it for ova, homogenize livers for 3 minutes in osterizer using 1.7% saline (1-2 inches of saline in bottom of osterizer cup). The 1.7% saline is used to prevent the eggs hatching.

(2) Pass homogenized mixture through both sieves (the coarse one on top) and rinse osterizer cup with more saline into a test cup.

(3) Allow to settle for 15 minutes at room temperature. Meanwhile set five culture tubes in a row on a rack.

(4) With a capillary pipette collect sediment from bottom of test cup and pour into first tube (collect enough sediment to fill first tube).

(5) Allow sediment in the tube to settle for three minutes and then transfer supernatant to second tube.

(6) Add fresh saline to first tube and again allow to settle for three minutes. Then transfer supernatant to third tube.

(7) Add saline again to first tube and repeat process.

(8) Use sediment in first tube as the antigen for the test (determine concentration first).

(9) Place one drop of serum and one drop of egg suspension on the slide, and cover with a vaseline-bordered cover slip.

(10) Incubate overnight at 37°C and read test under the microscope.

(11) Observe reaction around living eggs only (hyaline finger-like projections, sometimes septate), taking care to distinguish true precipitates from artefacts. Count all living ova and those with precipitate around them, noting the intensity of precipitation.

(12) Record results under following headings:

Name of patient or animal

Date of test

Positive control serum

Number of ova on slide

Number of positive ova

Intensity of reaction (strong, moderate, weak)

Remarks

**Results.** A positive reaction was obtained in 43 out of the 46 cases (93.4%). The test was repeated in the three cases in which negative results were obtained and the reactions were again negative. All three patients giving a negative reaction were known to have had bilharziasis for several years and had received repeated doses of stibophen (Fuadin). Two of them showed dead ova both in their stools and in rectal tissue, while the third patient had living ova in the stools and rectal tissue. No explanation for the negative circumoval test can be put forward at present.

It is expected that in the near future a larger number of circumoval tests will be performed in a bigger series of patients with and without bilharziasis. In this work, attention will also be paid to the possible value of the test as an additional criterion for assessing the results of treatment in human and experimental infections with *S. mansoni*.