Quantitative Determination of *Schistosoma Mansoni* Ova in Feces from Patients Under Treatment with Antimonial Drugs[1]

By JOSÉ OLIVER GONZÁLEZ and FEDERICO HERNÁNDEZ MORALES

From the Departments of Medical Zoology and Clinical Medicine, School of Tropical Medicine, San Juan, Puerto Rico

SEVERAL PROBLEMS still remain to be worked out concerning the number of live and dead *S. mansoni* ova passed in the feces of patients infected with this parasite. In the first place, no quantitative determinations have been made indicating the relationship that may exist between the number of live and dead ova passed in the feces of untreated patients. Neither is it known whether dead eggs, passed in feces, increase as a result of treatment or whether the adult worms are so affected that they lay dead eggs only.

Various methods for counting schistosome ova have been described, but these do not give consistent quantitative results and cannot be used to count living and dead eggs separately.[2] The method for counting *S. mansoni* eggs, described here, seems to meet the above requirements. With this technique, reasonable quantitative determinations can be made of dead and live ova which reveal, at the same time, interesting findings in the patients under treatment with antimonial drugs.

MATERIALS AND METHODS

The method used may be summarized as follows: the feces passed into an enamel pan during twenty-four hours is thoroughly commingled with a spatula. Into a "displacement" or Stoll flask, filled with water to the 56 ml. mark, enough feces from the original specimen is added to raise the level of the water to the 60 ml. mark. The weight of the feces, which displaces the 4 ml. of water, is approximately 4 g. Ten glass beads are then added to the flask, which is sealed with a No. 4 rubber stopper and shaken for ten minutes, or longer, until all the fecal material is well comminuted. The suspension is then passed through a wire sieve (50 meshes to the inch) into a large thick test-tube, graduated in 15 up to 45 ml. markings (Stoll

1. Received for publication May 10, 1946.

2. J. Allen Scott, Dilution egg counting in comparison with other methods for determining the incidence of *S. mansoni*. Am.J.Trop.Med., **25**:546–565, 1937.

tube), and allowed to sediment for at least 30 minutes. After sedimentation, the liquid above the 15 ml. mark is siphoned off, thus leaving the sediment suspended in only 15 ml. of liquid. The suspension is shaken and, immediately after, 0.075 ml. of the liquid is drawn off with a graduated pipette (Stoll pipette) from the middle of the tube, placed on a slide, and covered with a 22 x 22 mm. cover-glass. The total number of living and dead eggs is then counted. Live eggs are those in which motion of the flame cells can be observed under high dry magnification (x430).

Routinely, two samples are prepared from each tube and counted, the average of the two counts being considered the true value. With this procedure, the number of live and dead schistosome eggs can be calculated either per ml. of suspension, per g. or per ml. of stool, or per 24-hour specimen. The number of eggs obtained should be reduced to a formed-stool basis, multiplying the number of ova by 1, if the stool is formed; by 2 if the stool is mushy, and by 4 if the stool is liquid.

The number of eggs encountered in all specimens are expressed as eggs per ml. of stool. Since the number of eggs in 4 g. of feces are suspended in 15 ml. of the solution, the number of eggs per g. of feces can be thus calculated. The number of eggs per g. can be, in turn, converted to the number of eggs per ml. of stool by the use of the common specific gravity factor 1.04, which changes stool weights to cml.[3] According to Scott,[4] the egg output is less variable when expressed in terms of eggs per ml. of stool than in terms of eggs per unit of time.

The patients treated with the various antimonial drugs, on whom these egg counts were made, were hospitalized for periods of time ranging from two to three weeks. Their ages varied from eighteen to thirty-three years; they had lived most of their lives in a known schistosomiasis-endemic area. A total of 40 individuals were considered in this study: 11 were treated with neostibosan, 11 with stibanose, 12 with urea-stibamine, and 6 with the trivalent compound, anthiomaline. Before treatment commenced, three examinations were carried out on each patient and daily, thereafter, until he was dismissed from the hospital.

RESULTS

An evaluation of the efficacy of anthiomaline and urea-stibamine in the treatment of the schistosomiasis-infected patients, involved

3. N. R. Stoll and W. C. Hausheer, Concerning two options in dilution egg counting: small drop and displacement. Am.J.Hyg., **6**:134–145, 1926.

4. J. Allen Scott, The regularity of egg output of helminth infestations with special reference to *Schistosoma mansoni*. Am.J.Hyg., **27**:155–175, 1938.

in this study, has already been presented in two previous reports.[5] A similar report on the results of the neostibosan and stibanose treatments will appear later.

On the fourth to the tenth day of treatment, the stools of the patients treated with anthiomaline became negative simultaneously for live and dead eggs, that is, no difference could be observed in the time of their disappearance from the feces (Fig. 1). The stools continued negative during a period of study of six months. The results in the counts of the other five patients were essentially the same; there was no appreciable difference between the time in which living and dead ova disappeared from the feces.

At the end of treatment with urea-stibamine, living and dead schistosome eggs had disappeared from the stools of only 3 patients. Figure 2 illustrates the counts in one of these patients; in the other two, similar results were observed. One month later, however, the remaining 9 patients were also negative for both live and dead eggs. The feces of all 12 patients continued negative for a period of eight months.

Neostibosan and stibanose were less effective on schistosomiasis infections than either anthiomaline or urea stibamine. The results of the egg counts, made during treatment with these drugs, were, therefore, not taken into account when studying the rate of disappearance of live and dead eggs since both of these were found at the end of, or several months after, treatment in the stools of the majority of patients.

Table 1 shows the average percentage of living eggs encountered during six days for each of the 40 patients three days prior and three days after treatment was commenced. The counts obtained on the first three days of treatment did not differ significantly from those obtained before treatment, so that the average percentage of living and dead eggs, before the effect of the treatment was noticed, could be calculated from the sum of the counts for the six days. The average percentage of living eggs for each patient ranged from 9 to 79 percent. Eleven out of 40 (27.5 percent) individuals had 50, or more, and 29 (72.5 percent) had less than 50 percent as an average. The average percentage of live eggs for the total of 40 patients was 39.5.

---

5. F. Hernández Morales, R. M. Suárez, C. K. Pratt, and J. Oliver González, Treatment of schistosomiasis *Mansoni* with antimony lithium thiomalate (anthiomaline). Puerto Rico J. Pub.Health & Trop.Med., **21**:336–344, 1946.

F. Hernández Morales, J. Oliver González, and C. K. Pratt, The treatment of schistosomiasis *Mansoni* with urea stibamine. Am.J.Trop.Med., **26**:327–329, 1946.

6. J. Allen Scott, *op. cit.*

TABLE 1

Percentage of Live and Dead S. mansoni *Eggs per Patient, Calculated from Counts Made Three Days before and Three Days after the Beginning of Treatment with Various Antimonial Drugs*

Drug Administered	*Percentage of Live Eggs per Patient*												*Average Percentage for Each Group*		*Average Percentage for All Patients*	
													Live	*Dead*	*Live*	*Dead*
Stibanose	44	52	25	44	35	54	21	30	45	42	27		38	62	39.5	60.5
Neostibosan	43	52	65	32	24	28	25	50	41	24	46		39	61		
Urea stibamine	50	35	36	9	46	44	79	58	42	63	45	56	47	53		
Anthiomaline	53	22	33	28	35	33							34	66		

DISCUSSION

The simultaneous disappearance of living and dead *S. mansoni* eggs from the feces of patients, treated with anthiomaline and urea-stibamine, is a finding fraught with interesting implications. The prevailing conception is that dead eggs, recovered in feces, are those that have remained in the intestinal mucosa for long periods of time, where they are acted upon by the tissue and humoral body defenses, and are finally dislodged into the lumen of the intestine. During treatment with anthiomaline and urea stibamine, living and dead eggs disappeared simultaneously. It therefore seems likely to suppose that, normally, the adult worm is laying both live and dead eggs, and that the effect of the drug on the adult worm is such that, among other possible effects, oviposition of both types of eggs is interrupted.

There is also the possibility that the dead eggs found in the feces have died during their transit from the site of extrusion to the lumen of the intestine. Both suggestions, however, are compatible with the fact that living and dead eggs, found in feces, are not those trapped in the tissues. This theory is also supported by the fact that no dead eggs have appeared in the feces of the treated patients during follow-up examinations carried out monthly for varying periods of six to eight months. Eggs would have appeared in the feces during this time if those trapped in the tissues would have been dislodged into the lumen of the intestine.

Table 1 shows that the majority of the patients (29 out of 40, or 72.50 percent) passed a larger number of dead than of living eggs (11 out of 40, or 27.5 percent) during three days prior and after treatment, respectively. These results cannot be considered as the general rule, however, since, in the first place, all of the patients studied fell within the same age group and, in the second, they very likely acquired the infection during childhood. Such a long-standing infection may have produced tissue changes in the host, associated with the death of a great number of the eggs. Studies on the proportion of live to dead eggs should also be carried out on individuals from other age groups with more recent infections.

Using the above described technique, the daily output of eggs was found to be highly irregular. This fact has also been confirmed by other investigators. However, the coefficient of variation for any count of any stool, calculated by the application of the formula used by Scott[6] for a similar series, was not found to be significantly different from that calculated for a similar series by this same author ($48.9^{\pm 4.05}$ for the 40 cases as compared with $61.1^{\pm 5.7}$ found by Scott).

SUMMARY

1. A method is here described for counting live and dead *S. mansoni* ova in feces. The technique has proved satisfactory for studying the relationship between live and dead eggs in treated and untreated patients. Statistical analysis of the data has revealed that the method may be also used in the grouping of people according to the degree of infection. The coefficient of variation for any one count of any stool is not significantly different from the value obtained by other investigators working with similar data.

2. In 6 individuals infected with *S. mansoni* and treated with the trivalent antimony compound, anthiomaline, the live and dead eggs disappeared simultaneously from the feces. Dead eggs did not persist in the feces at all. Similarly, in 12 patients treated with the pentavalent antimony compound, urea stibamine, no live or dead eggs were found on 3 patients at the end of treatment nor on the remaining 9 patients one month after treatment. The patients treated with anthiomaline and urea stibamine have remained negative for periods of six and eight months, respectively, of follow-up.

3. The simultaneous disappearance of live and dead eggs after treatment with these drugs suggests that the dead eggs are either laid by the worm in this condition, or else that they die during their transit from the site of extrusion. Therefore, the dead eggs found in feces are not those previously caught in the tissues, which dislodge them into the lumen of the intestine. Originally, this site was supposed to be the main source of dead eggs appearing in feces.

4. In 29 out of 40 (72.5 percent) individuals, infected with *S. mansoni*, the number of dead eggs passed in feces was greater than the number of live ones. The relationship between live and dead eggs may be different in individuals within other age groups and with varying lengths of infection.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Mr. José L. Janer, of the Insular Health Department of Puerto Rico, for his assistance in the statistical analysis of these data.

FIG. 1

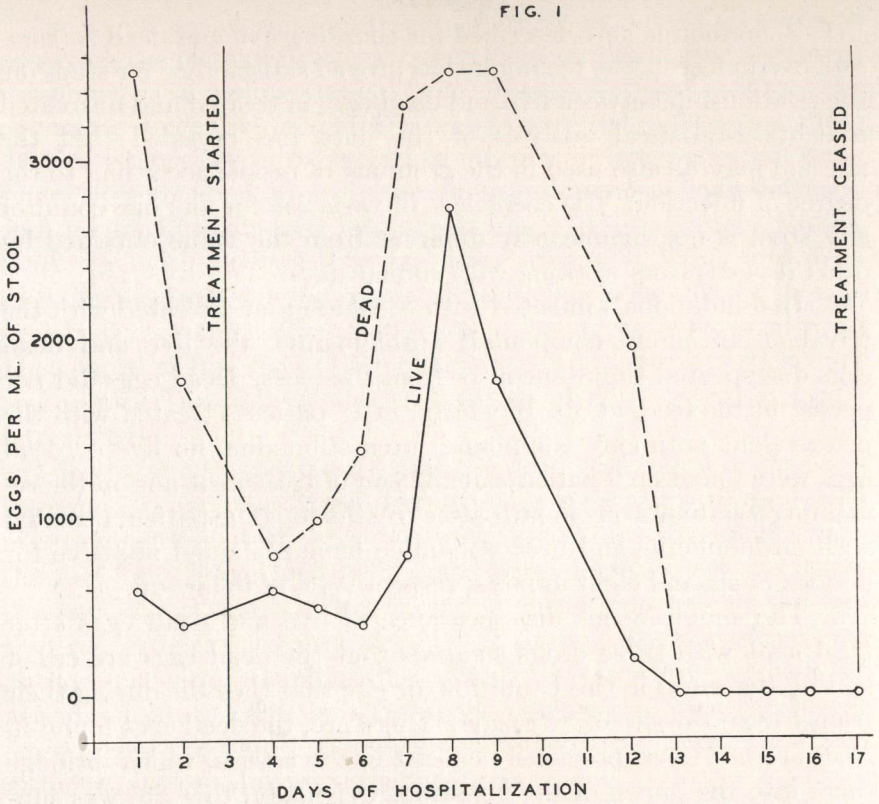
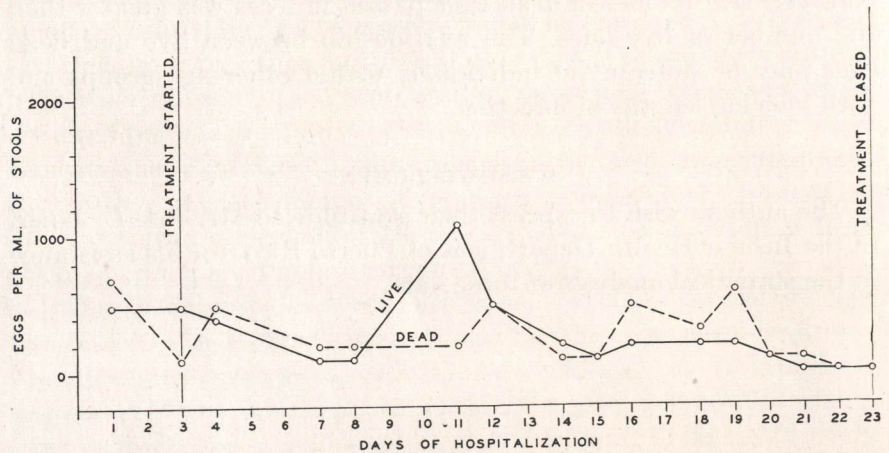

EGGS PER ML. OF STOOL
3000
2000
1000
0
TREATMENT STARTED
DEAD
LIVE
TREATMENT CEASED
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
DAYS OF HOSPITALIZATION

FIG. 2


EGGS PER ML. OF STOOLS
2000
1000
0
TREATMENT STARTED
LIVE
DEAD
TREATMENT CEASED
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
DAYS OF HOSPITALIZATION