THE SEDIMENTATION-CONCENTRATION METHOD IN SCHISTOSOMIASIS MANSONI *

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Schistosomiasis, due to infestations of *Schistosoma mansoni,* is not unimportant in certain regions of Puerto Rico, but its distribution and incidence still remain to be definitely determined.

The method usually relied upon for diagnosis is that of the routine smear examination. Owing to the fact, however, that under certain circumstances the presence of schistosome ova may not be disclosed, smear preparations inadequately serve an investigator to ascertain the approximate extent of schistosomiasis within a district or region. For this reason attempts were made to develop or test other devices for more accurate determination. Of these, the sedimentation method seemed the most promising. The principles underlying sedimentation have, of course, been in use a long time in the field of helminthology, especially as a demonstration of the presence of the ova of *Schistosoma haematobium.* This procedure has also been employed for the detection of hookworm ova, though it has long since been superseded by other methods.

It was at first believed by the authors that sedimentation had not previously been used for diagnosis in intestinal schistosomiasis, but after the completion of this work, several references pertaining to the subject were encountered. Faust and Meleney [1] recommend the principle; however, they, as well as Miyiari [2], advocate its use for the demonstration of miracidia of *Schistosoma japonicum,* not for the ova. Nazmi [3] in a recent paper mentions reports of Fouad and Helmy. The former depends upon brine flotation, which process deleteriously affects schistosome ova. The procedure of Helmy apparently bears some similarity to that developed by the present authors, but owing to the inaccessibility of his publication, a detailed comparison cannot be made.

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The idea suggested itself to the senior author in connection with this problem through his having employed sedimentation several years before in *Fasciola hepatica* infestations, his original object being merely to find a better and more certain way to demonstrate schistosome eggs in feces. Initially, a quantity of excreta estimated to be about 2 grams was placed in an 8-ounce photographic graduate and water added to the 2-ounce level. The fecal sample was left to soften from 15 to 30 minutes, as its consistency demanded. Longer softening periods were at times considered desirable if time were available. The mixture was then comminuted, the vessel filled with water, and the mixture passed through an 80-100 mesh sieve into another graduate. During the development period of the procedure the water was decanted at least twice, or until the liquid showed little turbidity. Decantation tends to cause the loss of ova, some of which can be demonstrated adhering to débris at the surface or to the sides of the vessel.

With accumulated experience the method underwent various modifications. At present, 1-gram samples are used. Urine sedimentation glasses have replaced graduates as final receptacles after passage of the material through the sieve. Substitution of these glasses permits increased concentration of ova, due to the greatly narrowed base, though the slope of the vessel retards or prevents sedimentation of ova to some extent. Sedimentation may be somewhat accelerated by gentle stirring of the liquid and by lightly tapping the container. Passage of comminuted material through the sieve should be accomplished in as short a time as possible; if the process is drawn out, the eggs may be distributed uniformly throughout the sediment, and a large number may lie even above the sediment. The water is not changed, though the upper half is carefully decanted before removal of the sample for examination, to enable the tip of a Stoll pipette to reach the bottom of the glass [4]. Before insertion of the pipette into the sediment the air is removed by bulb pressure. Sediment is permitted to rise to the 0.075 ml. level and is then placed upon a slide, covered with a square 22 mm. cover-slip, and the eggs counted. Twice the amount of sediment, that is, 0.15 ml. placed beneath a 22 × 40 mm. cover-slip, consistently discloses less than double that demonstrated by the lesser quantity.

The use of more than one gram of feces in conjunction with the examination of 0.075 ml. of sedimented material often results in the presence of an excessive proportion of relatively coarse particles. This condition may also cause obstruction of the pipette and thereby prevent removal of the desired quantity of sediment.

Water containing large numbers of air bubbles should never be employed, since many eggs are forced to the surface and remain there. To prevent this, containers can be filled with tap water which may be used after the bubbles have risen and disappeared. The bubbles are retained to a great extent in a mixture of this type of water and feces, this, apparently, being due to greater surface tension.

Unsatisfactory results are sometimes noted with certain types of feces, i. e. those of a very oily or greasy nature.

In lieu of wire mesh and Stoll pipettes, several thicknesses of cheesecloth and ordinary tapering pipettes may be substituted. When coarse particles occur in abundance, the latter type of pipette may be preferable.

The superiority of the sedimentation-concentration method over the routine smear becomes quite evident when data pertaining to comparative tests are studied (Table I). Fecal samples of 220 residents, chiefly school children of the Barranquitas district (situated in the hilly interior of the island), were examined. Schistosome ova were demonstrated in 30 (13.18%) by smears; in 73 (33.18%) by sedimentation. Apparently many of the cases found negative in smears harboured infestations of a low grade. In a few instances when ova were very rare in smear preparations, many could be demonstrated in concentrations. Three samples found to be negative in routine examinations yielded 127, 131 and 204 eggs respectively, in concentrations. On three occasions both gave equal results, one egg each, and in such cases it is probable that the factor of chance played a part, fecal particles containing one or more ova having been selected. Since the results of sedimentation tests are based upon the employment of a much larger quantity of material, the probability of finding ova should be proportionately greater, though the possibility of missing very light infestations or infestations in which but few eggs are being passed with excreta should be taken into consideration.

Sedimentation-concentration may also aid in disclosing the

presence of other intestinal parasites whose existence in some cases under observation is not indicated by routine examinations. Cysts of amebae can be demonstrated in samples of sediment. Cysts of *Giardia lamblia,* larvae of *Strongyloides*

TABLE I

COMPARISON OF SMEAR AND SEDIMENTATION-CONCENTRATION

No. of Ova		No. of Ova	
Smear	Concentration	Smear	Concentration
0	7	3	4
0	19	3	21
0	2	3	5
0	12	0	2
0	1	1	1
0	37	1	65
2	16	0	4
3	16	3	22
4	109	0	13
1	59	0	5
0	2	0	17
0	41	0	1
0	4	0	1
1	10	0	131
1	1	0	4
3	12	0	5
3	24	0	4
0	1	3	22
1	7	0	11
0	3	+	10
0	7	1	33
1	4	2	25
0	23	1	4
0	4	0	1
0	7	0	49
2	9	1	7
0	7	0	1
2	16	0	67
1	1	0	127
0	3	3	89
0	204	0	7
1	2	0	23
1	11	0	3
10	241	2	12
0	7	0	1
1	183	0	36
		0	3

stercoralis, and ova of *Ascaris lumbricoides* and *Trichuris trichiura* are often concentrated in great numbers by sedimentation. It is not recommended that sedimentation supplant the methods now used for the detection of these para-

sites. These findings are mentioned as incidental to the other observations recorded.

Not only does the concentration method serve to give a more accurate estimate of the incidence of schistosomiasis in a district, but in addition, the effects of treatment, in so far as relative abundance of ova is concerned, can to some extent also be determined. This is illustrated by Table II, which shows the results of a 35-day study of the feces of a heavily infested boy. It includes a 7-day preliminary period before treatment with Fouadin, followed by one of 28 days during which the therapeutic agent was administered intramuscularly 12 times. Two concentrations were prepared from each thoroughly mixed 24-hour fecal output. Usually duplicate counts were made of each concentration, one, by one of the authors (J. L. J.), the duplicate, by the senior author. The same pipette was employed throughout the course of the study; it was washed every time after use by removing the bulb and permitting a stream of water to run through the inner and over the outer surface; similarly, the wire mesh was exposed to a strong stream of water.

The figures (Table II) relating to sedimentation counts do not represent all the ova from a gram of feces, but merely those encountered in every 0.075 ml. sample examined. Several repeated counts from additional 0.075 ml. samples from one-gram concentrations indicate that such withdrawals had by no means removed all the eggs. Duplicate counts have in the main coincided fairly closely with the initial counts, usually not varying more than 30 per cent, the maximum difference between two counts from the same source being 48 per cent. When, because of treatment, the output of eggs was reduced to less than 200 per 0.075 ml., great discrepancies began to appear. The data presented should not be considered in the light of a strictly mathematical interpretation of the findings, but rather as a semi-quantitative means of comparison.

Reduction in the number of eggs passed occurred after administration of the fourth injection of Fouadin and a clinical improvement could be noted after the third had been given. This is in consonance with our findings in other cases similarly treated.

In this series duplicate Stoll counts (by weight) were

TABLE II

DAILY RECORD OF EGG COUNTS

Day	Weight in grams of daily fecal output	Routine		Stoll Count 3 gram samples				Concentrations 1 gram samples							
				1		2		1		2		1		2	
		A	D	A	D	A	D	A	D	A	D	A	D	A	D
1	510	14	8	23	15	23	16	507	150	597	125	650	201	512	210
2	500	14	16	10	9	12	6	329	182	323	180	221	120	344	191
3	660	5	9	6	10	4	5	297	133	251	120	228	115	286	128
4	345	8	5	12	5	12	8	281	119	179	69	274	159	149	85
5	470	9	6	10	11	10	17	455	246	477	159	454	180	416	137
6	340	10	13	8	11	7	14	287	222	234	219	256	189	214	198
7	550	10	5	13	7	.....	.....	300	138	.....	.....	236	101	.....	.....
8*	180	9	8	22	13	18	12	342	179	352	188	245	126	297	161
9*	640	7	8	9	4	4	3	232	80	164	77	228	58	242	81
10*	395	9	7	4	10	6	10	256	180	305	197	265	157	415	252
11	345	4	3	14	19	24	18	383	182	617	279	420	131	299	215
12*	600	13	8	10	10	5	11	238	68	293	74	487	117	395	98
13	320	9	5	10	10	18	8	428	169	361	173	517	183	455	170
14*	600	6	4	10	8	.....	.....	235	78	.....	.....	298	112	.....	.....
15	410	5	1	6	1	6	2	137	48	139	38	168	64	215	43
16*	370	2	5	4	3	4	2	108	16	165	23	109	20	136	21
17	360	12	4	17	5	14	3	138	13	108	14	100	13	163	20
18*	370	3	2	5	2	4	1	125	14	70	8	56	9	81	7
19	330	4	2	4	2	.....	.....	42	116	.....	.....	39	63	.....	.....
20*	280	0	8	7	1	5	1	31	6	72	8	75	9	108	20
21	480	1	1	1	1	.....	.....	47	17	.....	.....	38	17	.....	.....
22*	200	0	0	2	4	2	3	17	5	40	13	18	6	45	6
23	480	1	0	3	1	3	0	67	9	62	9	65	4	74	8
24	210	0	0	0	1	1	0	17	4	24	2	24	1	25	5
25	140	0	0	0	0	0	0	7	3	12	4	17	1	13	3
26	80	0	1	1	0	1	0	4	0	6	1	9	1	4	0
27	480	0	0	0	0	0	1	1	0	3	0	7	0	1	0
28	305	0	0	0	0	.....	.....	0	0	.....	.....	3	0	.....	.....
29*	590	0	0	0	0	0	0	1	0	0	0	0	1	0	0
30	340	0	0	0	0	0	0	2	0	0	0	1	1	0	0
31*	150	0	1	0	0	0	0	2	0	1	0	0	1	1	0
32	225	0	0	0	0	0	0	2	0	0	1	0	0	0	0
33*	300	0	0	0	0	0	0	0	1	1	1	1	0	0	0
34	360	0	0	0	0	0	0	1	1	0	0	0	0	.....	.....
35	350	0	0	0	0	.....	.....	1	0	.....	.....	0	0	.....	.....

* Indicates days of treatment.
A—alive.
D—dead.

made. Our procedure differed in one important particular from that usually followed by investigators in making counts of hookworm ova, namely, that water was used instead of a 0.1N solution of NaOH. This chemical at times seemed to adversely affect schistosome ova. The number of eggs recovered, as a rule, did not exceed those found in smear preparations. As treatment proceeded, eggs could not be demonstrated later than by routine examinations. Therefore, it appears that the Stoll count possesses no appreciable advantage over the smear.

Occasionally there is encountered in relevant literature a statement to the effect that antimony treatment produces a deleterious effect upon schistosome ova. It would seem that the concentration method might be employed to prove or disprove this assertion. The aforementioned patient supplied the material for the study of this aspect of the problem. Every egg was examined (Table II) to determine whether it was alive or dead. Movement or non-movement of the external cilia, or those of the flame cells, constituted the criteria upon which viability was based. A small percentage of empty shells was encountered in most preparations. These were considered as viable ova unless the miracidium was attached to the shell and its condition indicated it had been dead before the splitting of the shell. A study of the table suggests that the ratio of dead to viable eggs is on the whole no greater during the course of treatment than during the pre-treatment period. As treatment progresses the adult schistosomes are presumably killed. The high percentage of dead ova that may be found in the feces near the termination of a series of injections with a schistosomacide might be explained by the theory that some eggs are deposited in capillaries situated relatively distant from the intestinal lumen, or have experienced difficulty in passing through the tissues; and even when these ova have finally succeeded in reaching the intestine their miracidia have already perished. One case cannot be considered as adducing conclusive evidence in regard to this aspect of schistosomiasis, though definite indications are here presented which signify that the ova of *S. mansoni* are apparently not noticeably affected by Fouadin.

The method here described is not without defects. Considerable time and a fair quantity of relatively bulky equipment are required for the concurrent examination of several

fecal samples. However, the superior results obtained in diagnosis may justify the use of additional impedimenta.

These tests can by no means be considered exact agents of quantitative diagnosis, for the adult *Schistosoma mansoni* does not occur in the intestinal lumen; many of the ova fail to reach that destination; neither can the number of adult schistosomes be estimated in a patient. In the Stoll egg count these obstacles are not encountered. Furthermore, in the latter, the eggs can be evenly distributed in a liquid medium, whereas in sedimentation they are concentrated in the bottom of a container, the degree of concentration depending in no small part upon the consistency and specific gravity of the feces. In addition, no two urine sedimentation glasses possess identical inner basal contours, some tapering more markedly than others at the bottom. Undoubtedly the tip of the pipette does not always reach the site of greatest abundance of ova, which is probably influenced in a different manner by various types of excreta. With these defects to be considered it seems remarkable that the findings coincide to the extent that they do.

CONCLUSIONS

From tests made and investigations carried out by the authors over a determined period of time, it seems that:

The sedimentation-concentration method is superior to the routine smear for the detection of eggs of *Schistosoma mansoni* in feces.

It may also be employed as an adjunct to indicate the progress of therapy in schistosomiasis mansoni.

It may aid in disclosing the presence of other intestinal parasites.

Some evidence has also been advanced to suggest that all antimony compounds do not injure the ova of *S. mansoni* situated within the host.

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