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Evaluation of the Treatment of Human *Schistosoma mansoni* Infection by the Quantitative Oogram Technique*

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Egg output is the only measure available for quantitative assessment of the activity of chemotherapeutic agents in Schistosoma mansoni *infection. In the light of eight years' experience in the preparations of oograms, the authors suggest a simplified classification of* S. mansoni *eggs and certain improvements in the oogram technique by which quantitative data are obtained for comparison before and after treatment.*

Ten cases, taken from clinical trials on a variety of schistosomicidal compounds, are presented to illustrate the use of the quantitative oogram and the types of result obtained with active, partially active and inactive drugs.

CLASSIFICATION OF THE EGGS OF *SCHISTOSOMA MANSONI*

When the larvae of *Schistosoma mansoni*, a trematode inhabiting the portal venous system, reach the liver, they begin to feed and develop. As they attain sexual maturity, they migrate in pairs, the females characteristically held by the males, to their definitive "habitat", which in man are the venules of the mucosa of the descending colon, sigmoid and rectum, where the females oviposit. This is the reason why the rectum is one of the organs where the eggs of the parasite are more often found in the tissue and in greater quantities than in other intestinal segments or the liver, as shown by Koppisch (1940) in necropsy material, and confirmed by others (Ottolina & Atencio, 1943; Jaffé & Ferro, 1946; Hollands & Palmer, 1946).

It is generally agreed that the females deposit their eggs in the venules of the submucosal layer of the intestine in a continuous way, each female producing about 300 eggs per day, according to Moore & Sandground (1956). While the ova stay in the submucosal layer of the bowel they go through several evolutive changes before finding their way into the lumen of the gut. From the moment of oviposition, while immature, the eggs pass through the first, second, third and fourth stages; at the end of the sixth day they become mature ova containing a fully developed miracidium (see Fig. 1).

In this phase, the mature ovum remains alive in the tissues of the intestine for a maximum period of 12 days, at the end of which it dies if it has not been passed in the stools. Various morphological types of dead eggs are also formed, because the egg may die at any phase of its evolution from immature to mature ovum (see Fig. 2 and 3).

Microscopic examination of fragments of the rectal mucosa taken by biopsy, or of material collected by scraping the rectal surface with a special curet, shows numerous ova which, counted and classified, constitute the "oogram". A quantitative oogram is based on the global and the differential counts of the ova of *S. mansoni* in the material removed from the rectal mucosa by biopsy and scraping.

The study of the oogram, both for the total number of eggs and for enumeration of the various types present, especially the viable ova, is an easy and reliable method of evaluating in man the therapeutic value of antischistosomal drugs.

Several classifications of the ova of *S. mansoni* based on their morphological evolution have been proposed by different authors—e.g., Vogel (1942), da Silva (1948), Pereira & Netto (1949), Prata (1957). Since 1956, in this department, we have adopted in

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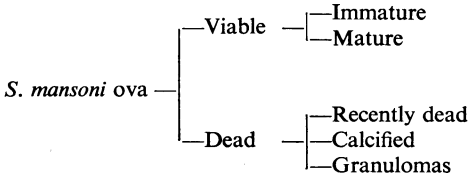
our daily work the last two methods, especially the valuable classification of Prata (1957), which is based on animal experimentation confirmed by Pellegrino et al. (1962), who studied the oogram in mice experimentally infected with *S. mansoni*.

Prata (1957) classifies the several schistosomal elements found in the fragments of the intestine of mice, according to their stage of development and morphological aspects, into: (*a*) eggs, infertile and fertile (viable and dead); (*b*) shells, and (*c*) parasitic nodules. Of both viable and dead eggs many types are described.

With the exception of infertile eggs, all the other elements can be found by direct examination of human rectal snips between two glass slides.

During the past eight years we have made over 5000 oograms, mostly for the purpose of assessing the therapeutic value of more than 50 new drugs tried in human *S. mansoni* infection. On the basis of the experience thus gathered we feel that, for assessing the therapeutic value of a new drug in this infection, the classification of *S. mansoni* ova can be simplified and the technique of the oogram improved by weighing the material removed from the rectum, so that quantitative data are available for comparison before and after treatment.

We classify the eggs of *S. mansoni* in two types—viable and dead:



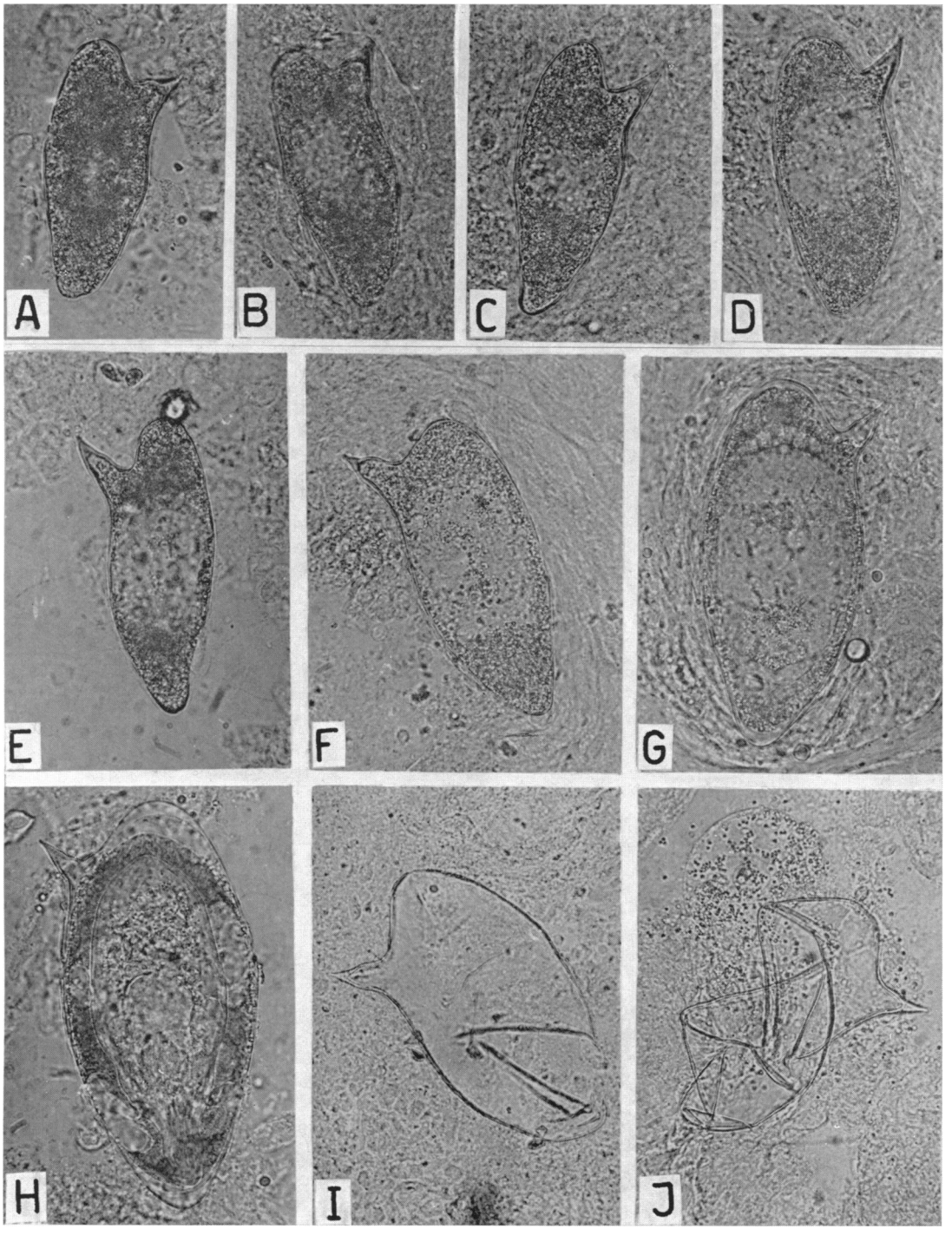
Viable eggs may be either immature or mature. The immature eggs have been classified, according to the development of the embryo, into immature of first, second, third or fourth stage, exactly like Vogel's classification of *S. japonicum*. The egg in the first stage has a small embryo, of about one-third of the transverse egg diameter (Fig. 1, A and B). When the embryo becomes slightly larger than half the transverse egg diameter, the immature egg is considered to be in the second stage (Fig. 1, C and D); and when it is two-thirds of the longitudinal diameter of the egg, it is in the third stage (Fig. 1, E and F). In the fourth stage the embryo occupies the whole of the egg shell (Fig. 1, G). However, since it is irrelevant, when evaluating drug activity, to distinguish each of these four stages—which are completed in six days (the time necessary for the embryo to reach the miracidial stage)—we count them all as *immature* eggs.

The mature egg (Fig. 1, H) has a fully developed miracidium, and signs of miracidial vitality are visible (flame cells in activity, miracidial contractions and beating of cilia). We also include as mature eggs the rarely found eggs with disturbed embryonic development, which have a shell the size of a mature egg but show an impaired internal structure. The embryo often lies on one side of the egg, frequently showing active flame cells. Also included in the category of mature eggs are those egg shells which remain after hatching of the miracidium, or its ecdysis, since they represent mature eggs from which the miracidium has been liberated (Fig. 1, I and J). It must be also noted that in the material we work with the escape of the miracidium may be caused by the mechanical compression of the two slides. These shells, contrary to the shells which remain after absorption or destruction of the egg contents, have an oblique cleft, are clear and distinct, and often the miracidium can be seen in its vicinity.

It is also important, for the sake of evaluating the activity of a drug on the worms (see Table 3) to recognize the various types of dead eggs, especially the recently dead ones, because they are the first of the dead eggs to disappear in treated patients. We therefore classify dead eggs as recently dead, calcified or granulomas. Though the last are not really eggs, since granulomas may or may not contain eggs, they represent a tissue reaction to the mature egg which dies this way. There are many types of recently dead egg, because the death of the embryo or of the miracidium may occur at any stage of development. When it occurs in the phase of immaturity, we have the hemitransparent, the granular and the eggs with retracted embryo. Hemitransparent eggs show a clear longitudinal half, whereas the other half, on the spine side, is dark (Fig. 2, E, F and G). Granular eggs (Fig. 2, A and B) contain small granules of a fatty nature (Vogel, 1942). Eggs with retracted embryos (Fig. 2, C and D) are those in which after death the embryo retracts within the egg shell, showing a clear and irregular outline. When death occurs after maturation is completed, this produces eggs with a dim structure, eggs with retracted miracidia and coarsely granular eggs. Eggs with a dim structure (Fig. 2, H) are those in which the miracidium becomes turbid and shows an indistinct outline and in which there is no motility of the larva, of the cilia and of the flame cells, i.e., no sign of vitality. Coarsely granular

FIG. 1

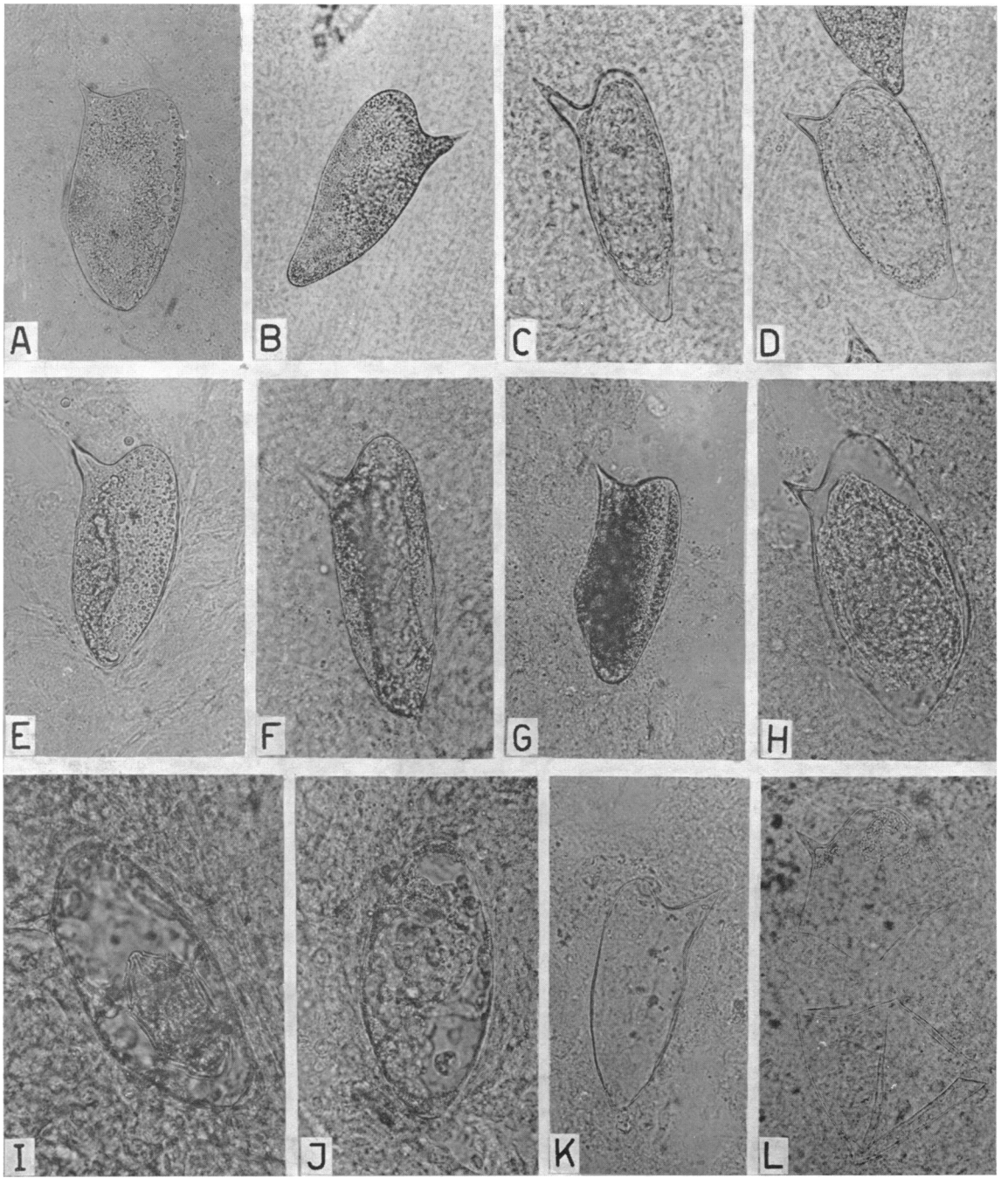
PRESS PREPARATIONS OF VIABLE *S. MANSONI* EGGS REMOVED BY BIOPSY AND SCRAPING FROM RECTAL MUCOSA OF PATIENTS



A and B: Immature first-stage eggs.
C and D: Immature second-stage eggs.
E and F: Immature third-stage eggs.
G: Immature fourth-stage eggs.
H: Mature egg.
I and J: Shells after ecdysis.

FIG. 2

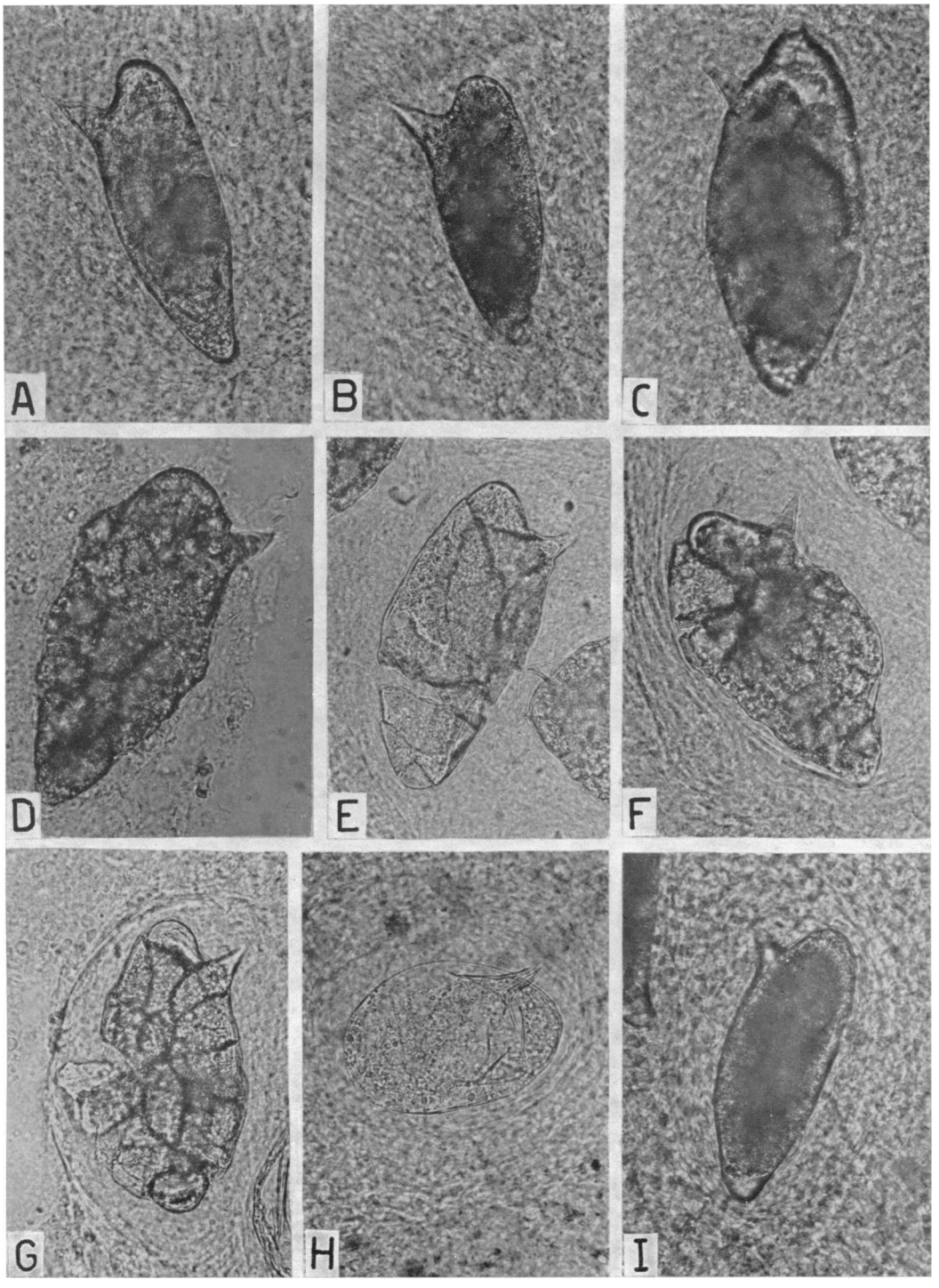
PRESS PREPARATIONS OF RECENTLY DEAD *S. MANSONI* EGGS REMOVED BY BIOPSY AND SCRAPING FROM RECTAL MUCOSA OF PATIENTS



A and B: Immature granular eggs.
C and D: Immature eggs with retracted embryo.
E, F and G: Immature hemitransparent eggs.
H: Mature egg with dim miracidial structure.
I: Mature egg with retracted miracidium.
J: Mature coarsely granular egg.
K and L: Shells after absorption and disintegration of contents.

FIG. 3

PRESS PREPARATIONS OF DEAD *S. MANSONI* EGGS (CALCIFIED EGGS AND GRANULOMAS) REMOVED BY BIOPSY AND SCRAPING FROM RECTAL MUCOSA OF PATIENTS



A and B: Darkened immature eggs.

C, D, E, F and G: Calcified mature eggs.
H and I: Granulomas with eggs.

eggs are self-descriptive (Fig. 2, J). Here also we include the shells that result from absorption or destruction of the egg contents (Fig. 2, K and L) whether the egg be immature or mature. These are shells with different forms, usually smaller than those which result from the ecdysis, fragmented, twisted, with a slight yellow tinge resulting from their long stay in the tissue.

Calcified eggs (Fig. 3) are highly refractive and often show a glassy or stony appearance. Their structure is entirely darkened, which signifies a long stay in the tissue. They may be calcified as mature (Fig. 3, C, D, E, F and G) or as immature eggs, the last being the so-called darkened eggs, with an entirely black appearance (Fig. 3, A and B).

The granulomas may (Fig. 3, H and I) or may not contain eggs.

TECHNIQUE OF RECTAL BIOPSY AND SCRAPING, AND OF QUANTITATIVE OOGRAM

The technique and instruments for rectal biopsy and curettage used in this department since 1956 have been described in detail elsewhere (Hadad, 1957; Cançado et al., 1957; Cunha, 1961, 1962, 1963).

We use the Sass Wolf rectoscope, 20 cm long and 2 cm in diameter, with distal illumination. The biopsy forceps is 30 cm long and cuts specimens about the size of a grape seed. The curette is 25 cm long, the spoon measuring 9 mm in diameter with not very sharp edges.

Before examination the patient is advised to empty the bowel. No cleansing enema is used since this could render the scraping ineffective by washing out the superficial mucus and with it the eggs.

The patient being in the knee-elbow position the instrument with the obturator in place is introduced in the usual way, after lubrification with liquid soap, which does not interfere with the microscope examination. The obturator is removed and the optical system then fitted.

At first, we note the condition of the lower rectal and anal mucosa. We look for congestion, pigmentary changes, petechiae, ulcerations, tumours, varicosities, etc. Under direct vision, the instrument is inserted further, to examine the inferior, middle and superior Houston's valves. We then proceed to collect specimens for examination. We begin by scraping the superior valve itself or its vicinity. Any pathological change is also curetted. With the instrument still on the superior valve we take a biopsy specimen of its mucosa, cutting away two or three fragments. The same operation is repeated on the middle and on the inferior valve. We generally remove six to nine fragments of rectal tissue and three portions of scraping. The illumination system is then disconnected, the obturator applied and the instrument removed. The examination lasts from five to six minutes, though it may last three minutes more if the rectum contains faeces. In that case, we introduce through the rectoscope, with the biopsy forceps or the curette, some pieces of surgical gauze which are pushed with the stools to the sigmoid, so that at least the middle valve may be uncovered.

Before starting the examination, two pairs of glass slides, marked 1 and 2 and previously weighed on a Mettler balance accurate to the nearest 0.1 mg, are set aside. To one of the slides of pair 1 we transfer, with the help of a wooden toothpick, the biopsy fragments and to one of the slides of pair 2, the scraped portions. These slides are then covered with their respective pair and squeezed by digital compression according to their resistance. Both pairs of slides (1, biopsy; 2, scraping), which should be handled with care to avoid breakage as this would alter the weight, are then immediately weighed again. The difference between the weights after and before the addition of the specimen gives the weight of each specimen. Adding the weights of the biopsy sample (1) and the scraping sample (2) we get the weight of the sample of the examined material, from which the data per gram are deducted. A table simplifies the calculation.

To make a quantitative oogram we examine the slides directly in the microscope by transparency. This is done reading across and down, and the different morphological types of eggs described above are counted. The results are noted per gram of examined material.

We usually make one oogram just before starting treatment and several oograms afterwards, the number and intervals being previously determined according to the nature of the drug used.

CLINICAL TRIALS

To show the significance of the quantitative oogram in the evaluation of treatment of *S. mansoni* infection, we shall present data relating to patients recently treated in this department with some new drugs as well as with a placebo and an antimonial. These drugs are 2-dehydroemetine, amphotalide, TAC pamoate and bis-(β-carbhydrazido-ethyl) sulfone.

These are cases from clinical trials carried out by us with the quantitative oogram technique. These trials will be reported on in a separate publication.

Placebo. Patient 1, a 13-year-old boy, heavily infected, was treated with a placebo (starch), two tablets of 250 mg each being given orally per day for 20 days. Neither the patient nor the doctor who made the oograms knew the nature of the substance under trial. Results are shown in Table 1.

Antimonial. Trivalent antimonials can cure *S. mansoni* infection. An illustration of this by the quantitative oogram technique is the case of patient 2, a 16-year-old boy weighing 53 kg who received sodium antimonyl gluconate intravenously (Table 2). This drug, in a total dose of 20 mg/kg/day, daily for six days, will cure about 80% of patients, as has been shown by one of us (Cunha, 1961, 1963).

2-dehydroemetine. Though natural emetine was used in the past for treating bilharziasis, it was later abandoned. Recently, Gelfand et al. (1962) used the emetine derivative 2-dehydroemetine in *S. haematobium* infection, and Gouveia & Teixeira (1963) for *S. mansoni.* We are now completing a clinical trial with the racemic 2-dehydroemetine (Hoffmann La Roche) in ampoules of 2 ml, containing 40 mg of the drug, for parenteral use. The examples of four patients (Table 3) show the effects of this drug in relation to dose and period of treatment.

Amphotalide

Amphotalide [1] (1-*p*-aminophenoxy-5-phthalimidopentane) belongs to the *p*-aminophenoxyalkanes, which have aroused much interest in the experimental evaluation of structure activity relationships but have proved elusive in the development of an effective schistosomicide for human infections (Collins et al., 1954, 1959; Raison & Standen, 1954; Collins et al., 1959; Schneider & Sansarricq, 1959; Larivière et al., 1960; El Bitash et al., 1961; Alves et al., 1961; Hocquet et al., 1963; Silva & Prata, 1962).

We used this drug as supplied by Rhodia under the name of R.P. 6171, in capsules of 250 mg, in doses of 40 mg/kg/day, orally for six days (Table 4).

TAC pamoate

Tris (*p*-aminophenyl) carbonium pamoate (TAC pamoate) and its chloride salt have been used by Eslager et al. (1961) and Thompson et al. (1962) in experimental infection, and also by Burnett & Wagner (1961) and Hitman & Wagner (1962). TAC pamoate was used by da Silva et al. (1964) in human

[1] Proposed International Non-Proprietary Name.

TABLE 1

QUANTITATIVE OOGRAMS OF PATIENT 1, TREATED WITH PLACEBO (STARCH), 500 mg DAILY, ORALLY FOR 20 DAYS IN DOUBLE-BLIND TEST

Type of eggs	Number of eggs per gram				
	Before treatment	Days after beginning treatment			
		7	14	21	28
Viable					
Immature	3 509	3 440	1 654	2 935	2 150
Mature	952	9 250	4 717	7 115	5 299
Dead					
Recently dead	1 197	1 880	1 054	1 303	1 805
Calcified	762	414	166	348	595
Granulomas	0	0	0	40	0
Total	6 420	14 984	7 591	11 741	9 849
Weight of sample (g)	0.0367	0.0530	0.0899	0.0741	0.0518

TABLE 2

QUANTITATIVE OOGRAMS OF PATIENT 2, TREATED WITH SODIUM ANTIMONYL GLUCONATE INTRAVENOUSLY, IN A TOTAL DOSE OF 20 mg/kg (1060 mg), DIVIDED IN SIX DAILY DOSES OF 176 mg

Type of eggs	Number of eggs per gram			
	Before treatment	Days after beginning treatment		
		7	30	261
Viable				
Immature	1 208	0	0	0
Mature	732	191	0	0
Dead				
Recently dead	128	628	0	0
Calcified	95	164	15	0
Granulomas	0	0	0	0
Total	2 163	983	15	0
Weight of sample (g)	0.0314	0.0366	0.0634	0.0583

TABLE 3

QUANTITATIVE OOGRAMS OF FOUR PATIENTS TREATED INTRAVENOUSLY WITH 2-DEHYROEMETINE

Type of eggs	Number of eggs per gram					
Patient 3 : 0.8 mg/kg daily for 10 days						
	Before treatment	Days after beginning treatment				
		12	20	27		
Viable						
Immature	1 460	0	72	1 570		
Mature	400	18	0	196		
Dead						
Recently dead	120	315	228	287		
Calcified	60	87	84	151		
Granulomas	0	0	0	0		
Total	2 040	420	384	2 204		
Weight of sample (g)	0.0500	0.0570	0.0835	0.0660		
Patient 4 : 1 mg/kg daily for 10 days						
	Before treatment	Days after beginning treatment				
		11	19	26	47	
Viable						
Immature	2 550	0	0	0	163	
Mature	1 750	98	0	21	104	
Dead						
Recently dead	250	65	282	218	148	
Calcified	1 100	0	371	166	252	
Granulomas	0	17	77	0	74	
Total	5 650	180	730	485	741	
Weight of sample (g)	0.0200	0.0610	0.0780	0.0960	0.0675	
Patient 5 : 1.5 mg/kg daily for 10 days						
	Before treatment	Days after beginning treatment				
		12	16	33	111	
Viable						
Immature	2 289	0	0	0	0	
Mature	2 142	29	0	0	87	
Dead						
Recently dead	441	29	0	17	35	
Calcified	147	43	24	102	9	
Granulomas	0	0	0	0	0	
Total	5 019	101	24	119	131	
Weight of sample (g)	0.0475	0.0692	0.0845	0.0585	0.1140	
Patient 6 : 2 series of 1 mg/kg daily for 10 days, with 10-day interval						
	Before treatment	Days after beginning treatment				
		11	19	35	42	113	125
Viable						
Immature	2 226	0	0	0	0	0	0
Mature	1 476	260	30	0	0	0	0
Dead						
Recently dead	1 206	1 178	1 094	1 009	959	0	70
Calcified	121	275	426	453	633	227	93
Granulomas	0	0	0	0	0	0	0
Total	5 029	1 713	1 550	1 462	1 592	227	163
Weight of sample (g)	0.0412	0.0655	0.0440	0.0485	0.0550	0.0530	0.0860

TABLE 4
QUANTITATIVE OOGRAMS OF PATIENT 7, TREATED WITH AMPHOTALIDE, 40 mg/kg ORALLY DAILY FOR SIX DAYS

Type of eggs	Number of eggs per gram		
	Before treatment	Days after treatment	
		5	34
Viable			
Immature	1 032	849	3 244
Mature	1 848	1 274	1 958
Dead			
Recently dead	312	1 108	1 040
Calcified	360	0	306
Granulomas	0	0	0
Total	3 552	3 231	6 548
Weight of sample (g)	0.0417	0.0361	0.0326

infection with *S. mansoni*. We have used TAC pamoate (CI-403A; Parke, Davis & Co.) in capsules of 175 mg; Table 5 shows representative results.

Bis-(β-carbhydrazido-ethyl) sulfone

This drug (compound S 201 of Farbwerke Hoechst A.G.) was shown by Lämmler (1963) to be highly effective against *S. mansoni* infections in mice, golden hamsters and monkeys, following subcutaneous and intravenous application. Tests of its acute and chronic toxicity in mice, golden hamsters, monkeys, rats, dogs and in a few human volunteers revealed tolerance. We have started a clinical trial with the drug; patients 9 and 10 are illustrations of the results (Table 6).

INTERPRETATION OF QUANTITATIVE OOGRAM

Experimental work with schistosomes has clarified many basic points on drug susceptibility.

Antischistosomal drugs seem to act initially on the reproductive organs of the worms (Fairley, 1926; Bang & Hairston, 1946; Vogel & Minning, 1947, 1948; Kikuth & Gönnert, 1948). Variable alterations of the gonads occur, and egg-laying is immediately stopped. Little is known about the toxic effect of antimony on the parasite, although much valuable information has emerged in recent years on the biochemistry of schistosome glycolysis. The action appears to be directly on the intense metabolic processes of the reproductive system (Kikuth & Gönnert, 1948). Trivalent antimonials reduce the utilization of carbohydrates by schistosomes. The nature of this interference in glycolysis has been shown to be a selective action upon phosphofructokinase, an enzyme responsible as catalyst in the phosphorylation of fructose-6-phosphate by

TABLE 5
QUANTITATIVE OOGRAMS OF PATIENT 8, TREATED WITH TAC PAMOATE, 40 mg/kg ORALLY DAILY FOR 18 DAYS, REPEATED AFTER AN INTERVAL OF 7 DAYS

Type of eggs	Number of eggs per gram						
	Before treatment	Days after beginning treatment					
		19	62	76	106	136	236
Viable							
Immature	12 669	0	0	0	0	208	87
Mature	4 480	159	0	0	0	0	319
Dead							
Recently dead	3 296	8 177	2 999	2 036	40	176	304
Calcified	1 286	1 540	688	391	60	64	43
Granulomas	0	1 221	76	78	0	0	0
Total	21 731	11 097	3 763	2 505	100	448	753
Weight of sample (g)	0.0194	0.0188	0.0522	0.0383	0.0496	0.0622	0.0685

TABLE 6
QUANTITATIVE OOGRAMS OF TWO PATIENTS TREATED INTRAVENOUSLY WITH BIS-(β-CARBHYDRAZIDO-ETHYL) SULFONE

Type of eggs	Number of eggs per gram			
Patient 9 : 75 mg/kg daily for 6 days				
	Before treatment	Days after beginning treatment		
		11	18	38
Viable				
Immature	3 718	0	0	0
Mature	2 556	725	0	0
Dead				
Recently dead	1 162	93	76	197
Calcified	697	167	25	131
Granulomas	0	56	0	33
Total	8 133	1 041	101	361
Weight of sample (g)	0.0301	0.0537	0.0396	0.0609

Patient 10 : 100 mg/kg daily for 6 days					
	Before treatment	Days after beginning treatment			
		12	22	36	43
Viable					
Immature	3 613	21	49	562	266
Mature	2 563	1 009	0	207	126
Dead					
Recently dead	712	93	97	77	42
Calcified	89	1 442	97	180	140
Granulomas	0	10	16	0	14
Total	6 977	2 575	259	1 026	588
Weight of sample (g)	0.0561	0.0967	0.0616	0.0389	0.0690

adenosine triphosphate to fructose-1, 6-diphosphate (Bueding, 1950, 1959; Mansour & Bueding, 1954).

However, there may be a general effect on the worms and secondarily on their reproductive system. Standen (1962) shows that 1:7 bis (*p*-aminophenoxy) heptane, both *in vitro* and *in vivo*, induces remarkable degenerative changes in the cuticle which, in turn, appear to present a focal point of attraction for phagocytes. Furthermore, the mode of action of this drug and its analogues follows the well-known pattern common to most schistosomicides—namely, passive shift of the worms from the mesenteric veins to the liver, followed by ensheathment in inflammatory tissue and subsequent phagocytosis (Schubert, 1948; Standen, 1953, 1955; Brener, 1962). In the case of the diaminodiphenoxyalkanes there was, however, one new effect in that the shift to the liver was never followed by remigration to the mesenteric veins (Standen, 1963).

The therapeutic effects of schistosomicides have an initial phase (shift of worms from mesenteric veins to the liver) and a second phase (ensheathment, degeneration and phagocytosis in the liver) which depends upon the time the worms are in the liver. If the worms are not killed, remigration to the mesenteric veins will occur. Since there is normally a continuity in the process of egg-laying by females, the first result of an active drug is to cause interruption of oviposition with consequent alterations in the oogram. Because no drug acts on the eggs themselves they continue their development in the tissue up to maturation and elimination in the stools. After some time no immature egg is observed. If the effect of the drug is of short duration or temporary—just sufficient to produce a major shift of the worms from mesenteric veins to the liver—but does not kill the worms, remigration to the mesenteric veins occurs and the oogram will show re-establishment of oviposition some time later, with reappearance of viable eggs, giving a picture of clinical relapse. If this does not occur and the effect is definitive, there is cure.

When the drug does not act on the worms—i.e., when it is inactive—no alteration of the distribution of the worms in the portal system is produced and the oogram will not change; this is treatment failure.

Finally, some drugs are " partially active ". During treatment some worms which did not shift to the liver may remain in the intestinal venules and maintain oviposition, which, of course, will be reduced. In any population of worms some are more resistant to a drug than others; this is why a quantitative criterion in the determination of therapeutic activity in experimental bilharziasis has been adopted (Brener, 1962). In these circumstances, though interruption of oviposition does not show in the oograms, the number of eggs is notably reduced, especially the viable ova, both immature and mature. Despite the fact that the distribution of the ova in

the wall of the rectal ampulla is not uniform and that the quantity of material taken by the curette and the biopsy forceps varies from case to case, it is clear that a very great number of viable eggs (immature and mature) must correspond to a great number of ovipositing females. Therefore, if the material is weighed, the total number of eggs per gram of material can easily be reckoned, providing a standard basis for comparison before and after treatment.

That this line of reasoning is valid can be seen from patients receiving a placebo (starch), on whom quantitative oograms were made once a week, in a double-blind test. As an example, one such case has been cited above (Table 1). Although variations in the total number of eggs per gram occurred they were never significant, as they were with the case of drugs active against the parasites (see Table 2, for instance).

The antimonial (Table 2) caused interruption of oviposition immediately after the end of treatment; this is characteristic of an active substance. This effect was definitive, i.e., parasitological cure was achieved. It is also to be noted that as time elapsed after treatment and egg-laying had stopped, the total number of eggs diminished gradually. The recently dead eggs disappeared first, then the calcified eggs (and granulomas), their absorption being more difficult. At a later date, biopsy and scrapings became negative.

Evaluation by the quantitative oogram method of the activity of 2-dehydroemetine in *S. mansoni* infection has been illustrated in Table 3, which shows the effects produced by raising the dose, and by raising the dose and at the same time prolonging the period of treatment. The dose of 0.8 mg/kg/day did not interrupt oviposition though an evident reduction may be seen in the total amount of eggs, especially the viable ones—and the situation returned later to what it was before treatment. With the dose increased to 1 mg/kg/day and 1.5 mg/kg/day there was interruption of oviposition as well as a marked reduction in the total number of eggs, but this effect was temporary, since in both cases a relapse occurred. It is to be noted, however, that in both cases the quantities of eggs as a whole, and of the viable eggs in particular, did not reach pre-treatment levels. These quantitative oogram findings support the belief that either a large proportion of the parasite population was destroyed by the drug, the few remaining females being able to reinitiate oviposition (characteristic of a still active disease), or the drug caused a considerable loss in the egg-laying capacity of the worms. Whatever the reason, if the role of the egg in the pathogenesis of the lesions in bilharziasis is important, a partially active drug may be useful. When the period of treatment was prolonged, by repeating a new series after 10 days, interruption of oviposition was obtained, and persisted for four months after treatment, as is shown by the disappearance of viable eggs and the great reduction in the total number of eggs present. This case exemplifies another aspect of the quantitative oogram particularly well—namely, the importance of identifying the various types of dead eggs. Though no viable egg was seen in the oogram four months after treatment, and the total number of eggs had been considerably reduced, we cannot say that the patient was cured. On the contrary, it is very likely that he was not completely cured because in the last oogram a great proportion of the dead eggs belonged to the category of recently dead eggs. Further examination will help to clarify this point.

Amphotalide (R.P. 6171), given in the conditions shown in Table 4, illustrates the trend of the quantitative oogram in treatment failure. The oograms are much the same as those following administration of a placebo (Table 1).

TAC pamoate, in the conditions in which it was used, was active, producing interruption of oviposition and enormous reduction in the total number of eggs (Table 5). This effect lasted for some time (about two months) and the next oogram showed viable eggs, meaning a relapse, but, as in previous cases, the considerable reduction in the total number of eggs, though these are still present 6½ months after completion of treatment, is to be noted.

At a dose of 75 mg/kg/day, which in the case of patient 9 meant 5.25 g per day and a total dose of 31.5 g, bis-(β-carbhydrazino-ethyl) sulfone (S 201) was active (Table 6). Oviposition was interrupted, with a great reduction in the total number of eggs still present on the day of the last examination, 32 days after completion of treatment. However, at a dose of 100 mg/kg/day, corresponding to a daily dose of 4.4 g and a total dose of 26.4 g, there was no interruption of oviposition, but a great reduction in the total number of eggs, including viable ones. This effect was still demonstrable 37 days after the end of treatment. In this case the drug was only partially active, with a subcurative effect. It caused cessation of egg-laying in most of the females or else it affected in some way the function of oviposition in them all.

An active drug, whatever its chemical structure may be, shows in the oograms an interruption of oviposition with a disappearance of immature eggs, and consequently a great reduction in the total number of eggs. As time goes on, the subsequent oograms may reflect one of two situations. First, the effect of the drug may be definitive, resulting in parasitological cure. After the viable eggs, the dead eggs also disappear. Of these, the recently dead ones disappear first and then the calcified progressively diminish in number until the examination becomes negative. Secondly, some time after completion of treatment, viable eggs may reappear, thus indicating a relapse. However, quantitative oograms make it possible to analyse a relapse more closely. If the total number of eggs is considerably and permanently reduced, owing probably to a reduction in the parasite population, this means a partial relapse as the drug has had a subcurative effect; the substance or dose in question may nevertheless be useful in the therapy of bilharziasis. On the other hand, the post-treatment oograms may show an initial reduction in the number of eggs with a later return to previous levels, revealing a total relapse (see Table 3).

Finally, inactive drugs behave in the same manner as a placebo. There are no significant changes in the oograms, either in the differential or in the global counts. These oograms are characteristic of total treatment failure.

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RÉSUMÉ

La plupart des médicaments actifs sur les schistosomes chassent le parasite des veines mésentériques vers le foie où il est inclus dans un tissu inflammatoire puis phagocyté; les produits stibiés, en inhibant la phospho-fructokinase, réduisent le métabolisme glucidique du parasite; le *p*-amino-phénoxy 1 : 7 bis heptane provoque sur sa cuticule des lésions dégénératives qui créent un point d'appel pour les phagocytes. Si l'action n'est que temporaire, les parasites retournent dans les veines mésentériques. Le premier résultat de l'action d'un médicament efficace est l'arrêt de la ponte des œufs dans la sous-muqueuse de l'intestin, aussi l'étude de l'oogramme sur des prélèvements de muqueuse rectale du malade permet-elle de juger l'efficacité d'un traitement.

Les auteurs qui ont effectué plus de 5000 oogrammes en huit ans, le plus souvent pour l'évaluation thérapeutique de plus de 50 produits nouveaux, exposent leur technique pour établir un oogramme quantitatif au cours d'un traitement dans une infection humaine à *Schistosoma mansoni*. Les observations de dix malades traités par un placebo, un composé stibié, 4 dosages différents de 2-dihydroémétine, le *p*-aminophénoxy-1-phtalimido-5 pentane, le pamoate de pararosaniline et la bis-(β carbhydrazido-éthyl) sulfone, illustrent l'application de cette technique.

On prélève un fragment de muqueuse rectale, par biopsie, et de la mucosité, par raclage, sous contrôle rectoscopique qui permet de noter l'étendue et la nature des lésions. Cet échantillon est pesé de façon à exprimer les numérations d'œufs en nombre par gramme de matière. Les chiffres avant traitement, au cours de celui-ci et après son arrêt sont comparés. Les auteurs proposent une classification simplifiée des œufs de *S. mansoni*: œufs viables, d'abord immatures avec quatre stades, donnant en six jours un œuf mûr (contenant un miracidium vivant); œufs morts, récemment, calcifiés ou inclus dans un granulome. Aucun schistosomicide n'agissant sur les œufs, ceux qui sont dans les tissus continueront leur évolution jusqu'à être éliminés dans les selles lorsqu'ils sont mûrs. Si le médicament est actif, l'oogramme montrera successivement la disparition des œufs immatures, des œufs mûrs et enfin des œufs morts. La réapparition d'œufs immatures est le signe d'une récidive; l'action du médicament n'était que temporaire. Un échec du traitement se traduit par des oogrammes sans modification; les variations normales de l'émission d'œufs en dehors de toute thérapeutique ne sont pas significatives. Certains produits, partiellement actifs, réduisent le nombre des œufs, surtout des œufs viables: l'oogramme quantitatif permet d'évaluer leur action.

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