

## SERUM ANTIBODY AND EOSINOPHIL CHANGES AFTER TREATMENT OF HUMAN MANSON'S SCHISTOSOMIASIS WITH NIRIDAZOLE OR HYCANTHONE

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### SUMMARY

Serum antibody and eosinophil changes were studied in 23 patients with Manson's schistosomiasis before and after treatment with Niridazole or Hycanthonone.

An increase of precipitin lines was detected by double immuno-diffusion at the end of the administration of Niridazole and reached its maximum at the 15th day post-therapy. After Hycanthonone the maximum increase was observed between the second and the third weeks. The same trend was observed with the eosinophil counts.

Immunofluorescence (F.A.T.) and hemagglutination (H.A.) tests showed a significant increase of titres, particularly between the second and the fourth week after the use of either drug.

It is concluded that F.A.T. is less sensitive than the other tests to detect immunological changes produced by the schistosomicidal effect of a drug. Neither test, however, seems to be useful as a criterion of cure. Such aspect is under investigation.

### INTRODUCTION

The appearance or the increase in serum antibodies to worm antigens have been shown in patients with schistosomiasis submitted to specific therapy, mainly by immunodiffusion<sup>3</sup>, hemagglutination<sup>6</sup> and immunofluorescence<sup>6</sup>.

The increased level of antibodies was attributed to the death of worms when specific drugs act upon them.

As far as eosinophil counts are concerned, similar results have been found<sup>15</sup>.

Most papers, however, report the evolution of antibodies either through a single technique or in a short term interval.

This paper shows our experience on a long term follow-up of patients with schistosomiasis mansoni submitted to Niridazole or Hycanthonone therapy. Blood and sera were simultaneously checked by eosinophil counts, immunodiffusion, immunofluorescence and hemagglutination tests.

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#### MATERIAL AND METHODS

From 23 patients with Manson's schistosomiasis, 17 were submitted to Niridazole (group A) and 6 patients to Hycanthon therapy (group B).

Hepatointestinal and hepatosplenic forms of the disease were present in both groups. Age varied from 8 to 42 years.

All selected patients passed viable eggs in the stools and were studied monthly for at least 6 months after treatment. Nine patients of the group A were followed-up for more than one year.

Most of the patients were submitted to a new series of treatment. The results will be reported in a future paper.

Antischistosomal drugs were given as shown in Table I.

Immunodiffusion was basically the OUCHTERLONY method<sup>10</sup>, using microscope slide<sup>13</sup>, slide<sup>13</sup>.

Fluorescence and hemagglutination techniques<sup>1, 5</sup> were performed on sera submitted to serial doubling dilutions, beginning respectively from 1:20 and 1:40. Titers (x) were normalized (n) through the equation  $x = 2^n \times 5^{(6)}$ . Medium titers, maximum and minimum values are graphically presented in Figs. 3 and 4. An increase of 2 dilu-

tions or more after treatment was considered as significant<sup>6</sup>. As for the eosinophil counts, only an increase of twice the highest pre-treatment value was considered.

#### RESULTS

*Immunodiffusion* — Positive results before treatment were observed in only 6 out of 16 patients (37.5%). However, after Niridazole all of the 10 patients but one showed positive results (Table II), with a predominance of high number of precipitin bands (3 or more) (Fig. 1). Similar results were observed after Hycanthon.

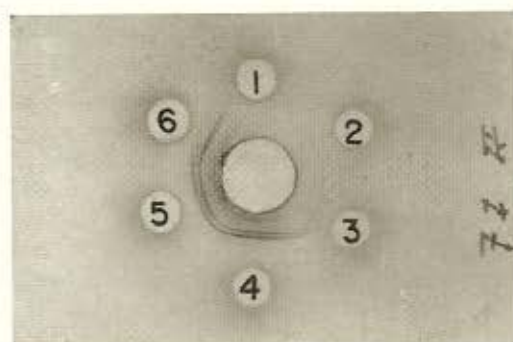


Fig. 1 — Double diffusion test in sera from one patient, before (no. 1), at the end (no. 2), and some weeks after treatment with Niridazole (nos. 3, 4, 5 and 6 correspond to 1, 2, 3 and 4 weeks respectively)

TABLE I

Schedule for chemotherapy and number of cured patients

Schedule	Drug	Dosage	Number of patients	Cured *
A <sub>1</sub>	Niridazole	25 mg/kg/day (7 days)	10	8 **
A <sub>2</sub>	Niridazole	15 mg/kg/day (15 days)	7	4 **
B <sub>1</sub>	Hycanthon (oral)	3 mg/kg/day (3 days)	3	3
B <sub>2</sub>	Hycanthon (intramuscular)	2.5 mg/kg/day	3	3

\* Parasitological cure was based on monthly negative stool examinations, for at least 6 months, and on negative (no viable eggs) rectal biopsies.

\*\* Irregular intake of the drug because of side-effects in 2 patients of the schedule A<sub>1</sub> and in 1 of the A<sub>2</sub>.

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TABLE II

Changes in immunofluorescence (F.A.T.) and hemagglutination (H.A.) titres, in immunodiffusion (I.D.) and in eosinophil (Eo) counts after treatment \*

Drug	F.A.T.		H.A.		I.D.		Eo	
	increase	no increase	increase	no increase	increase	no increase	increase	no increase
Niridazole **	10	6	9	0	9	1	6	1
Hycanthoner ***	3	3	5	1	5	0	6	0
Total of cases	13 (59.1%)	9	14 (93.3%)	1	14 (93.3%)	1	12 (92.3%)	1

\* Only patients with weakly or monthly determinations were included

\*\* Schedule A<sub>1</sub> or A<sub>2</sub> (see Table I)

\*\*\* Schedule B<sub>1</sub> or B<sub>2</sub> (see Table I)

The greatest increase of bands was observed between the second and fourth weeks after treatment with either drug. After the 30th week all but two patients became negative.

As the period of treatment varied considerably (see Table I, schedules A<sub>1</sub>, A<sub>2</sub>, B<sub>1</sub> and B<sub>2</sub>), it would be interesting to see whether the appearance of the peak of precipitin line varied accordingly. As we can see in Tables III and IV, the small number of cases and the lack of data in some periods did not allow us to conclude definitely, but we can observe that the increase of lines may start early, that is, at the end of the administration of Niridazole (patients no. 1, 2, 5, 8 and 10, Table III) and that it reached its maximum at the 15th day.

As far as Hycanthoner is concerned (Table IV) some increase may be observed at the first week after treatment, with the peak between the second and the third weeks.

#### Immunofluorescence and hemagglutination

— Fluorescence antibody test (F.A.T.) showed an increase of titers in 13 out of 22 patients (59.1%) and hemagglutination (H.A.) in 14 out of 15 patients (93.3%) after treatment (Table II). The maximum

increase was observed between the second and fourth weeks after the use of either drug (Figs. 3 and 4). Afterwards, a gradual decrease was observed, and at the end of one year the antibody levels were comparable or slightly below the pretreatment ones. A progressive decrease of titers was observed in most patients followed-up for a longer period (Table V).

Table III shows that the increase of F.A.T. and H.A. titers starts at the first week after Niridazole (schedules A<sub>1</sub> and A<sub>2</sub>). In all but two patients (M.V.N. and J.A.F.), a

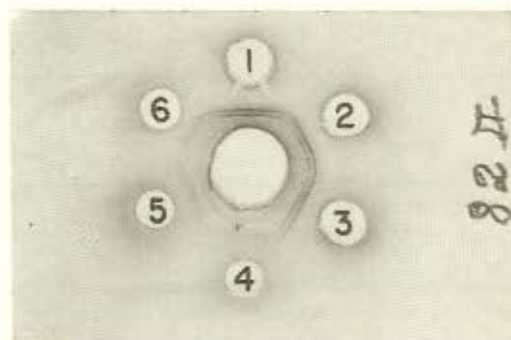


Fig. 2 — Same patient as in Fig. 1, from 9 to 14 weeks after treatment (nos. 1, 2, 3, 4, 5 and 6, respectively)

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TABLE III

Immunofluorescence (a) and hemagglutination (b) titers, precipitin lines (c) and eosinophil counts (d) before, during and soon after treatment with Niridazole (weekly determinations)

Patients	Before treatment		During treatment		After treatment				Schedule
	2nd. w.	1st. w.	1st. w.	2nd. w.	1st. w.	2nd. w.	3rd. w.	4-5th. w.	
1-O.P.R. (a)	160	160	160	—	640	1,280		1,280	A <sub>1</sub>
(b)	2,560	2,560	2,560	—	20,480	40,960		40,960	
(c)	1	1	2	—	4	4		4	
(d)	2905	1736	3600	—	3120	5208		2706	
2-A.P.R. (a)	160	160	80	—	160	640		320	A <sub>1</sub>
(b)	10,240	5,120	5,120	—	40,960	81,920		40,960	
(c)	1	1	2	—	4	5		4	
(d)	1,624	1,743	2,948	—	10,260	7,695		3666	
3-E.P. (a)	40		—	—	10	80	80	160	A <sub>1</sub>
(c)	0		—	—	0	4	4	4	
4-M.D.S. (a)	160	80	160	160	160		160		A <sub>2</sub>
(b)	640	640	640	640	2,560	10,240			
(c)	0	0	0	0			2	2	
(d)	663	774	561	1440	3080		2501		
5-M.V.N. (a)	320	640	160	640	1,280	320		320	A <sub>2</sub>
(b)	320	1,280	20,480	20,480	2,560	5,120		5,120	
(c)	0	0	0	1	2	2		1	
(d)	750	780	1,264	3570	1728	979		544	
6-S.M.P. (a)	80	160	160	160		320		160	A <sub>2</sub>
(*) (b)	1,280	1,280	1,280	1,280		20,480		5,120	
(c)	0	0	0	0		2		2	
(d)	980	583	1,122	2,964		5929		770	
7-O.V.S. (a)	40	40	80	80			160	160	A <sub>2</sub>
(*) (b)	640	1,280	640	2,560			5,120	5,120	
(c)	0	0	0	0			1	2	
(d)	0	0	0	0			70	0	
8-J.A.F. (a)	160	160	160	160		320		320	A <sub>2</sub>
(b)	640	640	1,280	5,120		5,120		10,240	
(c)	0	0		1		1		1	
9-C.A.S.D. (a)		40	40	40	160	320	2,560	1,280	
(b)		80	80	160	320	2,560	20,480	10,240	
(c)		0	0	0	1	4	4	3	
10-A.A.S. (a)	80	40	40	80		160		160	A <sub>2</sub>
(b)	160	160	160	320		640		640	
(c)	2	2	2	4		3		3	

(\*) Still with some viable eggs in the stools. Patient O.V.S. took the drug irregularly

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TABLE IV

Immunofluorescence (a) and Hemagglutination (b) titers, precipitin lines (c) and eosinophil-counts (d) before and soon after treatment with Hycanthon (weekly determinations)

Patients	Before treatment		After treatment				Schedule	
	2nd. w.	1st. w.	1st. w.	2nd. w.	3rd. w.	4-5th. w.		
11-M.P.R.	(a)		10	20	40	40	B <sub>1</sub>	
	(b)		40	80	160	160		
	(c)		0	0	0	0		
	(d)	1120	960	5100	4104	4266		2242
12-C.S.N.	(a)	160		160		160	B <sub>1</sub>	
	(b)	640		320		640		1,280
	(c)	1		1		2		2
	(d)	2,352	1820	4,032		10,100		3,564
13-H.S.N.	(a)	80	80	80	160	160	B <sub>1</sub>	
	(b)	160	160	160	320	640		320
	(c)	0	0	0	2	2		2
	(d)	2,040	2916	2,376	6477	6,407		3,720
14-M.G.S.N.	(a)	320	320	640	1,280	2,560	B <sub>2</sub>	
	(b)	320	320	640	2,560	20,480		1,280
	(c)	1	1	2	3	3		3
	(d)	2,774	2550	6,800	12,768	8,805		3,850
15-J.S.N.	(a)	320	320	320	640	320	B <sub>2</sub>	
	(b)	1,280	1,280	2,560	10,240	5,120		5,120
	(c)	0	0	0	1	1		0
	(d)	2,400	2040	3,048	2,826	4,805		3,100
16-L.S.N.	(a)	160		160	320	160	B <sub>2</sub>	
	(b)	5,120		2,560	20,480	20,480		10,240
	(c)	1		1	3	3		2
	(d)	1,176	1440	4,266	16,080	6,615		2352

significant increase is not observed at the end of treatment. Patient M. V. N. had been under treatment with two single doses of Niridazole, suppository form, some weeks before oral route therapy.

After Hycanthon (Table IV) the increase of titers starts at the second week.

*Eosinophils* — An increase of eosinophils was observed in 12 out of 13 patients (92.3%). The maximum increase was observed at the second week after treatment with either drug (Figs. 2 and 3).

Table III shows that the increase of eosinophils starts early, that is, at the end of administration of Niridazole and reaches its maximum up to the 15th day.

As for the Hycanthon (Table IV) the increase is observed at the first week after drug administration with the peak between the second and the third weeks.

Despite the gradual decrease of eosinophils in the following months, a level only slightly below that of the pretreatment period was observed at the 12th month.

TABLE V

A long term immunological study of schistosomotic patients submitted to Niridazole

Patients	I.F. *					H.A. *				I.D. *		
	Before	After	74 ws.	100 ws.	152 ws.	Before	After	74 ws.	100 ws.	Before	After	74 ws.
1 — O.P.R.	160 **	1,280	20			2,560	40,960	640		1***	4	0
2 — A.P.R.	160	640	40			10,240	81,920	160		1	5	1
3 — E.P.	40	640	80					640		0	4	0
5 — M.V.N.	640	1,280	80			1,280	20,480	640		0	2	0
8 — J.A.F.	160	320	80			640	10,240	2,560		0	1	0
17 — M.L.S.	80	640	40	20	40			160	160			
18 — R.A.	160	320	40	40				160				
19 — M.M.C.	80	160	20	20	40							
20 — F.A.M.	320	1,280	320	80	80			640	640			
21 — S.M.C.S. §	80	640	40									

\* Before treatment, highest value after treatment, and weeks after treatment

\*\* Titres

\*\*\* no. of arcs

§ Non-cured patient (viable eggs in the stools)

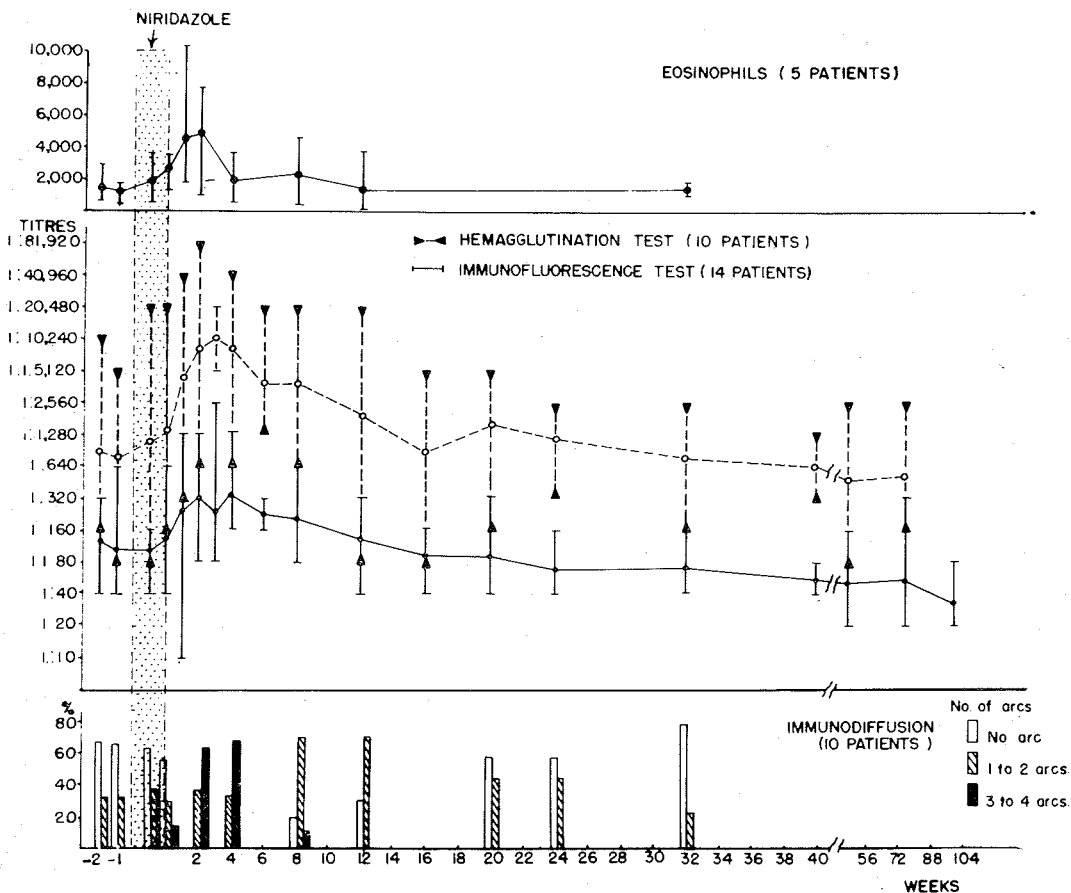


Fig. 3 — Eosinophil counts, hemagglutination and immunofluorescence titres and immunodiffusion, before, during and after treatment of patients with Niridazole

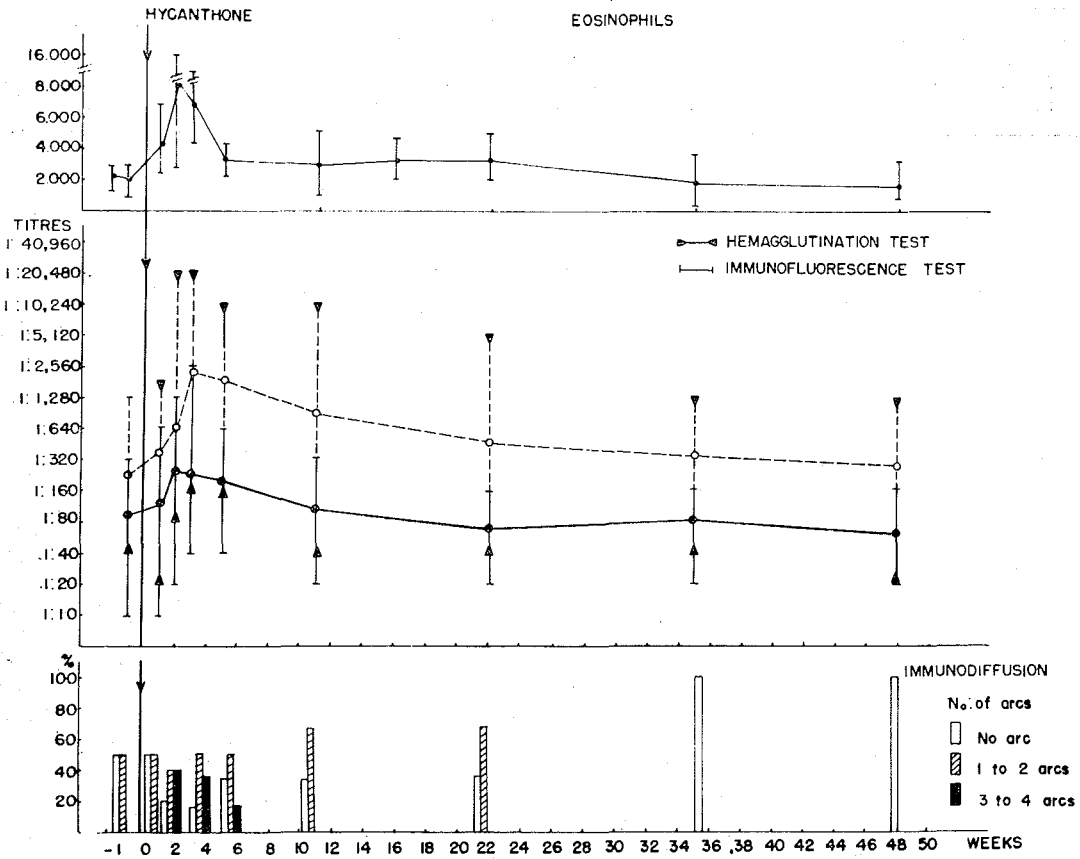


Fig. 4 — Eosinophil counts, hemagglutination and immunofluorescence titres and immunodiffusion, before and after treatment of 6 patients with Hycanthone

**PATIENT C.A.S.D**

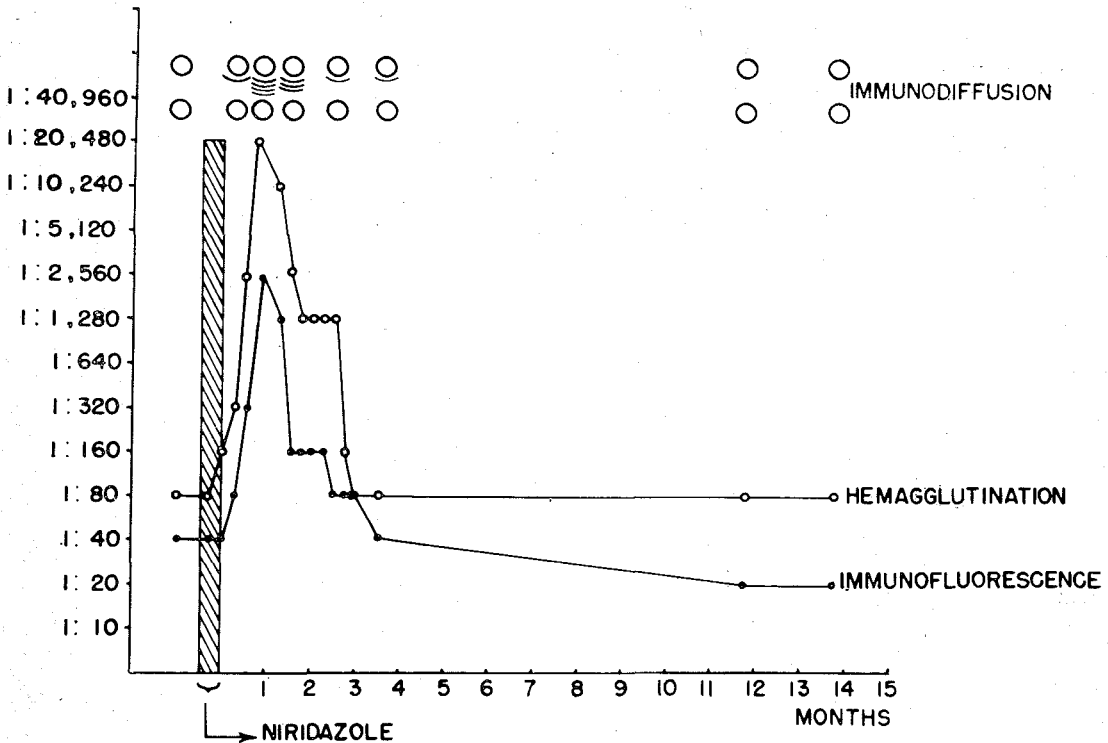


Fig. 5 — Immunodiffusion, hemagglutination and immunofluorescence before, during and after treatment with Niridazole (Patient C.A.S.D., no. 9)

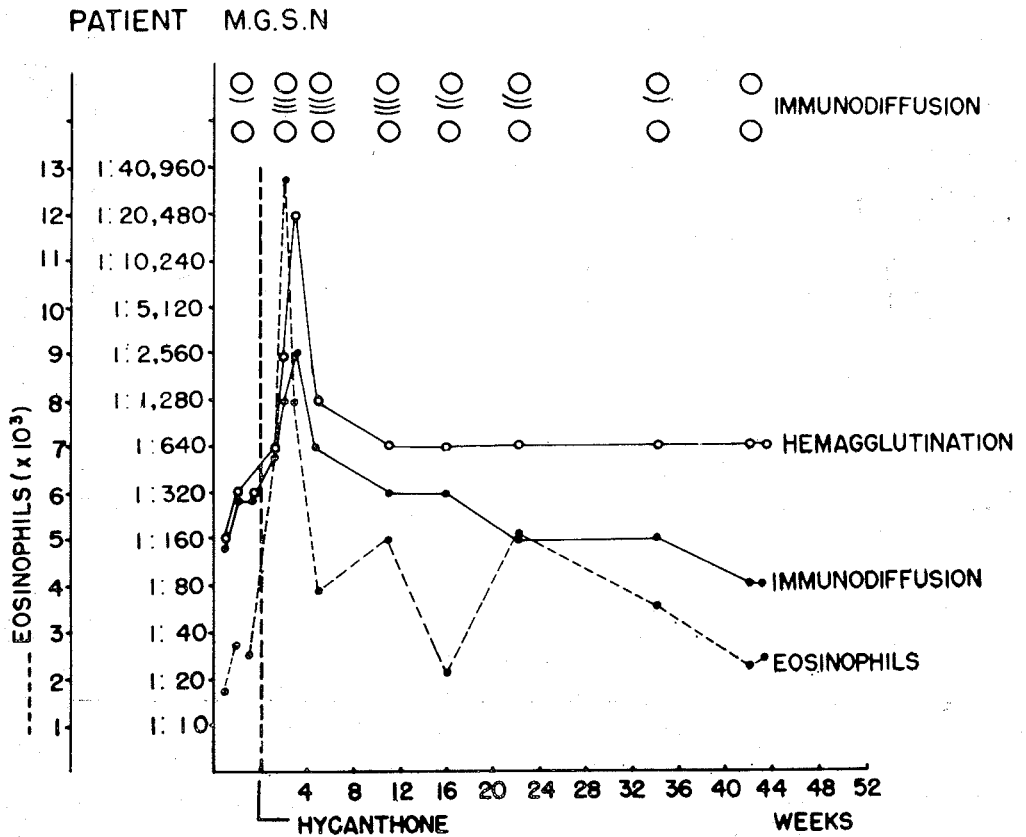


Fig. 6 — Immunodiffusion, hemagglutination, immunofluorescence and eosinophil counts before and after treatment with Hycanthone (Patient M.G.S.N., no. 14)

Only one patient (O.V.S., Table III) showed no increase in the eosinophil counts after treatment, despite the evident changes in all of the three immunological tests.

As to the non cured patients similar results with all tests were observed.

Illustrative cases of evolution of antibodies with either drug are shown in Figs. 5 and 6.

#### DISCUSSION

Significant increases of the eosinophil counts and of the levels of antibodies to schistosoma antigens as studied by immunofluorescence, hemagglutination and immunodiffusion were observed in a period between the second and the fourth weeks after treatment of schistosomotic patients with Niridazole or Hycanthone.

Fluorescence antibody test (F.A.T.) showed a significant increase of titers in only

59.1% of treated patients. The highest titers were maintained for one to two months and then decreased gradually (Figs. 3 and 4).

The same pattern was observed by some Authors<sup>7, 11</sup>. Contrarily to POTHIER<sup>11</sup>, however, we did not observe an increase of titers immediately after treatment except in two patients.

The elevation of titers may be attributed to the death of schistosoma worms and so the F.A.T. could be used to evaluate the therapeutic efficiency of a drug<sup>11</sup>. For this purpose, however, other immunological tests as hemagglutination and immunodiffusion and even the eosinophil counting are more sensitive.

According to RIFAAT et al.<sup>12</sup>, F.A.T. remains positive up to 10 years after effective treatment and so cannot be used as a criterion of cure.



Positive results more than one year after treatment have been observed by us (Table V) but the titers were lower than the pre-treatment ones in most patients. Though all of them were parasitologically cured, such low values were also observed in one non-cured patient.

Hemagglutination test (H. A.) showed a similar pattern but the increase of titers was much more frequent (93.3%).

This test is very useful for the evaluation of the efficiency of a drug. The increase of titers started at the first week after Niridazole and at the second week after Hycanthone. The maximum increase was observed between the second and the fourth week after either drug.

As far as immunodiffusion test (I. D.) is concerned our results are comparable to those of DODIN et al.<sup>3</sup> and GENTILINI et al.<sup>4</sup> though the precipitin systems found by the last Authors and by CAPRON et al.<sup>2</sup> were more numerous.

GENTILINI et al.<sup>4</sup> observed precipitation in 4 out of 5 patients one year after treatment. Only two of our patients showed positive immunodiffusion test after 30 weeks.

The maximum increase of precipitin lines is often observed one week before the peak of F. T. A. and H. A. titers (Tables III and IV).

A great sensitivity was also observed with the eosinophil countings. Thus, a striking increase was seen in all but one patient between the second and third weeks after treatment.

Our results agree with those of ZANANDREA et al.<sup>13</sup> and MACIEL<sup>8</sup>. These Authors observed a normalization of eosinophilia 60 days after drug administration. In our cases, however, some patients showed eosinophilia after one year, in spite of negative stool examinations. This persisting eosinophilia was also observed by MAINZER<sup>9</sup>.

According to some Authors<sup>14</sup> the increase of eosinophils after drug administration lacks specificity, as it was found in non-schistosomal patients. Such results do not agree with our experience in "cured" patients submitted to new series of chemotherapy (to be published later).

The magnitude of antibody and eosinophil changes after Niridazole and Hycanthone seemed to be comparable. Some slight differences in the beginning of such changes may depend on the period of time used in each schedule (see Tables III and IV).

A more detailed study of the immunological changes is imperative and should include patients with the same clinical form of schistosomiasis and in an uniform age group.

Summing up, the H. A. and I. D. tests and eosinophil counting are very useful to study schistosomal patients submitted to chemotherapy. F. A. T. is less sensitive.

If we consider the negative search for viable eggs in fecal and/or rectal biopsy materials as the only valid criteria of cure, neither test seemed to be useful in this aspect.

Thus, F. A. T. and H. A. tests showed positive results for long periods (more than one year) after treatment, and persistent eosinophilia was also observed in some "cured" patients. On the other hand, negative I. D. tests may be found in patients passing viable eggs in the stools.

The simultaneous use of such tests after new series of chemotherapy in "parasitologically" cured patients will be the subject of another paper.

#### RESUMO

#### *Alterações dos eosinófilos e dos anticorpos séricos após tratamento da esquistossomose mansônica humana com Niridazole ou Hycanthone*

Os Autores estudaram as alterações dos eosinófilos e dos anticorpos contra antígenos de *Schistosoma mansoni* em 23 pacientes, antes e após administração de Niridazole ou de Hycanthone.

Pela imunodifusão, observaram um aumento das linhas de precipitação a partir do fim do tratamento com Niridazole e que atingiu o máximo em torno dos 15.º dia. Após Hycanthone, aquele máximo foi observado entre a segunda e terceira semanas. A eosinofilia pós-tratamento, mostrou a mesma tendência.

Um significante aumento de títulos foi observado através da imunofluorescência (F. A.T.) e da hemaglutinação (H. A.), particularmente entre a segunda e a quarta semanas após o uso de cada droga.

Os Autores concluem que a imunofluorescência é menos sensível que as outras reações para demonstrar as alterações imunológicas conseqüentes ao uso de drogas esquistossomicidas. Por outro lado, nenhuma prova parece ser útil como critério de cura.

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