

THE CIRCUMOVAL PRECIPITIN TEST AS A CONTROL OF CURE IN THE EXPERIMENTAL SCHISTOSOMIASIS MANSONI (1)

Rômulo Teixeira de MELLO, Leógenes H. PEREIRA and J. PELLEGRINO (2)

S U M M A R Y

The circumoval precipitin test (COPT) was performed on sera from *Cebus* monkeys and mice, previously infected with *Schistosoma mansoni* and treated with oxamniquine. A progressive lowering of titres was observed after drug administration and COPT became negative after 240 and 280 days in monkeys and 240 days in mice. The control of parasitological cure was assessed by the oogram, and all animals produced no viable eggs after the first month following treatment. COPT is suggested as an additional control of cure on experimental chemotherapy of infected monkeys with promising compounds.

I N T R O D U C T I O N

The circumoval precipitin test (COPT) was first described by OLIVER-GONZALEZ⁷ using *Schistosoma mansoni* eggs as antigen. After 24 hours incubation at 37°C, the serum-egg mixture was examined microscopically for antibody precipitates around the antigen. The reaction is inhibited by previous absorption of the sera with living or lyophilized eggs but not with absorption with cercariae or adult worms. Then COPT antibodies are very specific for parasite egg antigens. These observations were confirmed by other investigators, including COKER & OLIVER-GONZALEZ³, BRUIJNING¹, FARIA & PELLEGRINO⁴, and PELLEGRINO & KATZ¹⁰, showing that COPT only becomes positive 42-60 days after experimental infections, i.e. after the oviposition is started.

From all immunological tests, the COPT is the reaction that presents the fastest lowering of titres after the curative treatment. However, there is no agreement among the authors on the time required for the titer changes.

RODRIGUEZ-MOLINA et al.¹³ found positive reactions up to 210 days after antimonial treatment of infected laboratory mice. BRUIJNING¹ and CANCIO et al.² found the beginning of titer reduction 15-60 days following treatment. KLOETZEL⁶, SADUN et al.¹⁴, SHOEB et al.¹⁵, and RIFAAT et al.¹¹ found changes in the circumoval precipitins from 15 days to 10 months after cure.

The *Cebus* monkey is a primate very much used for experimental schistosomiasis mansoni purposes, since it becomes easily infected and shows no usual self-cure of the disease. Such animals are not routinely sacrificed after experimental chemotherapy studies because the control of cure is estimated on stool examinations and rectal biopsies. Nevertheless, and additional serological control may also be helpful because many apparent cures show later relapses and in some cases following the treatment the worms are not killed at all, becoming only sterile. This paper presents a long-term study of COPT titres after experimental

(1) This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Financiadora de Estudos e Projetos (FINEP), Brazil; and by the World Health Organization (WHO), Switzerland.

Contribution number 101 from the Schistosomiasis Research Unit.

Address for reprints: Departamento de Parasitologia, Caixa Postal 4286, 30000 Belo Horizonte, Brasil.

(2) Deceased February, 1977.

treatment of *Cebus* monkeys and laboratory mice in correlation with rectal biopsies and necropsy findings.

MATERIAL AND METHODS

Animals and infections — *Cebus* monkeys maintained in the laboratory, fed with fresh vegetables and fruits, were infected percutaneously with about 200 *Schistosoma mansoni* cercariae (LE strain, Belo Horizonte, Brazil), shed by laboratory reared-and-infected *Biomphalaria glabrata*. The same food diet was maintained throughout the experiment.

Outbred albino mice, weighing 18-22 gm were infected by subcutaneous injection of about 40 cercariae.

Blood collection — *Cebus* monkeys were punctured, at different time intervals, in the saphenous vein to collect 1.5 — 2.0 ml of blood. Mice were anesthetized (groups of 5 animals) with ether, then the thorax was opened and blood collected directly by heart puncture, and then sacrificed with additional ether exposure. Sera were obtained from blood and kept at -20°C in the same day.

Drug treatment — *Cebus* monkeys received 40 mg/kg oxamniquine, i.m. injections, sin-

gle doses. Mice were treated the same way with 200 mg/kg.

Parasitological control — Control of infection was carried out with the oogram (PELLEGRINO & FARIA⁹). Rectal snips were removed from monkeys (KATZ, PELLEGRINO & MEMÓRIA⁵) and intestine samples collected from mice previously sacrificed for blood collections.

Circumoval precipitin test (COPT) — Infected hamsters were sacrificed after 45-50 days of infection and, the liver and intestine disrupted in a homogenator. Eggs were separated from tissues according to RITCHIE & BERRIOS-DURAN¹², and later concentrated to 100-150 eggs per 0.05 ml. They were maintained in 2.0% saline solution and stored at 4°C for no more than 4 hours, until the beginning of the reaction. The test was performed according to OLIVER-GONZALEZ's⁷ technique, with the modifications recommended by PELLEGRINO & ANDRADE⁸. The r values (scores) were considered positive if equal or higher than 5.

RESULTS

Data are summarized in Fig. 1 (*Cebus* monkeys) and Fig. 2 (mice).

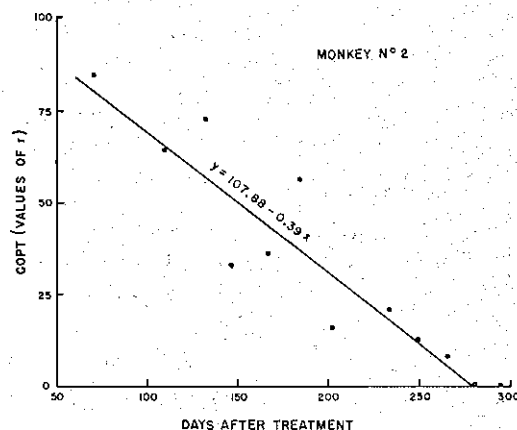
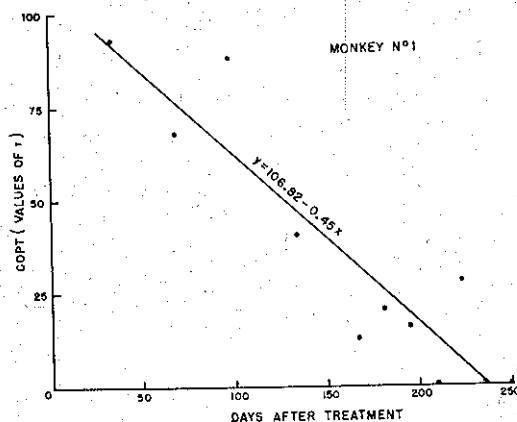


Fig. 1 — Circumoval precipitin tests in sera from *S. mansoni* infected and oxamniquine treated *Cebus* monkeys. Titres of reactions performed at different time intervals.

As can be seen, a progressive lowering of titres was observed in the two monkeys, as well as in mice, after chemotherapy. Reactions became negative after 240 and 280 days following drug administration, respectively to mon-

keys no. 1 e no. 2, and after 240 days to mice. The oogram performed several times in rectal biopsies of monkeys and intestine samples from different groups of sacrificed mice, showed no viable eggs after the first month after treatment.

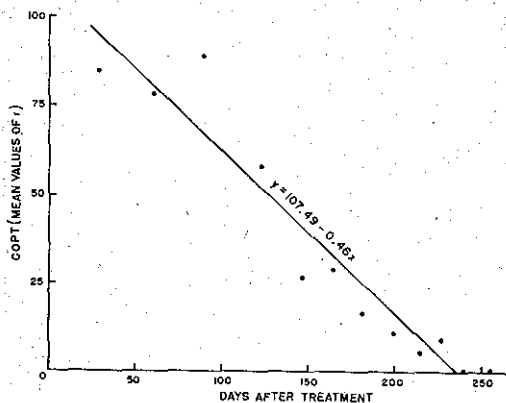


Fig. 2 — Circumoval precipitin tests in sera from *S. mansoni* infected and oxamniquine treated laboratory mice. Titres of reactions performed at different time intervals.

DISCUSSION

The circumoval precipitin test is a very specific reaction form schistosome egg antigens, and possibly this test presents the faster lowering of titres after curative treatment. Thus, this technique was suggested as an immunological control of cure of the human disease, but limitations due mainly to the laborious preparation of the antigen, as well as the long time consumed for reading the reactions, discourages routine work.

However, for immunological studies this test is employed. It can also be an additional procedure for control of cure of experimental infection and treatment. Obviously, it will be not recommended for screening studies, but for further investigations on active compounds in primates this technique seems to be appropriate, mainly considering apparent cures may lead to later relapses. If a drug only sterilizes the worms, by re-starting their oviposition, eggs may be excreted in small numbers and parasitological control will present some difficulties. The COPT may be an useful tool in such circumstances.

RESUMO

A reação peri-ovular como controle de cura na esquistossomose mansoni experimental

A reação peri-ovular (RPO) foi feita nos soros de macacos *Cebus* e camundongos infectados com *Schistosoma mansoni* e tratados

com oxamniquine. Uma queda progressiva dos títulos foi observada após a administração do composto, sendo que nos macacos a RPO tornou-se negativa 240 e 280 dias após a cura, ao passo que nos camundongos este período foi de 240 dias. O controle parasitológico de cura foi feito através do oograma, e os animais não apresentaram nenhum ovo viável a partir do 30.^o dia após o tratamento.

A RPO apresenta-se, pois, como um controle adicional de cura, quando se utilizam macacos *Cebus* na quimioterapia experimental da esquistossomose mansoni.

REFERENCES

- BRUIJNING, C. F. A. — The circumoval precipitin test in experimental *Schistosoma mansoni* infections. *Trop. Geog. Med.* 13: 378-387, 1961.
- CANCIO, M.; RIVERA DE SALA, A.; ARELLANO, G. R. & RODRIGUEZ-MOLINA, R. — Circumoval antibodies. Measurement during treatment of experimental schistosomiasis. *Amer. J. Trop. Med. Hyg.* 16: 729-734, 1967.
- COKER, C. M. & OLIVER-GONZALEZ, J. — Experiments on anti-schistosome circumoval precipitating antibody in mice. *Amer. J. Trop. Med. & Hyg.* 7: 390, 1957.
- FARIA, J. & PELLEGRINO, J. — Observações sobre a infecção experimental do hamster (*Cricetus auratus*) pelo *Schistosoma mansoni*. *Rev. Inst. Med. trop. São Paulo* 5: 281-286, 1963.
- KATZ, N.; PELLEGRINO, J. & MEMÓRIA, J. M. P. — Quantitative oogram method in *Cebus* monkeys experimentally infected with *Schistosoma mansoni*. *J. Parasit.* 52: 917-919, 1966.
- KLOETZEL, K. — A reação de precipitação peri-ovular na esquistossomose. II — Correlação aos dados clínicos. *Rev. Inst. Med. trop. São Paulo* 1: 129-137, 1959.
- OLIVER-GONZALEZ, J. — Anti-egg precipitins in the serum of humans infected with *Schistosoma mansoni*. *J. Infect. Dis.* 95: 86-91, 1954.
- PELLEGRINO, J. & ANDRADE, F. O. — A reação peri-ovular no diagnóstico da esquistossomose mansônica. *Rev. Inst. Med. trop. São Paulo* 4: 163-169, 1962.
- PELLEGRINO, J. & FARIA, J. — The oogram method for the screening of drugs in schistosomiasis mansoni. *Amer. J. Trop. Med. Hyg.* 14: 363-369, 1965.
- PELLEGRINO, J. & KATZ, N. — Cercarial slide flocculation and circumoval precipitin tests on *Cebus* monkeys experimentally infected with *Schistosoma mansoni*. *J. Parasit.* 53: 216-217, 1967.

11. RIFAAT, M. A.; ISMAIL, I.; EL MAHALLAWY, M. N.; AWWAAD, S. & ESSAWY, M. — A comparative study of some immunological tests for schistosomiasis before and after treatment. *Trans. Roy. Soc. Trop. Med. Hyg.* 63: 338-342, 1969.
12. RITCHIE, L. S. & BERRIOS-DURAN, L. A. — A simple procedure for recovering schistosome eggs in mass from tissues. *J. Parasit.* 47: 363-365, 1961.
13. RODRIGUEZ-MOLINA, R.; LICHTENBERG, F.; OLIVER-GONZALEZ, J. & RIVERA DE SALA, A. — Studies on immunity to *Schistosoma mansoni*. II — The circumoval precipitin reaction to *S. mansoni* in mice treated with Stibophen. *Amer. J. Trop. Med. Hyg.* 8: 565-569, 1959.
14. SADUN, E. H.; ANDERSON, R. I.; DE WITT, W. B. & JACHOWSKI, A. — Serologic reactions to *Schistosoma mansoni*. II — Quantitative studies in human patients treated with Stibophen. *Amer. J. Hyg.* 77: 146-149, 1963.
15. SHOEB, S. M.; BASMY, K.; HASEEB, N. M.; RIFAAT, M. A. & KHALIL, H. M. — The value of the circumoval precipitin test (COPT) in the assessment of cure from bilharziasis and in the evaluation of antibilharzial chemotherapy. *J. Egypt. Med. Ass.* 50: 29-38, 1967.

Recebido para publicação em 26/4/1978.