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OVIPOSITION BY *Schistosoma mansoni* DURING *IN VITRO* CULTIVATION

Leo Roberto BARTH(1), Ana Paula Morais FERNANDES(1) & Vanderlei RODRIGUES(1)

SUMMARY

Observation of *Schistosoma mansoni* oviposition during *in vitro* culture of adult worms for a maximum period of 10 days showed three well distinct phases in the kinetics of oviposition: an initial phase with low egg production, a period of maximum oviposition and finally a progressive reduction in the number of eggs during the late phases of culture. The kinetics of oviposition and the number of eggs laid by the parasites are influenced by the number of worm pairs per amount of RPMI 1640 medium, time of parasite development in the vertebrate host and type of serum utilized in the culture medium.

KEYWORDS: *Schistosoma mansoni*; *in vitro* cultivation; Oviposition.

INTRODUCTION

The first attempts to cultivate *Schistosoma in vitro* were made at the end of the 1930's. The lack of an appropriate medium led to the use of different types of substances in an attempt to satisfy the metabolic requirements of the parasite. Some investigators were relatively successful with the use of animal sera, also in terms of *in vitro* oviposition[2,4]. SENFT & SENFT[10] were the first to use a chemically defined artificial medium. SCHILLER et al.[9], using a diphasic medium originally described for the cultivation of *Hymenolepis diminuta* were able to obtain prolonged maintenance of *Schistosoma mansoni in vitro*. After a comparative analysis of the major culture media used for the *in vitro* maintenance of *Schistosoma mansoni* MERCER[5] noted that RMPI 1640 medium, 199 medium and essential Eagle medium presented similar results in terms of oviposition rates and number of mating pairs after 30 days of cultivation.

Quantitative differences have been observed in *in vitro* oviposition when the cultures are supplemented with peripheral serum and portal animal serum. The existence of active components probably stimulating oviposition has been demonstrated in the latter, this being one of the factors that possibly influences the preference of *Schistosoma* for the portal-hepatic system[11].

However, prolonged maintenance of the parasite *in vitro* causes lesions of the tegument such as ruptures in the apices of the tubercles of the tegument, vesicle formation, the appearance of villi, and regional removal of the tegument with exposure of the underlying muscles, as shown by scanning electron microscopy[1]. Ultrastructural analysis of the reproductive apparatus showed ovary degeneration in females, a fact that may account for the rapid loss of ovipositing ability during *in vitro* cultivation[3].

(1) Department of Parasitology, Microbiology and Immunology. Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brasil.
Correspondence to: Vanderlei Rodrigues. Departamento de Parasitologia, Microbiologia e Imunologia. Faculdade de Medicina de Ribeirão Preto. Av. Bandeirantes 3900, 14049-900 Ribeirão Preto, São Paulo, Brasil. Fax: (016) 6336631.

In the present study we analyzed the conditions of *in vitro* cultivation of *Schistosoma mansoni* for short periods of time as a model to estimate the viability of the parasite, especially in terms of oviposition, for drug assays and for the study of factors involved in the maintenance of parasite pairing.

MATERIAL AND METHODS

Balb/c mice weighing 20 to 30 g were inoculated intradermally with 150 cercariae of the LE strain of *Schistosoma mansoni* and adult worms were recovered 50 and 85 days later. The mice were sacrificed by excess ether inhalation and the abdominal and thoracic cavities were opened under aseptic conditions. Parasite pairs were directly collected from the portal-hepatic system with the aid of a sterile Pasteur pipette and transferred to Petri dishes (0.2 x 15 cm) at 37°C containing RPMI 1640 medium supplemented with 20 µM HEPES, pH 7.5, penicillin (100 units/ml) and streptomycin (100 µg/ml). The parasites that did not come out spontaneously after section of the portal vein were removed by intracardiac injection of sterile saline at 37°C.

Five experimental groups with ten mice each one were used and approximately twenty adult worms were recupered per mice after perfution. Parasite pairs were gently transferred to polystyrene plates (10 x 35 mm) containing 2 ml of the above medium supplemented with fetal calf serum 10%, rabbit portal serum 10% or 20% and maintained at 37°C in the presence of 5% CO_2 (Table 1).

The viability (motility and pairing) of the parasites was observed daily and the pairs that for some reason were found to be separated were discarded. Egg production of six experimental determinations was quantified during the same period of time by manual counting under an inverted light microscope (Leitz, Diavert).

RESULTS

In vitro oviposition using one or two worm pairs per 2 ml RPMI did not show significant differences in kinetics, as shown in Figure 1. However, a marked difference in the number of eggs deposited per pair was observed, especially on the sixth and eighth days of cultivation. Characteristically, there was an initial phase during the first 2 days of cultivation in which the number of eggs was small, followed by one elevation in ovipositing rate, which was even more evident when one parasite pair per 2 ml RPMI was used. Starting on the sixth day of cultivation, when oviposition reached a maximum, there was a progressive reduction of the process that culminated in a low ovipositing rate on the tenth day of cultivation. A periodic change of medium at regular intervals (each two days) did not change the total number of eggs or the kinetics of oviposition.

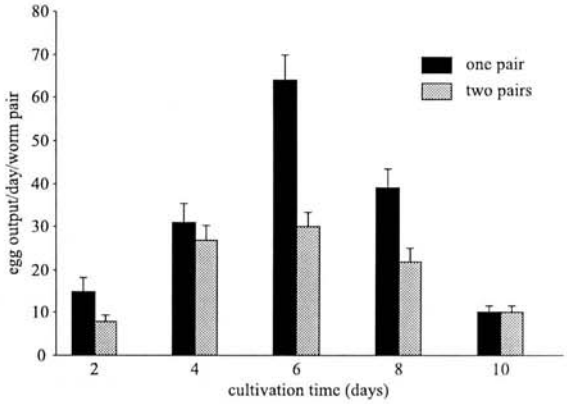

Fig. 1 – Egg-laying by *Schistosoma mansoni*. One and two pairs of adult worms were incubated *in vitro* with 2.0 ml of RPMI 1640 medium and FCS 10% (groups 1 and 3). The number of eggs was counted daily during 10 days of cultivation. Values represent number of eggs per day, means of six experimental determinations ± SD.

TABLE 1

Influence of number of worm pairs, time of parasite development and type of serum on the total amount of eggs produced during 10 days of cultivation in RPMI medium at 37°C in the presence of 5% CO_2

Experimental group	Pairs of worms in 2 ml RPMI	Perfusion time (days)	Serum* (%)	Total eggs/pair 10 days ± SD
1	Two	50	FCS 10%	98.64 ± 6.34
2	One	85	FCS 10%	74.05 ± 8.98
3	One	50	FCS 10%	161.00 ± 8.68
4	One	50	RPS 10%	189.75 ± 19.97
5	One	50	RPS 20%	171.20 ± 29.11

* FCS = Fetal Calf Serum
RPS = Rabbit Portal Serum

Throughout cultivation, the pairs stayed fully joined (the female inside the gynecophore canal of the male) and showed good motility, indicating that other, unknown factors influence the oviposition process.

Figure 2 shows the oviposition data obtained when parasite pairs were obtained after 50 and 85 days of development in the vertebrate host. The parasites with 85 days of development showed complete absence of the kinetics characteristic of the oviposition observed in the parasites with 50 days of development. Oviposition by worms with 85 days of development was zero during the first two days of cultivation, later becoming practically uniform and never exceeding 20 eggs per day throughout cultivation.

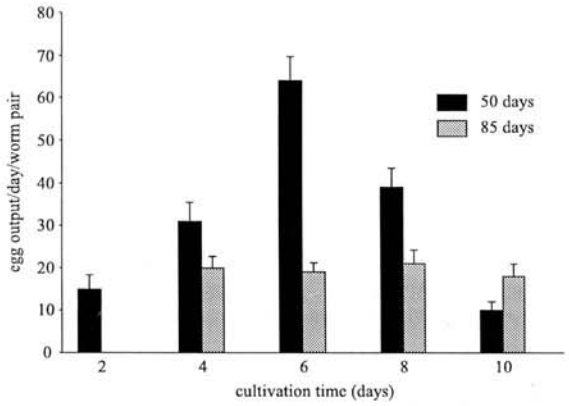

Fig. 2 – Effect of development time of *Schistosoma mansoni* on egg-laying *in vitro*. One pair of parasites with 50 or 85 days of development in a vertebrate host was maintained *in vitro* with 2.0 ml of RPMI medium and FCS 10% (groups 1 and 2). Values represent number of eggs per day, means of six experimental determinations ± SD.

The results presented in Figure 3 show that the presence of rabbit portal serum in the culture medium considerably reduces the early phase of low ovipositing rate observed with fetal calf serum. At 10% concentration in the culture medium, rabbit portal serum induced an ovipositing rate of 30 to 50 eggs per pair during the 10 days of cultivation, without the occurrence of an initial phase of low egg production during the first days of cultivation, as is the case for fetal calf serum.

When 20% rabbit portal serum was used, again, no low ovipositing rate was observed during the first two days of cultivation and the maximum ovipositing rate occurred earlier, i.e. during the first 4 days of cultivation, and was followed by a progressive reduction in oviposition until the end of the 10th day of cultivation.

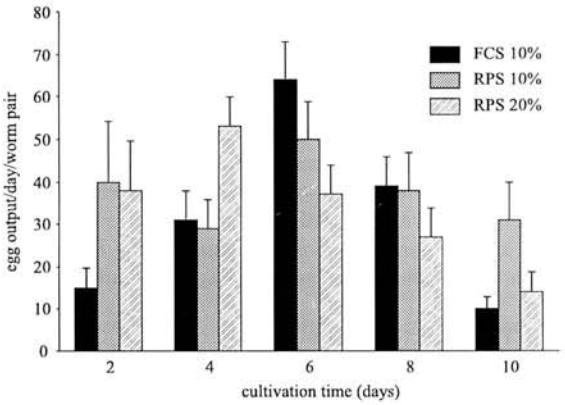

Fig. 3 – Effect of kind of serum on egg-laying by *Schistosoma mansoni*. One pair of parasites with 50 days of development in a vertebrate host was incubated *in vitro* with 2.0 ml of RPMI medium in the presence of FCS 10%, RPS 10% and 20% (groups, 3, 4 and 5). Values represent number of eggs per day, means of six experimental determinations ± SD.

In addition, the results in Table 1 show that the number of pairs per dish (2 ml RPMI) and time of parasite development in the vertebrate host had an effect on the total number of eggs produced during the 10 days of cultivation. In this case, the highest egg production was obtained for parasites with 50 days of development and cultivated at the rate of one pair per dish. With respect to the type of serum used, there was a perceptible increase in number of eggs produced in the presence of 10% rabbit portal serum, confirming a previous report about the existence of possible factors that induce oviposition in the hepatic portal system[11].

DISCUSSION

The kinetic pattern of *in vitro* oviposition (Figure 1) was similar in certain aspects to the curves obtained by other investigators, with the occurrence of 3 well distinct phases: an initial phase with low egg production, a period of maximum oviposition and finally a progressive reduction in the number of eggs produced[6, 8, 9]. In addition, a clear effect of number of pairs per culture dish on ovipositing rate was observed. A maximum rate of approximately 63 eggs was obtained on the sixth day of cultivation with one pair per dish, whereas this rate was reduced to approximately 30 eggs per pair when the dish contained two worm pairs.

Maximum oviposition by a single parasite pair occurred on the sixth day of cultivation, in agreement with the curve reported by SCHILLER et al.[9], although there is divergence about the peak of maximum oviposition. MERCER & CHAPPELL[6] have reported a maximum

peak between the third and fourth day of *in vitro* cultivation.

Previous works showed that the rate of *in vitro* oviposition is influenced by the composition of the medium and the kind of animal serum. Rates between 24.3 and 680 eggs per pair were obtained with several medium[6,7,8,9] although Mercer[5] showed that the oviposition rate was very similar during *in vitro* incubation using RPMI medium, 199 medium and essential Eagle medium.

The reduction in oviposition observed during the late phases of *in vitro* cultivation seems to be related to a degenerative process of the female reproductive apparatus, especially the ovary, which leads to a progressive loss in the ability to oviposition and to elimination of vitellin cells into the culture medium[3].

It is known that during the chronic phase of schistosomiasis there is a progressive decrease in number of eggs eliminated in the vertebrate host's feces. This fact was observed *in vitro* when the oviposition of parasites with 50 days of development in the vertebrate host was compared to that of parasites with 85 days of development (Figure 2). In the former the number of eggs was approximately 63 per pair on the sixth day of cultivation, while in the latter the number of eggs never exceeded 20 per pair.

The use of rabbit portal serum led to an earlier occurrence of the maximum ovipositing rate, markedly masking the initial phase of low egg production obtained with fetal calf serum (Figure 3). In addition, an increase in the total number of eggs obtained throughout cultivation was observed with the use of rabbit portal serum (Table 1).

WU et al.[11] have reported the presence of possible factors that only exist in portal serum, which were able to stimulate *Schistosoma mansoni* oviposition during *in vitro* cultivation. This fact may explain the preference of the parasite for the portal-hepatic venous system of its vertebrate hosts.

RESUMO

Ovoposição pelo *Schistosoma mansoni* durante o cultivo *in vitro*

A ovoposição *in vitro* do *Schistosoma mansoni* durante o cultivo de vermes adultos, durante um período de 10 dias mostrou que a cinética da ovoposição apresenta caracteristicamente 3 fases bem distintas. Uma fase inicial com baixa produção de ovos, um período de máxima ovoposição e finalmente uma progressiva queda no número de ovos nas fases tardias do cultivo.

A cinética da ovoposição, bem como o número de ovos depositados pelos parasitas são influenciados pelo número de casais por quantidade de meio RPMI 1640, como também pelo tempo de desenvolvimento do parasita no interior do hospedeiro vertebrado e finalmente pelo tipo de soro utilizado no meio de cultivo.

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REFERENCES

1. CARLISLE, S.; WEISBERG, I. & BENTLEY, A.G. – *Schistosoma mansoni*: morphologic changes induced by maintenance *in vitro*. **J. Parasit., 62**:319-334, 1983.
2. CHU, M.J. – Certain behavior reactions of *Schistosoma japonicum* and *Chlomorchis simensis in vitro*. **Clin. Med., 2**:411-417, 1938.
3. IRIE, Y.; BASCH, P.F. & BEACH, N. – Reproductive ultrastructure of adult *Schistosoma mansoni* grow *in vitro*. **J. Parasit., 69**:559-566, 1983.
4. LEE, C.V. & CHU, H.J. – Simple techniques for studying *Schistosome* worm *in vitro* **Proc. Soc. exp. Biol. (N.Y.), 32**:1397-1400, 1935.
5. MERCER, J.G. – *Schistosoma mansoni*: biological and biochemical studies on adult worms *in vitro*. Scotland, 1984. (**PhD Thesis, University of Aberdeen**).
6. MERCER, J.G. & CHAPELL, L.M. – *Schistosoma mansoni*: effect of maintenance *in vitro* on the physiology and biochemistry of adult worms. **Parasitology, 90**:339-349, 1985.
7. MICHAELS, R.M. – Mating of *Schistosoma mansoni in vitro*. **Exp. Parasit., 25**:58-71, 1969.
8. MICHAELS, R.M. & PRATA, A. – Evolution and characteristics of *Schistosoma mansoni* eggs laid *in vitro*. **J. Parasit., 54**:921-930, 1968.
9. SCHILLER, E.L.; BUEDING, E.; TURNER, V.M. & FISCHER, J. – Aerobic and anaerobic carbohydrate metabolism and egg production by *Schistosoma mansoni in vitro*. **J. Parasit., 61**:385-389, 1975.
10. SENFT, A.N. & SENFT, D.G. – Chemically defined medium for the maintenance of *Schistosoma mansoni*. **J. Parasit., 48**:551-554, 1962.
11. WU, G.; WU, C.H.; DUNN, M.A. & KAMEL, R. – Stimulation of *Schistosoma mansoni* oviposition *in vitro* by animal and human portal serum. **Amer. J. trop. Med. Hyg., 34**:750-753, 1985.

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