Rev. Inst. Med. trop. São Paulo
21(3):115-118, *maio-junho*, 1979

UDC 616.995.122

CULTIVATION OF SCHISTOSOMA MANSONI CERCARIAL BODIES TO ADULT WORMS

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SUMMARY

Cercarial bodies of **Schistosoma mansoni** develop to adult forms **in vitro** at the same rate reported for schistosomules. When compared with worms obtained from mice the initial rate of development of both is the same but it becomes slower at later stages.

INTRODUCTION

The cultivation of **S. mansoni** cercarial bodies has been previously reported (COLLEY & WIKEL[3]; TIBA et al.[7]). In this paper, the pattern of development of cercarial bodies to adult worms **in vitro** is described.

MATERIALS AND METHODS

Cercarial bodies were prepared as follows (RAMALHO-PINTO et al.[6]): 10 ml-aliquots of concentrated intact cercariae were pipetted into 15-ml glass conical centrifuge tubes and cooled in an ice bath for 10 min to reduce motility of the organism. Following centrifugation at low speed for 1 min the supernatant fluid was decanted. The packed cercariae (10,000-20,000) were suspended in 2 ml of cold sterile Earle's balanced saline containing 0.5% lactalbumin hydrolysate, 200 units/ml of penicillin, 200 μg/ml of streptomycin (Elac) and whorled for 45-60 sec in a Vortex Jr. mixer (Scientific Industries Inc., Queens Village, N.Y.). Isolation of cercarial bodies from tails was accomplished by transferring a 2.0-ml suspension to another glass conical centrifuge tube containing 8.0 ml of Elac; after 3 min at room temperature the tail-rich supernatant was decanted off and the sedimented bodies were washed 3 times Elac. The bodies thus obtained were transferred to a culture medium (200 organisms/ml) consisting of Elac plus 50% heat-inactivated rabbit serum, and 1% rabbit red cells (CLEGG[2]) and maintained in this medium in 20-ml screw-top tube at 37°C in an atmosphere of 5% CO_2-95% air. The medium was replaced every 3-4 days when the parasites were observed and counted in an inverted microscope at 100 x magnification.

Morphological criteria as described by BARBOSA et al.[1] were used to classify the parasites in relation to their stage. For technical facilities the gastric caecum shape was chosen according to FAUST et al.[4]: **stage 1** — schistosomula presenting only a light stain which stands for the beginning of the caecum; **stage 2** — a darker stain now bifurcating but not bypassing the acetabulum; **stage 3** — the dark stain bypasses the acetabulum and its branches link themselves later on: **stage 4** — the dark bifurcated stain after reconnection grows to the parasite end, but not longer than the bifurcated caecum; **stage 5** — the final linked caecum grows longer than its bifurcated section, but shorter than three times of its lenght; **stage 6** — their linked caecum grows three times longer than the bifurcated caecum (mature adults). Proteolytic activity and protein assays were performed in the extracts as previously described (GAZZINELLI et al.[5]).

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This work was supported by CNPq, SIP 08-099

RESULTS

First of all an attempt to increase the recovery of the body was made: mixed cercarial bodies and tails were sedimented for a longer period of time and this procedure repeated 2-3 times. As can be seen in Table I this manipulation increased the death rate in the culture medium. By the standard procedure as described in Materials and Methods the bodies presented a mortality rate of 3.44%/day during the first 10 days which fell to 0.81%/day on subsequent days (Fig. 1). Under these conditions more than 90% parasites become water sensitive in about 2 hr. However, the elimination of the preacetabular glands as measured by the residual proteolytic activity was completed only about four days later (Fig. 1 insert). This did not seem to interfere with the development rate of the parasite for as shown in Table II the initial rate of development was the same **in vitro** and in the white mouse, when identical criteria were used to classify the stages of the parasites. Addition of 0.6% of linolenic, or linoleic acid to the culture media did not stimulate the glandular secretion.

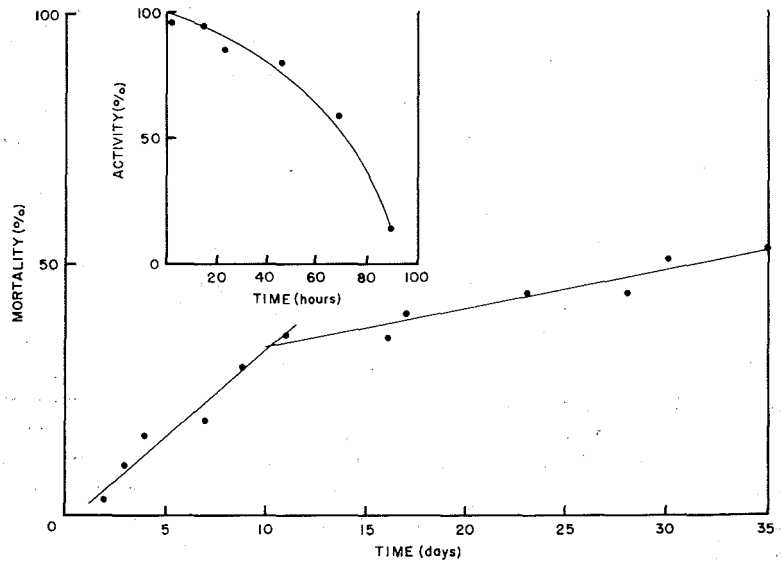

Fig. 1 — Mortality rate of cercarial bodies in culture media. The points represent the mean of ten tubes containing about 200 cercarial bodies each at day zero. Insert: remnant proteolytic activity in cercarial bodies cultured under the same conditions.

TABLE I

Death rate of cercarial bodies during the first four days in culture media

Cercarial bodies preparations (*)	Percent mortality on day			
	1	2	3	4
Three min sedimentation (1 time)	3.0	4.1	4.9	7.1
Ten min sedimentation (3 times)	2.0	7.8	18.2	26.0

(*) Cercarial suspensions were agitated in a vortex mixer and the bodies separated from the tail by gravity sedimentation

In vitro development as compared to **in vivo** took place at the same rate up to the stage 2 and then slowed down so that only 33% parasites reached the stage 4 after 34 days in the culture. At this time 69% of the parasites have already attained maturity (stage 6) in the mouse (Table II). This pattern of development was highly reproducible if conditions such as pH and number of bodies per tube were maintained strictly constant. After 50 days of experiment half of the parasites were still alive although assynchronic growth and development were always observed.

TABLE II

Development of **S. mansoni** cercarial bodies **in vitro** compared with optimum development in the white mouse

Days	Stages	PERCENT OF LIVING PARASITES											
		1		2		3		4		5		6	
		in vivo(*)	**in vitro**	**in vivo**	**in vitro**	**in vivo**	**in vitro**	**in vivo**	**in vitro**	**in vivo**	**in vitro**	**in vivo**	**in vitro**
7		100	2	0	98	0	0	0	0	0	0	0	0
9		67	0	33	100	0	0	0	0	0	0	0	0
13		14	0	32	82	50	18	4	0	0	0	0	0
22		5	0	9	31	19	51	56	18	11	0	0	0
34		0	0	1	19	3	46	6	33	30	2	60	0
46		0	0	3	0	1	0	3	0	13	0	80	0
49		0	0	0	4	0	36	0	40	0	20	0	0

(*) Data obtained from BARBOSA et al.[1] for the same strain of parasites used in this work (L.E. strain, Belo Horizonte)

DISCUSSION

Schistosoma mansoni can be cultured **in vitro** from cercarial bodies to a stage of development at which worms are nearly sexually mature. The preparation of the bodies is very simple and they can be obtained in a large amount. Cercarial bodies developed **in vitro** at the same rate and to same stage as those reported by CLEGG[2] for schistosomula. A deviation from the optimum growth rate observed for schistosomules in the mouse has also been noted when dealing with cercarial bodies (Table II). Since the preparation of cercarial bodies is simpler than of schistosomula it might be adventageous to use cercarial bodies for culture studies.

RESUMO

Desenvolvimento em cultura de corpos cercarianos do Schistosoma mansoni

Estudou-se o desenvolvimento de corpos cercarianos **in vitro.** O comprimento do ceco gástrico, de fácil observação, foi utilizado como critério para medida do desenvolvimento. Constatou-se que rapidamente os corpos cercarianos se transformam em esquistossômulos a julgar pelo aparecimento da sensibilidade a soluções hipotônicas e pela eliminação da atividade proteolítica das glândulas cefálicas. Verificou-se, ainda, que o desenvolvimento dos corpos cercarianos são assincrônicos, seguindo o mesmo curso de desenvolvimento do esquistossômulo cultivado **in vitro.** Por outro lado, ambos, corpos cercarianos e esquistossômulos, sofrem um desvio do crescimento ótimo quando comparados com o parasita desenvolvido no camundongo.

ACKNOWLEDGEMENTS

We thank José de Souza Filho and Rita de Cassia Soares for technical assistance.

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Recebido para publicação em 22/9/1978.