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ISOLATION AND MAINTENANCE OF A STRAIN OF ENTAMOEBA HISTOLYTICA SCHAUDINN, 1903 AND THE POSSIBILITY OF ITS UTILIZATION IN SCREENING OF DRUGS

Geraldo CHAIA (1), Edward Felix da SILVA (2), Célia Carvalho VILELA (1) and Lourenço CHIARI (1)

SUMMARY

A strain of **Entamoeba histolytica** was isolated from a patient suffering from acute intestinal amebiasis and the degree of virulence of this strain was studied in some rodents. Infection rates of 96 and 100% were observed on the 8th day after the infection in the unfed rats and hamsters with cecum traumatized. The subcutaneous infection rates obtained in hamsters, rats and mice, on the 5th day after the infection were 100, 50 and 48% respectively. From 392 hamsters submitted to intrahepatic infection 391 became infected (99.9%). The Authors discuss and conclude that hamsters with intrahepatic infection are the suitable animals for the preliminary screenings of drugs in laboratory.

INTRODUCTION

Many strains of **Entamoeba histolytica** have been isolated an used in experimental work under laboratory condition. Several researchers, among them ATCHLEY [1], CHIANG [3], TSUCHIYA [20], JONES [6], NEAL [10], PHILIPS [11], REINERTSON & THOMPSON [15], SARKISYAN [16], RAETHER [13], WITTNER & ROSENBAUM [21], tried to infect animals in order to observe the degree of virulence of those strains.

In the present paper the Authors isolated a strain of **Entamoeba histolytica,** observed its degree of virulence in infections of hamsters, mice and rats and point out the possibility of using the above mentioned strain in screening of drugs for the therapy of amebiasis.

MATERIAL AND METHODS

a) **Isolation of a strain of Entamoeba histolytica** — This strain was isolated in Belo Horizonte, Minas Gerais, Brazil, from a 28 year old woman suffering from severe dysentery with approximately 15-20 daily evacuations.

The rectosigmoidoscopy revealed ulcerations in the rectosigmoid. No extra intestinal amebic infections were observed through other routine tests. The material obtained from intestinal ulcerations, containing a large amount of trophozoites with active motility, was placed into a test tube with 10 ml of PAVLOVA [12] medium plus rice-starch (modified by JONES[6], DE CARNIERI[4], SILVA [17]) and stored at 36.5°C (± 0.5°C).

This strain of ameba that was designated Eh 1 was associated to **Escherichia coli** and has shown successful development and has been maintained under laboratory condition for three years with sucessive passages every 48 hours.

b) **Experimental infection in laboratory animals** — b.1 — **Cecal infection** — 251 Wistar rats (90-100 g) and 261 **Mesocricetus auratus** hamsters (60 g) of both sexes were individually infected with an average of 100,000 trophozoites. The rats and hamsters were divided into groups of fed and unfed (during 24 hours before the infection).

(1) Research Institute Johnson & Johnson for Endemic Diseases P.O. Box 136, 01515 São Paulo, S.P., Brazil
(2) Department of Zoology and Parasitology — Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, M.G., Brasil

I — **Unfed animals** — Of 145 rats, 85 (group A) and of 141 hamsters, 96 (group E) had the cecum traumatized. The remaining rodents, 60 rats (group B) and 45 hamsters (group F) were normally infected without trauma in the cecum.

II — **Fed animals** — Of 106 rats, 55 (group C) and of 120 hamsters, 60 (group G) had also the cecum traumatized. The remaining animals, 51 rats (group D) and 60 hamsters (group H) were normally infected without traumatization in the cecum.

Both groups of fed and unfed animals were subdivided into 3 groups that were sacrificed on the 4th, 8th and 15th days after the infection (Tables I and II).

b.2 — **Hepatic infection** — 392 hamsters (60-80 g) of both sexes were inoculated in the left hepatic lobule with approximately 40,000 trophozoites. The animals were mechanically sacrificed and examined five days after the infection.

b.3 — **Dorsal infection** — 70 randomly bred mice (20 g), 40 rats (100 g) and 80 hamsters (60 g) of both sexes, were subcutaneously infected in the dorsum with an average of 30,000 trophozoites. These animals were subdivided into two groups that were slaughtered respectively on the 5th and 15th days after the infection (Table III).

c. **Procedures** — c.1 — **Preparation of rice starch suspension** — Rice starch (Matarazzo) was dessicated during an hour daily for three consecutive days at 100°C. Two hundred and seventy ml of the modified PAVLOVA [12] medium were added to 6 g of rice starch.

TABLE I

Results obtained in rats intracecally infected with 100,000 trophozoites of a virulent strain of **Entamoeba histolytica**

GROUP	Sub-group	Condition at time of infection: animals	Condition at time of infection: cecum	Days after infection	Animals: Number submitted to infection	Animals: Number dead	Examination: Presence of lesion		Examination: Presence of trophozoites	
A	a.1	Unfed	t	4	10	0/10	9/10	(90%)	9/10	(90%)
	a.2			8	55	5/55	49/50	(98%)	48/50	(96%)
	a.3			15	20	0/20	7/20	(35%)	11/20	(55%)
B	b.1	Unfed	w.t	4	20	0/20	0/20	(0%)	7/20	(35%)
	b.2			8	20	3/20	0/17	(0%)	4/17	(23%)
	b.3			15	20	4/20	0/16	(0%)	5/16	(31%)
C	c.1	Fed	t	4	20	0/20	8/20	(40%)	11/20	(55%)
	c.2			8	20	4/20	2/16	(12%)	9/16	(56%)
	c.3			15	15	2/15	2/13	(15%)	7/13	(54%)
D	d.1	Fed	w.t	4	18	4/18	2/14	(14%)	8/14	(57%)
	d.2			8	18	2/18	1/16	(6%)	7/16	(44%)
	d.3			15	15	3/15	5/12	(42%)	6/12	(50%)

t = with trauma

w.t. = without trauma

TABLE II

Results obtained in hamsters intracecally infected with 100,000 trophozoites of a virulent strain of **Entamoeba histolytica**

GROUP	sub-group	Condition at time of infection: animals	Condition at time of infection: cecum	Days after infection	Animals: Number submitted to infection	Animals: Number dead	Examination: Presence of lesion	Examination: Presence of trophozoites
E	e.1	Unfed	t	4	67	11/67	54/56 (96%)	51/56 (91%)
	e.2			8	18	5/18	10/13 (77%)	13/13 (100%)
	e.3			15	11	5/11	1/6 (17%)	4/6 (67%)
F	f.1	Unfed	w.t.	4	18	0/18	15/18 (83%)	16/18 (89%)
	f.2			8	16	0/16	4/16 (25%)	5/16 (31%)
	f.3			15	11	0/11	0/11 (0%)	0/11 (0%)
G	g.1	Fed	t	4	20	0/20	8/20 (40%)	7/20 (35%)
	g.2			8	20	1/20	5/19 (26%)	8/19 (42%)
	g.3			15	20	2/20	3/18 (16%)	7/18 (39%)
H	h.1	Fed	w.t	4	20	0/20	5/20 (25%)	7/20 (35%)
	h.2			8	20	0/20	4/20 (20%)	5/20 (25%)
	h.3			15	20	4/20	1/16 (6%)	6/16 (37%)

t = with trauma
w.t. = without trauma

TABLE III

Results obtained in mice, rats and hamsters infected in dorsum with 30,000 trophozoites of a virulent strain of **Entamoeba histolytica**

GROUP	sub-group	animals	Days after infection	Animals: Number submitted to infection	Animals: Number dead	Examination: Presence of lesion	Examination: Presence of trophozoites
I	i.1	mice	5	50	0/50	50/50 (100%)	24/50 (48%)
	i.2		15	20	0/20	16/20 (80%)	6/20 (30%)
J	j.1	rats	5	20	0/20	20/20 (100%)	10/20 (50%)
	j.2		15	20	0/20	19/20 (95%)	4/20 (20%)
K	k.1	hamsters	5	40	0/40	40/40 (100%)	40/40 (100%)
	k.2		15	40	6/40	32/40 (80%)	24/40 (60%)

c.2 — **Obtaining and counting of trophozoites** — Three days before the infection of the rodents trophozoites from 3 maintenance tubes were transferred to three erlenmeyers (125 ml) containing each vial 50 ml of the modified PAVLOVA [12] medium plus 1 ml of the starch suspension and maintained at 36.5°C (± 0.5°C). Three days later the erlenmeyers were manually shaken and the medium was distributed into centrifuge tubes (15 ml), then they were centrifuged at 1,100 rpm for 6 minutes. The supernatant was discarded and the sediment of the tubes containing trophozoites was collected and poured in one single vial. Six samples of 0.005 ml of this sediment containing trophozoites were placed in individual slides covered with cover slips (18X18) and counted under a microscope (X 400) thus providing the mean number of trophozoites in each 0.005 ml sample.

c.3 — **Infection of the animals** — The trophozoites were inoculated with an insuline syringe (1 ml) with a BD 10.5 needle. The maximum volume inoculated in each animal was 0.005 ml in the liver and 0.1 ml in the cecum or dorsum. The operation site was prepared by cleasing with alcoholic iodine solution (4%) before and after infection. The infections were induced by direct injection either into the left hepatic lobule or cecum following laparotomy under ether anesthesia using the technique described by REINERTSON & THOMPSON [15].

The body wall was fastened with a stapling machine (RET-LIT MO13). The dorsal infection was carried out by subcutaneous injection.

c.4 — **Examination of the animals** — The material for examination was obtained by scrapping ulceration of dorsum and cecum or amebic hepatic abscess by means of a cover slip (18X18), and placed on a slide containing saline (0.8%) at 37°C, covered with a cover slip (18X18) and then examined under a microscope (X 100), being the motility of the trophozoites easily observed. In the hepatic infection the material was collected from the area surrounding the division between the normal tissue and the abscess. The presence of lesions produced in dorsum, cecum and liver were scored from macroscopic examination.

RESULTS

I — **Cecal infection** — As it can be observed in the groups of unfed animals, either traumatized or not, the infection rates of the rats were respectively 90, 96, 55% and 35, 23 and 31%, of hamsters were 91, 100 and 67% and 89, 31 and 0%. In the groups of fed traumatized and untraumatized the infection rates of rats were respectively 55, 56, 54% and 57, 44 and 50%, and of hamsters were 35, 42 and 39% and 35, 25 and 37%. These results are summarized on Tables I and II.

II — **Hepatic infection** — 391 out of 392 hamsters inoculated became infected thus obtaining an infection rate of 99.7%.

III — **Dorsal infection** — The infection rates obtained in mice were 48 and 30%, in rats 50 and 20% and in hamsters 100 and 60%. The animals were sacrificed respectively on the 5th and 15th day after the infection. These results are summarized on Table III.

DISCUSSION

Many researchers have been concerned about the complex problem of experimental infections of laboratory animals with different strains of **Entamoeba histolytica** for a long time. ATCHLEY [1], CHIANG [3] and TSUCHIYA [20], were able to produce only a low grade infection in cecum of rats. ELLEMBERG [5], suggested that symbiosis with bacteria may be essential to amebic pathogenicity. PHILIPS [11] reported the essential participation of bacteria in the etiology of amebiasis in experimentally infected guinea pigs. SARKYSIAN [16] related that **Escherichia coli** had not had any influence in the course of infection in rats but when **Bacillus subtilis** and **Clostridium perfringens** were associated to this strain there was a significant increase in the infection of these animals.

NEAL [9] concluded that bacterias from a virulent strain when associated to an avirulent one did not modify the virulence of this strain. RAETHER [13] was able to obtain high infection rates in hamsters by intrahepatic route using a monoxenic strain (ameba-crithidia). Nevertheless, CHAIA [2] in spite of all his efforts was not able to infect this type of rodent with this strain. WITTNER & ROSEN-

BAUM [21] inoculated ameba from axenic cultures into the liver of hamsters and they did not produce amebic hepatitis; however, the researchers were successful when they associated bacterias to this strain. NEAL [10] reports that strain isolated from acute relapsing cases are all virulent to rats, but those from contact carriers may be virulent or avirulent. RAO & PADMA [14] demonstrated that strains isolated from patients either symptomatic or symptomless could be virulent or not. NEAL [8] reported that animals submitted to a diet defficient in vitamins had an increase in the severity of the lesions. SINGH, SRIVASTAVA & DUTTA [18] observed that when cholesterol was added to a culture of **Entamoeba histolytica** the avirulent strains became virulent. SARKYSIAN [16] stated that it is important to activate the intestinal tract of the animals at the time of infection. PHILIPS [11] obtained higher infection rates in guinea pigs when the cecum of these animals was traumatized. On the other hand JONES [7] carried out inoculations without traumatizing the tissues and the number of cecal lesions was markedly reduced.

As it can be observed many attempts have been made in order to obtain better results in the experimental infections of animals and for this reason the Authors decided to observe the behaviour of a new strain in different types of rodents using different inoculation routes. Tables I and II show that virtually all the unfed and traumatized rats (group A) and hamsters (group E) presented higher rates of cecal infection and cecal lesions. On the other hand there was practically no difference in results obtained among the groups of fed animals either traumatized or not. Perhaps this is due to the following: a) in the fed animals the cecum remains full of feces thus causing difficulties in irritating the intestinal epithelium by means of mechanical traumatization; b) the traumatization could be performed in a stronger manner but then there would be the risk of perforating the cecum thus inducing peritonitis and consequently the animal's death. Therefore based on results obtained the Authors stress the necessity of traumatizing the intestine in order to obtain higher infection rates with this strain.

The experimental animals should present the following characteristics in order to facilitate the routine work for the laboratory technician on screening for active drugs: I) to be highly susceptible to infection; II) the lesions formed have to be highly visible and should contain a large amount of trophozoites. However, this did not happen in hamster and rats either dorsally or cecally inoculated although high infection rates were obtained.

On the other hand, hamsters when intrahepatically infected become the animals of choice because this rodents besides presenting amebic hepatic abscess easily visible (Fig. 1) they are also highly susceptible to infection

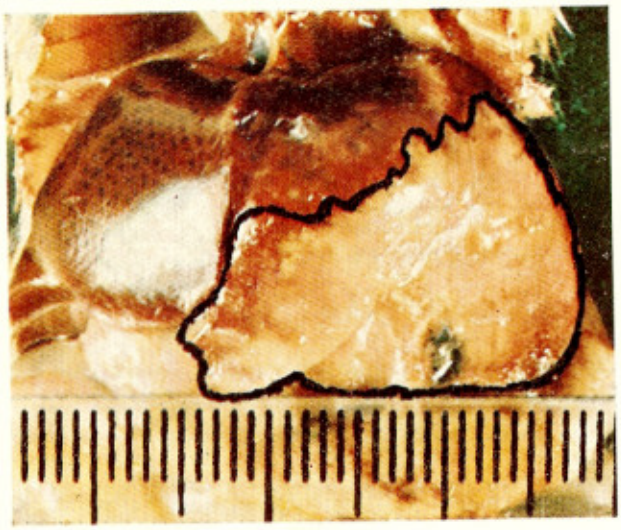

Fig. 1 — Amebic hepatitis in hamster 5 days after intrahepatic injection with 40,000 amebae

(99.7%) and another fact that confirms the suitability of this animal for the screening of new drugs was described by THOMPSON [19] who verified that drugs of known amebicide activity were highly effective in suppressing amebic hepatitis in hamsters.

RESUMO

Isolamento e manutenção de uma cepa de Entamoeba histolytica **Schaudinn, 1903 e a possibilidade de seu uso para seleção de drogas.**

Uma cepa de **Entamoeba histolytica** foi isolada de uma paciente com 28 anos, portadora de amebíase intestinal aguda, sofrendo de severa disenteria com aproximadamente 15 a 20 evacuações diárias. O grau de virulência da referida cepa foi estudado em roedores (ratos, hamsters e camundongos). Percentuais de infecção (96 a 100%) foram observados no 8.º dia após a infecção em hamsters e ratos, com o ceco traumatizado e mantidos em jejum 24 horas antes da infecção. As porcentagens de infecção subcutânea obtidas em hamsters, ratos e camundongos, no 5.º dia após a infecção foram de 100, 50 a 48 respectivamente. Dos 392 hamsters que foram submetidos a infecção, intra-hepática, 391 infectaram-se (99,9%).

Os Autores discutem e concluem que o hamster com infecção intra-hepática é o animal de escolha para seleções preliminares de drogas.

REFERENCES

1. ATCHLEY, F. O. — Experimental infections of rats with **Endamoeba histolytica. Amer. J. Hyg. 23**: 410, 1936 **in** JONES, W. R. — The experimental infection of rats with **Entamoeba histolytica**; with a method for evaluation the anti-amoebic properties of new compounds. **Ann. Trop. Med. Parasit. 40**: 130-140, 1946.

2. CHAIA, G. — Unpublished data 1971.

3. CHIANG, S. F. — The rats as possible carrier of the dysentery amoeba. **Proc. Nat. Acad. Sci. Wash. 11**: 399, 1925 **in** JONES, W. R. — The experimental infection of rats with **Entamoeba histolytica**; with a method for evaluation the anti-amoebic properties of new compounds. **Ann. Trop. Med. Parasit. 40**: 130-140, 1946.

4. DE CARNERI, I. — **Entamoeba invadens** Rhodain, 1934 ed **Entamoeba histolytica** Schaudinn, 1903; Studio comparato della sensibillita **in vitro** a 16 farmaci; e problemi connessi con l'uso della prima come modello per ricerche di chemioterapia dell' amebiase. **Riv. Parassit. 18**: 133-153, 1957.

5. ELLEMBERG, M. — Amoebiasis: the role of bacteria in Symptomatology. **Amer. J. Dig. Dis. 13**: 356-360, 1946.

6. JONES, W. R. — Experimental attempt to induce drug resistance in **Entamoeba histolytica. J. Exp. Parasit. 1**: 118-128, 1952.

7. JONES, W. R. — The experimental infection of rats with **Entamoeba histolytica** with a method for evaluating the anti-amebic properties of new compounds. **Ann. Trop. Med. Parasit. 40**: 130-140, 1946.

8. NEAL, R. A. — The pathogenicity of **Entamoeba histolytica. Proc. 6th Int. Congr. Trop. Med. Malar. 3**: 5-13, 1958.

9. NEAL, R. A. — Virulence in **Entamoeba histolytica. Trans. Roy. Soc. Trop. Med. Hyg. 45**: 313-319, 1957.

10. NEAL, R. A. — Some observations on the variation of virulence and response to chemotherapy of strains of **Entamoeba histolytica** in rats. **Trans. Roy. Soc. Trop. Med. Hyg. 44**: 439-452, 1951.

11. PHILIPS, P. B. — Studies on the amoeba-bacteria relationship in amebiasis. III. Induced amebic lesions in the germfree guinea pig. **Amer. J. Trop. Med. & Hyg. 13**: 391-395, 1964.

12. PAVLOVA, E. A. — Sur les methodes de la culture d'**Entamoeba histolytica. Med. Parasit. Dis.** (Moscow) **7**: 224-227, 1938 (in Russian). French Summary **in Trop. Dis. Bull. 36**: 286, 1939.

13. RAETHER, W. — Intrahepatikale Infectioversuche am Goldhamster mit **Entamoeba histolytica** — crithidia — Kulturen mit und ohne Beteilung von Bakterien. **Ztschr. Parasitkde 36**: 335-345, 1971 English Summary **in Trop. Dis. Bull. 68**: 1065, 1971.

14. RAO, V. G. & PADMA, C. M. — Some observations on the pathogenicity of strains of **Entamoeba histolytica. Trans. Roy. Soc. Trop. Med. Hyg. 65**: 606-616, 1971.

15. REINERTSON, J. W. & THOMPSON, P. E. — Experimental amebic hepatitis in hamsters. **Proc. Soc. Exp. Biol. & Med. 76**: 518-521, 1951.

16. SARKISYAN, M. A. — On the role of bacteria in experimental amoebiasis. **Medskaya Parazit. 39**: 671-674, 1970. English Summary **in Trop. Dis. Bull. 68**: 583, 1971.

17. SILVA, E. F. — Estudos da **Entamoeba moshkovskii**. II — Novos focos difundidos em áreas hídricas do Brasil e Uruguai. **Rev. Inst. Med. trop. São Paulo 16**: 203-221, 1974.

CHAIA, G.; SILVA, E. F. da; VILELA, C. C. & CHIARI, L. — Isolation and maintenance of a strain of **Entamoeba histolytica** SCHAUDINN, 1903 and the possibility of its utilization in screening of drugs. **Rev. Inst. Med. trop. São Paulo 19**:195-201, 1977.

18. SINGH, B. N.; SRIVASTAVA, R. V. M. & DUTTA, G. P. — Virulence of strains of **Entamoeba histolytica** to rats and the effect of cholesterol, rat cecal and hamster liver passage on the virulence of non invasive strain. **Indian J. Exp. Biol. 9:** 21-27, 1971.

19. THOMPSON, E. P. — Chemotherapy of amebic hepatitis in hamsters with emetine, chloroquine, amodiaquin (camoquin), quinacrine and other drugs. **Amer. J. Trop. Med. 31:** 707-717, 1951.

20. TSUCHIYA, H. — Experimental amebiasis in rats with cysts of human carriers with especial reference to a probable mechanism involved. **Amer. J. Trop. Med. 19:** 151, 1939 in NEAL, R. A. — The duration and epidemiological significance of **E. histolytica** infections in rats. **Trans. Roy. Soc. Trop. Med. Hyg. 45:** 363-370, 1951.

21. WITTNER, M. & ROSENBAUM, M. — Role of bacteria in modifying virulence of **Entamoeba histolytica** — Studies of amebae from axenic cultures. **Amer. J. Trop. Med. Hyg. 19:** 755-761, 1970.

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