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Influence of pH on the growth rates of *Entamoeba histolytica* in cultures

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The growth rates of Entamoeba histolytica isolates were studied in two diphasic, one monophasic, and one synthetic culture media. The effect of varying pH on the growths of E. histolytica was measured. Dobell and Laidlaw's modified medium was found to be the most suitable for the crude cultivation of E. histolytica. The longest survival period and highest growth rates were observed in Hallman, Michaelson and Delamater's synthetic medium. The optimal pH was found to be 6.5. Little evidence of propagation was found at pH 7.5 and none at PH 8.0.

INTRODUCTION

The in vitro cultivation of Entamoeba histolytica was developed by Boeck and Drbohlav (1) using inspissated whole egg slants with serum-glucose-Locke-egg solution. Dobell and Laidlaw (2) conducted a detailed study on the growth of E. histolytica and other amoebae from human in Boeck and Drbohlav's medium (1) substituting rice starch for glucose. Most of the media developed since the work of Dobell and Laidlaw (2) contain rice starch to promote optimal growth of E. histolytica and other amoebae. In 1952, Shaffer (3) studied the nature of some factors that affect the propagation of E. histolytica and finds that pH plays an important role for the growth of amoebae. In the present paper, the rate of growth of different isolates of E. histolytica in different culture media adjusted to specified pH levels has been studied to have a better understanding of pH in the cultivation of E. histolytica.

MATERIALS AND METHODS

Experimental design

The first phase of the experiment was to test the growth rates of five E. histolytica isolates in four different culture media to assess the suitability of the media. Each of the five isolates was inoculated into three tube containing similar culture medium viz. Dobell and Laidaw (2), Hallman et al. (4), Pavlova (5) and Rao (6). Growth aliquots were sampled every 24 hour and the number of amoebae counted in a Fuchs-Rosenthal counting chamber. Triplicate readings taken at each interval were averaged. After the selection of media and isolates for the experiment proper, the rate of propagtion was measured at seven pH levels. Amoebae were counted at 12-hour intervals upto 72 hours.

Strains of amoebae

Five E. histolytica isolates of human origin were studied. They were obtained from (i) human cases of amoebic dysentery (Isolates AD1, AD2, AD3, AD4) and (ii) a human case of amoebic vaginitis (Isolate AV1).

Culture methods

Two diphasic media (2,6), one monophasic medium (5) and one synthetic medium (4) were used in the study.

Technique for subculturing

After warming the media at 37°C for 30 min., inoculations were made from the sediment of a positive culture using a Pasteur pipette. Approximately 1 ml aliquots were deposited slowly at the bottom of each fresh medium tube avoiding the transfer of bubbles.

Counting of amoebae

In order to assess the growth rate of amoebe inculture, a method of estimating the number of amoebae at different intervals was necessary. The Fuchs-Rosenthal counting chamber, usually employed for enumerating white blood cells in cerebro-spinal fluid, was used. This method was found by Griffin and McCarten (7) to be more accurate than the original method described by Paulson (8). After inverting the culture three to five times in order to obtain an homogenous suspension of the organisms, a sample was removed from the mid-zone of the liquid and deposited in the two sections of the counting chamber. Each section has 16 squares with a total area of 16 sq. mm and a depth of 0.2 mm. The amoebae in each section were counted separately, and then the chamber was cleaned, refilled and the count repeated. The formula used for the calculation of the amoebae per cubic mm. is shown as follows:

$$A+B+C+D \times \frac{5}{64} = \text{number of amoebae/mm}^3$$

where A,B,C,D = number of organisms counted in each section

$$5 = \frac{1}{0.2} \text{ mm}$$

64 = 16 x 4 squares

Throughout the experiment, the inoculum size, the amount of starch and the age of the stock cultures were kept as constant as possible, for these factors have been found to be the major sources of variation in cultures of entozoic amoebae (7).

Adjustment of pH

The pH of culture media were adjusted to appropriate level with 1N NaOH or 1N HCl using a Beckman pH meter Model 72. One set of media was adjusted to pH 5.0, the second to 5.5, and so on at increments of 0.5, to the seventh set which was adjusted to pH 8.0. The supernatant fluid from each tube was used to make up a series of 9 tubes of culture medium. Each of the resulting 63 tubes of the medium was then inoculated with a standard number of amoebae contained in 1.0 ml of the fluid medium. The inoculum was standardized by the method of Shaffer (3) with one minor modification, that is using a 48-hr culture of the appropriate isolate of amoeba diluted to provide approximately 2,000 amoeba/ml of the inoculated medium. The pH of one culture of each series was check-measured immediately and the remainder layered with vaseline and incubated at 37°C. Counts of the amoebae were done thereafter on the representative cultures of each series at 12-hr intervals upto 72 hours. The pH of each culture tube was also check-measured immediately after the count.

RESULTS AND DISCUSSION

The growth rates of E. histolytica isolates in different culture media are shown in Table 1. The greatest number of organisms was found in Dobell and Laidlaw's modified medium, while Rao's (6) medium gave the lowest number. It was also found that the growth rate differed with the isolates and the medium used. The highest growth rate was found in isolate AD4 with Dobell's medium, the lowest rate in isolate AV1 with Rao's medium and intermediate results with Hallman's (4) synthetic and Pavlova's (5) monophasic media. Harinasuta and Harinasuta (9) grew three strains of E. histolytica in Locke-egg serum (10), liver infusion agar (11), inspissated horse serum (2) and aqueous egg-yolk infusion (12). They observed

Table 1. The rates of growth of E. histolytica in four different culture media

Isolate	Time (hr)	Trophozoites/mm^3			
		Dephasic media		Monophasic Synthetic	
		Dobell & Laidlaw[2]	Rao[6]	Pavlova[5]	Hallman et al.[4]
AD1	0	20	20	20	20
	24	50	30	40	45
	48	130	65	75	95
AD2	0	20	20	20	20
	24	45	30	35	40
	48	125	60	75	80
AD3	0	20	20	20	20
	24	55	35	45	50
	48	135	80	100	115
AD4	0	20	20	20	20
	24	60	40	50	55
	48	160	85	115	125
AV1	0	20	20	20	20
	24	45	35	40	40
	48	120	70	95	100

that all strains grow best in Boeck and Drbohlav's medium, moderately in Dobell and Laidlaw's (2) medium and very poorly in Balamuth's (12) egg-yolk infusion medium. In 1961, Botero(13) studied the rate of growth of E. histolytica (p-A[1] strain) in six different culture media. He also find that the growth cycle differs with the medium used; the greatest number of organisms being obtained with Dobell and Laidlaw's (2) medium, a finding supported by the present work.

The growth curve of E. histolytica isolate AD4 is shown in Fig 1. It is seen that the longest period of survival was obtained in Hallman's (4) synthetic medium and the shortest in Pavlova's(5) monophasic medium. The highest amoeba count was obtained in Dobell's medium (2) after 48hr. The longest survival time of amoebae in Hallman's synthetic

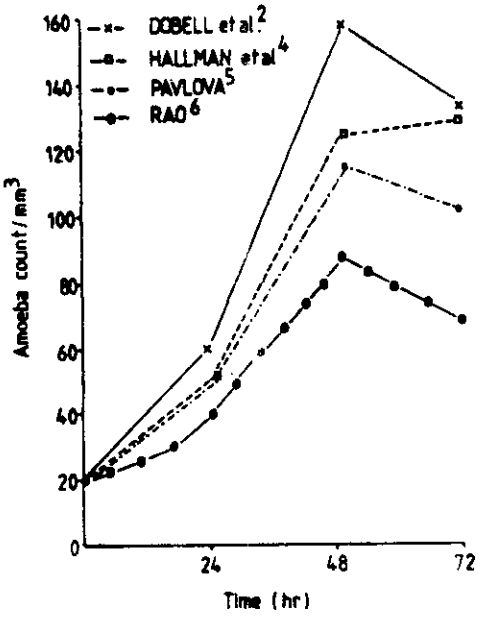

Fig. 1. The growth curves of E. histolytica isolate AD4 in four different culture media

medium might be due to the absence of serum and the substitution of amino acids and vitamins; Amoebae propagate well in this medium with a very good survival time and subcultivations could therefore be made at 96-hr intervals. Pavlova's monophasic medium has been used only effectively to transport material containing Trichomonas vaginalis from women. The growth of amoebae in Pavlova's medium is acceptable but subculturing is required after 48 hrs.

Table 2. Growth of E. histolytica isolate AD4 in two media adjusted to specified pH levels

pH	Amoeba count 10^3/ml			
	Dobell and Laidlaw[2]		Hallman et al.[4]	
	24 hr	48 hr	24 hr	48 hr
5.0	3.0	3.5	2.5	2.6
5.5	3.5	4.5	3.0	3.7
6.0	6.2	10.0	5.8	9.0
6.5	8.5	16.5	7.5	12.0
7.0	7.2	14.0	6.0	10.5
7.5	5.5	9.0	5.5	7.0
8.0	3.0	3.5	2.9	3.0

Table 2 shows the amoeba counts in Dobell's and Hallman's media respectively adjusted to the pH levels specified prior to inoculation. The amoeba counts were performed at 0, 24, and 48 hr respectively. It is seen from the table that the highest counts are at pH 6.5 for these two media.

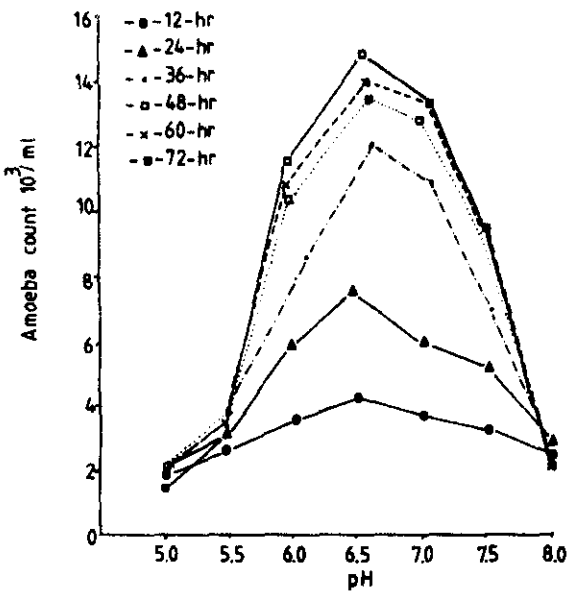

Fig.2-Growth of E.histolytica isolate AD4 in Hallman et al 4 synthetic medium adjusted to specified pH levels prior to inoculation.

Figure 2 shows the growth curves of isolate AD4 in Hallman's (4) synthetic medium. The growth curves reveal that variations in pH had a marked effect on the ability of E. histolytica to propagate. A pH of 6.5 was optimal for isolates of E. histolytica. In all series, the pH remained constant throughout the period of observation, deviating not more than 0.1 from the original tube checked at 0-hr.

Chang (14) reported that, in his hands, E. histolytica grew freely between pH 6.6 and 7.3. He made no comment on the ability of the amoebae to propagate at 6.0, but stated that a minimal growth occurred as the pH levels approached 5.5 and 8.0. The medium he used was not mentioned in his paper.

But in 1952, Shaffer (3) made a study on the effect of varying inocula and of pH on the growth of E. histolytica in Shaffer-Frye (15) medium. He described growth curves of the NIH 200 and the Luna strains(16) of E. histolytica in S-F medium adjusted to specified pH levels. He found that the NIH 200 strain propagate well over the range of pH 6.0 to 6.9 but do so very poorly at pH 5.1, 5.4 and 7.4. He also propagate amoebae in S-F medium at pH 8.0. Growth of E. histolytica has also been reported at pH 5.4, 6.0 and 8.5 (10). Rees, Key and Shaffer (17) again reported that E. histolytica grown in association with penicillin-inhibited streptobacillus propagate well at pH 6.0 to 6.5, a finding in agreement with the present study in which all isolates were found to propagate best at pH 6.5. The differences between the results of Shaffer's (3) and the present study could be attributed to differences in the culture media used. Isolates of E.histolytica tested in the present work propagated well at pH 6.5 and litle evidence of propagation was seen in the two media adjusted to pH 7.5, and none in that adjusted to pH 8.0. It is therefore concluded that higher pH levels did not favour the growth of E. histolytica in any medium.

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