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Survival Time and Critical Temperatures of Various Strains of *Entamoeba histolytica*

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Reports of studies conducted on the survival time of the cysts of *Entamoeba histolytica* are frequently found in the literature of this parasite (Yorke and Adams, 1926, Jones and Newton, 1950, and Chang, 1950), but the survival time of the trophozoites of *E. histolytica* in culture is seldom discussed (Tsuchiya, 1945). The work reported here was therefore undertaken in the hope that a detailed temperature study might reveal some other specific and hitherto unknown characteristic of the biology of this parasite.

Materials and Methods

The amebae were grown in Ringer-Egg-Locke (REL) medium dispensed in screw capped culture tubes (15 mm x 150 mm). The medium was prepared as follows:

Eggs were rinsed with alcohol and then broken into a Waring Blendor. Twelve and one-half ml of sterile Ringer's solution were added for each egg used. This mixture was shaken in the Blendor until a homogenous suspension was obtained. Two ml of this egg-Ringer mixture was dispensed into each culture tube. The tubes were slanted and the egg was coagulated by heat, after which 5 ml of Stone-Locke's solution (Stone, 1935) was added to each slant. These culture tubes were autoclaved twice at 15 lb pressure (121°C) for 25 minutes and then incubated overnight to check for contamination. A small amount of rice starch was added to each tube by means of a small platinum spatula. Stock cultures

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were transplanted every 48 hours by transferring 0.3 ml of the sediment into a fresh slant.

The following amebic strains were used in this study:

1. University of Chicago strain (UC).
2. 201 strain, derivative of NIH 200, cultured with organism "t", which was a clostridium-like organism. NIH 200 with organism "t" was inoculated intrahepatically into hamsters in March 1951, and when recovered later, was found to contain an additional bacterial contaminant, which was a diptheroid-like organism.
3. 202 strain had *Alkaligenes fecalis*, both rough and smooth colonies.
4. HK9 strain. This strain was isolated from a North Korean prisoner of war.
5. HK14. Same as above.
6. F22 SB strain had spore forming bacilli.
7. The single *Entamoeba coli* strain used was the So strain. This strain had *Aerobacter aerogenes*.

Experimental

Survival Time

The experiments were repeated twice. In most cases, the results were identical, but in a few the survival time varied slightly. When this occurred, an average of the three trials was taken as the survival time, as shown in Table I.

Forty-eight-hour old cultures were used as stock to seed the experimental culture tubes. One ml of the sediment from each tube was removed, placed in a sterile tube, and mixed well, thus providing a uniform suspension. Three-tenths ml of this stock suspension was inoculated into fresh, warm REL tubes, which were in turn placed immediately at the desired temperature. Every 24 hours a tube from each one of the temperatures below 37°C was removed and incubated at 37°C for 48 hours and then examined for living amebae. Tubes at temperatures above 37°C were removed, examined immediately, and if no amebae could be found, a transplant was made and examined at the end of 48 hours.

The two species of amebae, *E. histolytica* and *E. coli*, were exposed to temperatures of 3, 18, 25, 32, 33, 37, 41, and 45°C.

Controls at 37°C were consistently run simultaneously. These were inoculated from the same stock suspension used in the experimental tubes.

TABLE I

Survival Time of Amebae in Days. Results of Individual Experiments with Mean in Parentheses

Strain	3°C	18°C	25°C	32°C	37°C	41°C	45°C
							1 hr.
UC	3, 3, 3 (3)	3, 3, 3 (3)	3, 4, 3 (3.3)	9, 9, 9 (9)	7, 6, 8 (7)	4, 4, 4 (4)	1 hr (1)
							1 hr.
							1.5 hr
201	4, 4, 4 (4)	4, 4, 5 (4.3)	5, 5, 5 (5)	11, 11, 12 (11.3)	9, 9, 11 (9.7)	5, 5, 5 (5)	1.5 hr. (1.5)
							1.5 hr.
							1.5 hr.
202	3, 3, 2 (2.7)	4, 4, 5 (4.3)	5, 5, 5 (5)	10, 9, 11 (10)	8, 9, 10 (9)	8, 9 ,9 (8.7)	1.5 hr. (1.5)
							1.5 hr.
F22 SB	1, 1, 1 (1)	2, 2, 2 (2)	2, 2, 2 (2)	3, 3, 3 (3)	8, 9, 6 (8)	4, 4, 4 (4)	No exper. done
K9	2, 2, 2 (2)	2, 2, 2 (2)	2, 2, 2 (2)	5, 5, 5 (5)	4, 4, 4 (4)	4, 4, 4 (4)	No exper. done
K14	2, 2, 2 (2)	2, 2, 2 (2)	2, 2, 2 (2)	4, 4, 4 (4)	4, 3, 4 (3.7)	4, 4, 4 (4)	No exper. done
							3 hr.
So	8–10 hr.	8–10 hr.	1, 1, 2 (1.3)	7, 6, 8 (7)	8, 7, 7 (7.3)	11, 11, 12 (11.3)	3 hr. (3 hr.) 3.5 hr.

In studying Table I, it can be seen that *E. coli* (So) had a survival time of less than a day at 3°C and 18°C. This was investigated more thoroughly and it was found that this species disappeared within 8 to 10 hours at these two temperatures. This finding seems to confirm that of Dobell and Laidlaw, who in 1926 reported that *E. coli* was very sensitive to cold, while *E. histolytica* was less easily killed by lower temperatures. For example, at 3°C, while *E. coli* survived less than a day, strains K9 and K14, two recently isolated strains of *E. histolytica* which grow poorly in culture, had a survival time of 2 days. Even strain F22 SB, a monobacterial culture of *E. histolytica*, which has a comparatively poor growth and is extremely sensitive to any environmental change, had a survival time of one day at 3°C.

At the higher temperatures the outcome was reversed: at 41°C, *E. coli* had a survival time of 11 days, while of the *E. histolytica* group, strain 202 showed the longest survival time, 8½ days.

At 45°C, *E. coli* was found to survive for 3 hours, while *E. histolytica* had only an hour and a half survival time.

The optimal temperature, that is to say, the normal growth temperature, for both species studied thus far has consistently been reported to be 37–38°C. However, at 33°C all the strains under the present investi-

gation gave forth a luxuriant growth, and at 32°C all the *histolytica* strains, with the exception of F22 SB, had a longer survival time than at 37°C. At 41°C *E. coli* had a longer survival time than at 37°C.

The bacterial flora of *E. histolytica* strain 201 and *E. coli* strain So were isolated and added to all the other strains. These amebae were subjected to some of the temperatures mentioned above. The purpose of this step was to discover whether the bacterial flora has any effect on the survival time of the different strains of amebae. (Table II)

With the exception of strain F22 SB, changing the bacterial flora of the different strains of *E. histolytica* seemed to produce no significant changes in the survival time of the amebae. F22 SB, a monobacterial strain, showed an increase in length of survival upon addition of bacterial flora isolated from strain 201 of *E. histolytica*. The other strains showed

TABLE II

Survival Time (Days) of Trophozoites with Mixed Bacterial Flora. Results of Individual Experiments with Means in Parentheses

Strain	3°C	25°C	41°C
UCC*	5, 3, 4 (4)	4, 4, 4 (4)	3, 5, 5 (4.3)
201C	4, 4, 4 (4)	4, 4, 4 (4)	5, 5, 5 (5)
SoC	Less than a day	1, 2, 1 (1.3)	10, 9, 11 (10)
UCB*	2, 3, 3 (2.6)	2, 2, 3 (2.3)	5, 5, 5 (5)
201B	5, 4, 3 (4)	4, 4, 4 (4)	6, 4, 5 (5)
202B	2, 3, 2 (2.3)	4, 4, 4 (4)	6, 9, 6 (7)
F22 SB	1, 1, 1 (1)	2, 2, 2 (2)	4, 4, 4 (4)
F22 SBB	3, 3, 3 (3)	3, 3, 3 (3)	5, 5, 5 (5)
K14B	2, 2, 2 (2)	2, 2, 2 (2)	4, 4, 4 (4)
K9B	2, 2, 2 (2)	2, 2, 2 (2)	3, 5, 4 (4)
SoB	Less than a day	1, 2, 1 (1.3)	9, 9, 9 (9)

* Suffix C = Bacterial flora of *E. coli* (So) added.
Suffix B = Bacterial flora of 201 strain of *E. histolytica* added.

slight changes in the length of survival time, which do not warrant a detailed description here.

An important factor not to be overlooked is that at all temperatures there are differences among the various strains. It is possible for one strain to survive for only 1 day at 3°C, while another strain could live 4 to 5 days at the same temperature. For this reason, when discussing survival time, the strain or strains used should be designated, and generalizations should be avoided.

Critical Temperatures

Strain So of *E. coli* and strains UC, 201, and 202 of *E. histolytica* were investigated. The above strains of amebae containing the bacterial flora of strains 201 and So were also studied. It was soon apparent that all strains of both species grew well at 33°C. The growth at this temperature was luxuriant and the amebae appeared to be in good health. Vacuolated amebae were observed upon several occasions, but they were few in number. The cultures as a whole were as good as those kept at 37°C. These cultures were carried for ten transfers, after which it was concluded that cultivation was possible at this temperature.

At 32°C the number of trophozoites of *E. coli* diminished with each transfer, and after 8 days (four transfers) no trophozoites were found. The strains of *E. histolytica* grew poorly at first, and there were many amebae containing large vacuoles. Examination of UC strain showed no amebae for two transfers; however, after two or three successive transfers, this strain reappeared and all other strains improved in appearance.

Lowering the temperature a fraction of a degree (less than 0.5°C) from 32°C resulted in the death of strains UC, 202, UCC, 202C, UCB, and 202B, but not of strains 201 and 201C. The critical temperature for these last two strains was found to be 31.5°C.

As for the high critical temperature, it was found that all strains of both *E. coli* and *E. histolytica* grew well at 40°C and 41°C. Cultures were maintained at these temperatures for a month. At 41.3°C strains 201, UC, 201C, 201B, UCC, and UCB soon disappeared, but strains 202, 202B, 202C, So, SoC, and SoB continued to grow. It should be mentioned, however, that at this high temperature, strains So, SoC, and SoB behaved peculiarly in that upon examination of the cultures every 48 hours, they showed only one to two amebae which were ragged and very unhealthy in appearance. These strains were nevertheless kept

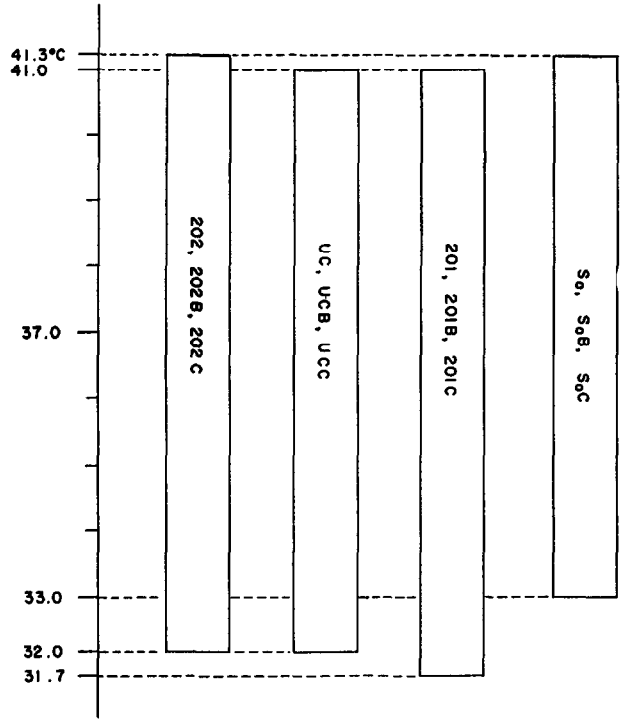

FIG. 1. Temperature ranges permitting growth of different strains of amebae.

at this temperature (41.3°C) for a month, after which they still maintained the unhealthy appearance and the same poor growth. On the other hand, the 202, 202B, and 202C strains grew poorly at first, but soon improved, and by the end of the month, were growing very well. At 41.5°C there was no growth in any of the strains. The results of this study can be more easily visualized by consulting Fig. 1.

DISCUSSION

It seems that the survival time for different strains of *E. histolytica* at different temperatures vary. The same is true for the critical temperatures of different strains. These differences seem to depend on the amebae themselves and not on the accompanying flora, since interchanging the bacterial flora of different strains had very little effect on the length of the survival time at different temperatures.

More significant, however, was the fact that the critical temperatures for the different strains were not altered when their bacterial flora was

interchanged. We may conclude that the capacity of some strains to grow at higher or lower temperatures than other strains is an intrinsic property of the amebae, in which the bacteria take very little part.

Summary

Six strains of *Entamoeba histolytica* and one strain of *Entamoeba coli* were subjected to different temperatures. The single strain of *E. coli* tested proved to be more resistant to the high temperatures and more susceptible to the low temperatures than all the *E. histolytica* strains under study. Individual strains of *E. histolytica* differed in the length of their survival time at varying temperatures.

Interchanging the bacterial flora of some of the strains produced no significant changes in the survival times of the amebae except for one monobacterial strain which benefited by the increase in bacterial flora.

The critical temperature of three strains of *E. histolytica* and one strain of *E. coli* was carefully investigated. There were marked differences in the critical temperature among all strains. However, interchanging the bacterial flora of the strains did not alter their critical temperature.

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References

Chang, S. L. 1950. Kinetics in the thermo-destruction of cysts of *Endamoeba histolytica* in water. *Am. J. Hyg.* **52,** 82–90.

Dobell, E., and Laidlaw, P. P. 1926. On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae. *Parasitology* **18,** 283–318.

Jones, M. F., and Newton, W. L. 1950. The survival of cysts of *Endamoeba histolytica* in water at temperatures between 45°C. and 55°C. *Am. J. Trop. Med.* **30,** 53–58.

Stone, W. S. 1935. A Method of producing encystment in cultures of *Endamoeba histolytica*. *Am. J. Trop. Med.* **15,** 681–684.

Tsuchiya, H. 1945. Survival time of trophozoites of *Endamoeba histolytica* and its practical significance in diagnosis. *Am. J. Trop. Med.* **25,** 277–279.

Yorke, W., and Adams, A. R. D. 1926. Observations on *Endamoeba histolytica*. II. Longevity of the cysts in vitro and their resistance to heat and to various drugs and chemicals. *Ann. Trop. Med. Parasitol.* **20,** 317–326.