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Temperature Adaptation of *Entamoeba histolytica* and Its Effect on Virulence[1]

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Cabrera and Porter (1958) found that the maximum culture temperatures for three strains of *Entamoeba histolytica* (UC, 201 and 202) were 41.0, 41.0, and 41.3°C and the minimum were 32.0, 32.0, and 31.7°C. In the present investigation, by progressive gradual elevation of the culture temperature, these three strains grown in Ringer-Egg-Locke (REL) medium were adapted to 42°C, and one of them to 42.5°C. Similarly, by lowering the temperature, all three strains were adapted to continuous cultivation at 30°C and one of them to 29°C. The adaptation of *E. histolytica* to high and low temperatures presented a very logical question: Are these adapted strains similar in behavior to the unadapted ones? To find the answer to this question a detailed study of the adapted strains compared with the unadapted strains was undertaken.

Methods and Results

The amebae were cultured in Ringer-Egg-Locke (REL) medium dispensed in screw capped culture tubes (15 mm x 150 mm). While investigating the critical temperatures of *E. histolytica*, it was found that the three strains under investigation grew at 32°C. At the end of a month the temperature was lowered to about 31.7°C. This had no effect on the strains which had been at 32°C for a month, but when tubes were inoculated from the control cultures kept at 37°C, no growth took place in some cultures, while it occurred in others. Further lowering of the

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temperature to 31.5°C produced death in all the strains of amebae inoculated from the control cultures kept at 37°C, but had no effect on the preconditioned or adapted strains. Periodic lowering of the temperature, always less than 0.5°C each time, was performed every two weeks. Several strains were kept growing at 29°C for longer than 18 months.

Adaptation of these organisms to high temperatures was also accomplished. All three strains of *E. histolytica* grew at a little above 42°C, but when the temperature was raised to 42.5°C, only strain 202 survived, while at 43°C all adapted strains failed to grow. During these experiments the temperature was raised serially about 0.3°C and the strains were kept at a set temperature for 2 to 3 weeks before it was again increased. At 42.5°C control strains which had been growing at 37°C survived for less than 48 hours.

At this point in the study the question arose as to whether the failure of the controls to grow at the higher temperatures was caused by the abrupt shock suffered by the amebae which in turn resulted in their death. Perhaps if the controls had been placed less abruptly into this high temperature, they might have survived and grown. To answer this question, controls were kept at 41°C for two transfers, where they grew well, and then were placed at 42°C, where they failed to grow. Adapted amebae were subjected to the same treatment with no impairment to their growth. This result would seem to indicate that adaptation enabled these amebae to survive at 42°C.

Tubes of these amebae adapted to high and low temperature were returned to 37°C, which may be considered to be the normal growth temperature. After 48 hours at 37°C, the tubes were examined and it was found that of the three strains adapted to 42°C, one (UC) contained a large number of cysts, and the other strains showed good growth but no cysts. On the other hand, when the strains adapted to low temperature were returned to 37°C, another strain (201) showed a large number of cysts, while the other two strains contained only trophozoites. This test was repeated several times with the same results. The cysts of strains UC and 201 were kept in the refrigerator for three weeks and excystation was attempted at 30, 37, and 42°C. Both strains excysted at 37°C, but not at 30 or 42°C.

This work has been summarized in Fig. 1. It is believed that during the course of this study *E. histolytica* had for the first time been adapted to high and low temperatures. It should be recalled, however, that Cutler in 1918 reported that he had cultured *E. histolytica* at 28–30°C;

thus it is possible that he had unknowingly adapted these organisms to low temperature.

Following the adaptation of *E. histolytica* to high and low temperatures, the next step was to study the virulence of the adapted strains, as compared with that of the unadapted strains. Strain 202 adapted to 42.5°C was first investigated. Mice were inoculated intrahepatically with about 30,000 amebae in 0.05 ml of inoculum. The count was made according to Paulson's method (1932). All the mice inoculated with the adapted strains of amebae died from bacterial liver abscesses within 3 days. It seemed that perhaps the bacteria accompanying the strain of amebae adapted to high temperature had become very virulent. For this reason it was decided to test strains 201 and 202 which had been adapted to 30°C.

White mice were abandoned as experimental animals and instead golden hamsters (*Cricetus auratus*) weighing between thirty-five and fifty grams were used. Their diet consisted of a standard commercial laboratory chow and water.

Amebae of strains 201 and 202, from 48-hour old cultures, were washed once with sterile saline. Inoculations were done intrahepatically. The hamsters were anesthetized with ether and the operation site was swabbed with alcohol. The skin was incised, but no abdominal incision was needed, since after the skin incision the liver was clearly outlined and the inocula were introduced into the liver. The inocula of amebae

TABLE I

Summary of Experiments on Virulence Using Strains 201 and 202

Substrain	Number hamsters	Number infected	Number not infected	% Infectivity
Unadapted amebae strain 201	20	0	20	0
Amebae adapted to 30°C strain 201	20	18	2	90
Unadapted amebae 201 plus adapted bacteria 201	35	12	23	34
Amebae 201 adapted to 30°C, returned to 37°C	15	1	3	80
Unadapted amebae strain 202	15	1	14	6.6
Amebae adapted to 30°C, strain 202	15	9	6	60

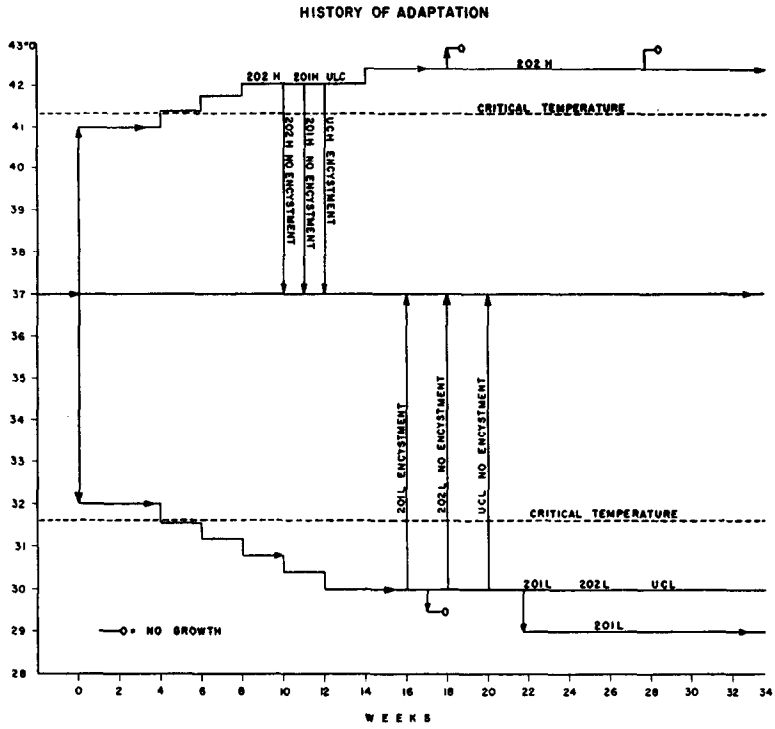

FIG. 1

grown at 30°C contained only 1,500 to 2,000 amebae, since growth at 30°C was not abundant. The other inocula grown at 37°C contained 25,000 to 30,000 amebae in 0.05 ml. of suspension. At the end of 72 hours the animals were sacrificed and examined.

From the results given in Table I and Fig. 1, it can be seen that before adaptation, the two strains of *E. histolytica* studied had a low degree of virulence, strain 202 producing one liver abscess in 15 hamsters inoculated. After adaptation, however, the virulence of the amebae was decidedly increased.

Intrahepatic inoculation of 20 hamsters with strain 201 adapted to 30°C produced large liver abscesses in 18 of the hamsters. Adapted strain 202 produced liver abscesses in 9 out of 15 inoculated hamsters. Trophozoites of *E. histolytica* in large numbers were recovered from all these abscesses. These results apparently demonstrate that adaptation to low temperature was associated with increased virulence of the strain.

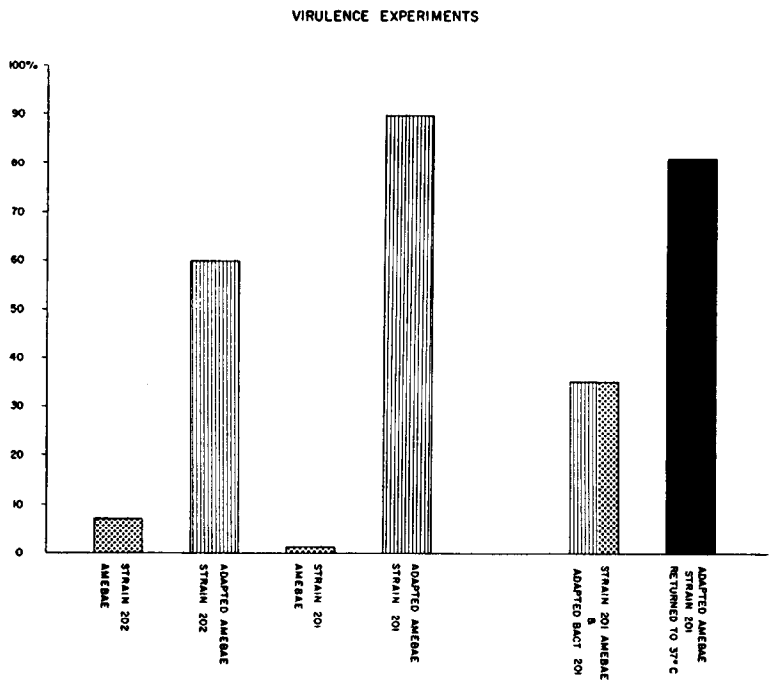

FIG. 2

That the accompanying bacteria might play an important part in this enhanced virulence was suggested when the isolated bacteria of the adapted strain was added to the control strain of *E. histolytica.* A moderate number (12 infections out of 35) of the inoculated hamsters developed amebic liver abscesses.

When adapted strain 201 was returned to 37°C and maintained at this temperature for 6 weeks, then tested for virulence, there was found to be a slight decrease in the virulence of the strain (Fig. 2). This possibly indicates that prolonged cultivation at 37°C might result in loss of virulence, and reversion of the strain to its original condition of low pathogenic index.

DISCUSSION

Temperature Adaptation

Interest in the possible adaptation of microorganisms dates back to 1887 when Dallinger made his first report on microorganisms adapted to grow at high temperature.

It is a well known fact that continued growth of bacteria at temperatures higher than their optimum results in physiological changes. *Bacterium prodigiosum* fails to produce its red pigment when grown at temperatures higher than 30°C, and the anthrax bacillus when grown for several transfers at 42°C becomes avirulent.

Suboptimal temperatures have no demonstrable effect on the physiology of the bacteria, and at temperatures below the minimal norm the organism becomes dormant.

Our findings with *E. histolytica* seem to be in contrast with the above facts, for the virulence of this parasite was enhanced when grown at subnormal temperatures.

Encystment

Although the actual mechanism of encystment is still obscure, many conditions conducive to this process have been observed. Those which Cleveland and Sanders (1930a) believed necessary to bring about encystment of trophozoites are as follows:

1. Accelerated growth of amebae for 24 hours or more.
2. Addition of rice starch to rice-free cultures.
3. The presence of certain (undetermined) bacteria in the medium.

In his studies on *E. histolytica* Chang (1946) reported that after inoculation there was a drop in the oxidation-reduction potential which reached its lowest point within 6 hours. This was followed by a gradual rise of potential for 16 to 18 hours, which in turn was followed by a period of 10 to 16 hours in which the potential rose sharply. Mass encystment occurred at the beginning of this sharp rise in the oxidation-reduction potential, although this did not always produce encystment. Cultures which originally had a high rate of multiplication did not encyst at the "sharp rise". This suggested that there must be an accelerated rate of multiplication of the trophozoites before encystment occurred. The "sharp rise" was not observed in cultures of bacteria alone, and the oxidation-reduction potential was always higher in cultures of amebae and bacteria. He concluded that the bacterial activities were greater during the first ten hours after inoculation, causing a drop in the potential. However, as the growth of amebae became richer, there was a depressing effect of the bacterial activity, thus giving a rise in the potential.

In this work encystment has been produced in two strains of amebae by three different procedures. However, it should be borne in mind

that a procedure which would produce encystment of one strain, may not necessarily do so when a different strain is used.

The three methods employed in the present studies which resulted in encystment were as follows:

1. Strain 201 adapted to 30°C encysted when returned to its original normal growth temperature of 37°C.
2. Strain UC adapted to 42°C encysted when returned to its original normal growth temperature of 37°C.
3. Encystment was also obtained when the bacteria from strain 201 adapted to 30°C were isolated and then added to the unadapted 201 strain of amebae growing at 37°C.

Associated with the first and second methods which were apparently responsible for encystment were the observations that the rate of multiplication of the trophozoites in the adapted cultures at high and low temperatures was only about one-tenth that of the rate at 37°C. With this in mind we may assume that returning these cultures to 37°C brought an immediate drop in potential caused by the metabolic activities of the bacteria. However, this drop was soon overcome by the luxuriant growth of amebae. When this prolific rate of multiplication was at its maximum, it is possible that the "sharp rise" of potential accompanied by mass encystation took place. It is also possible (Everritt, 1950) that the prolific rate of multiplication led to overpopulation and accumulation of large amounts of metabolic by-products which in turn led to encystation.

Encystment accomplished by the third method can be explained on a different basis. It is possible that the adapted bacteria added to the unadapted strain of amebae overgrew some of the bacteria originally associated with the amebae, resulting in environmental conditions which were unfavorable to the existence of trophozoites, thus inducing encystment.

Virulence

Although much work has been done on the virulence of *E. histolytica*, the role that the amebae and the bacteria play together, and/or independently, is not completely understood. Cleveland and Sanders (1930b), Deschiens (1938) and Westphal (1937) found that bacteria play an important role in the virulence of *E. histolytica*. On the other hand, Faust and Swartzwelder (1935), after failing to elicit any response in three dogs inoculated with highly pathogenic bacteria which had been isolated

from the dysenteric stools of amebic infections, concluded that the bacteria were not primarily responsible or enhancing amebic infections.

From another viewpoint, Meleney and Frye (1933) and Frye and Meleney (1933, 1936), after a long series of experiments, suggested that strains differ in their pathogenicity and that this pathogenicity is in great part an intrinsic characteristic of the amebae.

The present investigations would indicate that different strains of amebae differ in their pathogenicity, and that the pathogenicity of a single strain can be modified by environmental changes: i.e., by adaptation to a lower temperature. That the bacteria may play an important role may be concluded from the following. A strain of low virulence was adapted to 30°C.; the virulence of the strain increased greatly. The bacteria of this now highly virulent strain, when added to the original parent strain, produced a moderate increase in the pathogenicity of the strain. However, it should be kept in mind that the same bacteria were present in the original parent strain as in the adapted strain. Therefore, when the bacteria of the highly virulent strain of amebae were added to the original strain, no new species were being introduced. Nevertheless, this addition of bacteria did cause an increase in virulence. It is possible that the bacteria had been affected by the adaptation, although in what way, is not known.

Summary

1. Three strains (UC, 201, and 202) of *E. histolytica* were adapted to grow at low (29°C) and high (42.5°C) temperatures.

2. Encystment of strains 201 and UC was produced by returning the trophozoites from the temperatures of adaptation to the original normal growth temperature of 37°C.

3. Unadapted strains 201 and 202 of *E. histolytica* had a low degree of virulence for livers of hamsters, while the same strains adapted to low temperature were highly virulent.

4. The virulence of these adapted amebae was not lost by returning these parasites to their original normal growth temperature of 37°C for 6 weeks.

5. By adding the bacteria of the adapted culture to the unadapted amebae, a moderate increase in the virulence was produced.

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