EXPERIMENTAL BIOLOGY

Study of the Dynamics of *Blastocystis Hominis* Reproduction *In Vitro*

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The dynamics of *Blastocystis hominis* reproduction *in vitro* in Pavlova's and Nelson—Jones media was studied. The time of generation in these media was 21.5 and 16.7 h, respectively. The duration of the lag phase was 1 day, of log phase 2 days, and of the stationary phase 6 days in both cases. The cell count in the logarithmic phase increased at the expense of the vacuolar forms proliferation. During the stationary phase, the granular forms quantitatively predominated over vacuolar forms, the share of the granular forms reaching almost 100% at late stages of the subculture development.

Key Words: *Blastocystis hominis; protozoan culture; culture growth curve; morphological polymorphism*

Blastocystis hominis are protozoa parasitizing in human intestine. The level of population infection with this microorganism is very high [2]. They are characterized by a wide morphological polymorphism. The vacuolar, granular, and ameboid forms are studied best of all [1,5,10,13]. Blastocysts most often multiply by binary division, but endodiogeny, plasmotomy, budding, and schizogeny were also described [12,13].

One of approaches to the study of *B. hominis* is culturing in different media [1,5-8,10-13]. However, the data on morphological forms developing *in vitro* are contradictory. In addition, we failed to find reports on systematic studies of the dynamics of morphological forms and its correlation with culture growth curves.

Organization of individual time is one of the types of time organization of a biological system [4]. Individual time of a system of cells multiplying *in vitro* can be regarded as a period of culture existence from reactivation in fresh nutrient medium until natural degeneration; in other words, it is characterized by this culture growth curve and by the dynamics of morphology and function of the cell system related to reproduction phases of these cells. We studied the time organization of *B. hominis* culture.

MATERIALS AND METHODS

B. hominis polyxenic strain was isolated in Pavlova's medium (PM) [3] from feces of a parasite carrier and was maintained for 2 months by reinoculation in this medium, after which the experiment was carried out.

Samples containing 4.5 million blastocyst cells each were put into two culture tubes. Pavlova's medium (10 ml) was added to one sample, Nelson—Jones' medium (NJM; 10 ml) into the other [9]. Both culture media contained 10% bovine serum and a loop of sterile rice starch. The tubes were incubated at 37°C. Specimens of culture were collected with a pipette after shaking every day over the first 2 weeks and then at 1-3-day periods. The concentration of protozoan cells

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was evaluated in a Goryaev's chamber. The measurements were carried out over 39 days after the start of the experiment. The results were used to plot culture growth curves for both media. The time of generation in the logarithmic growth phase (GT) was estimated by the formula:

$$GT=t/(3.3 \log (b/B),$$

where t is log-phase duration, b cell concentration at the beginning of this phase, and B cell concentration at the end of this phase.

The specimens collected from the tubes were fixed in 10% formalin. Smears on slides were prepared from fixed material and stained with Meyer's hematoxylin. The smears were examined under an immersion microscope (×900). A total of 200 cells were examined per term. The percentage of three morphological forms (vacuolar, granular, ameboid) was evaluated. Vacuolar form is presented by round or slightly flattened cells with a mean diameter of 4-15 μ, with central vacuole and a peripheral cytoplasmic rim containing several punctate dark granules [5,10,13]. In contrast to the vacuolar form, the granular form is presented by cells with numerous granules in the central body and peripheral cytoplasm [5,10,13]. Ameboid cells are round or irregularly shaped, reached 3-8 μ in diameter, and contain one or two nuclei; the cytoplasm contains phagocytic vacuoles and often forms 1-2 pseudopodias [5,10,13]. The data were used to characterize the dynamics of these morphological forms in correlation with the culture growth phases.

RESULTS

B. hominis culture growth curves for PM and NJM are presented (Fig. 1). The reproduction of the protozoa in both media is characterized by similar dynamics. The lag phase took 1 day and the logarithmic growth phase 2 days in both cases; the stationary phase was poorly pronounced, its duration being about 6 days. The degeneration phase started after this phase (after 9 days of observation). According to some authors [7,8,12], the maximum cell count in *B. hominis* culture is observed on days 3-5 of culturing. However, the count of protozoa in the degeneration phase in NJM sharply decreased and reached trace levels on day 18 of culturing, while in PM cell count gradually decreased, but remained high until the last term of observation. The time of generation in the logarithmic growth phase was 21.5 h in PM and 16.7 h in NJM.

Hence, the dynamics of proliferative activity of cells is similar during culturing of *B. hominis* in both media. However, NJM with a composition more accurately meeting the metabolic requirements of the colorectal protozoa provides more rapid reproduction in exponential growth of the subculture.

Examination of stained smears detected solitary ameboid *B. hominis* cells only on day 2 of culturing, and therefore the contribution of this form was neglected. In our experiment, the cell system morphologically consisted of vacuolar and granular forms, which was in agreement with other reports [1,10,13]. However, a sufficiently high percentage of ameboid cells (5.5%), in addition to the vacuolar and granular forms, was detected in culturing of monkey blastocysts [5]. Other authors [8] observed the development of vacuolar and ameboid, but not granular forms in culturing of *B. hominis*. Presumably, the morphology of blastocyst culture is specific of the protozoan strain and depends on maintenance conditions.

We detected figures of binary division in just solitary cases. Presumably, cell division was very short.

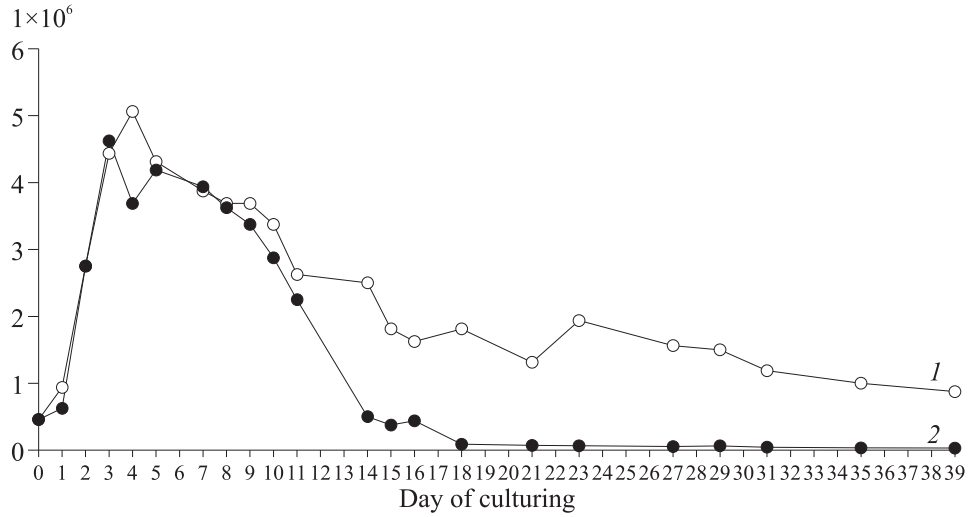

Fig. 1. *Blastocystis hominis* culture growth. *1*) culturing in PM; *2*) in NJM. Ordinate: number of cells/ml medium.

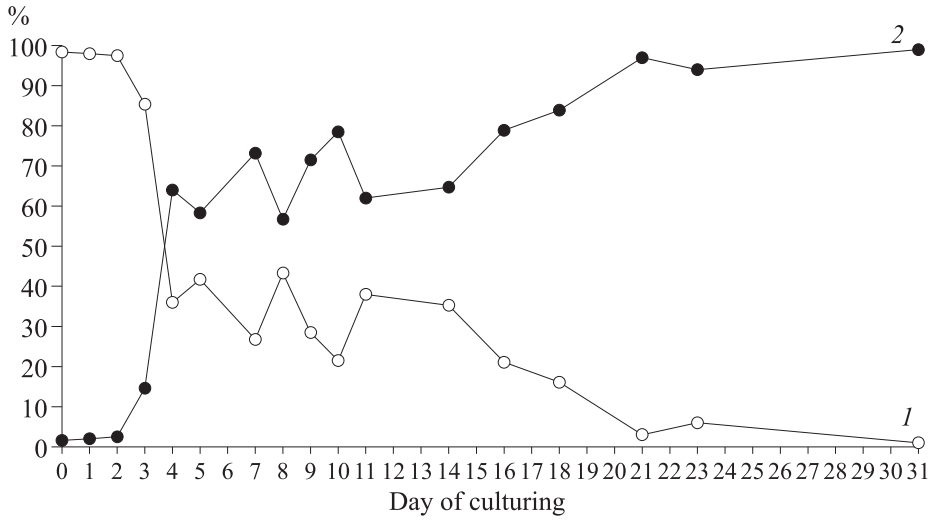

Fig. 2. Dynamics of the percentage of *Blastocystis hominis* morphological forms during culturing in PM. Here and in Fig. 3: *1*) percentage of vacuolar forms; *2*) percentage of granular forms.

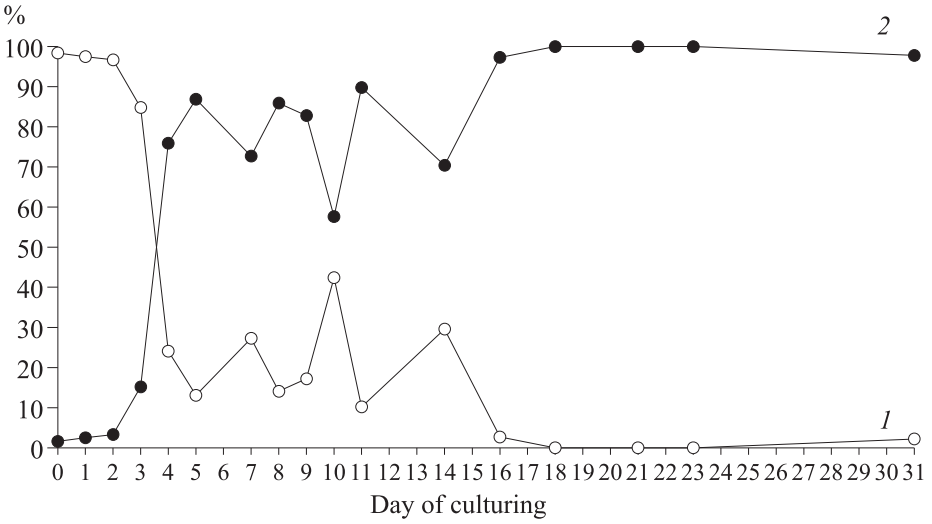

Fig. 3. Dynamics of the percentage of *Blastocystis hominis* morphological forms during culturing in NJM.

Changes in the percentage of vacuolar and granular forms during culturing in PM and NJM over 31 days are presented in Figures 2 and 3. Similarity of curves can be observed in both cases. Initially, the subculture virtually completely consisted of vacuolar blastocysts. Their percentage remained high throughout the logarithmic phase, but started to decrease gradually, which was paralleled by an increase in the percentage of granular forms. The onset of the stationary phase was characterized by a drop of the percentage of vacuolar blastocysts in parallel with sharp increase in the percentage of granular forms. Granular blastocysts predominated over vacuolar ones throughout this phase and during the first 5 days of the degeneration phase. The percentage of granular forms fluctuated about the mean level (66.1% for PM and 77.7% for NJM) over 10 days. This was followed by a steady increase in the percentage of granular blastocysts and a reduction in the percentage of vacuolar ones, which virtually completely disappeared during the late periods of observation.

These data suggest that reproduction of *B. hominis* in our experiment was largely due to multiplication of the vacuolar forms. The detected dynamics of the subculture morphotype indicates transition of the vacuolar forms into granular ones with aging of this cell system, which is confirmed by observations of other authors [10,11]. Virtually complete transition is observed during the degeneration phase, the granular forms being retained for a long time in PM.

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