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ULTRASMALL FORMS M. BOVIS-DISSOCIATIVE 117 AND 118 OPTIONS

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Мікобактерії туберкульозу володіють великою пристосованістю до зовнішніх умов і мінливістю, яка виражається непостійністю форми, величини, культуральних, ферментативних реакцій, а також біологічних властивостей. Саме дисоціація і є одним з видів неспадкової фенотипової мінливості, яка обумовлена впливом внутрішньо- і позаклітинних факторів на прояв генотипу. У процесі дисоціації одночасно з конфігурацією морфологічних ознак, змінюються біохімічні, антигенні, патогенні властивості бактерій, а отже, й стійкість їх до фізичних і хімічних факторів зовнішнього середовища. Наведено результати експериментальних досліджень ультрадрібних форм M. bovis-дисоціантів, які підтверджують їх безпосередню участь ультрадрібних форм (елементарних тілець) в біологічному циклі розвитку M. bovis-дисоціантів. Доведено, що субкультури M. bovis-дисоціантів містять ультрадрібні форми, частота виділення яких підвищується залежно від кількості генерацій (пасажів). Ультрадрібні некислотостійкі форми при посіві на елективні середовища і культивуванні утворюють колонії як поодинокі, так і у вигляді суцільного росту (результат злиття окремих колоній), проте ріст культури спостерігається в кілька разів повільніше, ніж у контролі. За мікроскопії культур ультрадрібних форм M. bovis-дисоціантів, отриманих при фільтрації через пори 0,1 мкм та 0,05 мкм, виділяли культури, які відрізнялися за морфологічними ознаками від вихідних (без фільтрації) M. bovis-дисоціантів. Наявність ультрадрібних форм у популяції мікобактерій і їх здатність до генерації культур із зміненими морфологічними ознаками підтверджують їх важливе значення в біологічному циклі розвитку дослідженого виду збудника туберкульозу.

Ключові слова: *M. bovis-дисоціанти, біологічний цикл, ультрадрібні форми, елементарні тільця, мінливість мікобактерій.*

The article presents experimental results which claim a direct part ultrasmall forms (elementary cells) in the biological cycle of dissociative options of *M. bovis*. It is proved that such forms generated by rod options of Mycobacterium.

The prolonged existence of the problem of tuberculosis animals and humans, even after the opening of the pathogen and its focused, multilateral knowledge stipulates probably, the variety of biological properties (conversion and reversion). Intensive work scientists of 50–70-ies last century and the relevant results on the subject, not completed implementation of their results into production in the future.

The system of prevention and eradication of tuberculosis animals only considers typi-

cal an acid resistant coli pathogens. That they built and developed a system of prevention and eradication of animal diseases. Such forms of the pathogen as ultrasmall (filtering), granular, L-shape, not acid resistant sticks and filamentous forms, which have been reported for decades because their properties are not taken into practice. These forms will undoubtedly determine the efficiency of diagnosis, of struggle and tension of epizootic process tuberculosis of animals in a given farm in general.

The problem of tuberculosis has existed since ancient times to now, even though in the knowledge has achieved many successes. It is certainly significantly affects provided quality implementation of the results of research on the effectiveness of infection prevention and its eradication. Meanwhile a series of questions at the present time, are insufficiently studied and controversial. The first group includes ultrasmall forms of mycobacteria, their value in some populations of microorganisms in the development of infection and the biological cycle of mycobacteria. Messages authors [1, 3] in the 70's of last century and in recent years [2] shows that ultrasmall forms and basic biological cells identical in nature, which, moreover, is not cultivated (cultured bad) on ordinary nutrient media . It specifies the complexity of their study. At the same time dissociative forms of mycobacteria, including ultrasmall (elementary bodies), as evidenced by our investigation of previous years [4], cultivated by 3 °C in ordinary nutrient media of pH 6,5 and 7,1 in the case of in particular of significant number replanting. This has determined the opportunity to explore the availability ultrasmall (elementary bodies) forms of dissociate population of *M. bovis*.

Objective: to identify ultrasmall forms of dissociate population *M. bovis* in the dynamics numerous passages through dense nutrient medium.

Materials and methods. For experiments have used Mycobacterium subculture that was stored in the laboratory of epizootology and infectious of animal diseases, museum pathogenic strain of 124th generation of *M. bovis* and dissociative forms that had flaked 117th (*a*, *b*, *c*) and 118th replanting (from 60ies to 110th subculture).

The paper have used for membrane syringe filter holders filter model of DH 25PWT1-1, filter the diameter of 25 mm and filter holders material – Teflon, manufacturer AWL-Tech (Czech Republic).

The filters diaphragm disc type of Vladipor MFAS-B1 and MFAS-B2, membrane material – microporous membranous prepared from a mixture of cellulose acetate with a pore size of 0,1 and 0,05 microns and the total porosity of 80–85 %, the producer ZAO STC "Vladipor" (Volodymyr, Russian Federation).

The suspension of Mycobacterium test samples were prepared by selection of the bacterial masses with bacteriological loop, over the flame of burner in terms of boxing, and placed in a sterile pounder. Using a pestle we were homogenized the bacterial mass in a pounder with the addition of sterile isotonic solution of sodium chloride. On receipt of the bacterial masses we prepareted the suspended matter which contained the mycobacteriums (1 mg/cm^3), of every of the experimental samples, separately.

The filtration of the suspended matter by testing samples of mycobacteria of different morphological forms was conducted using a syringe connected to the principle Luer-cone in dimensional sterile tubes. For the filtration process we are unscrew the housing of filters holders, on resisting mesh (leveling head) was placed microfiltrative membrane, wrench the housing and connects with a syringe.

The filtrate 1 (filter MFAS-B1 0,1 microns) and the filtrate 2 (filter MFAS-B2 0,05 microns), every testing sample were seeded with bacteriological loop in four bacteriological test tubes with eggs nutrient medium for the cultivation of mycobacteria and cultured in temperature of 3 °C, within 90 days. Accounting of the growth of mycobacteria cultures the first seven days was conducted each day, and in followed by once a week during the experiment.

In cultures have studied the growth rate of in nutrient medium of the eggs, the appearance, the emulsification, the pigment formation and the morphology in the smears, the tinctorial abilities of mycobacteria which painted by the method of Tsyl-Nielsen.

The smear microscopy was performed of primary cultures and of subcultures which derived by the replanting them before and after filtration.

Results. It was established that the research of dissociative forms *M. bovis* of 117th b ver-

sion shown the following (Fig. 1). The initial yellow-orange culture (Fig. 1.1) formed with the not acid resistant single grains, sticks and a large number of granular filamentous (branching) forms, some of which break up into separate parts (Fig. 1.2).

But, after conducting their filtering and exploring the prepared smears in immersion, we were not found any forms of mycobacteria, and 3-month cultural investigation and obtained sown on nutrient medium filtrate have not given positive results.

At the same time after conducting the same research with the 110th subculture of mycobacterium by 117 b version, was installed the opposite results. So, in Fig. 1.3 shows that the original yellow 110th subculture was formed by not acid resistant grains, and individual (as an exception) grainy sticks (Fig. 1.4). At the same time in the filtrate (0,1 micron pores) are found rare and not acid resistant grains and, sometimes, the short thin sticks (Fig. 1.5).

Meanwhile, following seeding the filtrate on artificial culture medium and cultivating it by 3 °C, was revealed the growth of culture (from individual colonies) at 23–26 day (Fig. 1.6), which was formed not acid resistant grainy sticks (Fig. 1.7).

So ultrasmall forms (elementary bodies) often found in Mycobacterium subcultures that many times seeding through dense nutrient medium and generating, the rod not acid resistant dissociative forms. Perhaps this is due to adaptive properties ultrasmall forms (mostly elementary bodies) to elective nutrient medium, which over time by repeated replanting, adapt to it. However ultrasmall forms in an artificial environment do not generate own kind in morphology descendants, and form not acid resistant rod versions of mycobacteria.

Our researches of previous years, as well as other authors showed that elementary bodies almost don`t cultivate on ordinary nutrient media [1–3]. However, this work is making sure claims that dissociative elementary bodies can be cultivated along with other forms of preserving the possibility of reversion in not acid resistant forms Mycobacterium tuberculosis, that said, Mycobacterium those clones continuously formed from elementary bodies pathogen rod forms, that they are an integral part of the biological development cycle even though the phenomenon is not always observed in each population mycobacteria one or another strain. Perhaps this feature is determined by the stage of development one or another mycobacterium strain and, of course, the environmental conditions.

However, the literature reported that L-forms mycobacteria cultured on selective nutrient medium despite its large size, compared to the typical sticks, change cell wall components, stretching, pass through bacterial filters. To clarify this issue, the features, including the presence of elementary bodies investigated L- and other forms of the dissociative 118th generation (Fig. 2).

The results of researches showed that in 20 months finding 60th 118th generations of culture in the positive low temperature almost have been no changes appearance (Fig. 2.1).

At the same time the morphology of mycobacteria and their tinctorial properties during the period fundamentally were changed. The initial culture was formed (Fig. 2.2) from not acid resistant small grains (isolated), coccus, grainy – short and more longer rods, filaments are formed by the grains and L-forms (ovals) from which are exempt granular formation.

After 20 months (in terms of a refrigerator), the microscopy of the same subculture found another morphology and the tinctorial properties of mycobacteria. In this case (Fig. 2.3) only single L-forms stay not acid resistant, while the vast major-

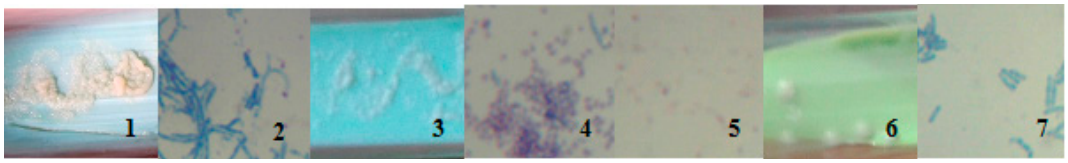

Fig. 1. The cultural properties and morphology of M. bovis version 117 b.
Culture: *1 – 60th generation (output); 3 – 110th generation;*
6 – 111th generation (with ultrasmall forms);
Morphology: *2 – 60th generation; 4 – 110th generation; 5 – ultrasmall 110th forms generation;*
7 – M. bovis derived from ultrasmall 110th forms generation × 1600

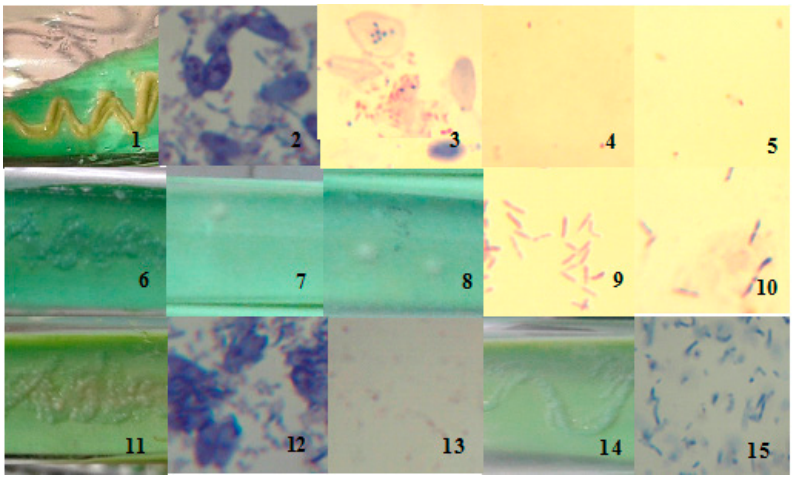

Fig. 2. The cultural properties and morphology M. bovis118th option.
Culture: *1 – 60th generation; 6 – 61st generation (control); 7–8 – with 60th ultrasmall forms of generation (pores 0,1 and 0,05 microns); 11 – 110th generation; 14 – of ultrasmall forms;*
Morphology: *2 – 60th generation (native); 3 – 60th generation (suspended matter); 4, 5 – 61st ultrasmall forms of generation (pores 0,1 and 0,05 microns); 9, 10 – M. bovis from ultrasmall forms 60th generation; 12 – 110th generation; 13 – 110th ultrasmall forms of generation (pores 0,1 micron); 15 – M. bovis from ultrasmall 111th forms generation* × 1600

ity of them acquired a reddish color with mostly blue grains in the middle. These grains – but most of reddish color, pushed (exempt) through the shell. The seeds of red and blue color, both of them, dominate in the field of the microscope. The sticks (not acid resistant) revealed only singly.

So, long stay L- and other forms of Mycobacterium of the 60th generation in conditions of the positive low temperature was accompanied mostly with transformation of the not acid resistant sticks and single grains in acid. The L-shapes, to the same, have a clear tendency towards destruction.

After the filtering of the suspended matter of mycobacteria which was examined under immersion after 20 months of storage for 3 degrees through filters with pores of 0,1 and 0,05 microns in diameter and again examined with the microscope had been installed in the first and in the second case (Fig. 2.4 and 2.5) only a few small not acid resistant grains.

In the control (Fig. 2.6), without filtering, found growing at 8–10 day a large number of the colonies. Meanwhile when it was seeded, the filtrate mycobacteria, in culture medium and cultivating for 3 °C was detected on day 23 the simultaneous growth of individual colonies (1 of 3 in vitro), without reference to the diameter of the pores and of the filter (Fig. 2.7 and 2.8).

However, having prepared the smear of the cultures obtained under the immersion, was established acid (partially) short and long granular sticks, and detached single grains (Fig. 2.9). In another culture (Fig. 2.10) which was obtained from the filtrate (the filter with pores of 0,05 microns) under immersion was detected the acid long, with rounded edges and grain in the middle, sticks.

The same time was identified also submicroscopic (at the limit of visibility) the acid single grains (elementary bodies). Obviously, the different pores` diameter of the filter passes the ultrasmall forms (elementary bodies) of various sizes, with a some potential capacity to generate acid or not acid resistant forms of the mycobacterium. It maintains the diversity (possibly) of their biological significance.

Exploring the culture of 110th replanting (Fig. 2.11) of the same strain of dissociative mycobacteria under the immersion found (Fig. 2.12) not acid resistant forms, identical 60th generation: the short sticks (rather coccus), the

elongated L-forms with dark grains inside. Of the various of L-forms exempt the grains. In the filtrate of prepared the suspended matter of mycobacteria, (filter with pores of 0,1 microns) under the immersion was detected (Fig. 2.13) the submicroscopic grains.

After the sowing filtered mycobacteria on the nutrient medium after 20 days was obtained the culture of continuous growth (Fig. 2.14), and the smear, which prepared from the resulting culture, found (Fig. 2.15) only not acid resistant grains, coccus, short and long sticks in the absence of granular L-forms .

Exploring the pathogenic *M. bovis* strain 124th parents` generation (Fig. 3) was revealed the numerous, small and medium sizes, the correct form colonies matte yellow and white color (Fig. 3.1), and under immersion (Fig. 3.2) – acid sticks: short thin, straight with rounded edges, located both singly and accumulations (cultivation temperature only 37 °C).

After the filtering of, which was examined under the immersion, pathogenic *M. bovis* strain of 124th generation and reviewed the smear, that prepared by us from the filtrate, is not detected the forms of mycobacteria, the culture growth is not obtained (3-month incubation).

However, it should be noted that all investigated dissociative forms of *M. bovis*, except

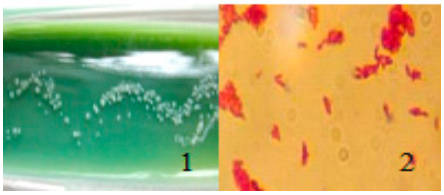

Fig. 3. The subculture (1) and the morphology (2) virulent M. Bovis of 124th passage ×1600

of pathogenic strain, not cultivated at 37 °C.

Consequently, the researching of dissociative L- and other forms witnessed undeniable dynamics of changes of biological characteristics, that indicate that in numerous passages through artificial nutrient medium increased the frequency of the formation of the ultrasmall forms and their adaptation to the environment. However, this is accompanied by the absence (usually) generation of the same forms mycobacteria in these subcultures: scilicet, of elementary cells (with it only isolated) in remote terms was formed not acid resistant rod forms. It maintains a natural part of the ultrasmall forms in the biological cycle of the mycobacteria, because they generate rod-form of Mycobacterium tuberculosis. The researched L-forms do not penetrate through the bacterial filters, even though they have a modified in biochemical terms, plastic cell wall.

Conclusions

1. The subcultures of species dissociative of M. bovis contain the filterable forms, their frequency of excretion increases depending on the number of generations (passages).

2. The ultrasmall not acid resistant forms, by the sowing in elective medium for cultivation mycobacteria, multiply to form colonies both a single and a continuous growth (as a result of the merger of separate colonies) culture in several times slower than in control test.

3. In cultures, which obtained from the filtrate, under the immersion are found the different, from those cultures in nutrient medium, morphological forms.

4. The presence of the ultrasmall forms in the populations of mycobacteria, and their ability to generate the morphologically modified microorganisms in the subcultures, is convincingly argues their indisputable importance in the biological development cycle of the examined species Mycobacterium tuberculosis.

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