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BIOLOGIC PROPERTIES OF DISSOCIATIVE L- AND OTHER FORMS OF M. BOVIS

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M. bovis with different biologic properties in comparison with pathogenic strains has been studied in the present work. The race of modified forms of M. bovis was selected. L-forms after passaging through dense nutrient medium at 3° C change morphologically: transform into the acid-nonproof rod-shaped, coccus forms; at 37° C transform into the acid-nonproof (sometime acid-proof) elementary bodies (grains). In the first generation the culture-revertant isolated from organs of Guinea pigs on the second day produces an orange culture and synthesize novel short-chained free fatty acids that were not detected in the virulent mycobacteria of the maternal culture

Dissociative forms of M. bovis, lipids, medium, temperature

Introduction. Mycobacteria and M. bovis in particular have an ability to change significantly their phenotype or genotype with loss of some antigens and appearance of others that mainly are not novel for mycobacteria [1]. Such changes can take place under environment factors and independently that determines the lability of microbe genome with somnolent or active or resuscitation promoting factors (rpf) [2-6]. They deretmine a particular ability of mycobacteria at a certain developmental stage to remain high probability of reversion in the initial or conversion into a new form, with clearly marked constant genetic or temporary phenotypic properties: morphology of mycobacteria, cultural, biochemical and other properties, change of the lipid composition [7-15]. In spite of general understanding of such mechanisms there are a lot of unclarified problems related firstly to the morphology of microbe cell, its biologic activity, capability of acquirement of properties of atypical mycobacteria.

The investigation of L- and other forms of M. bovis dissociants with redused/ lost sensibilizative ability that are reproduced in dynamics of passages through dense medium at 3º C is an actual task because there are no publications related to the problem involved in available literature.

Biologic properties of L- and other forms of M. bovis dissociants cultivated at temperature 3 and 37° C have been studied in this research.

Experimental. Hemerodiaphorous forms of one strain of M. bovis that was obtained in 118 passage trough Lowenstein-Jensen medium (pH 7.1–7.2) at 37º C and self-restrained with an initial growth of single colonies (118 subculture) at 2–3º C during 20 months have been used. Isolated colonies of next 60 reseedings were investigated. Namely, the growth rate, pigmentation upon change of medium' pH, morphological signs, tinctorial properties (smears were stained by Ziehl-Neelsen method) of changed forms of microorganisms cultivated at 3 and 37º C, the growth of culture of various generations of mycobacteria through simple nutrient media.

Dehydrogenase and catalase activity of mycobacteria of initial cultures and cultures-revertants isolated from Guinea pigs organs in that were inoculated microorganisms involved have been investigated by usual methods.

Mycobacteria of initial virulent cultures (2; 59; 100 passages) cultivated at 37º C and dissociative forms cultivated at 3 and 37º C were investigated by chromatography for qualitative and quantitative determination of free fatty acids of lipids [10].

In order to establish sensibilizative ability and other features of mycobacteria in long-term experiment using simultaneous probe (PPD- for mammals and allergen from atypical mycobacteria (AAM) reseeding microorganisms cumulated through the dense medium at 3º C were inoculated (single dose of 1 $mg \cdot cm^{-3}$) for 4 Guinea pigs, two of which were euthanized after three and the other after nine months from the beginning of the experiment, the next step was a bacteriological examination of biologic material from them and reinoculation of isolated cultures (revertant) for

laboratory animals (Guinea pigs).

The possibility of mycobacteria involved to cause tuberculosis infectious process was studied by the double inoculation of mycobacteria cultivated at 3 and 37º C (1 mg·cm^{-3} with an interval of 1.5 months) for animals. The macroorganism reaction was estimated every 10 days: the weight, the term of canker occurrence in the place of suspension introduction, delayed sensitivity progress (simultaneous test with PPD- for mammals every 30 days) and postmortem changes (after three months) were studied. M. bovis maternal strain was used as reference. Methods stipulated for respective procedure were used [10].

Results and discussions. Studying of mycobacteria growth on the dense egg medium of one tube with pH 7.1–7.2 at 3º C after 118 passages allows one to determine that after 20 months under low positive temperature conditions of cultivation the nature of cultures and their qualitative composition have been changed significantly. In particular, before culture was placed in conditions with low temperature only 5 small colonies were observed, after 20 months film, one large rough colony and 10 small colonies have been observed. By smear microscopy, prepared from individual colonies that were formed by the cultivation at 37 and 3º C, acid-proof short, thick and thin, straight and curved with rounded ends and pronounced grains bacilli that were placed in clusters, were observed. After seeding of mycobacteria suspension made from isolated colonies on the nutrient medium and cultivation at 3º C on twelfth day the weak growth of single small well shaped smooth gray-white colonies (second generation of subculture) has been observed. Lately, these colonies have formed solid growth on sowing line (Fig. 1).

Fig.1.**The culture of M. bovis of second generation (3ºC)**

The smear microscopy showed acid-nonproof ovals (L-forms) with various surface optical density and single acid-proof elementary bodies (Fig. 2). There was no observed growth of M. bovis of

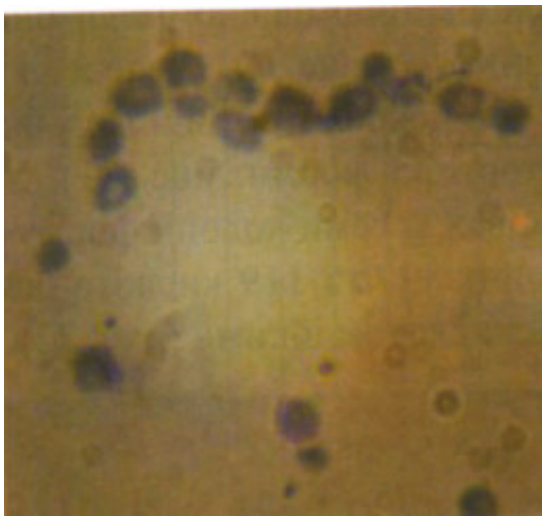


Fig. 2. **L-forms of of M. bovis of second generation. ×1500**

100 subculture of maternal pathogenic strain at 3ºC.

Then a seeding of mycobacteria suspension on the media of six test tubes and the cultivation at 3 and 37º C were done. The primary growth of moist cream colonies was found on 11 and 25 days of investigation, respectively. By smear microscopy acid-nonproof ovals with various surface optical density (L-forms) and acid-proof elementary bodies were identified.

The investigation of pathogenic and sensibilizative properties of obtained cultures at 3 and 37º C on Guinea pigs showed that mycobacteria involved do not cause pathological-anatomical changes typical for tuberculosis and do not cause the sensibilization of animals to PPD- for mammals.

Then microorganisms involved were passaged seven times and cultivated at 3 and 37º C. At 3º C the growth was observed on 5th day, while at 37º C on fourth. In the first case mycobacteria were orange colored and in another case they were cream. By smear microscopy acid-nonproof large and short, thick and thin, straight and curved with rounded ends and pronounced grains bacilli and acid-roof elementary bodies and L-forms were identified (Fig. 3). It should be noted that at early

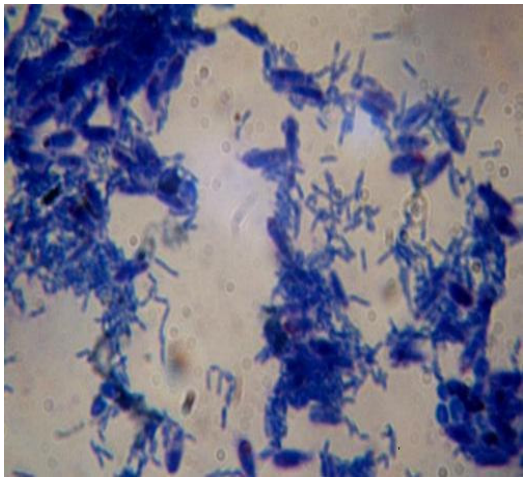
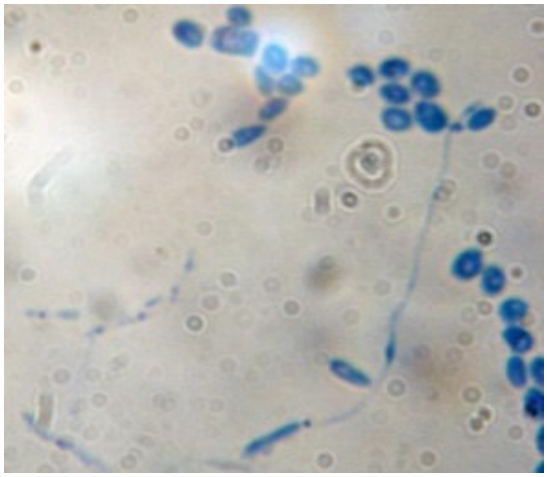
Fig.3a  Fig.3b 

Fig. 3. **L- and other forms of M. bovis cultivated at 3º C (a) and 37º C (b). ×1000.**

stages of morphogenesis of maternal culture its acid-nonproof fibers have released acid-proof grains that can produce typical M. bovis cells. This fact has attached our attention in 2008 [12]. Lately, after 60-80 passages of acid-nonproof forms of this causative agent variant (as can be seen from Fig. 3b) fiber microorgamisms release acid-nonproof structure elements that make L-forms (longed at first) with various surface optical densities.

The investigation of pathogenic, sensibilizative properties of subcultures of Mycobacteria of 10th generation, obtained at different cultivation temperatures, showed that Guinea pigs remain alive and do not react to PPD- for mammals during the three-month experiment.

Then still ten passages of mycobacteria of subculture involved through dense nutrient medium were done. The forming of colonies was significantly higher than in tenth generation only at 37º C (two days). By smear microscopy acid-nonproof large and short, thin, straight and curved with rounded ends and pronounced grains bacilli, L-forms (ovals) and slightly red single elementary bodies were identified at 3º C, while at 37º C acid-nonproof mainly short with pronounced grains straight and curved bacilli, L-forms and acid-proof elementary bodies were identified.

The following investigations of mycobacteria passaged at different temperatures (15th generation) were made on simple nutrient media: meat infusion agar and broth.

In reference sample (100 subculture of investigated variant) pathogenic M. bovis on Lowenstein-Jensen medium at 37º C grew on 23rd day, while at 3º C during three months growth was not observed. However, on meat infusion agar and broth mycobacteria of pathogenic strain did not grow, while modified microorganisms grew on 2-3 day of investigation. On agar solid growth of light grey culture on sowing line was observed. In broth at the beginning light grey film and turbidity with following precipitation were observed on surface. After three weeks level of sediment became 4-6 times greater.

After one week by smear microscopy acid-nonproof large and short, thick and thin, straight and curved with rounded ends and pronounced grains bacilli, acid-nonproof grais and L-forms were identified at 3º C, while at 37º C acid-nonproof elementary bodies and L-forms were identified. At 3º C in culture grown on meat infusion agar acid-nonproof large and short, thick, straight and curved with rounded ends and pronounced grains bacilli and acid-nonproof grains, while at 37º C acid-nonproof elementary bodies and L-forms were identified.

After three weeks by smear microscopy made of film and deposit of meat infusion broth were obtained practically same compared to weekly culture morphological and tinctorial properties. However, in the deposit at 3º C various shaped bacilli were acid-nonproof, in the film they were mixed, while at 37º C only acid-proof.

Interesting data were obtained at parallel passage of L-forms through dense Lowenstein-Jensen medium at different temperatures of cultivation. In this way, by smear microscopy (Fig. 4) of some orange colored subculture of first generation at 3º

Fig.4a

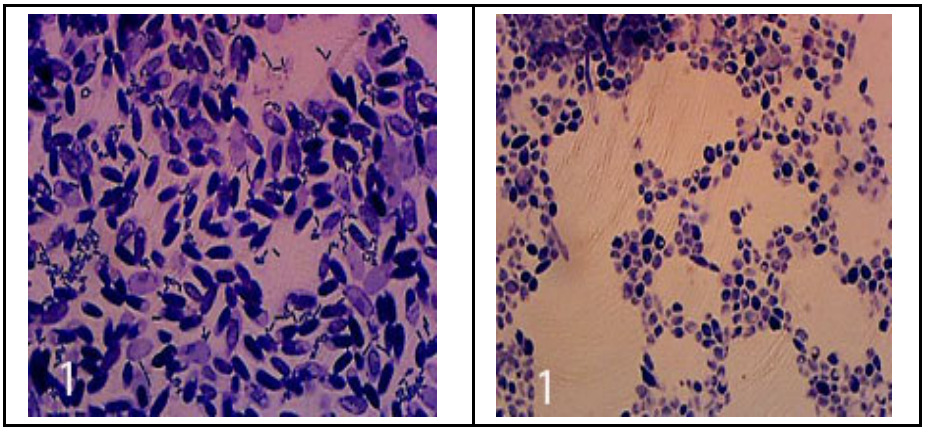


Fig.4b

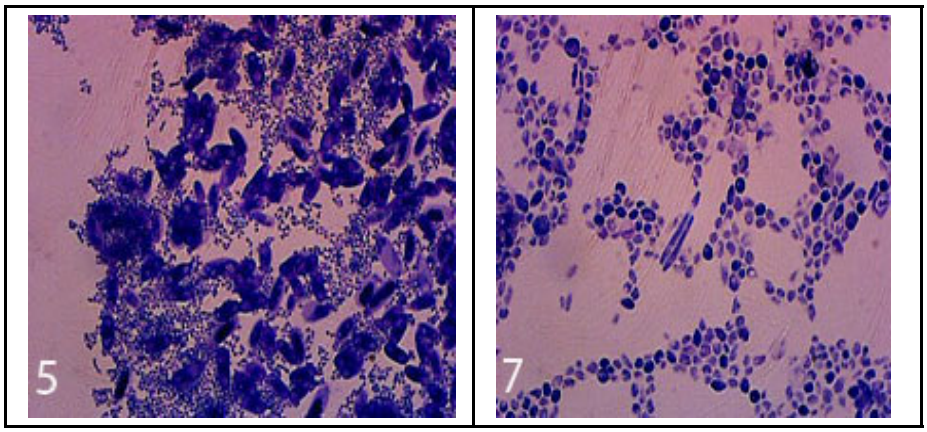


Fig.4c

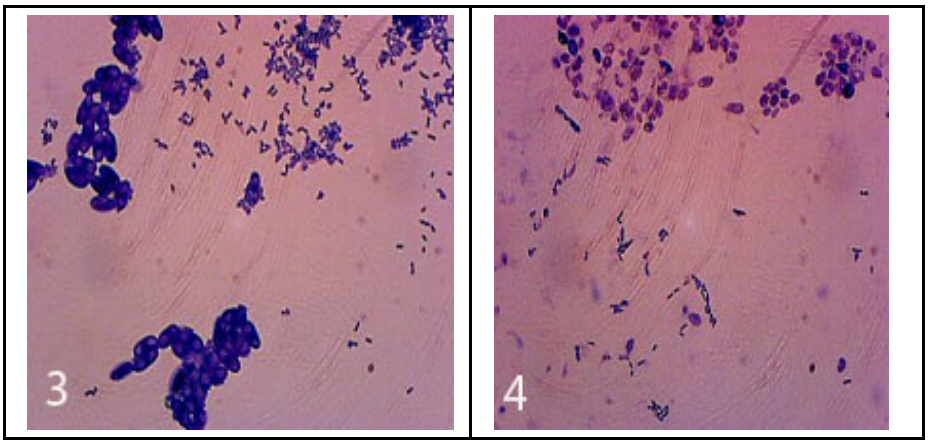


Fig.4d

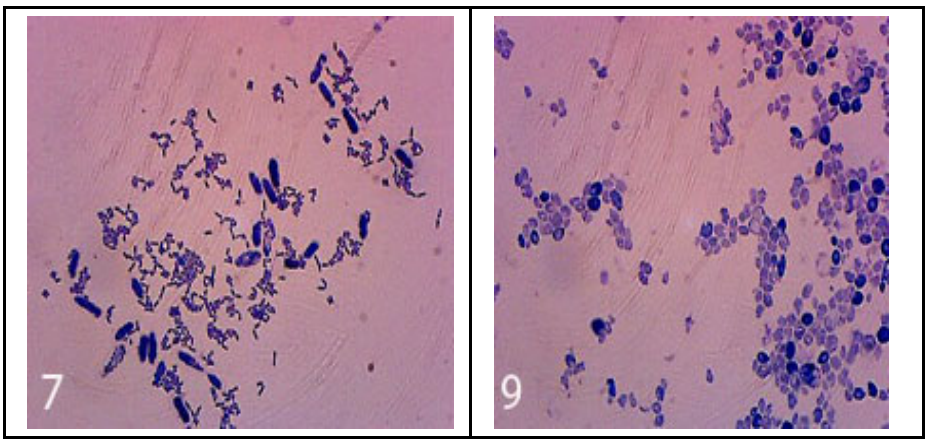


Fig.4. **L- and other forms of M. bovis passaged through the dense nutrient medium at 3º C (on the left) and 37º C (on the right). ×1000**

Fig.4e

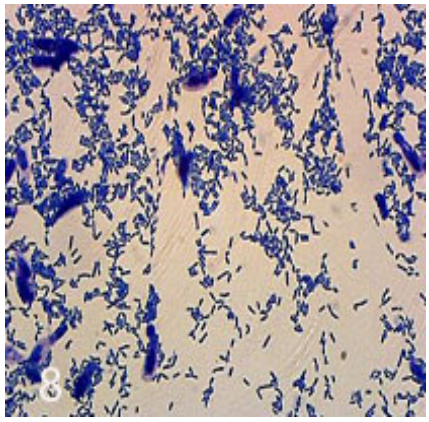


Fig.4f

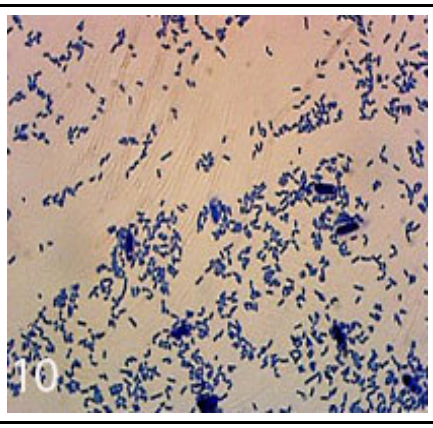


Fig.4g

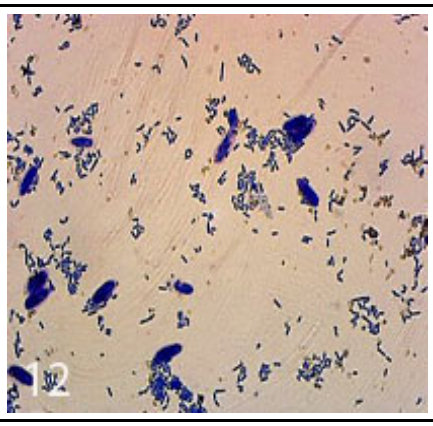


Fig.4h

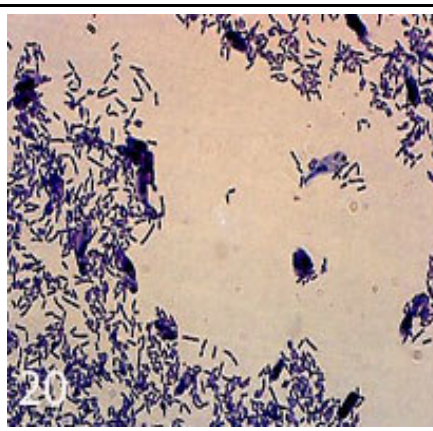


Fig.4. **L- and other forms of M. bovis passaged through the dense nutrient medium at 3° C (on the left) and 37° C (on the right). ×1000**

C L-forms (longed ovals, some of them have released grains) and grained bacilli (short and larger) were identified, while at 37º C (yellow easily emulsificated subculture) only L-forms with various surface optical density (practically rounded shape) and single grained bacilli were observed. In the third generation at 3º C subculture was made of L-forms (longed ovals), bacilli and grains, in the fourth at 37º C L-forms (rounded) and single rod-shaped formations were observed.

The fifth generation at 3º C was made of longed L-forms and rod-shaped and grained acid-nonproof elements. The seventh subculture (37º C) features practically by L-forms (rounded) and single thick grained short and large bacilli.

By smear microscopy of seventh subculture longed L-forms from that grained forms were released and short bacilli were identified. In the ninth subculture at 37º C by smear microscopy only L-forms, single round-shaped reddish elementary bodies were observed. The smear of eighth subculture (3º C) was identical to seventh subculture smear. Be smear microscopy of twelfth subculture at 37º C L-forms and elementary bodies (grains) with reddish color were identified.

There were no observed significant changes in subcultures of tenth (3º C) and 18th (37º C) generations. However, in these subcultures grained bacilli were observed.

In twelfth subculture (3º C) longed L-forms from that acid-nonproof grains released, short and large bacilli and single reddish elementary bodies were identified; in the 19th subculture (37º C) L-forms, large and short grained bacilli and reddish elementary bodies were observed. The twentieth (3º C) and 21st (37º C) generations have same morphological forms as 12th and 19th.

Summarizing the dynamics of morphological signs, tinctorial properties and nature of culture growth of modified M. bovis (including L- forms), one can mark an undeniable fact that at low positive temperature (3º C) acid-nonproof grains, grained bacilli, L-forms are formed, while at 37º acid-nonproof grains, short and rod-shaped bacilli and single reddish elementary bodies occurred that, probably, influence on a nature of culture growth. Namely, in the absence of elementary bodies the culture intensively grows on sowing line of mycobacteria suspension, while in the presence of elementary bodies after 4-5 days of cultivation, it sinked under their pressure in the medium: solid growth became thicker and after 2-4 weeks medium flows down, indicating unusual properties of these forms of M. bovis.

At the same time our previous investigations [7-12] repeatedly showed that reproduction rate of strains involved, decrease of virulence, changes in qualitative and quantitative composition of free fatty acids in lipids significantly affected by pH of medium. Therefore, in this connection was necessary to study the influence of acid gram-equivalents in the medium on the pigmentation, morphology and tinctorial properties of mycobacteria cultivated at 3º C (at 37º C in this stage of investigation mycobacteria lost the ability to grow on the medium).

As it can be seen from Fig. 5, the slightly orange culture in 27-37th generation on medium with pH 6.5 after 2-3 weeks gained more intensive pigmentation, while on medium with pH 7.1-7.2 re-

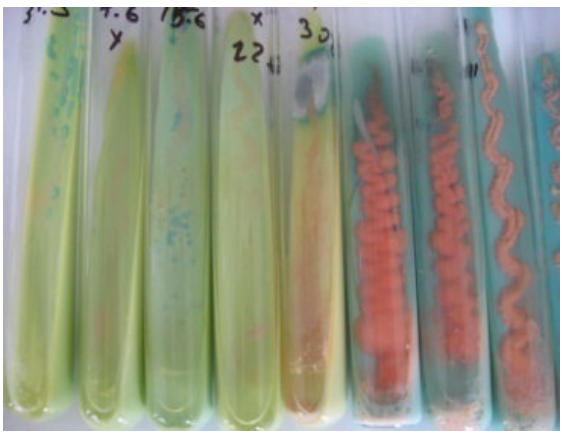
Fig.5a  Fig.5b 

Fig. 5. **The culture of L- and other forms of M. bovis cultivated on the medium with pH 7.1-7.2 (a) and 6.5 (b).**

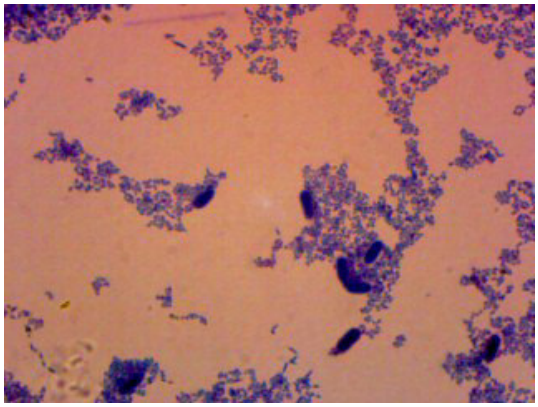
Fig.6a 

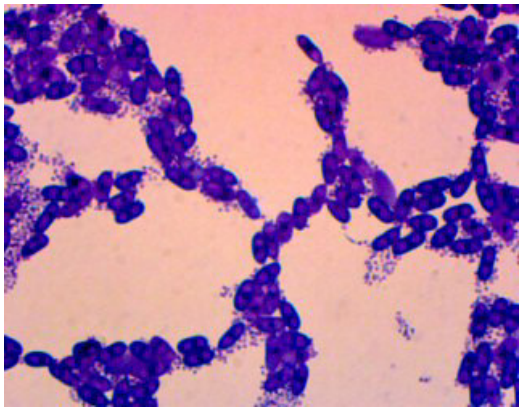
Fig.6b 

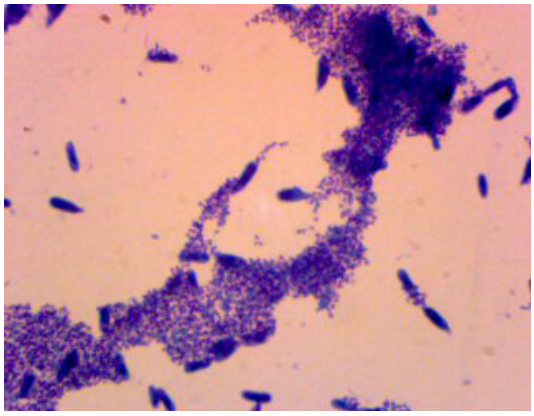
Fig.6c 

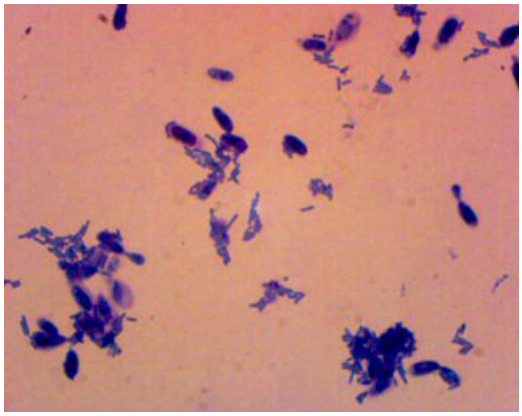
Fig.6d 

Fig.6. **L- and other forms of M. bovis: 26th generation, pH 7.1-7.2 (a); 27th generation, pH 6.5 (b); 33rd generation, pH 6.5 (c); 37th generation, pH 6.5 (d). ×1000**

mained the same during three months of cultivation.

By smear microscopy of culture from 26th generation (Fig. 6) mainly acid-nonproof grained forms, rarely acid-proof bacilli and thick (15-20 μm) dark blue bacilli with same surface optical density were identified. First seeding of such different forms from 26th generation on the medium with pH 6.5 accompanied by appearance of large number of blue ovaloid forms (with dark grains in the middle) with various surface optical density; small bacilli, sometimes with reddish color that were situated only near or around blue ovals. This evidences the releasing of grains from ovals. The same effect was observed in the smear made of culture from 33rd and 37th generations. However, not large amount of blue formations that lately transformed into thick blue ovaloid grained bacilli has been observed. Around of these bacilli small acid-nonproof (rarely acid-proof) grained forms (0.1-0.2 μm) have been situated. Bacilli with typical shape for tuberculosis causative agent have not been observed in this and following generations.

After still ten passages it was established that in the first five passages (from 41st till 45th) culture growth was observed on a 4th day usually like a cream film on the sowing line with following formation of single large dry orange colonies (Fig. 7). At the same time 45th subculture was mucinous and viscous. By smear microscopy acid-nonproof grained and large (twelve times bigger than bacilli) longed ovals with the same surface optical density that released from short bacilli were observed (Fig. 8).

After following five seedings the culture remained mucinous, its growth occurred on 1-6th day; pigment changed from orange to yellow (Fig. 9).

By smear microscopy of the culture from 50th passage practically same forms of microorganisms: both acid-nonproof grained ovals (L-forms) and acid-nonproof grains that placed between ovals were identified (Fig. 10). On the 60th passage the culture was identical to that, obtained for 50th.

Thus, the cultivation of dissociative forms of

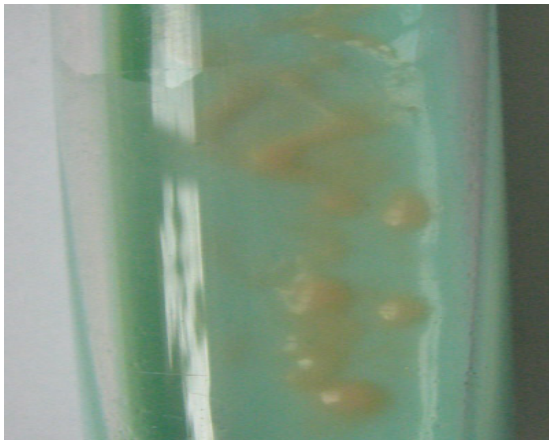


Fig.7.**The culture of modified M. bovis (45th passage at 3º C)**

Fig.8. **L- and other forms of M. bovis (45th passage at 3º C). ×1000**

Fig. 9. **The culture of dissociative forms of M. bovis of 118 variant (50th passage at 3º C)**

M. bovis at low positive temperature (3º C) on the medium with different pH, has been accompanied by maintenance of morphology.

It was necessary to investigate possible morphologic changes in long-time preservation of culture. Therefore, microscopy of the smear prepared from unmodified 27th subculture at 3º C, has been held after 15 months. Acid-nonproof short and larger bacilli and acid-proof elementary bodies (grained forms) were observed (Fig. 11).

On the fourth day orange-reddish solid growth on the sowing line (Fig. 12) morphology and tinctorial properties of L-forms on Lowenstein-Jensen medium without sodium salicylate at 3º C were investigated. Acid-nonproof formations (Fig. 13) with lightly red capsule and dark grains that are situated in the middle of ovaloid longed form (rod-shaped) were observed. Lightly red grains

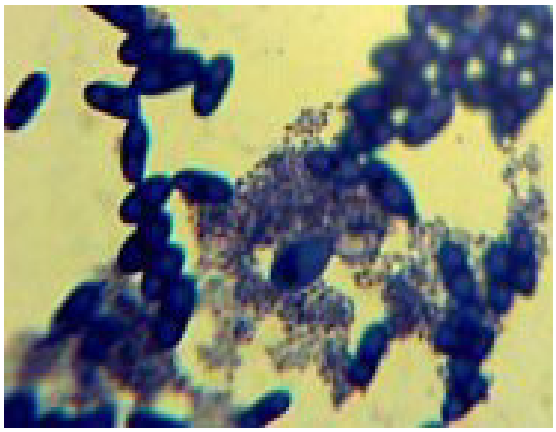


Fig.10. **M. bovis of dissociative forms of 118 variant (50th passage at 3º C). ×1000**

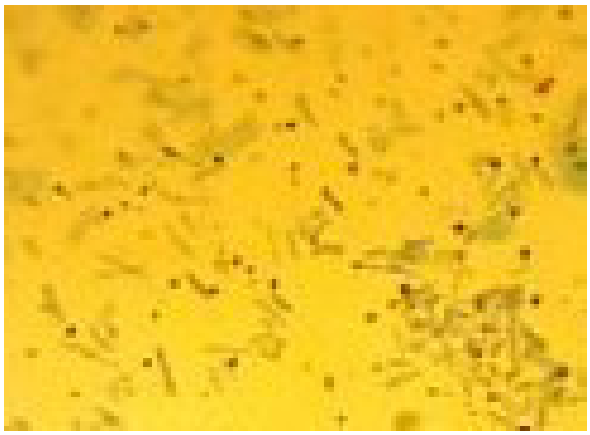


Fig.11**. Elementary bodies and acid-nonproof forms of bacilli of M. bovis (cultivated during 15 months at 3º C). × 1300**

Fig.12. **The culture of L-forms of M. bovis (reference sample)**

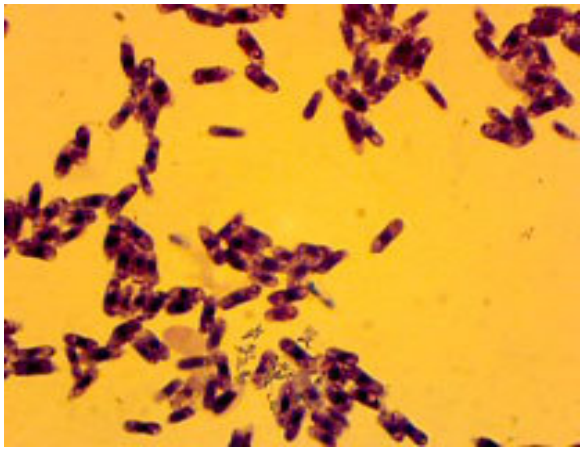


Fig.13. **L- and other forms of M. bovis. × 1500**

and bacilli have been released from some of them.

At the same time in initial modified forms and in cultures-revertants an increase of redox reactions (dehydrogenase and catalase activity) and decrease of virulence were observed.

Thus, results obtained in the present paper evidenced that L- and other forms gained properties representative for atypical mycobacteria.

According to data obtained by investigation of free fatty acids content in M. bovis of typical virulent and modified avirulent forms the last features by appearance of novel short-chained hendecanoic acid. Its synthesis was 15.6 times more intensive at 3º C compared to 37º C. At the same time at 3º C the synthesis of lauric and arachic acid was very weak, while in virulent mycobacteria their quantity can be determined. At 37º C only lauric acid has been synthesized in small amount. It evidences deep changes in the metabolism of microbe cell that absolutely influenced by surviving under low positive temperature and accompanied by the formation and reproduction of colonies.

The quantitative content of free fatty acids in modified mycobacteria has been significantly changed compared to the reference virulent maternal culture. At the same time skeleton free fatty acids (palmitic, oleic, stearic) in mycobacteria of dissociative forms remained on the high level that evidence their stability regardless of changing in properties of mycobacteria, their biological activity. In other words, change of cultural, tinctorial, virulent, biochemical and other properties and morphological signs does not result in significant change on content of skeleton free fatty acids.

At the same time ratio of short- and long-chained free fatty acids in virulent subcultures (average 100 and 124) and in avirulent mycobacteria (L- and other forms) was 9.94:1; 5.51:1 (3º C) and 5.31:1 (37º C), respectively. This is contrary to observations that have been made because virulent mycobacteria have significantly lower level of short-chained free fatty acids compared to long-chained. Perhaps, because such studies of dissociative forms obtained at low positive temperatures have been held for the first time, this pattern ratio of short- and long-chained acids inherent for so many passaged and genetically modified mycobacteria and determined by their metabolism. For virulent mycobacteria of maternal cultures (2^{nd} culture) this ratio was significantly different 1.84:1. However, mycobacteria of 59^{th} subculture ratio of these acids was 4.89:1. This suggests change in the ratio of acid synthesis during passages through artificial nutrient medium.

Meanwhile, the content of unsaturated free fatty acids that characterize the adaptive metabolism of microbe cells (palmitoleic, oleic and linoleic+linolenic) was lower in the first and second and much higher in the third than in the virulent maternal culture.

Probably, these particular contents of unsaturated fatty acids in the dissociative forms of mycobacteria (L- and other forms) determine the degree of virulence that can be characterized by long-chained acids as decreasing of amount of unsaturated acids that are responsible for the leukoprotease inhibition [16], i.e. their low level

increases the activity of protective factors of microorganism that provides neutralization of long-chained free fatty acids and possibly determines the total avirulence of studied forms of mycobacteria, in which the content of unsaturated fatty acids remains practically the same while in pathogenic mycobacteria the level of long-chained free fatty acids is high. The ratio of long-chained to unsaturated acids in pathogenic strains is 4.63:1, in avirulent 3.34:1 (3º C) and 2.69:1 (37º C).

It should be noted also that in the second generation of maternal culture of investigated strain analyzed factors were 1:1.26, notably content of long-chained free fatty acids was sensibly higher than content of unsaturated acids. However, in the 59th subculture this ratio was 2.38:1; this determines consistent, tendentious changes of synthesized systems of microbial cells that are influenced by its adaptation to changing of environmental conditions. In this not only chemical but also physical links of cell components (lipids, proteins, carbohydrates) are interrupted that leads to the appearance of complexes with new qualities and properties and probably to changes in the genotype.

It was established that persistence of investigated microorganisms (27th subculture acid-nonproof bacilli and L-forms) in Guinea pig organism last nine (term of the investigation) and more months. However, from suspension made from macroscopic nonmodified organs of laboratory animals acid-proof elementary bodies (grains) and bacilli with typical morphologic forms, that produce orange culture on the third day of cultivation, were isolated.

The inoculation of isolated acid-proof mycobacteria (culture-revertant) for Guinea pigs (1 mgcm^{-3}) was not accompanied by development of allergic status (allergic reaction on tuberculin and AAM after 30, 60 and 90 days was negative), but from organs of euthanized after three months experimental animals acid-proof bacilli that formed an orange culture on 3rd day of cultivation were isolated.

Obviously, multiple passages through artificial dense medium and long-term maintenance (20 months) at low positive temperature have changed the genetic balance that has provided them surviving as a result of the loss of some (specific for the pathogenic microorganism) and the appearance of novel properties particularly representative to other mycobacteria including atypical. At the same time, the persistence in the organism of Guinea pigs of typical morphologic acid-proof forms (bacilli) that were reversed from L-forms has not accompanied by the development of the disease. They remained chromogenic and retain the ability to form colonies (culture-revertant) on dense nutrient medium beginning from the first generation (from biologic material of Guinea pigs) on the second day of cultivation.

The loss of sensibilizative ability in mycobacteria that many times passaged and persistened in the organism of Guinea pigs may evidence, in some limits, the loss of immunogenic capacity, as it is known that the development of allergic (tuberculin) reaction, besides its intensity, indicates immunological restructuring of macroorganism (development of infection) with parallel acquisition of specific resistance.

However, the results of the experiment have shown that one of a variety of indicators of clinical manifestations of infection – the change in the body weight of Guinea pigs, in which organism 1 mgcm^{-3} of studied mycobacteria was inoculated, has a pattern of dynamics: on the tenth and twentieth day of the experiment the body weight increased from 25 till 45 g, respectively, on 35th day it decreased compared to the 20th day on 35 g with following increase to 50 grams per 46th day. However, after 50 days, at the second injection of 1 mg·cm^{-3} of such forms of mycobacteria, such decrease in body weight was observed after 15-20 days with following smoothing of the positive dynamics of the test target. This may indicate the residual virulence of investigated modified forms of M. bovis with the possible formation of specific anti-tuberculosis immunity without developing of the necessary level that can be detected by diagnostics, allergic status (and ulceration in the area of injection of mycobacteria suspension).

Touch smears bacterioscopy from organs of euthanized (after 80 days) animals showed acid-nonproof bacilli and grains-bodies.

In the reference sample the body weight has been increased tendentiously and touch smears bacterioscopy was negative.

Obviously, mycobacteria with new, genetically fixed properties have different ability to stimulate

benign infectious process without the required level of allergy to PPD for mammals and AAM.

Also it should be noted that probably an attenuation of activity of genes responsible for pathogenic properties that are defined by redox reactions (dehydrogenase and catalase activity and others) and genes that were activated (appeared in the dormant state) stimulate the metabolic processes of pigmentation with inhibition of factors (toxins) of pathogenicity.

At the same time we found no relationship between the rate of reproduction (day of formation of colonies) and pathogenicity, as the initial maternal culture (third generation) of investigated modified forms of M. bovis have had high virulence and formed colonies on the second or third day without pigmentation and have not had dehydrogenase and catalase activity [13].

Thus, the race of modified forms of mycobacteria with special properties that may be promising for the design of anti-tuberculosis vaccine was selcted.

Conclusions. The race of modified forms of M. bovis was selected. L-forms after passaging through dense nutrient medium at 3º C change morphologically: transform into the acid-nonproof rod-shaped, coccus forms; at 37º C transform into the acid-nonproof (sometime acid-proof) elementary bodies (grains). At 3º C the growth of orange culture on dense selective nutrient medium takes place on second-third day of cultivation (sometime on 24th hour), at 37º C the growth of yellow culture takes place in the same termin, but after 20 passages the weak growth of culture occures and lately dissapears. On meat infusion agar and broth investigated mycobacteria grow on first-second day of cultivation. They have marked dehydrogenase and catalase activity, are avirulent: during nine months partically reverse into the acid-proof morphologic forms without causing an tuberculosis of Guinea pigs, have a low or absent sensibilizative property (for PPD- for mammals and AAM), do not cause an ulceration in the area of injection of mycobacteria suspension. In the first generation the culture-revertant isolated from organs of Guinea pigs on the second day produces an orange culture and synthesised novel short-chained free fatty acids that were not detected in the virulent mycobacteria of the maternal culture.

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БИОЛОГИЧЕСКИЕ СВОЙСТВА ДИССОЦИАТИВНЫХ L- И ДРУГИХ ФОРМ M. BOVIS

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В данной работе были изучены M. bovis с отличными от патогенных штаммов биологическими свойствами. Селекционирована новая раса модифицированных форм M. bovis. Установлено, что L-формы при пассажировании через плотную питательную среду при 3 ° С изменяются морфологически: превращаются в некислотостойкие палочко-, коккоподобные формы; при 37 ° С - некислотостойкие (иногда кислотостойкие) элементарные тельца (зерна). В первом поколении культура-ревертант, изолированная из органов морских свинок, на второй день продуцирует колонии оранжевой окраски и синтезирует новые короткоцепочные свободные жирные кислоты, которые не были обнаружены у вирулентных микобактерий материнской культуры

Ключевые слова: *диссоциативные L- формы M. bovis, липиды, температура*

БІОЛОГІЧНІ ВЛАСТИВОСТІ ДИСОЦІАТИВНИХ L- ТА ІНШИХ ФОРМ M. BOVIS

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Показано, що L-форми за пасажів через щільне середовище за температури 3 °С змінюючись морфологічно переходять в некислотостійкі паличко- та кокоподібні форми, за 37° С – в некислотостійкі (інколи кислотостійкі) елементарні тільця (зерна); за температури 3 °С ріст на щільному елективному живильному середовищі помаранчевої культури відбувається на другу-третю добу культивування (інколи на 24 годину), за 37 °С – жовтуватої культури в такі ж строки але після 20 пересівів відбувається слабкий ріст культури за цієї температури і з часом зникає; на МПА та МПБ – ростуть (3 і 37 °С, останні субкультури з (26-ї генерації) тільки за 3 °С) на першу-другу добу культивування; на середовищі із натрієм саліциловокислим за низької плюсової температури ростуть на шосту добу, а за 37 °С – на 15 добу; володіють вираженою дегідрогеназною й каталазою активністю; авірулентні – впродовж дев'яти місяців частково реверсують у кислотостійкі морфологічні форми не викликаючи туберкульоз у морських свинок, володіють низькою або не володіють взагалі сенсибілізуючою здатністю (щодо ППД- для ссавців, в тому числі ААМ), не утворюють виразки у ділянці введення мікобактерій; в першій генерації культура-ревертант (виділена з органів морської свинки) на другу добу утворює помаранчеву культуру; синтезують нові коротколанцюгові вільні жирні кислоти, які не виявляються у вірулентних мікобактерій материнської культури

Ключові слова: *дисоціативні форми M. bovis, ліпіди, температура*