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СКРИНИНГ БАКТЕРИЙ РОДА *BACILLUS* ПРОДУЦЕНТОВ ЕКЗОПОЛИМЕРНИХ ВЕЩЕСТВ С ФЛОКУЛИРУЮЩЕЙ АКТИВНОСТЬЮ

Проведен скрининг среди 400 штаммов бактерий рода Bacillus продуцентов екзополимерних соединений с флокулирующими свойствами. Установлено, что среди всех исследованных штаммов бацилл 40,9% и 14,0% продуцируют экзополимеры полисахаридной и полиаминной природы соответственно при культивировании на агаризованных питательных средах. С наибольшей частотой продуценты полисахаридных и полиаминных веществ выделялись среди грунтовых штаммов бацилл – 55,34 и 47% от всех штаммов соответственно. Для дальнейших исследований отобраны перспективные штаммы, флокулирующих активность екзополимеров которых составляла 75-78%.

Ключевые слова: бактерии рода Bacillus, скрининг, биофлокулянты, экзополисахариды, полиамины.

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SCREENING OF BACTERIA OF THE GENUS BACILLUS PRODUCERS OF EXOPOLYMERIC SUBSTANCES WITH FLOCCULATING ACTIVITY

It was screened the producers of exopolymers with flocculating properties among 400 strains of bacteria of the genus Bacillus. It was found that under cultivation on agar nutrient mediums only 40,9 % and 14,0 % of all investigated strains produced exopolymers of polysaccharide and polyamine nature respectively. The producers of polysaccharide and polyamine substances were isolated with greatest frequency among soil strains of bacilli – 55 and 47 % of all strains, respectively. For further research were selected perspective strains with 75-78 % flocculated activity of exopolymers

Keywords: bacteria of the genus Bacillus, screening, bioflocculants, exopolysaccharides, polyamines.

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EXPERIMENTAL MODELLING OF AN ACID ESOPHAGUS BURN OF 2ND DEGREE IN IMMATURE RATS

In the experiments, we reproduced an acid esophageal burn model of 2nd degree in rats. The changes of basic blood biochemical parameters to confirm acid burn of esophagus in experimental animals. Our approach can be used for study molecular mechanisms of pathogenesis of 2nd degree.

Key words: an acid esophageal burn, biochemical parameters.

Introduction. Corrosive esophageal burns constitute a serious and widespread health problem during childhood [5,8]. The most common burns are oral of mucosa, pharynx, esophagus, especially in childhood. Acetic acid, vinegar essence, acid cleaning product are cause of almost 42,3% percent of an acid esophageal burns. Esophageal burns as a result of accidental swallowing of caustic material are seen frequently in children ages 1 to 8 years. [5,6].The depth of an esophageal burn depends on the concentration chemical substance, nature and the amount and contact time with the mucosal.

There are many pathologies and complications following an acid esophageal burns: swelling of the larynx, toxic shock syndrome [11, 14, 18], necrosis of esophageal and stomach tissue, dysmotility [2], scar stricture, esophageal deformations, corrosive esophagitis, gastroesophageal reflux [10, 19], candidiasis [12], malignization in a remote period [20] and others. Thereby the burn disease is an important issue that needs solution immediately.

Today there are a number of experimental models of an acid esophagus burns (AEB) which require the use of chemicals for their creation where, in itself, they affect the certain biochemical parameters [13, 15, 16, 20], which complicates the interpretation and analysis of the experimental results. Therefore, in our researches it was a guideline to develop a new model of burn, which we could use for broad spectrum research of burns pathogenesis especially in children ages from 1 to 8 years.

Materials and methods. In our experiments we used nonlinear immature white rats (1 month) weighing 90-110g, which were kept on a standard vivarium diet. The animals were experimentally simulated with the AEB with 30% solvent of CCl_3COOH; it was injected into the esophagus via the broach. The broach was placed at a depth of 4.0 cm whereupon 0.2 ml of 30% CCl_3COOH solution was slowly injected through it. The control rats were administered once orally for injection of 0.2 ml water [9].

The control of the esophageal mucosa was carried out at 1, 3 and 7th day after injection of acid. The choice of terms was caused by the pathological process development in chemical burns of the esophagus [1]. At the end of the experiment the animals were sacrificed by the dislocation of cervical vertebrae. The resulting esophagus was cut longitudinally, washed with cold saline. We used slices of esophageal length of about 1.5 cm for morphological studies. They were fixed in 12% formalin solution and embedded in paraffin. Dewaxed sections of 5 microns thickness were stained with hematoxylin – eosin for Van Hizonom. The research of the esophagus status was carried out visually. The number and destruction area were determined grossly with a magnifying glass in bright light.

Biochemical indexes determined in the serum of blood. The biochemical parameters (total protein, albumin, the level of urea, creatinine, K+ ions and ALT, AST activity) were determined with analyzer Humalyser 3000 with use of appropriate sets.

Separation of protein fractions serum rats were determined by method disc electrophoresis in 10% polyacrylamide gels (PAGE) with sodium dodecyl sulfate (SDS) by method Lemmli with modifications [17]. For estimate the results of electrophoresis used the program Totallab 2.01

The statistical analysis of the obtained results was performed using the methods of variation statistics and correlation analysis using the computer program Excel. To determine the reliability of the differences between the two samples we used the Student test (t). Whereby differences $P < 0.05$ were deemed reliable.

Results and discussion. It is known that acetic acid, due to its chemical structure, has an expressed soluble effect on lipids as well as resorptive effect. The dissolution of intercellular lipids causes rapid penetration of acetic acid from the gastrointestinal tract into the vascular bed whereas the dissolution of lipids of cell membranes causes the penetration of acetic acid into the cell as a molecule where they are subjected to dissociation with the formation of acidic ions. The acetic acid effect on mucous tissue is protein folding, takes water from them and leads to the formation of coagulation necrosis with a dense surface crust [7].

We have shown that the 30 % CCl_3COOH penetrates the tissue and results in significant mucosal lesions of the esophagus (Figure 1). It was revealed edema, congestion, damage to the superficial layers of the epithelium, hemorrhages into submucosa.

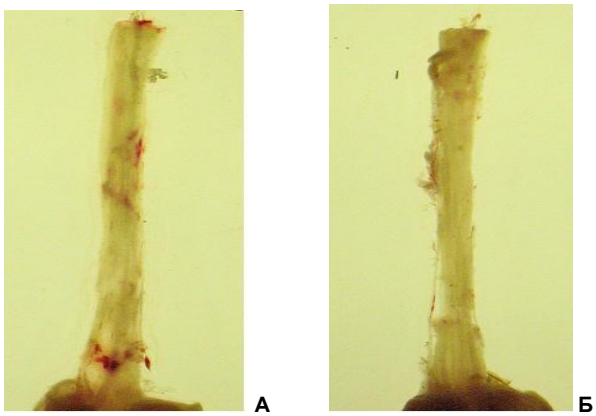

Figure 1. Macrophotography of the rats esophageal mucosa after experimental simulation of an acid burn with 30% CCl_3COOH (A-1st day, B – 7th days)

The micrography of the esophageal (Figure 2) mucosal lesions demonstrates damage to the esophagus tissues in rats under experimental simulation of AEB through 30% CCl_3COOH: fibrinous, erosive esophagitis (damage to the mucosa and submucosa). The comparison of the detected lesions and researches of status of the esophageal mucosa of children with 2nd degree burns specified the experimental model conformity [1].

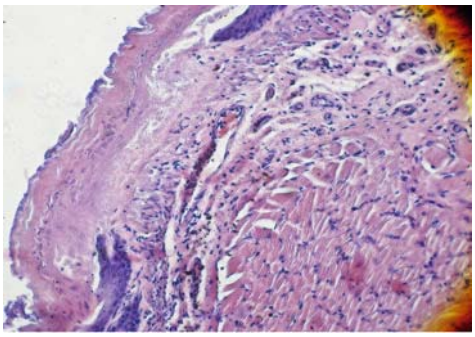

Figure 2. The micrograph of the rat esophageal mucosa after experimental simulation of an acid burn with 30% CCl_3COOH

It is known, the esophagus burn is accompanied with prolonged disturbances of carbohydrate, lipid and protein metabolism leading to the pathological changes into the functioning of various organs and systems [3]. Therefore, one of the major diagnostic criteria of the AEB severity is to determine biochemical parameters. We selected the following indicators as the level of total protein, albumin, urea, potassium ions, creatinine, AST, ALT, which are the main biochemical parameters of blood serum. They describe a functional state of the organism and assess the degree of metabolic disorders under studied pathology.

We have determined the concentration of total protein in blood serum of rats with chemical burns (30% CH3COOH) of the esophagus. In the process, we have obtained the following submitted data in table 1.

***Table* 1. Biochemical parameters in blood serum of rats under experimental modeling of an acid esophagus burn with 30% CCl_3COOH (M ± m, n = 10)**

Parameter	Control	AEB, 30% CCl3COOH		
		1st day	3rd day	7th day
Total protein,(g/l)	64,4±0,2	47,11±2,0*	48,13±1,01*	51,13±1,12*
Albumin, (g/l)	36,5±0,01	16,01±1,36*	20,19±1,9*	21,22±2,01*
Urea, (mmol/l)	8,9±0,11	19,32±2,01*	16,7±2,01*	14,1±1,23*
Creatinine, (μmol/l)	88,9±0,1	175,1±2,3*	171,9±2,1*	145,01±2,9*
K^+, (mmol/l)	5,1±0,01	13,8±1,3*	13,4±1,01*	8,0±1,1*
ALT, (Ul/l)	27,1±1,0	87,8±1,55*	74,1±0,93*	67,6±2,23*
AST, (Ul/l)	55,1±1,0	68,1±1,63*	63,1±1,85*	60,09±1,9*

**- P<0,05 compared to control.*

It was defined that the experimental burns of the esophagus through 30% CCl_3COOH leads to the significant changes in a total protein content. On the 1, 3 and 7 day of supervisions its content decreased in 1.4; 1.3 and 1.2 times respectively, comparing with control values.

It is defined that on the 1, 3 and 7 days there is decreasing of albumin in 2.3; 1.8 and 1.7 times respectively, comparing with control values. So there is hypoproteinemia, which occurs mainly by reducing the amount of albumin.

The definition of the content of the final products of protein metabolism plays an important role in the clinical laboratory diagnosis, which are viewed as a system of residual nitrogen. Almost a half of the components of this system are provided by urea. The rate of urea is critical for diagnosis of kidney disease in clinic. In the context of chemical burns through 30 % CCl_3COOH, we observed an increase: the level of urea increases on the 1, 3 and 7th day in 2.2; 1.9 and 1.6 times respectively, which may indicate the development of severe disease process. We have determined the level of creatinine in the blood serum of experimental animals. In the process of study we have obtained the following data (table 1), which indicates that the experimental esophageal burn on the 1, 3 and 7th days causes the significant changes of the creatinine, which is usually used as a marker of toxic body effect and may indicate kidney failure. The experimental burn of esophagus predetermined the increase of creatinine in blood serum in the course of the whole period of the study, on the 1, 3 and 7th days in 1.98; 1.93 and 1.63 times compared with control values corresponding literature data. So burn disease are characterized with an acute intoxication, violation of body water and salt metabolism, which often leads to kidney damage [17-19]. The research of the level of K^+ ions in blood serum of the rats with an acid esophagus burns permitted to determine its increase in 2.7; 2.6; 1.5 times at all time. Modeling esophageal burns the description of aminotransferase activity in blood serum of the rats showed that the ALT was raised in 3.2; 2.7 and 2.5 times as well as the activity of the AST increased in 1.2;1.1; and 0.9 times, respectively, on the 1, 3, 7 day of the experiment. In case of the acid burns the determined changes indicate significant carbohydrate, lipid and protein metabolism, which may be a proof of formation of a steady state of the 2nd degree-level esophagus burns in the animal experiments.

So, in terms of simulated the 2nd degree-level acid burns – the level of the studied biochemical parameters (total protein, albumin, urea, creatinine) and the activity of AST and ALT levels most varied on the 1st day of the research. Obtained results correspond to literature data and have shown significant damage of water-salt, nitrogen metabolisms and biochemical parameters of liver function at the shock stage (1 day). It can confirm of formation the stable state an acid esophagus burns of 2nd degree in experimental animals [4].

Electrophoretic analysis of serum protein composition of experimental animals showed in all studied samples, both in control and in conditions of AEB 2 degree, presence of protein fractions with molecular masses from 15 to 168 kDa (Table 2). Conducted researches did not show qualitative changes of protein levels in serum, but it is possible to determine their quantitative changes. Thus, we have shown a growth of protein levels in fractions with m.m. 167 kDa on the 1, 7, and 21st days of experimental acid burns by 15%, 40% and 19% respectively, and reduce on the 15th by 7%. Also, we have established an increase of protein levels in fraction with m.m. 150 kDa on the 7,15 and 21st days of the experiment by 24% and 37% and 52% respectively compared with control values. These protein stripes correspond to IgG fraction, and in view of obtained data on fluctuations of their levels, of some interest are analysis of their levels and a more detailed study. Increased level of this fraction of immunoglobulins indicates the activation of the immune system and appearance in the bloodstream of "defective" protein molecules, to which antibodies are formed. Analysis of electrophoretograms showed a growth of protein fraction levels with m.m. 130 kDa on the 1th day by 19%, and reduce on the 15th and 21st by 93% and 287% compared with control values after modeling of AEB. We have shown increased levels of the fraction m.m. 113 kDa on the 1th and 15th days by 8% and 13%, and compared with control values This fraction can correspond C-reactive protein, which is a central component of markers of the acute phase of inflammation. Elevated levels of the shown fraction on the 15th day indicate addition of bacterial infection or inflammation caused by non-infectious parts of necrotic tissues. Shown reduction in level of albumin fraction (67 kDa) after modeling of an acid esophagus burn on the 1, 7, 15 and 21st day by 13%, 71%, 50% and 20% respectively compared with control values. We have determined reduction of fraction 55 kDa after modeling of acid burns, which corresponds prealbumin on the 1, 7, 15 and 21st day by 80%, 69%, 125% and 86% respectively. Obtained data may indicate suppression of protein-synthetic function of the liver due to burn injuries. The results of investigations showed that in experimental animals after modeling of chemical burns of the esophagus, the level of postalbumin fraction ~ 40 kDa is reduced on the 1, 7, 15 and 21st days level of this fraction by 170%, 200%, 289% and 324% respectively compared with control values. Level of postalbumin fraction ~ 25 was significantly decreased on the 1, 7, 15 and 21st days by 97%, 104%, 293% and 204% respectively after modeling of acid burns. We have determined reduction level of postalbumin fraction ~ 15 kDa on the 1, 7,15 and 21st day by 40%, 311%, 310% and 251% respectively compared with control values.Quantitative changes in postalbumin

factions may be associated with increase of degradation processes of tissue proteins due to increase of activity of proteolytic enzymes of different specificity or traumatic effect of chemical factors.

So, after modeling of chemical burns of the esophagus one may talk of hypoproteinemia, which occurs mainly due to reduction in the amount of albumins and activation of humoral immune response.

***Table* 2. Relative content of proteins fraction in blood serum after modeling of an AEB (μg /mg protein) with 30% CCl_3COOH (μg /mg protein), (M±m, n=3)**

Proteins fraction m.m. (kDa)	Control	1st day	7th day	15th day	21st day
γ- globulin fraction – 168	86,8 ± 2,5	101,6±0,8*	143,0±6,9*	80,8±3,8*	106,0±4,2*
γ- globulin fraction –150	156,7±3,7	152,7±5,9	203.9±7,9*	247,0±8,1*	323,0±12,9*
130	449,2±28,9	548,9±20,3*	468,4±16,4	231,6±7,1*	115,9±3,2*
113	60,8±2,8	66,0±3,4	58,5±2,3	69,4±2,8*	30,6±1,4*
103	185,2±11,7	61,2±2,5*	42,8±1,7*	25,7±1,02*	46,6±1,9*
89	126,0±9,1	96,9±1,4*	84,0±0,2*	98,0±0,4*	46,0±1,8*
Albumin fraction –67	325,3±16,7	287,4±11,5*	189,3±7,6*	216,0±16,9*	270,0±10,8*
Postalbumin fraction ~ 55	221,0±9,7	122,6±4,9*	130,1±5,2*	97,8±3,9*	118,2±4,7*
Postalbumin fraction ~ 40	145,0±0,9	53,7±2,1 *	48,3±1,9*	37,2±0,69*	34,1±0,96*
Postalbumin fraction ~25	79,8±3,1	40,4±1,7*	39,1±2,6*	20,3±0,4*	26,2±0,05*
Postalbumin fraction ~15	74,5±1,9	53,2±2,1*	18,1±0,72 *	18,5±0,74 *	21,2±0,85 *

**p<0,05 compared with control value*

Conclusions. Thus, we have reproduced the model of 2nd degree AEB using 30% CCl_3COOH which was accompanied by corresponding morphological lesions of esophageal mucosa and changes in basic biochemical parameters. This approach may be used in the study of biochemical and immunological mechanisms of pathogenesis of the 2nd degree an acid esophageal burns of on animals.

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ЕКСПЕРИМЕНТАЛЬНЕ ВІДТВОРЕННЯ МОДЕЛІ КИСЛОТНОГО ОПІКУ СТРАВОХОДУ ІІ-ГО СТУПЕНЮ

В експерименті на щурах відтворено модель опіку стравоходу ІІ-го ступеню,що викликана введенням 30% CCl_3COOH. Модель опіку стравоходу підтверджена біохімічними показниками крові піддослідних тварин. Запропонований підхід може бути використаний у дослідженні біохімічних та імунологічних механізмів патогенезу опіку стравоходу.

Ключові слова: кислотний опік стравоходу, біохімічні показники.

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ЭКСПЕРИМЕНТАЛЬНОЕ ВОСПРОИЗВЕДЕНИЕ МОДЕЛИ КИСЛОТНОГО ОЖОГА ПИЩЕВОДА ІІ-Й СТЕПЕНИ

В эксперименте на крысах воспроизведена модель ожога пищевода II-й степени, вызванная введением 30% CCl_3COOH. Модель ожога пищевода подтверждена биохимическими показателями крови подопытных животных. Предложенный подход может быть использован в исследовании биохимических и иммунологических механизмов патогенеза ожога пищевода.

Ключевые слова: кислотный ожог пищевода, биохимические показатели.