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# A Method For Increasing The Directive Activity Of Different Cell Lines.

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**ABSTRACT**

Considering that metabolism, reproduction intensity, growth rate and proliferation depend not only on the cell line, but also to a greater extent on the characteristics of biological additives in growth media. We have carried out these studies, the purpose of which is to develop a method for increasing the virus-producing activity of MDBK cells by using in the growth medium a potential activator of the metabolism of the cells of the natural biopolymer-apiphy to preparation. The cell cultures used were MDBK, LEK, and VERO transplanted lines from the cryobank of the FTsTRB-VNIVI Federal State Budgetary Educational Institution. As test viruses, the vaccine strain TK-A (VIEW) - B-2, cattle RTIs viruses, the reference strain PTK-2 of cattle PG-3 virus and reovirus were obtained from the museum of the strains FTsTRB-VNIVI. Commercial chitosan was used as a standard polymer in pharmaceuticals, produced domestically produced by Pharmacor Production Ltd. (St. Petersburg). For the cultivation of cell culture used nutrient medium: 199, Needle MEM, DMEM, GLA with the addition of blood serum and antibiotics. As a stimulant for the reproduction of viruses, the Vita-Forza apifitopreparat developed by the FSBR-VNIVI FSBI staff was used. To activate the metabolism of viruses, ethanol apifito extract (AFE) was introduced into growth media at the rate of 1 g / l, on which test cell lines were seeded. Cells were grown on AFE-containing media for 48 hours, and after the indicated exposure, virus test strains (PG-3, RTI) were added to them. During the experiment, we conducted 5 consecutive passages of each virus. The level of accumulation of the virus in the cell culture was determined by the method of titration according to the method commonly used in virology. The results of the studies showed that the greatest amount of viral mass of RTIs and PG-3 was obtained when using 1 M / L of Eagle's Needle MEM apifitopreparam as a growth-stimulating additive in the growth medium.

**Keywords:** cell culture, viruses, proliferation, apifito extract, activation of metabolism, virus titers.

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## INTRODUCTION

At present, the development of the market for new biotechnological products obtained using transplantable cell lines is accompanied by an increase in the volumes of production and consumption of nutrient media, among which special attention is drawn to media based on products of animal and vegetable origin [1].

However, there is a real risk of infection by prions of drugs obtained using animal products, therefore government organizations controlling the production of therapeutic and prophylactic drugs have imposed a requirement to limit the use of animal substances in the production of vaccines [2].

At the same time, the use of high-molecular compounds (IUDs), biopolymers [4,7,8], from which natural biopolymers chitin and chitosan, obtained from crustaceans, have the highest biological activity, is a promising direction in the field of biotechnology, cell and genetic engineering. and insects (bees), containing proteins, carbohydrates, amino acids, micro- and macro elements and possessing metabolism-stimulating, growth-stimulating and bactericidal activity [3,9,10]. It was established that the introduction of biopolymers (wax moths) into growth (nutrient) media significantly increased the proliferation of cultured animal cells (lymphocytes and splenocytes) under in vitro conditions.

Considering that the combination of api products with phyto preparations leads to an enhancement of the biological effect of individual components [5], the staff of the Federal Center for Scientific and Technical Information of the Russian Federation "FTSTRB-VNIVI" developed the chitin-containing natural composition "Vita Forts" [6], which is unique in composition (more than 400 chemical compounds ), and biological action (metabolism-, growth-, immune stimulating, detoxifying, adaptogenic, antioxidant) in vitro, there is good reason to believe that this apiphytopreparation can be used as stve metabolism activator during cultivation of animal cells in vitro (in vitro) for the reproduction of viruses in the manufacture of vaccines.

However, studies on the use of apifito preparatov as activators of cell growth in vitro are rare and do not give a complete picture of the role of api-products in cell biotechnology. Due to the fact that the activation of cell metabolism is one of the urgent tasks of biotechnology and due to the lack of study of the effect of apiphyto products on the growth and development of animal cells under artificial culture conditions for viro logical research, as well as due to the urgency of the problem, we have conducted these studies

## MATERIALS AND METHODS

Commercial chitosan was used as a standard polymer in pharmaceuticals, produced domestically produced by Pharmacor Production Ltd. (St. Petersburg). The drug is a lyophilized, amorphous-crystalline encapsulated biopolymer containing 220 mg of chitosan in one capsule. The Chitosan-pharmacor supplement contains the biologically active substance chitosan obtained from the chitinous shells of red-legged Kamchatka crabs by deacylation.

Nutrient media were used to grow cell cultures: 199, Needle MEM, DMEM, GLA with the addition of blood serum to them, both adult cattle and cows at a concentration of 10%, antibiotics, Needle MEM on Earl's saline solution with irreplaceable amino acids; blood serum of cattle (cattle) qualification for cell cultures, made in FGBTN "FTsTRB-VNIVI"; Wednesday 199; microbiological standard media (meat-peptone broth (MPB), meat-peptone agar (MPA), Keith-Tarozzi medium, thioglycolic, Saburo).

As a virus reproduction stimulator, an apifito preparat was used on the basis of the biologically active drug Vita-Forza, which was made according to the technology developed by the staff of FTsTRB-VNIVI. To activate the metabolism of viruses, ethanol apifito extract (AFE) was introduced into growth media at the rate of 1 g / l, on which test cell lines were seeded. Cells were grown on AFE-containing media for 48 hours, and after the indicated exposure, virus test strains (PG-3, RTI) were added to them. Viruses were introduced into the tested cell cultures after the formation of a complete monolayer.

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## RESULTS AND DISCUSSION

Conducted experiments to assess the growth-stimulating activity of the drug from the class of natural biopolymers – apifito extract from the drug "Vita-forts".

The results of the study of the effect of AFE-on cell cultures of the MDBK line are presented in Figure.

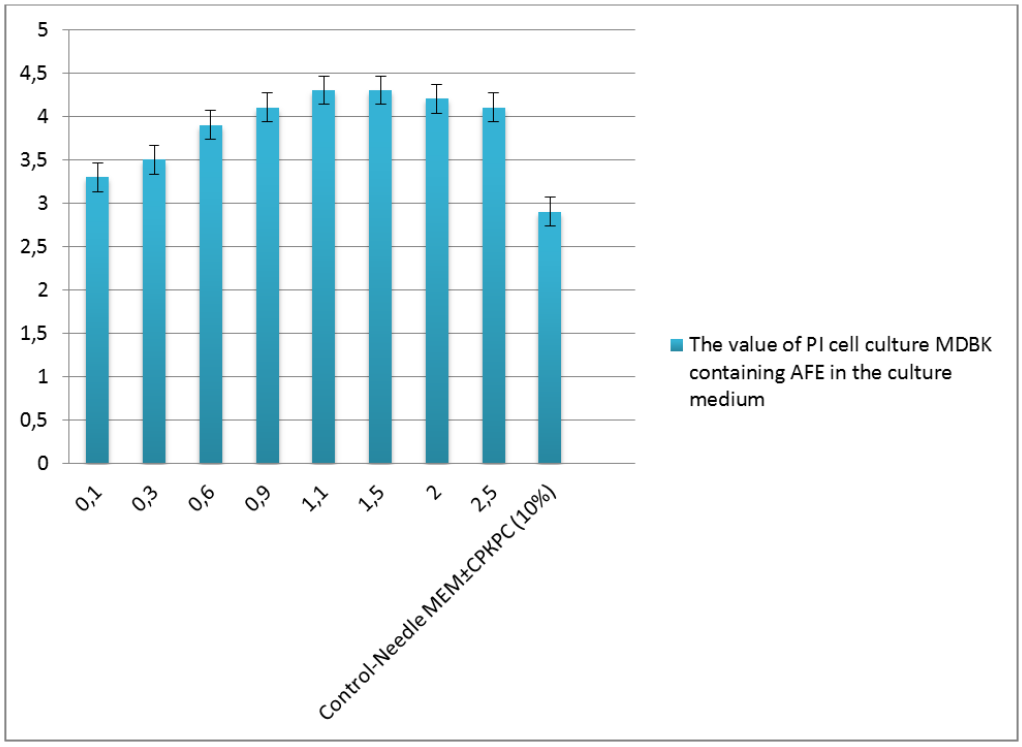


**Figure: The values of the proliferation index of cell culture MDBK in the nutrient medium Needle MEM with different concentrations of apitae extract "Vita-Forza"**

From the data of the figure it can be seen that the introduction of apifito extract in the amount of 0.9-1.5 mg / l to the composition of the cell culture has a pronounced stimulating effect, increasing the PI by 17% compared with the control. When the concentration of apifito extract in the medium is 0.1 mg / ml, the PI values of the MDBK cell culture are comparable to those in the Needle MEM medium with 10% SKRPC ($P > 0.05$).

Cells grown in an experimental medium with an apifito extract had a shape characteristic of this type of cells, with clearly defined boundaries, no signs of degeneration, and did not morphologically differ from cells grown in the control medium.

In the next series of experiments, the effect of AFE in the growth medium on the reproduction of RTIs and PG-3 viruses was studied.

The results of studies on the reproduction of RTIs and PG-3 viruses by MDBK, LEK and VERO lines in phytoextrax-containing and serum (control) growth media are presented in the table.

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**Table: Reproduction of viruses IRT and PG-3 on transplantable cell lines of MDBK, LEK, and VERO cells cultured in apifito preparat-containing growth medium Eagle's Needle MEM**

| Cell culture lines | Growth environment | Virus titers, lgТЦД$_{50/ml}$ | |
|---|---|---|---|
| | | IRT | PG-3 |
| MDBK | Needle MEM + AFE | 6,9 ± 0,3$^{xx}$ | 6,6±0,5 |
| | Control (SKKRS) | 6,7±0,1 | 6,5±0,7 |
| LEK | Needle MEM + AFE | 6,8±0,3 | 6,5±0,1 |
| | Control (SKKRS) | 6,7±0,1 | 6,5±0,3 |
| VERO | Needle MEM + AFE | 6,5±0,7 | 6,3±0,9 |
| | Control (SKKRS) | 6,7±0,1 | 6,5±0,5 |

AFE – apifito preparat; SKKRS serum of cattle

From the materials presented in the table, it can be seen that the greatest amount of viral mass of RTIs and PG-3 was obtained when using the Needle MEM Apifito extract from the Vita-Forza natural composition on the MDBK cell line as a growth-stimulating additive to the growth medium. At the same time, the ИРТ virus multiplies much better as compared with the PG-3 virus and its titer exceeded control values by 13%, PG-3 titer - by 14%.

When using the LEK cell line under these conditions, there was also a tendency to enhance the reproduction of RTIs. As can be seen from the table, the titers of the RTI virus in this variant of the experiment exceeded the control values by 1%. As for the PG-3 virus, the value of its titers were comparable to those of the control, having the same values of the amount of virus.

When using VERO cell lines, there was a slight weakening of the virus-producing ability of cells, which was 5% lower than the control, however, the difference in titers of control and experience was not significant. The same downward trend in the virus-producing ability of VERO cells was observed when using PG-3 virus as a test, which was less than the control values by 3%.

## CONCLUSION

Thus, as a result of the research conducted, the technology of obtaining apifito extract from the biologically active composition Vita-Forza was optimized, and a nutrient medium based on it was developed, suitable for cultivating MDBK, LEK, VERO cells and possessing biological properties comparable to the properties of the Eagla nutrient medium MEM.

Long passaging of used cell lines (MDBK, LEK, VERO) in an AFE-containing medium for 10 passages did not adversely affect the stability of the growth, cytomorphological, karyo logical, and genetic properties of animal cells.

Cultured RTIs and PG-3 viruses were cultured on nutrient media containing apifito extract (AFE) in the amount of 1 g / l, provided an increase in viral reproduction, increasing the titer of RTIs by 13% and PG-3 virus by 10% compared with the control.

The obtained apifitoextract of the chitin-containing composition is used in the laboratories of the FSBRI-VNIVI Federal State Budgetary Educational Institution to increase viral biomass in order to obtain vaccine preparations for the prevention of viral animal diseases. "The method of obtaining natural biopolymer Apizan and its use for the activation of animal cell cultures in vitro during the reproduction of viruses" received RF patent №2649360 02.April 2018.

## REFERENCES

[1] Dyakonov LP Animal cell in culture / Ed. L.P. Dyakonov. M .: Izd. Sputnik + 2009; 656
[2] Ivanov AV and others. Biological active feed additive "Vita-Forts" published on 20.05.2008; 14: RF patent №2324361 C1A23K 1/00
[3] Butler G B et al. Anionic Polymers DrugsVogl. Eds Wiley- Intersci. Publ. 1978;49-141
[4] Carraeber Ch E Biomedical and obental Applications of Polymere Plennen Press 1960; 226

[5] Chitin A Natural Product for the 21 st Century International Comission on Natural Health Product 1995
[6] Hood M A Chitin/chitosan Abst.First intern.Cong. 1977; 44-45
[7] Ingram D OThe Biosgynthesis of Macromlecules W.A. Benjamin Inc1985; 395
[8] Metods for preparation of media, supplements and substrate for serum-free animal cell culture Cell Cult.Mol and Cell Bio 1996; 1
[9] Train E et al. Proceden de preparare a serului stimulat de cal, pentru culture celulate 1982; Patent 80263, MRI3 A61K 35/22
[10] Pumper R et al. Multiplication of vaccinia virus in serum-free and serum-containing cell cultures Nature 1960; 185:123 – 124