

# The role of nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase in the activation of cytochromes

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**Abstract.** The aim of this theoretical study is to investigate the mechanisms of action of these enzymes in the context of metabolism in highly productive animals. The relevance is due to the active development of agriculture and increased interest in a detailed study of the cytochrome P450 system for obtaining high-quality livestock products. This work highlights the functional significance of the enzyme Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase in the processes of activation of cytochromes P450. An analysis of existing data on the interaction of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase with other activating enzymes is carried out. The work is based on the analytical processing of scientific publications, as well as the results of experiments and current statistical reports on the development of animal husbandry. It was established that the enzyme Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450-oxidoreductase ensures the course of a number of vital reactions. Analysis of data on the interaction of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450-oxidoreductase with other enzymes showed the complexity of the mechanisms of metabolism regulation in highly productive animals.

## 1 Introduction

Today, the agricultural sector in the economic and industrial sectors of Russia is one of the most actively developing and promising areas. In general, the country is experiencing an annual increase in production volumes at livestock enterprises and an increase in the level of demand among the Russian population for products of animal origin. According to official statistics from the Federal State Statistics Service [6] in the category "Agriculture", for the period from 2018 to 2023, the volume of production of meat products of cattle and poultry (in slaughter weight) per capita increased by 13.9%, and the volume of milk production per capita increased by 11.6% (Table 1), which is dictated, first of all, by the popularization of healthy, nutritious nutrition among the country's population, as well as the

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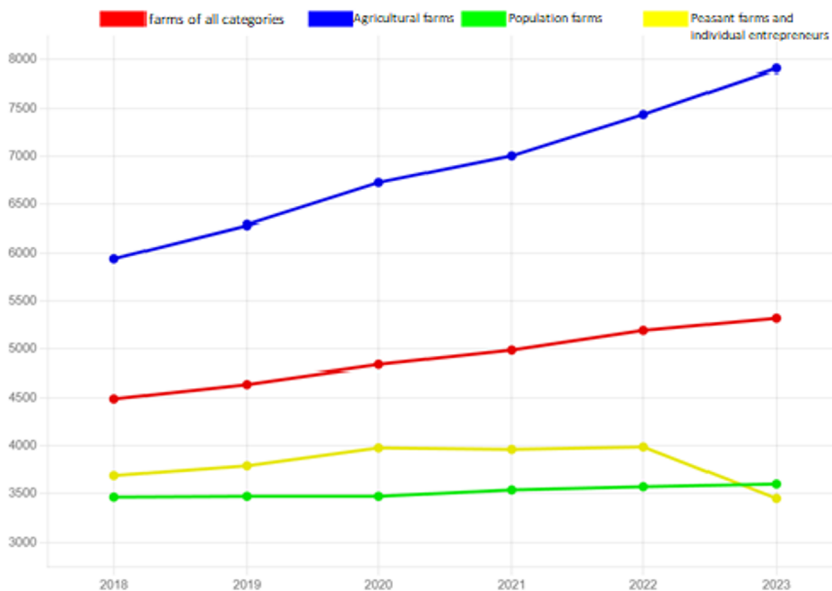
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active introduction of modern methods of using animal raw materials in the pharmaceutical industry and feed production.

**Table 1.** Livestock production per capita in Russia for the period 2018-2023 [6].

Type of product	2018	2019	2020	2021	2022	2023
Cattle and poultry for slaughter (slaughter weight), kg	72	73	76	77	80	82
Milk, kg	207	212	218	220	225	231

In order to saturate the market with full-fledged and safe livestock products in terms of veterinary and sanitary conditions and to meet demand among the population, it is necessary to ensure sustainable development of the Russian agricultural sector. At the current stage of scientific and technological progress, this goal is achieved through consistent intensification of production by organizing narrowly focused selection and breeding activities, practical application of achievements in the field of biotechnology and veterinary genetics, introduction of progressive technologies for keeping and raising animals, production of high-quality complete feed, comprehensive mechanization and automation of technological processes, modernization of livestock farms [2-3]. One of the key trends in the issue of modern intensification of production is the choice of highly productive breeds and lines of animals with good genetic potential. This is evidenced, for example, by the official statistical summary of the average productivity of dairy cattle (milk yield per 1 cow in kg) for the period 2018-2023. (Figure 1) in Russian farms of various types [6], which reflects positive dynamics when analyzing this parameter in organizations of all categories. In quantitative terms, the productivity of dairy cattle in farms of all categories has increased by an average of 18.5% since 2018.



**Fig. 1.** Milk yield per cow in Russian farms for the period 2018-2023 (in kg) [6].

Animals characterized by a relatively high level of productivity are also distinguished by an increased level of metabolism. The intensive flow of assimilation and dissimilation processes in the body of highly productive animals is due not only to the increased synthesis of organic products, but also to a significant load on the enzyme systems due to

the active use of antibiotics, biological stimulants and other veterinary drugs. Understanding the mechanisms of action of enzymes of various groups, the features of their structure and interaction with other biologically active substances at the molecular level will in the future make it possible to evaluate and regulate the state of metabolism of highly productive animals by influencing specific enzymes, their activators and/or the expression of genes encoding the phenotypic profile of the corresponding enzymes.

Currently, in pharmacology, biochemistry and other natural sciences, the central object of research is the enzymes of the cytochrome system, in particular cytochrome P450. Great interest in cytochrome P450 is due to their primary role in the processes of transformation of xenobiotics and inactivation of toxic compounds, including drugs. In the projection of veterinary science, the elimination of foreign substances from the animal's body means obtaining pure, non-toxic products, so the issue of a detailed study of not only the cytochrome system itself, but also its activators (primarily Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase), inhibitors is quite relevant.

The aim of this work is to conduct a theoretical study of the existing information regarding the functional role of the enzyme Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-ferrihemoprotein oxidoreductase (synonyms - Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase, Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-hemoprotein oxidoreductase) in the activation of cytochromes, as well as to review and analyze data on the interaction of this enzyme with other activating enzymes.

## 2 Materials and methods

The main sources for this theoretical study were scientific publications of not only domestic but also foreign figures, as well as the results of experiments on the issue under study and current statistical reports on the development of the livestock sector in the field of agriculture in Russia. The search for scientific literature was carried out in the main bibliographic databases (Elibrary, Pubmed, Scopus (Elsevier), Web of Science) by keywords. When selecting publications, their citation rate and relevance were taken into account, first of all. Scientific papers published before 2020 were included in the analysis if they contained critical information necessary for a full disclosure of the topic and missing in later publications. The main general scientific methods that were used in the framework of the presented study were the collection of scientific data from Russian and foreign authors on the issue under study, processing and grouping of information, as well as generalization, analysis and synthesis of the results obtained.

## 3 Results

Researchers from our and foreign countries have conducted experiments to study the importance of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase in the work of enzyme systems and in the metabolism of mammals in general. It was found that the enzyme Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-hemoprotein oxidoreductase ensures the course of a number of vital reactions. The importance of this enzyme in embryogenesis and ontogenesis is confirmed by its expression already at the two-cell stage of embryonic development, as well as by the lethal outcome in embryos of laboratory mice with knocked out Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-hemoprotein oxidoreductase. The results of the study of dead embryos showed a disorder of the general development of the neural tube, cardiovascular system, eyes, and limb rudiments [12]. Studies were conducted to disable Nicotinamide- $\beta$ -adenine

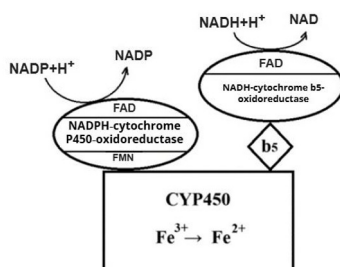
dinucleotide phosphate-H-cytochrome P450 oxidoreductase in individual tissues in order to determine with high accuracy what exactly was the cause of death in the case of a global block of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-hemoprotein oxidoreductase. During experiments to disable the target enzyme in the liver of mice, it was noted that, despite normal growth and fertility, the rodents exhibited a violation of the metabolism of steroid hormones and fatty acids, and the toxicity threshold for various groups of drugs was reduced [10, 12]. This indicates the primary role of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase in ensuring the normal course of metabolism of steroid and other compounds, which is a vital condition.

The enzyme Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-hemoprotein oxidoreductase (synonyms: Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase, Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-ferrihemoprotein oxidoreductase) is a flavoprotein or protein in its chemical structure, which contains flavin mononucleotide or flavin adenine dinucleotide as prosthetic groups, which can change from an oxidized state to a reduced state by adding two hydrogen atoms and two electrons, and vice versa. The functional spectrum of this enzyme includes, first of all, ensuring the possibility of the catalytic cycle of microsomal monooxygenases (cytochromes P450), in which Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-ferrihemoprotein oxidoreductase plays the role of an "electron supplier"; as well as direct participation in the transfer of electrons to such enzymes as cytochrome b5, squalene monooxygenase and heme oxygenase [14]. The electron transfer process itself is based on the stabilization of the one-electron reduced form of the cofactors flavin mononucleotide or flavin adenine dinucleotide.

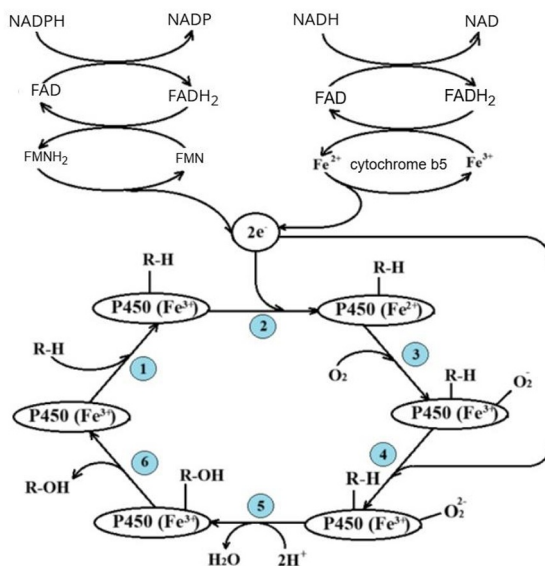
Cytochrome P450 enzymes are chemically complex enzymes of the monooxygenase family, which include a protein part (apoenzyme) and a prosthetic iron-containing configuration (heme), which, due to the property of the metal in the heme to change its valence, is capable of attaching oxygen molecules during active interaction with cofactors flavin adenine dinucleotide (flavin mononucleotide), Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H. The catalytic cycle of cytochromes, known as microsomal oxidation, is closely associated with the functional activity of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-ferrihemoprotein oxidoreductase. The key role of nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase in the catalytic cycle of microsomal oxidation is the reduction of ferric iron (Fe<sup>+3</sup>) to the divalent form (Fe<sup>+2</sup>) after cytochrome P450 binds the substrate in close proximity to the flavoprotein prosthetic group. This occurs through a sequential transfer of electrons from nicotinamide- $\beta$ -adenine dinucleotide phosphate-H to the heme center of cytochrome P450. In the divalent iron state, the heme of cytochrome P450 acquires the ability to subsequently attach an oxygen molecule, after which electron transfer and oxidation of the iron atom are initiated, which reduces the bound oxygen to peroxide. A hydroxyl ion is then separated from the intermediate product, subsequently forming a molecule of water and an activated form of oxygen. The reactive state of the oxygen atom enables it to act on the chemical bond between carbon and hydrogen atoms (C-H) in the fermented substrate to form a hydroxy group and release the reaction product. Cytochrome P450 is reorganized to its original state, and the catalytic cycle is closed. Thus, the main function of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase in the process of cytochrome P450 activation and the initiation of microsomal oxidation is to ensure a redox "partnership" between cytochromes P450 and Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H [14].

It should be noted that another enzyme activator, Nicotinamide- $\beta$ -adenine dinucleotide-H-cytochrome b5-oxidoreductase, also participates in the process of iron reduction in the heme of cytochrome P450. It interacts with Nicotinamide- $\beta$ -adenine dinucleotide

phosphate-H-cytochrome P450-oxidoreductase by the type of summation synergism. Nicotinamide-β-adenine dinucleotide-H-cytochrome b5-oxidoreductase, as well as Nicotinamide-β-adenine dinucleotide phosphate-H-ferrihemoprotein oxidoreductase, performs the function of electron transfer [10]. The tandem effect of these activators is presented in simplified versions in the diagrams (Figures 2-3). Nicotinamide-β-adenine dinucleotide-H-cytochrome b5 oxidoreductase catalyzes the transfer of reducing equivalents from the physiological electron donor Nicotinamide-β-adenine dinucleotide-H to two molecules of cytochrome b5, which, in turn, like the cytochrome P450 enzymes, is a hemoprotein [8, 10].



**Fig. 2.** Scheme of activation of cytochrome P450 enzymes by Nicotinamide-β-adenine dinucleotide-H-cytochrome b5-oxidoreductase and Nicotinamide-β-adenine dinucleotide phosphate-H-cytochrome P450-oxidoreductase.

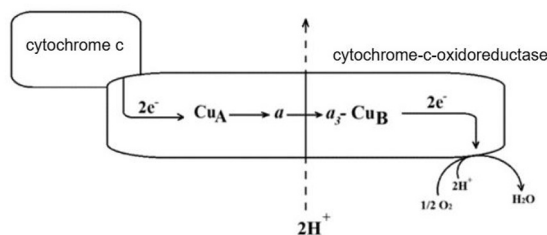


**Fig. 3.** Scheme of participation of Nicotinamide-β-adenine dinucleotide-H-cytochrome b5-oxidoreductase and Nicotinamide-β-adenine dinucleotide phosphate-H-cytochrome P450-oxidoreductase in the catalytic cycle of microsomal oxidation.

Microsomal oxidation as a complex process has the ability to induce: by selectively activating the synthesis of cytochromes P450, it is possible to increase the intensity of the process as a whole. Inducers are substances that initiate the transcription of the corresponding Matrix ribonucleic acid [5].

It should be noted that in addition to initiating the catalytic cycle of microsomal oxidation through the activation of cytochrome P450 enzymes, the enzyme Nicotinamide- $\beta$ -adenine dinucleotide phosphate-N-ferrihemoprotein oxidoreductase supplies reducing equivalents to other enzymes, such as heme oxygenase, squalene monooxygenase, and fatty acid elongase [12]. The function of heme oxygenase is to maintain heme homeostasis; this enzyme catalyzes the breakdown of heme into biliverdin, iron, and carbon monoxide. Squalene monooxygenase is involved in cholesterol biosynthesis processes, and experimental studies have confirmed its dependence on Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-ferrihemoprotein oxidoreductase [12]. Fatty acid elongases catalyze fatty acid chain elongation reactions. Thus, a number of different substances acting as electron-accepting "partners" of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-ferrihemoprotein oxidoreductase suggest that Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase plays a significant role in many physiological and toxicological processes.

Similar properties are possessed by oxidases of the aerobic respiratory chain of electron transfer, the purpose of which is the synthesis of adenosine triphosphate molecules. For example, cytochrome-c oxidoreductase (synonym – cytochrome aa3) is the terminal enzyme of the aerobic respiratory chain, which catalyzes the transfer of electrons from cytochrome c to oxygen with the formation of a water molecule, and also maintains the proton gradient in the mitochondrial matrix. As cofactor structures, cytochrome-c oxidoreductase includes three oxidation-reduction centers: heme a and the copper-containing center a<sub>3</sub>-CuB (it includes heme a<sub>3</sub> and the copper atom CuB) (Figure 4).



**Fig. 4.** Scheme of electron transfer from cytochrome c to oxygen with the formation of a water molecule with the participation of cytochrome c oxidoreductase.

Data on the role of the enzyme Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-ferrihemoprotein oxidoreductase in the activation of cytochromes P450 formed the basis for modern methods of studying the characteristics of cytochrome P450 isoforms, in particular, the electrochemical method, in which the function of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase is assumed by an electrode. Cyclic voltammetry or amperometric titration allow electrochemical screening of cytochrome P450 isoforms and establishing the profile of substrates and inhibitors for each individual form of the enzyme [11].

## 4 Discussion

The progressive growth of production volumes at enterprises producing products of animal origin is currently evidenced by official statistics. Popularization of agriculture, increasing demand for products among the population, dynamically developing animal breeding technologies, attraction of financing to the livestock sector - all these factors create a need for a scientifically based approach to providing farms with highly productive and healthy animals. In the conditions of modern organization of agriculture, the intensification

strategy, which is chosen by most owners of livestock enterprises, has contributed to the active development of veterinary genetics and biotechnology, deepening of selection and breeding work. In addition, there was a need to understand the key mechanisms for maintaining intensive metabolism at the molecular level in highly productive animal breeds. The natural science community has developed a great interest in systems that ensure consistently high productivity indicators, set artificially, with an excessively high toxicological load on the animal's body associated with the global deterioration of the ecological status and the active use of a large number of veterinary drugs.

The central object of study for modern researchers in the field of biochemistry, pharmacology, and molecular physiology of animals has become the enzyme systems of cytochrome P450 and the activators associated with them, primarily Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase. These enzymes play a key role in the transformation and elimination of xenobiotics from the body of mammals, which means a direct dependence of the quality of livestock products on the level of functional activity and the coherence of these systems. This emphasizes the need to develop comprehensive strategies that include not only selection and breeding work and technological innovations, but also taking into account the biochemical and physiological characteristics of animals with a high metabolism.

Based on the results of the analysis of scientific literature on the problematic issue, it was established that the enzyme Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase is of central importance in the context of cytochrome activation and the launch of the catalytic cycle of microsomal oxidation, which underlies the metabolism of xenobiotics and toxic compounds in the body of animals. The function of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase is closely associated with the work of Nicotinamide- $\beta$ -adenine dinucleotide-H-cytochrome b5 oxidoreductase, which is also a supplier of electrons for cytochromes P450. In order to study in more detail the mechanisms of action of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase and its interaction with other biological systems, it is necessary to conduct additional studies, including those aimed at detecting inhibitory and activating substances. Data on the interaction of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase with other activating enzymes suggest the possibility of regulating metabolism in highly productive animals through the effect on specific cytochrome P450 isoforms. This, in turn, may open new horizons for optimizing veterinary care for highly productive animals and improving the quality of livestock products in a broader context, and may also become a promising direction for the development of new approaches in veterinary science and practice.

## 5 Conclusion

As a result of the theoretical study, it was established that the enzyme Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase of mammals plays a primary role in the context of the processes of xenobiotic transformation and inactivation of toxic compounds in the body of animals through the activation of cytochromes P450. This fact is directly related to obtaining clean and toxicologically safe livestock products. Analysis of data on the interaction of Nicotinamide- $\beta$ -adenine dinucleotide phosphate-H-cytochrome P450 oxidoreductase with other activating enzymes, in particular with Nicotinamide- $\beta$ -adenine dinucleotide-H-cytochrome b5 oxidoreductase, showed the complexity of the mechanisms of metabolism regulation in highly productive animals. Thus, the study of mammalian enzymatic systems may in the future become the foundation for the development of new technologies and methods for increasing the productivity of

farm animals without consequences for their immediate health, as well as without deteriorating the quality of the resulting products.

## Acknowledgement

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## References

1. V.P. Geshel, E.D. Geshel, The Role of Intensification in the Development of the Economy of Agricultural Production. *Economy and Entrepreneurship*, **4(141)**, 277-289 (2022)
2. O.V. Nikolaev, O.I. Durseneva, The Main Directions for Improving the Economic Efficiency of Livestock Intensification in Modern Conditions. *Actual Problems of Economics, Finance and Education in the Context of Digitalization. Proceedings of the National Interuniversity Scientific and Practical Conference of Students, Postgraduates and Young Scientists, Russian State Agrarian Correspondence University, Balashikha, Russia* (2022)
3. L.L. Pashina, V.V. Reimer, Trends and Problems of Livestock Breeding Development in Russia. *Agro-industrial complex: Problems and Development Prospects, Proceedings of the All-Russian Scientific and Practical Conference, Far Eastern State Agrarian University, Blagoveshchensk, Russia*, **2** (2021)
4. V.S. Ponomarev, O.S. Popova, A.V. Kostrova, L.A. Agafonova, Clearance tests as a method for diagnosing hepatobiliary pathologies in animals. *Agrarian Science Euro-North-East* **24(6)**, 924-938 (2023)
5. D.A. Sychev, V.A. Otdelenov, N.P. Denisenko, V.V. Smirnov, Study of the activity of cytochrome P450 isoenzymes for predicting drug-drug interactions in polypharmacy. *Pharmacogenetics and pharmacogenomics*, **2**, 4-10 (2016)
6. Federal State Statistics Service. Agriculture, hunting and forestry, [https://rosstat.gov.ru/enterprise\\_economy](https://rosstat.gov.ru/enterprise_economy)
7. M.V. Fedorova, G.D. Sagaradze, D.A. Fedotov, V.S. Popov, A.Yu. Efimenko, E.V. Proskurnina. Activity of microsomal reductases of rat epididymis in experimental cryptorchidism, *International Journal of Applied and Fundamental Research*, **2**, 17-21 (2022) DOI: <https://doi.org/10.17513/mjpf.13352>
8. C.A. Davis, M.J. Barber, Cytochrome b5 oxidoreductase: expression and characterization of the original familial ideopathic methemoglobinemia mutations E255- and G291D, *Arch Biochem Biophys*, **425(2)**, 123-32 (2004) DOI 10.1016/j.abb.2003.12.041
9. D. Hamdane, C. Xia, S.C. Im, H. Zhang, J.J. Kim, L. Waskell, Structure and function of an NADPH-cytochrome P450 oxidoreductase in an open conformation capable of reducing cytochrome P450. *J Biol Chem*, **284(17)**, 11374-84 (2009) DOI 10.1074/jbc.M807868200
10. J.R. Kurian, S.U. Bajad, J.L. Miller, N.A. Chin, L.A. Trepanier, NADH cytochrome b5 reductase and cytochrome b5 catalyze the microsomal reduction of xenobiotic hydroxylamines and amidoximes in humans, *J Pharmacol Exp Ther*, **311(3)**, 1171-8 (2004) DOI 10.1124/jpet.104.072389

11. N.E. Moskaleva, V.G. Zgoda, Modern Methods of Cytochrome P450 Analysis, *Biomedical Chemistry*, **7(2)**, 124-135 (2013)
12. S.R. Polusani, R. Kar, M.A. Riquelme, B.S. Masters, S.P. Panda. Regulation of gap junction function and Connexin 43 expression by cytochrome P450 oxidoreductase (CYPOR). *Biochem Biophys Res Commun*, **411(3)**, 490-5 (2011) DOI 10.1016/j.bbrc.2011.06.132
13. D.S. Riddick, X. Ding, C.R. Wolf, T.D. Porter, A.V. Pandey, Q.Y. Zhang, J. Gu, R.D. Finn, S. Ronseaux, L.A. McLaughlin, C.J. Henderson, L. Zou, C.E. Flück, NADPH-cytochrome P450 oxidoreductase: roles in physiology, pharmacology, and toxicology. *Drug Metab Dispos*, **41(1)**, 12-23 (2013) DOI 10.1124/dmd.112.048991
14. Sh.J. Sadeghi, Breakthrough in P450 bioelectrochemistry and future perspectives. *Biochimica et Biophysica Acta*, **1814**, 237–248 (2011)