

**MINISTRY OF SCIENCE AND HIGHER EDUCATION
RUSSIAN FEDERATION**

**FEDERAL STATE AUTONOMOUS EDUCATIONAL INSTITUTION OF
HIGHER EDUCATION
NORTH-CAUCASUS FEDERAL UNIVERSITY**

Methodological Guidelines
for Students on arranging and performing independent work
in the Discipline of General and Medical Enzymology
for Students majoring (Specialty) in 31.05.01 General Medicine

Methodological Recommendations for Studying Theoretical Material

When performing the independent study of theoretical material, the student must follow the rules as set below. The working curriculum of the respective academic discipline shall be recommended to be used as the basis for such activity. According to the schedule of classroom lessons and independent work, a different number of hours is to be allocated for studying individual topics. A significant portion of the student's independent work should be allocated to mastering the material on the covered topic, writing a summary (notes) on the specified theoretical blocks, and searching for additional literature based on the NCFU library and via the Internet system. The lecture material shall not cover all theoretical material; the greater part of it must be mastered through independent work. Given the above, working with the recommended literature is mandatory. Special attention must be paid to the content of the main provisions and conclusions, the explanation of phenomena and facts, and the understanding of the practical application of the theoretical issues under consideration. In the process of this work, the student should strive to understand and memorize the main provisions of the material being considered, the examples illustrating it, as well as to understand the illustrative material. Preparation should be completed by drawing up a plan (summary/notes) on the material (issue) being studied. This allows for the creation of a concentrated, concise representation of the issues being studied. First, studying the recommended literature is to be done. Special attention must be paid to the content of the main provisions and conclusions, the explanation of phenomena and facts, and the understanding of the practical application of the theoretical issues under consideration.

During this work, the student should strive to understand and memorize the main provisions of the material being considered, the examples illustrating it, as well as to understand the illustrative material. Preparation should be completed by drawing up a plan (summary/notes) on the material (issue) being studied. This allows for the creation of a concentrated, concise representation of the issues being studied. If necessary, one should seek (advice) consultation from the instructor. When going to a consultation, it is necessary to thoroughly think through the questions that require clarification. Keeping records contributes to turning reading into an active process, mobilizing motor memory along with visual memory. The following should be born in mind: a student who systematically keeps records creates their own individual stock of auxiliary materials for a quick review of what has been read and for the mobilization of accumulated knowledge. Records are especially important and useful when they reflect the thoughts that arose during independent work.

It is important to develop the students' capacity to compare sources and to think through the material being studied.

Improving note-taking skills among students is of great importance.

The instructor may recommend the following main forms of recording to students: a plan (simple and detailed), excerpts, and theses (abstracts).

The results of note-taking can be presented in various forms.

- A plan is an outline of the read material, a brief (or detailed) list of questions reflecting the structure and sequence of the material.
- A detailed plan fully replaces a summary.
- A summary (notes) is a systematized, logical presentation of the source material. Four types of summaries are distinguished:
 - Outline-summary (Plan-notes) – this is an expanded, detailed plan in which sufficiently detailed records are provided for those points of the plan that need explanation.
 - Textual summary – this is the reproduction of the most important provisions and facts of the source.
 - Free summary – these are clearly and briefly formulated (stated) main provisions resulting from a deep understanding of the material. It may contain excerpts, quotations, and theses; part of the material may be represented by a plan.

- Theme-based summary – this is compiled on the basis of studying a number of sources and gives a more or less exhaustive answer according to a certain scheme (issue).

Methodological Guidelines for the Preparation for Practical Activities

When preparing for practical activities, the recommendation implies studying the control questions for the given lesson and the theoretical material from one's own notes.

The main form of work during the lessons is an interview (discussion). Each student must be ready to speak on all issues raised in the plan and demonstrate maximum activity during their consideration.

The speech must be constructed freely, convincingly, and with arguments. The instructor ensures that the speech is not reduced to a reproductive level (simple reproduction of the text); simple reading of the summary is also not allowed.

It is necessary for the speaker to show their own attitude towards what they are talking about, express their personal opinion and understanding, substantiate it, and be able to draw the correct conclusions from what has been said. In doing so, the student may refer to the entries in the summary, directly to the primary sources, use knowledge of previously studied disciplines, etc. In addition, to obtain a grade at an advanced level, students solve situational tasks based on the results of the mastered topics.

During the execution of practical works, a defense of practical skills is provided, the full list of which is specified in the fund of assessment tools.

Methodological Recommendations for the Preparation of Presentations and Reports

The report must contain elements of novelty; along with the fundamental aspect, an analysis of the current state of the problem under study must be carried out, as well as inclusion in regional issues. The report must include an introduction, a theoretical part, a conclusion, a list of literature, and a presentation that reveals the essence of the topic in graphs, pictures, diagrams, and formulas.

The report is recommended to be submitted on 1 printed sheet. The text of the work must be printed with 1.5 line spacing on one side of a standard sheet (A4) of white paper.

The text and other printed elements of the work must be black, and the contours of letters and signs must be clear, without halos and shading. The font must be Times New Roman, size 14. The titles of chapters and paragraphs are highlighted in bold. The sheet with text must have margins: left – 30 mm, right – 10 mm, top – 20 mm, bottom – 20 mm.

Page numbering of the text is done in the lower right corner of the sheet. It is necessary to number pages starting from the page where the Introduction is printed, on which the number 3 is placed. After that, all pages, including appendices, are numbered.

A space equal to two intervals is placed between the title of the chapter and the title of the paragraph of this chapter, and the title of the paragraph must not be separated from the text of this paragraph by a space. The titles of paragraphs shall be separated from the text of the previous paragraph by a space equal to two intervals. Each chapter, as well as the introduction, conclusions, appendices, and the list of used literature, begin on a new page. The word Chapter is not to be written. Chapters have ordinal numbers within the entire work, designated by Arabic numerals (for example: 1, 2, 3), followed by a period.

The word **PARAGRAPH** or the paragraph symbol (§) is not placed in the title. Paragraphs have ordinal numbers within the chapters, designated by Arabic numerals (for example: 1.1. and 1.2.).

Headings of chapters and paragraphs in the text must be centered; a period shall not be placed at the end of the title of the chapter and paragraph. It is not allowed to hyphenate (transfer) a part of a word in a heading. 4.5. The numbering of tables and figures can be running (continuous) or relate to the number of the chapter and paragraph. For example, if a table or a figure is included in the text of the first paragraph of the second chapter, the numbering is as follows: Table 2.1.1., Fig. 2.1.1.

The last digit means the ordinal number of the table (or figure) in this paragraph.
The table is to be placed as the next page after the first mention of it in the text.

1. List of Main and Additional Literature Required for Mastering the Discipline

1.1. List of Main Literature:

1. Andrusenko, S.F. General and medical enzymology : textbook / S.F. Andrusenko. Moscow : KNORUS Publ., 2023. 242 p.
2. Basic Biochemistry course // Edited by A.I. Glukhov, A.E. Gubareva. Moscow: GEOTAR- Media, 2020. 584 p.
3. Fedorin, D. N. Enzymes: structure, properties, classification / D. N. Fedorin, N. V. Selivanova, A. T. Eprintsev. Voronezh : VSU, 2020. 113 p. — Text : electronic // Lan : electronic library system. — URL: <https://e.lanbook.com/book/433136>

1.2. List of Additional Literature:

1. Rogozhin, V. V. Enzymology. Practicum : a textbook for universities / V. V. Rogozhin, T. V. Rogozhina. Saint Petersburg : Lan Publ., 2025. 340 p. ISBN 978-5-507-51488-5. — Text : electronic // Lan : electronic library system. — URL: <https://e.lanbook.com/book/450725>
2. Breshchenko, E. E. Biochemistry: biologically active substances. Vitamins, enzymes, hormones : a textbook for universities / E. E. Breschenko, K. I. Melkonyan ; edited by I. M. Bykov. — 3rd ed., ster. — St. Petersburg : Lan, 2024. — 136 p. — ISBN 978-5-507- 47791-3. — Text : electronic // Lan : electronic library system. — URL: <https://e.lanbook.com/book/419102>
3. Fundamentals of enzymology: a textbook / S. N. Afonina, I. V. Karnaukhova, E. N. Lebedeva [et al.]; edited by A.V. Sgibnev. Orenburg : OrGMU, 2022. 97 p. — Text : electronic // Lan : electronic library system. —URL:<https://e.lanbook.com/book/340538>
4. Avetisov, A. K. Applied catalysis : textbook / A. K. Avetisov, L. G. Bruk ; edited by O. N. Temkin. — Saint Petersburg : Lan, 2020. — 200 p. — ISBN 978-5-8114- 3854-9. — Text : electronic // Lan : electronic library system. — URL: <https://e.lanbook.com/book/126902>
5. Biochemistry: a textbook / A.V. Strygin, B. E. Tolkachev, A.M. Dotsenko, E. I. Morkovin. Volgograd : VolgSMU, 2022. 132 p. ISBN 978-5-9652-0820-3. Text : electronic // Lan : electronic library system. — URL: <https://e.lanbook.com/book/338252>
6. Karpenko, L. Y. Biological chemistry : a textbook / L. Y. Karpenko, A. A. Bakhta, A. I. Kozitsyna. — Saint Petersburg : SPbGUVU, 2022. — 228 p. — Text : electronic //Lan : electronic library system. — URL: <https://e.lanbook.com/book/366575>
7. Fundamentals of biochemical analysis: laboratory practice: a textbook / compiled by O. S. Kotlyarova, S. M. Chydym. Novosibirsk : NGAU, 2020. 77 p. — Text: electronic // Lan : electronic library system. — URL: <https://e.lanbook.com/book/257708>
8. Lelevich, S. V. Theory and practice of laboratory biochemical research : textbook manual for vocational education / S. V. Lelevich. — 4th ed., ster. — Saint Petersburg : Lan, 2024. — 304 p.— ISBN 978-5-507-50169-4. — Text : electronic // Lan : electronic library system. — URL: <https://e.lanbook.com/book/414779>
9. Kuznetsov, O. E. Laboratory tests in the clinic : a textbook for vocational education / O. E. Kuznetsov, S. A. Lyalikov. — 3rd ed., erased. — Saint Petersburg : Lan, 2024. — 500 p. — ISBN 978-5-507-51527-1. — Text : electronic // Lan : electronic library system.— URL: <https://e.lanbook.com/book/422633>
10. Laboratory workshop on biochemistry for students of medical and pediatric faculties : a textbook / O. L. Nosareva, E. A. Stepovaya, E. V. Shakhristova, L. V. Spirina. — 3rd ed., supplement. and ispr. — Tomsk : SibSMU, 2023. — 165 p. — Text : electronic // Lan : electronic library system. — URL: <https://e.lanbook.com/book/369047>

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Methodological Guidelines
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in the Discipline of General and Medical Enzymology
for Students majoring (Specialty) in 31.05.01 General Medicine

- Aim of mastering the discipline:
The purpose of mastering the discipline is to form part of the professional (PC-13) competencies of the future specialist 05.31.01 Medical practice.
- Objectives of mastering the discipline:
 - formation of an understanding of the connections between the structure, function and biological significance of enzymes.
 - formation of an idea about enzymes, the relationship of enzyme malfunction with the appearance of hereditary human diseases.
 - formation of skills in isolation and purification of enzymes, as well as techniques of modern biochemical analysis, methods of evaluation and selection of methods of analysis adequate to the task.
 - developing the ability to form a holistic understanding of the importance of enzymes in medicine.

Topic 1. Introduction to enzymology Prospects for the development of enzymology. Stages of development of enzymology. Ribozymes are catalysts of a non-protein nature: diversity, structural features, and mechanism of action.

Control questions

1. The procedure for working in the laboratory.
2. Formatting a laboratory journal.
3. Safety precautions when working in the laboratory.
4. Definition of the subject of enzymology and its tasks.
5. The significance of the work of domestic scientists in the development of enzymology.
6. The significance of the work of foreign scientists in the development of enzymology.
7. The biological role of enzymes.
8. The application of enzymes.
9. Recent advances in the study of enzymes.

Topic 2. Methods of isolation and research of enzymes Sources of enzymes Methods for determining enzymatic activity. Methods for determining amino acids in the active center of enzymes and determining their role in the catalytic action. The use of genetically engineered methods for the determination of amino acids in the active center of the enzyme

Control questions

1. Sources of raw materials for the extraction of enzymes of animal origin
2. Sources of raw materials for the extraction of enzymes of plant origin
3. Sources of raw materials for the extraction of enzymes from fungi and bacteria
4. Main methods of enzyme extraction and purification. Disintegration. Extraction. Separation of insoluble particles.
5. Removal of non-protein substances. Separation. Concentration
6. Enzyme purification. Salting out and dialysis.
7. Homogeneity assessment.
8. Enzyme drying and storage

Topic 3. General properties and structure of enzymes The structural organization of enzymes. One- and two-component enzymes. Principles of spatial organization of the apoenzyme. The structural organization of the active center of enzymes. The allosteric center

Control questions

1. Molecular weight of enzymes. Examples, units of measurement, methods of determination.

2. Enzyme centers. Active center, catalytic center, adsorption (binding) center. Characteristics of the active center. Allosteric centers.
3. Structural organization of enzymes

4. Apoenzyme?

5. Classification of coenzymes and cofactors. By mechanism of action. By chemical structure. Classification by mechanism of action.

6. Classification of coenzymes and cofactors. By mode of action. By chemical structure. Classification by mode of action.

Topic 4. Nomenclature and classification of enzymes Principles of enzyme classification. General characteristics of enzyme classes. Nomenclature of enzymes. Oxidoreductases. General characteristics of the class. Examples. Hydrolases. General characteristics of the class. Examples. Transferases. General characteristics of the class. Examples. Synthases (lyases). General characteristics of the class. Examples. Isomerases. General characteristics of the class. Examples. Synthetases (ligases). General characteristics of the class. Examples. Translocases. Examples. Comparative characteristics of classes

Control questions

1. Oxidoreductases. Subclasses, examples of reactions (at least 4)
2. Transferases. Subclasses, examples of reactions (at least 4)
3. Hydrolases. Subclasses, examples of reactions (at least 4)
4. Lyases. Subclasses, examples of reactions (at least 4)
5. Isomerases. Subclasses, examples of reactions (at least 4)
6. Ligases. Subclasses, examples of reactions (at least 4)
7. Translocases. Subclasses, examples (at least 4)
8. What is the catalytic activity of an enzyme. What is the international unit of activity (IU). Formulas. What is the SI unit of activity (katal). Formulas

Topic 5. Theories of enzyme action and mechanisms of enzymatic reactions The interaction of the enzyme with the substrate. Basic and transitional states. Formation of the enzyme-substrate complex and its role in catalysis. Multipoint binding of the substrate. Types of enzymatic catalysis and causes of high catalytic activity of enzymes. Effects of convergence and orientation. The effect of conformational conformity. Theory of conformational correspondence of enzyme and substrate and theory induced conformational conformity. Acid-base catalysis (specific and generalized). Intramolecular acid-base catalysis. Covalent catalysis. Nucleophilic catalysis. The mechanism of catalytic action of serine proteases. Chymotrypsin: activation mechanism and characterization of the stages of catalysis. Principles and features of functioning, organization and regulation of multi-enzyme complexes. Induction and repression of enzymes.

Control questions

1. Examples of enzyme regulation through covalent modification
2. Multienzyme systems and complexes. Examples
3. Non-branched multienzyme systems. Examples
4. Branched multi-enzyme systems. Examples
5. Phosphorylation – dephosphorylation of enzymes
6. Oxidative modification of enzymes
7. Protein-protein interaction
8. Genetic regulation (constitutive, inducible (adaptive), and repressible enzymes)
9. Compartmentalization. Compartment

Topic 6. Kinetics of enzymatic reactions Methods for determining enzymatic activity. Quantitative characteristics of enzymes. The rate of the enzymatic reaction. Factors affecting the reaction rate. The Michaelis constant. Graphs of the dependence of enzyme activity on substrate concentration described by various equations.

Control questions

1. What is the optimal pH value for the enzymatic activity of enzymes?
2. How to determine the change in amylase activity under the influence of pH? Characteristics of the optimal pH and its parameters for salivary amylase.
3. What are the physicochemical foundations of the influence of the optimal pH on enzyme activity?
4. What are the physicochemical foundations of the influence on enzyme activity of a shift in pH from the optimal zone in either direction?
5. Practical application of knowledge about the influence of pH on the rate of enzymatic reactions and processes.
6. Draw graphs showing the dependence of enzyme activity on the pH of the medium.
7. Thermolability of enzymes.
8. Name the optimal temperatures for enzymes of animal, plant, and microbial origin.
9. How does a decrease in temperature, from the optimal level to 0 °C and below, affect the rate of the enzymatic reaction? What can explain this effect?
10. How to determine the optimal temperature for enzyme activity?
11. Plot a graph showing the dependence of enzymatic activity on the influence of temperature.

Topic 7. Inhibitors and activators of enzymes Cofactors: coenzymes and prosthetic groups, their most important types and representatives. Cofactors of oxidoreductases. Characteristics of the main representatives. Transferase cofactors. Characteristics of the main representatives. Cofactors of synthesis and isomerization. Characteristics of the main representatives. The role of metals in the catalytic action of enzymes. Examples of metalloenzymes and enzymes activated by metals.

Control questions

1. What substances are called enzyme inhibitors?
2. List the types of enzyme inhibition.
3. Define irreversible enzyme inhibition.
4. Give an example of substances that are irreversible enzyme inhibitors.
5. Give examples of the use of irreversible enzyme inhibition in medicine.
6. Define reversible enzyme inhibition.
7. List the types of reversible inhibitors.
8. Define competitive enzyme inhibition.
9. Provide examples of substances that are competitive inhibitors.
10. Draw a Michaelis-Menten plot for competitive inhibition.
11. How do the Michaelis constant and the maximum rate of the enzymatic reaction change under competitive inhibition? Explain the reason for these changes.
12. Characterize non-competitive enzyme inhibitors.
13. Provide examples of substances that are non-competitive inhibitors.
14. The use of inhibitors in enzymology and medicine.
15. Retro-inhibition.

Topic 8. Enzymopathies. Enzyme diagnostics. Enzyme therapy Pathological changes in enzyme activity. Diseases in which, due to changes in the activity of enzymes, the course of the corresponding biochemical reactions in the body is disrupted and metabolic diseases develop. Diagnosis of a disease (or syndrome) based on the determination of enzyme activity in human biological material (biological fluids, tissues). Tasks of enzyme diagnostics. Methods of enzyme diagnostics. Objects of enzyme diagnostics: blood (serum, plasma), biological fluids (urine, cerebrospinal fluid, exudates, etc.), tissue biopsies. The use of enzymes as therapeutic drugs.

Enzyme complexes. Substitution therapy or an element of complex treatment for gastrointestinal diseases, thrombosis and thromboembolism, oncological diseases

Control questions

1. Blood enzymes.
2. Urine enzymes.
3. Classification of enzymopathies.
4. Enzymopathies of carbohydrate metabolism. Manifestations and treatment options.
5. Enzymopathies of protein metabolism. Manifestations and treatment options.
6. Enzymopathies of nucleic acid metabolism. Manifestations and treatment options.
7. Enzymopathies of lipid metabolism. Manifestations and treatment options.
8. Enzymopathies of heme-containing enzymes. Manifestations and treatment options.
9. Enzymopathies. Hereditary (primary) enzymopathies. Examples, causes
10. Acquired (secondary) enzymopathies. Examples, causes
11. Classification of hereditary enzymopathies depending on the degree of clinical manifestation. Examples
12. Classification of hereditary enzymopathies by type of metabolic disorder. Examples
13. Acquired enzyme disorders. Nutritional

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8. Lelevich, S. V. Theory and practice of laboratory biochemical research : textbook manual for vocational education / S. V. Lelevich. — 4th ed., ster. — Saint Petersburg : Lan, 2024. — 304 p.— ISBN 978-5-507-50169-4. — Text : electronic // Lan : electronic library system. — URL: <https://e.lanbook.com/book/414779>

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