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P89 Neurochemical methods of investigations in the clinic / V. K. Pozdeev, N. V. Pozdeyev. — Saint-Petersburg: Renome, 2013. — 312 p.

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This book describes modified by authors methods: measurement of aminothiols (glutathione, homocysteine, cysteine), neuroactive amino acids (glutamate, aspartate, GABA, taurine) by HPLC; ninhydrin method of determination of glutamate decarboxylase activity and free amino acids in cerebrospinal fluid and brain tissue using sulfocationites; determination of total globulins, albumins, peptides, amino acids using Sephadex; trioxyindole method of catecholamine measurement; fluorimetric method of detection of histamine, 5-hydroxytriptamine, 5-hydroxyindoleacetic acid, homo-vanillic acid; kynuramine method of monoamine oxidase activity measurement; and also methodology of clinical biochemical investigations.
For neurochemists, biochemists, neurologists, and also professionals in related specialties.
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Dedicated to young investigators capable of constructively contributing to the modern science

PREFACE

Analytical capabilities of modern biomedical chemistry are phenomenal. Clinical-diagnostic laboratories are equipped with flow cytometers, automatic immunochemical, hematological, and biochemical analyzers; pre-made kits of reagents are manufactured for the above mentioned equipment. The development of new and improvement of existing methods is a continuous, never ending process. It is a difficult task even for large groups of professionals to describe, and assess advantages and disadvantages of all neurochemical methods that exist at a current stage of scientific development. On the other side, preparation of biological samples, which is, to a large degree, a universal process that forms the basis of the modern detection methods and determines successful resolution of scientific and practical problems, is learned by an investigator from frequently error-prone personal and colleagues' experience. In this book we set a goal to share our long-term experience of performing neurochemical studies, acquaint the reader with modifications of several methods, careful ways to prepare biological objects. We also think of the methodology of these studies that is very important for successful performance.

Some research and clinical laboratories have high performance liquid chromatography systems with a good variety of detectors (including mass spectrometers). This facilitates, unifies, and ensures maximal sensitivity and reproducibility of the data, predetermines work using known technologies, and maximally simplifies the task of the instrument operator.

However, all above mentioned benefits have a reverse side undermining the need for being creative and independent. Methodology becomes unified, and the need to prepare reagents and the urge to understand physical and chemical basis of the methods disappears. However, when unique studies are needed, these qualities of the researcher become necessary. In addition, even when the most advanced methods of the separation and detection of complex

biological mixtures are used, preservation of sample's natural state remains paramount and very difficult task.

Tandems of HPLC/MS, MS/MS, and gas chromatography/MS ideally separate complex mixtures of substrates but are damaging to easily oxidizable compounds. Soft conditions of high performance liquid chromatography with fluorimetric or photometric detection allow measuring concentration of easily oxidizable thiols (glutathione, homocysteine, and cysteine) in biological media; although, when electrochemical detection is used, these metabolites lose their native state.

In this publication the authors present a series of methodological solutions, describes careful

manipulations with biological materials and, in appendix, lists physical and chemical characteristics of most frequently used reagents.

Modern biochemical science has accumulated large amount of data about pathogenesis of many diseases; therefore it becomes an important task to establish biochemical diagnosis in addition to clinical one. With biochemical (neurochemical in case of diseases of nervous system) diagnosis, a pharmacologic therapy based on the pathogenesis is used. Biochemical diagnosis allows individualized pharmacologic therapy of diseases with heterogenous pathogenesis.

Most of the results presented in this publication are related to the study of the pathogenesis of convulsive disorders (epilepsy). Paroxysmal nature of this disease allows observing the dynamics of brain neurotransmitter systems, helps uncover pathogenetic and compensatory mechanisms; on the other side it helps revealing universal clinical manifestation (generalized seizure activity) of various metabolic abnormalities. These clinical and biochemical studies helped us to formulate the concept of heterogeneity of the pathochemistry of the epilepsy in 1980s and established the need to make neurochemical diagnosis (excluding trial and error approach to anti-convulsant therapy).

A wide spectrum of biochemical methods is needed to establish the biochemical diagnosis. The most informative approach in case of central nervous system diseases is to study cerebrospinal fluid (CSF) since biochemically active compounds (neurotransmitters and neuromodulators) released from neurons, all to some degree reach CSF. Precursors and products of inactivation of biologically active compounds are also present in CSF and this allows investigation of their metabolism.

In this book we present our modifications of the following methods: determination of glutamic acid decarboxylase and monoamine oxidase activities, determination of aminothiols, monoamines, amino acids in CSF, blood, urine and brain tissue by high performance liquid chromatography. We describe ways to prepare reagents and biological samples. Procedures for storage, concentration, purification, and extraction of proteins, peptides, monoamines, amino acids from biological media are important even for modern methods of chromatography and detection as they ensure reproducibility and reliability of the data.

For convenience, we included in the appendix general information about chemicals, methods of their purification, density charts of aqueous solutions of bases and acids, relationships between SI units and other metric systems, various methods of concentration expression, and atomic weights of elements.

New methods to study proteins, aminothiols, amino acids, and oligopeptides help analyzing a wide spectrum of these compounds simultaneously. The creation of high performance systems of liquid and gas chromatography facilitates biochemical studies. Undoubtedly, in the near future clinical biochemical laboratories will be equipped with these systems and the problem of preserving analyzed compounds in their native state will always be important.
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Pozdeev Vladimir (born in 1938), MD, PhD in Neurology, Doctor of Science, Professor of Biochemistry. Dr. Pozdeev graduated from Irkutsk Medical University and did residency and PhD training in the Department of Neurology of Irkutsk Medical University. He worked as a Head of the Subdivision of Biochemistry of the Department of Neurophysiology of the Institute of Experimental Medicine of the Academy of Medical Sciences of the USSR in Saint-Petersburg. Since the year 2000 he holds a position of the lead scientist of the Department of Experimental Clinical Investigations and he is a Director of the Clinical Diagnostic Laboratory of the Research Institute of Influenza in Saint-Petersburg. His research interests are in the field of clinical and biochemical methods of the investigation of the pathogenesis of central nervous system diseases and, most recently, hepatitis C. Dr. Pozdeev is working on individualization of metabolic therapy. He established a concept of biochemical heterogeneity of epilepsy and discovered pathogenetic and compensatory mechanisms of paroxysmal disorders.

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