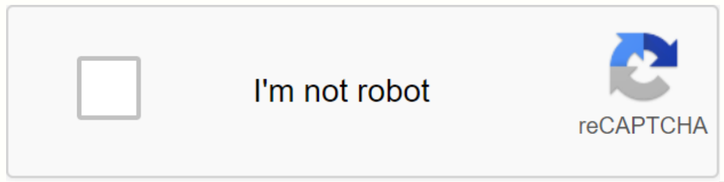

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Schoeff, MS, MT (ASCP) Professor and Director, Medical Laboratory Science Program University of Utah School of Medicine Education Consultant, ARUP Laboratories Salt Lake City, UtahAcquisitions Editor: John GoucherManaging Editor: Meredith BrittainMarketing Manager: Allison NoplockProject Manager: Rosanne HallowellDesigner: Stephen DrudingCompositor: ASI Maryland CompositionSixth EditionCopyright © 2010, 2000 5, 2000, 1996, 1992, 1985 by Lippincott Williams & Wilkins, and Wolters Kluwerbusiness.351 West Camden Street 530 Walnut StreetBaltimore, MD 21201 Philadelphia, PA 19106Printed in ChinaAll Rights Reserved. This book is copyrighted. No part of this book may be reproduced or transmitted in any form or by any means, including photocopies or scanned or other electroniccopies, or used by any information storage and retrieval system without the written permission of the copyright owner, except for brief citations contained in critical articles and reviews. 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However, authors, editors and publishers are not responsible for errors or non-credits or for any consequences arising from the use of the information in this book and make no warranty, expressed or implied, as to the currency, completeness or accuracy of the content of the publication. The application of this information in a particular situation remains the professional responsibility of the medical practitioner; described and recommended clinical treatment may considered as non-obsolete and universal recommendations. The authors, editors and publisher have made every effort to ensure that the choice of medicinal products and dosages referred to in this text are consistent with current recommendations and practices at the time of publication. 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Lippincott Williams & Wilkins customer service representatives are available from 8:30 a.m. to 6 p.m., EST. Sheile, Chris and Carson for their support and impatience. MLBTo Nancy, my wife, for her continued support and education. EPFTo my wife, Anita, for her continued support. LESForeword Providing health care and practice medicine and viduals access to the health care system as well as clinical laboratory science have gone through the exponential workforce that provides care. Change has been growing in the last decade. There have been, and continue to be, a lack of educated and trained clinical laboratories pre-be, many factors contributing to this change. Growing fessionals. This is the result of a number of factors, including costs and concerns about unequal access to the health of an aging workforce, reduced education programs, and care and the growing number of unincarested have made competitive career opportunities.health care one of the best challenges for this country. Politicians and regulators face the need to pre- as a result of all these issues, the role of clinical lab-vide accessible health care while trying to control costs. oratory of doctors is changing. We can no longer affordPolicy makers and payers to have an increased impact on making easy analysts who perform, report, and ensure themedical and diagnostic decisions through managed care. results of laboratory tests. Doctors recognize aConsumers and payers alike expect easy access and recognize the need to help select tests and result in high quality care at the most economical prices. Interpretation. It is essential that laboratory expertsReports on medical increased visibility for working with doctors in helping them testing the need for increased patient safety and quality possibilities and optimisation of clinical outcomes. So we can do dotives. The emphasis has shifted from simply diagnosing, and that, clinical laboratory physicians need to be educated in disease treatment to identifying and controlling diseases and trained not only in test performance and utilizing risk factors and maintaining health. There is increased, but also in pathophysiology, differential diagnosis, aconcern on issues of public health and bioterrorism, as well as how diagnostic information contributes to patient care as well as the environmental impact on health. The result and results.are the following and other factors, laboratory tests are expanding and play an increasingly valuable and important role in the sixth year of Clinical Chemistry: Techniques in the field of health care provision. Principles, correlation was written with these changing needs in mind. The text provides a comprehensive perspective Technological advances dramatically changed pathophysiology as it relates to clinical chemistry di-way to practice clinical laboratory science. Molecular di-agnostic testing. The emphasis on preanalytical, analyti-agnostic testing allows for earlier detection of the disease. analytical aspects of diagnostic testing. ThisAdvances in biotechnology, including omics-ge-text not only provides comprehensive information on butnomics, proteomics, and pharmocogenomics—they also have case studies and other strategies that will rise up to the advent of personalized medicine. We can improve critical thinking and problem-solving skills. Use of text and supporting materials willlor treatment options based on genetic makeup. Testing to enhance theoretical, technical and consulting skills de-techniques have shifted from tubes, beaks, and velopment. This text is not only an excellent source of oversized, automated analyzers of microanalytical systems (laboratory clinical laboratory and medical students, but also a foron chip) that allow for reduced sample size, fewer clinical laboratory physicians, physicians and other staff, and smaller tools. The walls of lab-health care providers are disappearing, with an increasing number of tests that can be done at the care site. Paula Garrott, EDM CLS (NCA) Director, Science Division The demographics of health care are also changing. As the College of Liberal Arts and Sciencesa due to an aging population and a longer life of ex-president and associate professor emeritus, we are seeing an increase in chronic diseases, clinical laboratory science departments that affects the health care system. These demo-University of Illinois at Springfieldgraphics not only affect the number and types of indi-Springfield, IllinoisivPrefaceClinical chemistry continues be one of the most rap-sionals who practice clinical chemistry and laboratory advancing field of laboratory medicine. Because the drug on a daily basis. The basic principles of publishing the first edition of this textbook in 1985, the analytical procedures discussed in the chapters reflect the small changes have taken place. New technologies and up-to-date or routine techniques have been introduced in analytical techniques with a dra-clinical chemical laboratory. Detailed procedures have a tactical impact on the practice of clinical chemistry and have been omitted due to the diversity of equipment and laboratory medicine. In addition, the health care system commercial kits used in today's clinical laboratories.is constantly changing. There is an increased emphasis on tool manuals and kit package inserts to improve the quality of patient care, the individual patient out—the most reliable reference to detailed guidance on cur-come, financial responsibility, and overall quality management-rental analytical procedures. All chapters material bolment. For this reason, editors have replaced updated, improved and regrouped for better follow-up procedures with techniques in the title to make it and readability. As a new offering, the website discusses the ongoing evolution of the lab's role in further case studies, reviewing issues, teaching re-in health care. Point-of-care testing (POCT) is also on resources, teaching tips, additional references, and teach-headed health care practices and has brought both ing aids to instructors and students available from above and to opportunities for clinical laboratorians. the publisher who assists in the use of this textbook (see Now more than ever, clinical laboratories must be the section that follows).deals with disease correlations, interpretations, problem solving, quality assurance and cost-effective additional ingenuity; need to know not only how the tests, but more importantly what, why and when. Clinical Chemistry: Techniques, Principles, Correlations, Sixth Edition, contains additional resources for both in-what's new in this edition of Structors and Students, available on the book companion website at thePoint.lww.com/Bishop6e.The Of Clinical Chemistry Editors: Techniques, Principles, Correlations have designed the sixth edition to be instructorsan an even more valuable resource for students and staff. Approved admission instructors will have access to the following additional resources: As in the previous five editions, the sixth edition of Clinical Chemistry: Techniques, Principles, Correlation is ■ Brownstone Test Generatorwise, up-to-date, and easy to understand for ■ Responses to student case studies at all levels. It is also designed for virtually ■ Answers to review questionsorganized source for instructors and practition- ■ Image Bankers. Editors tried to keep books read- ■ and further improve its content. Since clinical ■ Web links and teaching Tipslaboratorians to use their interpretive and analytical skills in the daily practice of clinical chemistry, efforts have been made to maintain an appropriate balance of interanalytical principles, techniques, and correlations of re-students who have purchased clinical chemistry: sults with disease states. Techniques, principles, correlations have access to fol-lowing additional resources: In this sixth edition, editors have made several significant changes in response to requests from our ■ Chapter Goalreaders, Students, Instructors and Practitioners. ■ Flash CardsAncillary materials have been updated and expanded ■ Quiz Bank (see Additional Resources, below). Chapters now in-clude current, more often encountered case stud-In addition, buyers of text have access to topics and practice questions or exercises. To provide searchable Full Text Online by going to Clinicalthorough, an up-to-date study of clinical chemistry, all chemistry: techniques, principles, correlation websites have been updated and reviewed by profess-based thePoint.lww.com/Bishop6e. For more information, please refer to the inner front cover of vvi PREFACEof this text, including the passcode we convey to our students, colleagues, in order to gain access to the website. teachers and mentors in the profession who helped shape our ideas about clinical chemical practice and ed-ACKNOWLEDGMENTS ucation. We also want to thank the many companies and professional organizations that have provided the product infor-A project as large as it requires help and vulture-waving and photography or granted permission to repro-port many individuals. Editors want to express diagrams and spreadsheets from their publications. An important source of information was also the many awards of contributors to this Sixth Institute of Clinical and Laboratory Standards (CLSI): techniques, principles, ments. Correlations – specialized laboratory experts These documents are directly mentioned in appropri and educators whose editors had the pleasure of eating chapters.to know and exchange ideas with over the years. These individuals have been selected for their expert- Editors would like to recognize contribu-ise in specific areas and their commitment to edu- tion and the efforts of all individuals for the previous edition.cation of clinical laboratories. Many of them have made their efforts provided a framework for many professional careers in the clinical laboratory, in the current chapters. Finally, we gratefully recognize the bench, teaching students, or consulting with doctors. cooperation and assistance staff in LippincottIn these frontline positions, they have developed per- Williams & Wilkins for their advice and support.spective about what is important for others klinických laboratórií. Redaktori sa neustále snažia zlepšovať budúce vydania tejto knihy. Opäť žiadame a vítame pripomienky našich čitateľov , kritiku a nápady na zlepšenie. PrispievateliaJohn J. Ancy, MA, RRT Julia C. Drees, PhDSenior klinický konzultant klinickej chémie FellowInstrumentation Laboratory San Francisco General HospitalDirector, Respiračné služby University of CaliforniaSt. Elizabeth's Hospital San Francisco, CaliforniaBelleville, Illinois Sharon S. Ehrmeyer, MDMichael J. Bennett, PhD, FRCPath, FACB profesor, patológia a laboratórna medicínaProfesor riaditeľ, lekárska technika / Klinické laboratórium Oddelenie patológie a laboratórnej medicínyUniversity pennsylvania vied a katedra patológie a laboratórnej medicíny Riaditeľ, Metabolické choroby Laboratory University of WisconsinChildren's Hospital of Philadelphia Madison, WisconsinPhiladelphia, Pennsylvania Jordan Erickson, BSMaria G. Boosalis, PhD, MPH, RD, LD Kvalita Analyst - Six Sigma Black BeltDirector, divízia klinickej výživy Katedra pediatrieCollege of Health Sciences University of North Carolina HealthcareUniversity of Kentucky Chapel Hill, Severná KarolínaLexington, Kentucky Edward P. Fody, MDLarry Broussard , PhD , DABCC , FACB lekársky riaditeľProfeszor oddelenie patologických klinických laboratórnych vied Holland HospitalLouisiana State University Health Sciences Center Holland, MichiganNew Orleans, Louisiana Elizabeth L. Frank, PhD, DBACCDean C. Carlow , MD, PhD odborný asistent - ClinicalAssistant profesor Katedra patológieDepartment patológie a laboratórnej medicíny University of Utah Health Sciences CenterUniversity z Pennsylvania School of Medicine Salt Lake City , UtahandMedical director, Klinická chémia Vicki S. Freeman, PhDDepartment patológie a klinickej chémie katedra stoličky a profesordeologickej nemocnice Philadelphia Klinické laboratórne vedyPhiladelphia, Pennsylvania University of Texas Lekárska pobočka Galveston, TexasEileen Carreiro-Lewandowski, MS, CLSProfessor Linda S. Gorman, PhDMedical Laboratory Laboratory Science CRS divízie DirectorUniversity Massachusetts-Dartmouth University of KentuckyN. Dartmouth, Massachusetts Lexington, KentuckyJanelle M. Chiasera, PhD, MT (ASCP) Marissa Grotzke, MDAssociate profesor a riaditeľ FellowSchool zdravotníckych profesií vnútorné lekárstvoUniversity z Alabamy v Birminghame divízie endokrinológie, metabolizmu a diabetesBirmingham, Alabama University of Utah Medical Center Salt Lake City, Utah viiviii prispievateľov Christopher R. McCudden, PhD asistent profesorRyan Hungerford, MD associate director Core LaboratoryFellow Director of Blood Gas and Special ChemistryEndocrinology Department of Pathology & Laboratory MedicineUniversity of Utah University of North Carolina-Chapel HillSalt Lake City, Utah School of Medicine Chapel Hill, North CarolinaKamisha Johnson-Davis, PhDClinical Chemistry Fellow Gwendolyn Appell McMillan, PhDDepartment of odborný asistent (Klinická)University of Utah Department of PathologySalt Lake City, Utah University of Utah Salt Lake City, UtahRobert Jones, MD aendokrinológia divízie Lekársky riaditeľUtah Diabetes Center toxikológie, stopové prvky, farmakogenetikaSalt Lake City, Utah ARUP Laboratória Salt Lake City, UtahThomas P. Knecht, MD, PhDMedical riaditeľ A. Wayne Meikle, MDAbbott Diabetes Starostlivosti ProfessorAlameda, California Katedra vnútorného lekárstva University of Utah School of MedicineRonald H. Laessig , PhD adirector, Wisconsin State Laboratory of Hygiene Staff PhysicianProfessor, Population Health Sciences University of Utah HospitalProfessor, Patológia a laboratórna medicína Salt Lake City, UtahSchool of Medicine and Public HealthUniversity of Wisconsin Susan Orton, PhD (ABMLI)Madison, Wisconsin assistant professor Division of Clinical Laboratory ScienceLouanne W. Lawrence, DrPH, CLS (NCA)

University of North CarolinaProfessor and Department Head Chapel Hill, North CarolinaClinical Laboratory SciencesLouisiana State University Health Sciences Center Joan E. Polancic , MSEd, CLS (NCA)New Orleans, Louisiana riaditeľ školstva a plánovania projektov Americkej spoločnosti pre klinické
laboratórne scienceKara L. Lynch, PhDClinical Chemistry Fellow (ASCLS)Department of Laboratory Medicine Bethesda, MarylandUniversity of California – San FranciscoSan Francisco General Hospital Elizabeth Porter, BS, MT (ASCP)San Francisco, California Technical Specialist Point of Care
TestingRuth McCall, BS, MT (ASCP) Quest DiagnosticsFormer Director, Phlebotomy and Clinical Laboratory Cincinnati Lynch , PhDClinical Chemistry Fellow (ASCLS)Department of Laboratory Medicine Bethesda, MarylandUniversity of California – San FranciscoSan Francisco General Hospital Elizabeth
Porter, BS, MT (ASCP)San Francisco, California Technical Specialist Point of Care TestingRuth McCall, BS, MT (ASCP) Quest DiagnosticsFormer Director, Phlebotomy and Clinical Laboratory Cincinnati Lynch, PhDClinical Chemistry Fellow (ASCLS)Department of Laboratory Medicine Bethesda,
MarylandUniversity of California – San FranciscoSan Francisco General Hospital Elizabeth Porter , BS, MT (ASCP)San Francisco, California Technical Specialist Point of Care TestingRuth McCall, BS, MT (ASCP) Quest DiagnosticsFormer Director, Phlebotomy and Clinical Laboratory Cincinnati Lynch, ,
Ohio Asistent programy Alan Remaley, MD, PhDCentral New Mexico Community College Senior StaffAlbuquerque, Nové Mexiko Laboratórna medicína National Institutes of Health Bethesda, MarylandCONTRIBUTORS ixWilliam L. Roberts, MD, PhD Cathee Tankersley, BS, MT (ASCP), CLS
(NCA)Profesor vo výslužbe Program DirectorDepartment patológie fakulty , emeritnýUniverzita Utah Phoenix CollegeSalt Lake City, Utah Phoenix, ArizonaandMedical riaditeľ David P. Thorne, PhD, MT (ASCP) Chémia Skupina Biomedicínske laboratórne diagnostiky ProgramARUP Laboratória Michigan
State UniversitySalt Lake City , Utah East Lansing, MichiganAlan Rockwood, PhD Lynda Tymchak, MEd, MT(ASCP)Docent (klinické) vzdelávacie službyRezort patológie Global Med TechnologiesUniversity z Utahu El Dorado Hills, CaliforniaandSection Director for Mass Spectrometry Tom WachterARUP
Laboratories Director of Corporate SafetySalt Lake City, Utah ARUP Laboratories, Inc. andMichael W. Rogers, MBA, MT(ASCP) University of Utah Medical CenterQuality Management Specialist University of UtahMcLendon Clinical Laboratories Salt Lake City CaliforniaandSection Director for Mass
Spectrometry Tom WachterARUP Laboratories Director of Corporate SafetySalt Lake City , Utah ARUP Laboratories, Inc. andMichael W. Rogers, MBA, MT (ASCP) University of Utah Medical CenterQuality Management Specialist University of UtahMcLendon Clinical Laboratories Salt Lake City
CaliforniaandSection Director for Mass Spectrometry Tom WachterARUP Laboratories Director of Corporate SafetySalt Lake City, Utah ARUP Laboratories, Inc. andMichael W. Rogers, MBA, MT(ASCP) University of Utah Medical CenterQuality Management Specialist University of UtahMcLendon Clinical
Laboratories Salt Lake City Californiaand , UtahUniversity of North Carolina HospitalsChapel Hill, Severná Karolína G. Russell Warnick, MS, MBA Berkeley Heartlab, Inc Michelle E. Russell Alameda, CaliforniaBiomedical Laboratory Laboratory Diagnostics ProgramMichigan State University Betty M.
White, MAT, MT (ASCP)East Lansing, Lansing, Odborný asistent Program DirectorAmar Akhtar Sethi, MD, PhD klinické laboratórium SciencePhysician Western Carolina UniversityLipoprotein Metabolizmus sekcie Cullowhee, Severná KarolínaNational Heart, pľúca, a Krvný ústavNárodné inštitúty zdravia
Monte S. Willis, MD, PhDBethesda, Maryland odborný asistent patológie a laboratórnej medicínyCarol J. Skarzynski, BA, SC (ASCP) University of North CarolineClinical Instructor andDepartment of Pathology and Laboratory Medicine Assistant DirectorHartford Hospital School of Allied Health Clinical
Chemistry (Core) LaboratoriesHartford, Connecticut McLendon Clinical Laboratories University of North Carolina HospitalsSusan King Strasinger, DA, MT (ASCP) Chapel Hill, North CarolinaFaculty AssociatealClinic Laboratory Sciences Alan H. B. Wu, PhDThe University of West Florida profesor
laboratórnej medicínyPensacola, Florida University of California - San Francisco a šéf, Klinická chémia Laboratórium San Francisco Všeobecnej nemocnice San Francisco, KaliforniaContentsPART 1 Zdroj a zloženie bloodbasic princípy a prax vzoriek / 41Clinical Chemistry / 1 typy vzoriek krvi / 41
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OF BIOLOGICAL SAFETY CABINET CABINETS APPLICATIONTYPE FACE AIRFLOW PATTERN RADIONUCLIDES / BIOSECURITY PRODUCTCLASS I ,* open front velocity TOXIC CHEMICALS LEVEL(S) PROTECTIONClass II Type A (IFPM) NoType B1 Yes 75 Front; rear and upper numbers 2, 3
YesType B2 via HEPA filter No. 2, 3Type B3 Yes 75 70% Recirculation via YesClass III HEPA; exhaust via HEPA 100 30 % recirculated via Yes 2,3 HEPA; exhaust through HEPA and (low levels/ 2.3 severely caducteral) 100 No recirculation; total exhaust Yes via HEPA and hard-ducted 100 Same as IIA,
but plenums under Yes 2, 3 vacuum in the room and exhaust air is powered by NA air intake inlets and exhaust Yes 3, 4 to 2 HEPA FILTERSSource:Centers for Disease Control and Prevention and National Health. Biosecurity in microbiological and biomedical laboratories.4. gloves may be added with
the release of air inlet pressures to allow work with chemicals/radionuclides. Nitrile gloves, for example, offer a wider range of com-BIOLOGIC SAFETYpatibility with organic solvents than latex gloves. Labcoats, preferably with knit-cuff sleeves, should be full of General Reflections length and priming and
made of liquid-resistant mate-rial. When performing manipulations prone to splashing All blood samples and other bodily fluids should be col-danger, laboratory sheath should be supplemented with lected, transported, manipulated and processed using strict stringable aprons and / or sleeve garters, built
from preventive measures. Gloves, clothes and face protection must be suitable material to protect against substances. Properly used if splashing or splashing is likely to occur.shoes are required; Footwear constructed of porous mate- Consistent and thorough hand washing is essentialrials, open-toed
shoes, or sandals are considered neeffec- component of infection control.tive against spilled dangerous liquids. Centrifugation of biological samples produced gently by respirators may be necessary for various procedures in dispersed aerosols, which are a high-risk source of infec-clinical laboratory.
Whether it is used for biological or biological. Ideally, samples should remain limited during chemical risks, the correct type of respirator must be centrifugation. As an additional preventive measure, use use for specific hazards. Respirators with a high centrifutor with an internal shield are recommended.
for example, when spilling directly with patients with tuberculosis (TB) or performing procedures that may aerosolise specific- Any blood, bodily fluid or other potentially infectious ma-mens of patients with suspected or confirmed cases of material leakage must be cleaned and the area or equipment of TB.
The training, maintenance and written protocol for use must be disinfected immediately. Cleaning includes respirators are necessary in accordance with respiratory recommendations: protective standard. ■ Wear suitable protective equipment. Each employer must provide (free of charge) laboratory
cladding, ■ Use mechanical equipment to collect broken glass beads or other protective equipment to all employees who may be exposed to biological or chemical risks. It is objects.is employer's responsibility to clean and maintain all ■ Absorb spills with paper towels, gauze pads, or PPE. All
contaminated PPE must be removed and disposed of before leaving the laboratory. Tissue. ■ Clean the spillage site with normal aqueous detergent. ■ Disinfect the leakage site disinfectant or 10% bleach using appropriate contact time. CHAPTER 3 ■ LABORATORY SAFETY AND REGULATIONS 79■
Rinse the leakage site with water. Transport■ Dispose of all materials in suitable biohazard Clinical Laboratories normally transport regulated material. Containers. The U.S. Department of Transportation (DOT) and the International Air Transport Association (IATA) have specific requirements for the
transportation of regulated materials. There are two types of classification of samples. KnownIn December 1991, OSHA issued a final rule for occupa- or suspected infectious samples are marked by infectious exposure to blood-borne pathogens. In order to minimise substances, if the pathogen can easily
be transferred to the exposure of the unemployed, each employer must have a written person or animals and there is no effective plan for controlling exposure to treatment. The plan must be available to all available. Diagnostic samples are tested aployees, whose reasonable presumed duties may result
in routine screening or initial diagnosis. Each type of occupying blood exposure or other potentially infec-sample has rules and packaging requirements. Thetious materials. The exposure control plan must be dis-DOT guidelines listed in the Code of Federal Regulationsjudied with all employees and be
available, while 49; IATA publishes its own manual, Dangerous Goods That Work. The staff member must be provided with the regulations.appropriate training of all the techniques described in the expo-sure control plan at the initial assignment of work and annually chemical safety thereafter. All
necessary equipment and supplies must be readily available and regularly inspected. Hazard Communication Clinical Lab workers are knowingly or un- In the August 1987 issue of the Federal Register, OSHAknowingly in frequent contact with potentially bio-published new Hazard Communication
Standardhazardous materials. In recent years, new and serious (Right to Know the Law). The right to know the law was a de-labor hazard for employees arose, and it veloped for employees who may have been exposed to a dangerous problem was complicated because of general chemicals. Staff must
be informed of the state of health of understanding epidemiology, the mechanisms of risk associated with these chemicals. The intention of transmitting the disease or inactivating the law is to ensure that health risks are evaluated for an alysous agent. Special precautions must be taken when chemicals
are produced and that this information is processed with all samples due to the continuous increase transmitted to employees.in infectious samples taken in the laboratory. Therefore, in practice, samples from patients with con- To comply with the Regulation, clinical laboratories confirmed or suspected of
hepatitis acquired immunodefi-must:ciency syndrome (AIDS), Creutzfeldt-Jakob disease or other potentially infectious diseases should be treated ■ and carry out carry out other hazards than other normal samples. The adoption of a policy program.standard precautions, which considers blood and other
bodily fluids from all patients to be potentially infec- ■ Obtain material safety data sheets (MSDS) for each native is required. hazardous compound present at the workplace and have MSDS easily accessible to employees. Airborne Pathogens ■ Educate all employees annually on how to interpret the TB
recovery, OSHA issued chemical labels, MSDSs, and health risk statements in 1993 that the agency will promote chemicals and how to safely work with chemist-CDC guidelines for preventing calve transfer. Tuberculosis in medical facilities. The purpose of the guidelines is to promote early detection,
isolation, ■ To store hazard warning labels on received containers and to process active cases. Tb exposure control or on-site control must be established.programme and risks to laboratory personnel must be assessed. In 1997, OSHA issued a proposed standard material safety data sheet (29 CFR
1910.1035, tuberculosis). The standard mandates the development of MSDS tuberculosis is the main source of safety information overexposion control plan of any device involved in diag-staff who can use hazardous materials in ichnosesis or treat cases of confirmed infectious TB. Profession. Employers
are responsible for obtaining TB isolation areas with specific ventilation controls from the chemical manufacturer or for the development of ANBE based in health facilities. For each hazardous agent used on pracovisku.sa, these MSDS workers may require high-risk workers to wear a respirator for a
standardised format, not mandatory, but all re-protection. All healthcare professionals who are considered to be healthcare professionals referred to in the act must be dealt with. The aggregate risk for TB infection must be investigated. Information requirements for MSDS include the following: ■ Product
name and identification ■ Hazardous ingredients80 PART 1 ■ BASIC PRINCIPLES AND PRACTICE OF CLINICAL CHEMISTRY■ Permissible exposure limit (PEL) exposure (i.e. short-term or single contact, versus■ Physical and chemical data long-term or prolonged, repeated contact). Almost any data
on the danger to health and the carcinogenic potential substance, even the most harmful, may endanger damage to a■ Primary routes of the inlet lungs, skin, eyes or mucous membranes of fol-■ The risk of fire and explosion may be low for a long time or short-term exposure and may be toxic if the
reactivity data are exceeded excessively. In addition, some chemicals are toxic at very low concentrations of escape and disposal. Exposure to toxic substances may be direct contact (absorption), inhalation, ingestion or■ Handling of vaccination/injection through PPE recommendations.■ Emergency and
first aid procedures■ Precautions for storage and In the clinical chemistry laboratory, the name, address and telephone of the chemical manufacturer should be conscious toxic fumes from chemical sol-vents such as acetone, chloroform, methanol or carbon number tetrachloride that do not give explicit
sensory irritation■ Special warnings of the information section, as well as bromide, ammonia and formaldehyde. Air sampling or routine monitoring may be necessary for MSDS to be printed in English and provide quantification of dangerous levels. Mercury is another often specific composed identity,
along with all the usual ignored sources of toxic fumes. It's highly named. All information sections must be filled in, and volatile and toxic and is quickly absorbed by the oversue that MSDS has been printed, it must be stated. skin and respiratory tract. Mercury leakage kits should beCOPIes of MSDS shall
be readily accessible by em- available in areas where mercury thermometers are used.ployees during all changes. Most laboratories gradually phase out the use of mercury and mercury-containing compounds. Laboratories should have a laboratory standardOSHA policy and method of legal disposal of
mer-cury. Laboratory engineering checks, PPE and proce-work exposure to hazardous chemicals during major controls must be adequate to protect personnel Laboratories, also known as laboratory standards, against these substances.were adopted in May 1990 to provide laboratories with special
guidelines for the handling of hazardous chemicals. Storage and handling of chemicalsThirsty OSHA standard requires that any laboratory that uses shazardous chemicals have written chemical hygiene To prevent accidents when handling chemicals, there is an im-plan. This plan shall provide procedures
and working practices to develop respect for all chemicals and to regulate and reduce the exposure of laboratory equipment to full knowledge of their properties. This is a particu-sonnel for dangerous chemicals. Hazardous chemicals are very important in the transport, dispensing or use of these
chemicals, which pose a physical or health risk to an acute or chemical that comes into contact with some other chemist-chronic exposure. Procedures describing how to protect icals could result in the formation of substances that are employees against teratogens (substances that affect whole-toxic,
flammable or explosive. For example, acetic acid development in the fetus or embryo), carcinogens, is incompatible with other acids such as chromium and ni-a and other toxic chemicals must be described in tric, tetrachloromethane is incompatible with sodium,plan. Training in the use of hazardous
chemicals for inclusion and flammable liquids is incompatible with the recognition of signs and symptoms of exposure to hydrogen, loca peroxide and nitric acid. Chemical hygienist on the quantities of chemicals needed and nature or must be designated for each laboratory using a dangerous type of
chemicals. storage is necessary to preventchemicals. The Protocol shall be reviewed annually and laboratory fires and accidents checked. Ideally,updated when regulations are modified or chemical in-store should be organized so that each class vengeance changes. Remember that practicing consistent
chemicals is isolated in an area that is not used for direction-and thorough hand washing is an essential part of tine work. An up-to-date inventory should be maintained to prevent chemical hygiene. indicates the location of chemicals, minimum/maximum required quantities, durability, etc. Some
chemicalsToxic effects of dangerous substances deteriorate over time and become dangerous (e.g. ether forms explosive peroxides). Storage should not be basedToxic substances have the potential to produce lubricated substances only in alphabetical order, since incompatible chemically harmful
effects (local or systemic) direct chemical effects can be stored side by side and react by chemists-or interference with the function of body systems. Call. They must be separated for storage as specified, may cause acute or chronic effects related to duration Table 3-2.CHAPTER 3 ■ LABORATORY
SAFETY AND REGULATIONS 81TABLE 3-2 STORAGE REQUIREMENTS STANDARDS FOR handling these substances. Benzidine is a common example of a known carcinogen. Where possible, a substance stored separately, a substitute chemical or other process should be flammable solids used to
prevent exposure to carcinogenic agents. For regu-flammable liquids Organic cotton acids (OSHA) and institutional safety requirements, the oxidisers laboratory must maintain an accurate list ofminer acids water-reactive substances carcinogens. Other Chemical stainsKaustika Strict attention to good
laboratory technique can help prevent chemical spillage. However, emergency procedures should be established for Folic acid to deal with any accidents. If a leak occurs, the first step should be to help/evacuate the person-air-reactive substance nel, and then incarceration and cleanup of the leak can
begin. There are several commercial spill kits availableHeat-reactive substances for neutralization and absorption of spilled chemical solutions requiring cooling (Fig. 3-2). However, no set is suitable for all types of spillage. Emergency procedures for spills should also include unstable substances reporting
system. (radiation sensitive to SAFETY impacts) Environmental protection Radiation safety policy should include environmentally flammable/flammable chemicals and protection of personnel. All areas in which radioactive flammable and flammable liquids used or stored in materials are used or stored
must be dispatched with numerous routine procedures, are among the most serious signs and transport in these areas should be limited to biological materials in the laboratory of clinical chemistry to basic personnel only. Regular and due to a possible fire or explosion. Are classified monitoring must be
(a) decontamination according to the flash point, which is the temperature in laboratory equipment, glass and work areas which are burning enough steam to ignite, should be planned as part of routine procedures.mixture with air. Flammable liquid has a flash point Records must be kept in terms of the
amount of ra-pod 37,8 °C (100 °F) and flammable liquids, defi-diactive material on the hand, as well as the amount that is required to have a flashpoint at or above 37,8 °C (100 °F). Some commonly used flammable and flammable sol- FIGURE 3-2. Spill cleaning kit.vents are acetone, benzene, ethanol,
heptane, iso-propanol, methanol, toluene, and xylene. It is important to remember that flammable chemicals also include cer-tain gases, such as hydrogen, and solids such as paraffin. Corrosive chemicals Corrosive chemicals are harmful to the skin or eyes through direct contact or tissue of the
respiratory and gastrointestinal tract if inhaled or ingested. Typical ex-amples include acid (acetic, sulfuric, nitric, and hy-drochloric) and base (ammonium hydroxide, potassium hydrohydroxide, and sodium hydroxide). Reactive chemicalsReactive chemicals are substances that, under certain conditions,
can spontaneously explode or ignite or unleash heat or flammable or explosive gases. Some strongacids or bases react with water to heat production (ex-mic reaction). Hydrogen is liberated when alkaline metals (sodium or potassium) are mixed with water or acids and spontaneous combustion may also
occur. A mixture of oxidising agents such as peroxides and reducing agents such as hydrogen generate heat and can be explosive. Carcinogenic chemicalsCarcinogens are substances found to be cancer-causing substances. OSHA has released lists of confirmed and suspected carcinogens and detailed
82 PART 1 ■ Basic principles and practice of clinical chemistry. The Nuclear Regulatory Commission (NRC) Unseeded Fuel License is required if the total amount of radioactive ma-reaction oxygenterial exceeds a certain level. Laboratory safety offi-cer must consult the Institutional Security Officer of Heat
on these requirements. FIGURE 3-3. Fire tetrahedron. Personal protection as a reaction chain in which the fire continues, and evenThing is necessary that only properly trained staff work accelerates. This is due to malfunction and recombination with radioisotopes and that users are monitored to ensure
that the nation molecules from burning material, that the maximum permissible radiation dose is not ex-oxygen in the atmosphere.ceeded. Radiation monitors must be regularly evaluated to check the degree of exposure to the laboratory staff. The fire triangle has been modified into three records to be
preserved during the employment-dimensional pyramid known as the tetrahedronment fire plus 30 years. (Fig. 3-3). This treatment does not eliminate fire-fighting procedures, but pro-liquorizing radiation additional means by which fires can be prevented or extinguished. The fire goes out if any of the
znonionizing forms of radiation are also a concern in three essential elements (heat, air, or fuel) being removed.by the clinical laboratory. The equipment often emits different wavelengths of electromagnetic radiation, which must be the classification of fires protected against fires by artificial shielding or the
use of PPE (Table 3-3). These energies have different biological fires have been divided into four classes based on on-effects, depending on wavelength, intensity of power, and ture of combustible material and requirements of forduration exposure. Laboratorians must be knowledge-extinguishing:capable
of the dangers posed by their equipment to protect themselves and auxiliary personnel. Class A: ordinary flammable solid materials such as paper, wood, plastics and fabricFIRE SAFETY Class B: flammable liquids / gases and flammable petro-Chemistry fire leum productsFire is essentially a chemical
reaction that includes Class C: energized electrical equipment oxidation of flammable material or fuel, with class D: flammable / reactive metals such as magnesium, subsequent release of heat and light. In a clini-cal chemical laboratory, all the elements necessary for sodium, and potassium fire to begin
with are present-fuel, heat or source of ignition, and oxygen (air). However, recent research suggests that the fourth factor is present. This factor was classifiedTABLE 3-3 EXAMPLES OF NON-IONIZING RADIATION IN CLINICAL LABORATORIESTYPE APPROXIMATE SOURCE OF EQUIPMENT
EXAMPLE PROTECTIVE MEASURESS LOW FREQUENCY WAVELENGTH Engineered shielding and 1 cm2 Radiofrequency coil in ICP-mass posted pacemaker warningMicro Wave Spectrometer Engineering Shielding 3 m-3 mmFrared Energy-beam microwave used to accelerate insulation and 750

nm-0.3 cm tissue staining in histology-prep processes appropriate warning labels Filters, diffusers, (a) thermal lamps, lasers not reflecting surfaces Eye and skin protection; UVVisible spectrum 400–750 nm General light and dazzle warning labelsUltraviolet 4–400 nm Germicidal lamps used in biological safety cabinetsCHAPTER 3 ■ LABORATORY SAFETY AND REGULATIONS 83Types and applications of fire extinguisher drills must be carried out regularly and with appropriate documentation. Just as fires have been divided into classes, fire extin-guishers are divided into classes that correspond to the control of other fire type hazards to be extinguished. Rest assured, choosing the same type-use of the wrong type of fire extinguisher may be an electrical hazard. For example, do not use water for burningliquids or electrical equipment. Most individuals are aware of the potential hazards associated with the use of electrical appliances and pressure water extinguishers, as well as foam and ment. The dangers of electricity can be direct and resulted byconscientious dry-chemical types, are used for class A in shock, or burns. Burns. hazards can result in fires. Multipurpose dry-chemical and carbon dioxide ex-fire or explosion. Therefore, there are many preventativechtinguishers are used for Class B and C fires. The halogenated procedures to be followed for the operation or operation of by-hydrocarbon fire extinguishers are particularly recom-electrical equipment:admonished for use with computer equipment. Class D fires present special problems and firefighting is left on ■ Use only explosion-resistant equipment with dangerously trained firefighters using special dry-chemical extin-atmospheres.guishers (Fig. 3-4). Staff should know the location and type of portable fire extinguisher near their work ■ Be especially careful when operating a high voltage area and know how to use a fire extinguisher before a fire occurs, such as electrophoresis. In the event of a fire, first evacuate all personnel, patients and visitors in immediate danger and ■ Use only properly grounded equipment (three spikes then activate the fire alarm, report the fire and try to connect).if possible, extinguish the fire. Staff should act as a team to carry out emergency procedures. Fire ■ Check for frayed electrical cables. ■ Immediately report any faults or devices that ring out the singles for repair. ■ Do not operate on live electrical equipment. CLASS FIRE TYPE FIRE EXTINGUISHER OPERATIONA Class A Fires ABC Use the following types of PULL fire extinguishers PIN Ordinary IM flammable substances: NOZZLE Wood, Paper, fabric, etc. SQUEEZE TRIGGER Pressurised water Dry ChemicalB Class B Fires Use these types of fire extinguishers ABC Dry Chemical Flammable Liquid Grease BC Carbon dioxide Petrol paints Oils, etc. C Halon ABC SWEEP Class C Fires NOZZLE BC Use these types of carbon dioxide Extinguisher housing with extinguisher (blade , sprinkle) Electrical Equipment Motors Switches Halon Dry Chemical Class D Fires Use this typeD reagent Metal X Flammable Metals MagnesiumFIGURE 3-4. Proper use of fire extinguishers. (Modified with the permission of the Clinical and Laboratory Safety Department, University of Texas Health Science Center in Houston.) 84 PART 1 ■ BASIC PRINCIPLES AND PRACTICE OF CLINICAL CHEMISTRY■ Never use electrical equipment with wet hands. minimize fluid loss due to evaporation by boiling- ■ Know the exact location of the electric control panel off and prevent connection and build-up of pressure. electricity to the work area. Mechanical hazards■ Use only approved extension cables and do not stretch- Except for physical hazards such as fire and electrical load circuits. (Some local regulations prohibit the use of shock, laboratory personnel should be informed of any extension cord.) mechanical hazards of equipment such as centrifuges,■ Carry out ground inspections and other regular preventive autoclaves and homogenizers. maintenance carried out by devices. Centrifups, for example, must be balanced by distributed-compressed gases risking ute loads as well. The operator should open the lid until the rotor stops completely. SafetyCompressive gases, which serve inoperable.in number of lock functions on the device and should never have been provided by the laboratory, represent inoperable.in unique combination of hazards in a clinical laboratory: fire danger, explosive- Laboratory glassware itself is another potential haz-zion, suffocation, or mechanical injury. There are ard. Substances, such as glass beads, should be added to helpseal general requirements for safe handling com-eliminate shocks / boilover when liquids are heated.Pressed gases: Pliers or gloves should be used to remove hot glass from furnaces, hot plates, or water baths. Glass pipettes■ Get to know the gas you will be using. which should be handled with extreme care, as well as sharp in-■ Store tanks in a vertical position. spores such as cork borehole borehole, needles, scalpel blades,■ Keep the cylinders secured at all times. and other tools. Flammable liquids and compressed gases should never be stored to detect signs of wear or fatigue that could contribute to breakage or injury. All infectious sharps in the same area. must be disposed of in osha-approved containers to re-use them■ Use the correct regulator for the type of gas used. risk of injury and infection.■ Do not attempt to control or shut down gas flow with the ergonomic hazard lightening regulator.■ Keep the removable protective caps in place until increased mechanisation and automation have created many lengthy and repetitive manual tasks, the cylinder is used. laboratory processes often require repeated handling■ Make sure that the acetylene tanks are properly drained from tools, containers and equipment. These physical actions can contribute to repeated stress over time (the gas is not compatible with the copper hose). orders such as tenosynovitis, bursitis and ganglion cysts.■ Do not make a frozen or stuck cylinder valve. Primary contributing factors associated with repetition-■ Use a hand truck to transport large tanks. stress disturbances are position/posture, applied force,■ Always check the tanks when received and then periodically and the frequency of repetition. Be sure to consider marking the hand tool (e.g. ergonomic pipettes), compliance with any problems such as leaks. ergonomically correct technique and position of the device-■ Make sure that the cylinder is correctly marked for ing when engaging in any repetitive task. The contents can identify chronic symp-toms of pain, numbness or tingling in the extremities. indicate the onset of disorders of the recurrent strain. Other haz-■ Empty tanks should be marked as empty. ards include acute musculoskeletal injuries. Do not forget to properly lift heavy objects, keep the load nearcryrogenic materials Risk the body and using the muscles of the legs, rather than the back. Gradually increase strength when or dragging,Liquid nitrogen is probably one of the most used and avoid pounding the action with liquids (liquefied gases) in the laboratory. However, there are several hazards associated with the use of disposal of hazardous materialsEntertain cryogenic material: fire or explosion, suffocation, build-up of pressure, matter and tissue Safe handling and disposal of chemicals and other damage similar to thermal burns. materials require a thorough knowledge of their proper links and hazards. Hazardous waste generators have only containers made of materials intended for moral and legal responsibility, as defined in the applicable refrain ultra-low temperatures should be used for cryo-local, state and federal regulations to protect both theogenic works. In addition to the use of eye/face protection, the individual and the environment in waste disposal.hand protection to protect against the risk of contact, there are four basic waste disposal techniques: flushing surfaces are recommended. Gloves made of impenetrable material should fit freely so that they can be released quickly if liquid is spilled on them or into them. Also, tominimize violent cooking /foaming and splashing, speci-mens to be frozen should always be inserted by rescheduling very slowly. Cryogenic liquids should be stored in well insulated but loosely sealed containers thatCHAPTER 3 ■ LABORATORY SAFETY AND REGULATIONS 85down drain to sewerage, incineration, landfill rules are any health care-related equipment, including, but notburial, and recycling. limited to outpatient surgical centres; blood banks and blood collection centres; clinics, including medical, dental, chemical waste and veterinary; clinical, diagnostic, pathological or biomedical research laboratories; emergency medical services; In some cases, it is allowed to flush hospitals soluble in water; long-term care facilities; smaller emergency cen-substances to drain with large amounts of ters; occupational health clinics and clinical laboratories;water. However, strong acids or bases should be neutral and professional offices of doctors and dentists before disposal. Foul-smelling chemicals should never be disposed of in drains. A possible reaction of medical waste is defined as a special waste of medical chemicals in the outflow and potential toxicity must be con-care equipment and is further defined as solid waste, which, ifsidered in deciding whether a particular chemical can be dis-treated or manipulated, can transmit infectious resolved or diluted and then flushed into sewers. For diseases. (For more information, see TJC Webexample, azid soda, used as a preservative, instead of: . It contains explosive salts with metals such as copper, animal waste, blood volume and blood products, microbio-tubes. Most institutions prohibit the use of soda due to logical waste, pathological waste and sharps. Approved for this hazard. Methods and management of medical waste are incineration, incineration, sterilization, burying, thermal inacti- Other liquid wastes, including flammable solvents, vation, chemical disinfection or encapsulation in solids, must be collected in approved containers and separate matrici.do compatible classes. If practical, solvents such as asxylene and acetone may be filtered or re-reduced for medical waste generators, they must be reused. If recycling is not feasible, disposal procedures should be carried out by specially trained personnel. Flammable material can also be burned in special ■ Employers of health care workers must set up anddemed incinerators with burners and washing machines to implement an infectious waste program.to remove toxic combustion products. ■ All biomedicine waste should be placed in a bag Also before disposal, hazardous substances that bear the symbol of biological hazard and then placed explosively (e.g. peroxides) and carcinogens should be transformed into less dangerous forms whenever possible in an impermeable container that is resistant to punctures. and fitted with a fixed, tight lid. All chemical wastes containing solids which are not suitable for incineration plants must be clearly labelled with the word biohazardous or buried in a landfill. But this practice has its symbol.created an environmental problem, and now there's a seism of safe places. ■ All sharp instruments, such as needles, blades and glass objects, should be placed in special containers resistant to puncture of radioactive waste before being placed in a bag and container. The method of use and disposal of isotopes is strictly regulated by the NRC and depends on the type of waste ■ Needles should not be transported, recapped, bent, or (soluble or non-permeable), its level of radioactivity, and broken by hand.radiotoxicity and half-life of the isotopes involved. The Irradiation Security Officer should always be consulted on ■ All biomedical waste must then be disposed of in accordance with the policies of the agreement dealing with the disposal of radioactive waste. Many of the recommended procedures.clinical laboratories transfer radioactive materials to an alicensed receiver for disposal. ■ Potentially biohazardous material such as blood or blood products and contaminated laboratory waste, biohazardous waste cannot be directly disposed of. Contaminated composted waste may be burned. ContaminatedOn November 2, 1988, President Reagan signed into non-compostable waste, such as glass, the Medical Waste Monitoring Act of 1988. His pur-autoclaved before the take-off. Particular attention has been paid to (1) the environmental protection levy, which should be paid to the disposal of syringes, needles, the agency responsible for setting up the programme and the broken glass, which could also cause accidental monitoring of medical waste from production to disposal, (2) cuttings or punctures. Suitable containers should befine medical waste, establish acceptable techniques for use in the disposal of these sharp sharp liquidation, and (4) establish a department with the power to enforce new laws. Several STATES have implemented federal guidelines and incorporated INVESTIGATIOOAddd additional requirements. Some entities involved in any accident with personal injuries, even minor ones, should be reported immediately to the supervisory authority. Under OSHA regulations, employers are required to keep records of accidents at work and illness86 PART 1 ■ BASIC PRINCIPLES AND PRACTICE OF CLINICAL CHEMISTRY FOR THE DURATION OF EMPLOYMENT PLUS 30 YEARS. Record- Information on each death at work, non-fatal oc-management requirements include the first accident report, cupational disease, biological or chemical exposure, andaccident investigation reports, and an annual summary of non-fatal work-related injuries that included con-loss, which is recorded on OSHA injury and disease log sciousness, work or movement limitation, transfer to-(Form 300). or medical treatment (other than first aid). The first accident report is used to inform the insurance company and human resources or employees, because it is important to determine why and how the department anlation that an injury occurred in the workplace. accident has occurred, accident investigations should bebased and the supervisory authority usually finish carrying out. Most accidents can be traced to one of tworeports that contains information about the employer and the root causes: the environment (dangerous conditions) or the injured person, as well as time and place, cause, and personal (dangerous acts). Environmental factors include the nature of the injury. The message is signed and dated; then adequate guarantees, the use of incorrect or defective equipment shall be transferred to the manager of the risk or insurance of the institution, to the dangers associated with the place or representative of poverty. Cleaning. Personal factors include inappropriate Labour-Tory attire, lack of skills or knowledge, specific physical Inquiry reports should include information or mental conditions, and attitude. The employee is a posi-on injured person, a description of what happened, tive motivation is important in all aspects of safety pre-cause accidents (environment or personal), movement and prevention of accidents.other contributing factors, witnesses, nature of the injury, and measures to be taken to prevent recurrence. It is especially important that the relevant author-this report has been signed and dated by a person to be notified immediately if any person is conducting an investigation. needle during blood collection or incision during subsequent processing or handling of the sample. In the amount - Every year a journal and a summary of accidents at work with recommendations for the protection of laboratories and diseases should be completed and sent, see Protection of laboratory workers from the U.S. Ministry of Labour, Office Statistics on infections acquired at work; Approved guideline, OSHA Injury and Disease Protocol (Form 300). 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Pre-analytical and analytical aspects Proficiency testing Determining whether or not to determine ■ QUALITY MANAGEMENT Quality improvement reference intervals: Lean Six Sigma Benchmark Analysis Improvement Methodology-DMAIC Data Analysis to Create Metric Reference Interval-Lean and Six Sigma Data Analysis to Verify Perspective Reference Interval: Patient Safety, Lean Six Sigma and (Transmission) Laboratory practical use Six Sigma Metrics■ DIAGNOSTIC EFFICIENCY ■ PRACTICAL PROBLEMS WITH PRACTICE DIAGNOSTIC EFFICACY MEASURES ■ ONLINE RESOURCES ■ REFERENCES■ EVALUATION OF METHODS Regulatory aspects of method evaluation (alphabet soup) Method Selection method Evaluation First things First: Determine inaccuracy and inaccuracyIt is widely accepted that most medical deci-verify the acceptability of new methods before message-sions are made using Data. It is therefore about patient outcomes. When a method is critical to the results produced by the laboratory, it is essential that the laboratory ensure this. Determination and maintenance of accuracy required remains valid over time; This is achieved by a process that can be expended on effort and cost, which requires the use of a series known as quality control (QC). All concept approaches depending on the complexity of the test. fall under the austy of quality management (QM),To get started, you need to appreciate what is the quality and how the whole test process is measured with thequality. To this end, it is essential that the sub-overall objective of improving the accuracy of laboratory re-stand basic statistical concepts that allow laborato-sults. This chapter describes the basic statistical concepts for measuring quality. Before introducing a new test and providing an overview of the necessary procedures, it is important to determine whether the test is capable of introducing a new method and ensuring its acceptable performance. the evaluation method is used for accuracy.88CHAPTER 4 ■ METHOD EVALUATION AND QUALITY MANAGEMENT 89BASIC CONCEPTS SOURCES OF ANALYTICAL VARIABILITYYYYYach day, high-volume clinical laboratories generate operator conditions environmentmiliflower results. This amount of clinical laboratory technique (e.g. temperature, humidity)data must be summarised to monitor the formancia of the test. The basis for monitoring performance reagents (known as QCs) is descriptive statistics. Differences Surge SurgeDescriptive Statistics: Measures Center, Test Accessories Matrix effects (hemolysis, Propagation, and shape of lipemia contamination, serum proteins)When carefully examined, the collection of seeming similarities always has at least slight differences for a given average is calculated by the sum of observations and characteristic (e.g. smoothness, size, color, weight, vol-division by number of observations.ume, potency). Similarly, laboratory data shall have at least differences in light measurement. For example, if glucose Σ Add . . . on a given sample is measured 100 times in a row, there would be a range of data. Such differences in laboratory n data points (x) and . . . values can be the result of different sources. Σ xi divides the total number Although measurements will vary, their values are data points (n)patterns that can be visualized and analysed iɕ1tively. Laboratorians view and describe these patterns byasying graphic representations and descriptive statis- Σ / ntics (Fig. 4-1). xi n When comparing and analysing collections or iɕ1laboratory data, the patterns may be described by their centre, layout and shape. Although comparison center Σ/x– ɕ n xi n find average (x bar) (Eq. 4-1) data are most common, comparison spread can still be iɕ1more strong. Data dispersion allows laboratories to reassess predictability (and lack thereof) laboratory The sum sign Σ stands for (x1 Ƨ x2test or measurement. Ƨ x3 Ƨ . . Ƨ xn) and is used in many statistical formulas. Often, the mean of a specific dataset is referred to as centering measures x–or x bar. The three most commonly used center descriptions (Fig. 4-2) are mean, median, and mode. The median is the center of the data after the havemean data is most commonly used and often referred to as the order ordered. This is the value that divides the data inaverage. The median is the median point of the data and half. For example, due to a sample of 5, 4, 6, 5, 3, 7 and 5,it is often used with skewed data. The mode is rarely used as a ranking ranking of points: 3, 4, 5, 5, 5, 6, 7. Since the area measures the data center, but is more often used for the odd number of values in the sample, select middledescribe data that appear to have two centers (i.e. bimodal). value: 5. Number 5 divides the data in half. Since other samples with an even number of values: 5, 4, 6, 8, 9 and 7, again rank points: 4, 5, 6, 7, 8, 9. Select two mean values: 6 and 7. Then add the SpreadFIGURE 4-1 Center format. Basic data includes center, distribution, and shape.90 PART 1 ■ THE BASIC PRINCIPLES AND PRACTICE OF CLINICAL CHEMISTRY Center ance represent the average distance from the center of the data (average) and each value in the dataset. CVFIGURE 4-2. The center can be defined by medium (x-bar charac- allows the lab to compare SDs with different units.ter), median, or mode. A scope is one description of the dissemination of data. It is: 6 Ƨ 7 ɕ 13; and divide 2: 13/2 ɕ 6.5. As before, simply the difference between the highest and lowest6.5 splits the data in half. data points: range ɕ high Ⱦ low. For samples 5, 4, 6, 5, 3, 7 and 5, the range is 7 Ⱦ 3 ɕ 4. Scope's mode is the most frequently occurring value in an often good dispersion rate for small sampledataset. Although it is rarely used to describe data, it is data. It has a serious disadvantage; range is sus-referred to when referring to the shape of data, bi-ceptible to extreme values or outliers.modal distributions, for example. In the sample: 3, 4, 5, 5,5, 6 and 7, the value that occurs most frequently is 5. In order to calculate the SD dataset, it is easiest to tomod this set is then 5th first to determine variance (s2). The deviation is similar in diameter in that it is average. Variance is average When describing the center of a dataset, the square distances of all values from the intermediate value are very used to show how the data is distributed (spread). Thespread represents the relationship of all data points to the Σ /n (Eq. 4-2)average (Fig. 4-3). There are three most commonly used propagation letters: (1) range, (2) SD and (3) coeffi-s2 ɕ (xi Ⱦ x–)2 n Ⱦ 1cient variation (CV). The simplest measure of iɕ1understand is range. Range simply the largest value in the data minus the smallest value that repre- As a measure of variance, variance represents the dif-sents extremes of data one might encounter. The standard difference between each value and the data.deviation mean (also referred to as s, SD or ☐) is the most fre- Considering the values of 5, 4, 6, 5, 3, 7 and 5, the variance can be used immediately to scale the variation. Although the calculation calculated as below screenshot shown: SD may seem a little intimidating, the concept is very difficult. The SD and, more specifically, the vari- x– (5 Ƨ 4 Ƨ 6 Ƨ 5 Ƨ 3 Ƨ 7 Ƨ 5)/7 ɕ 5 (xi Ⱦ x–) (5 Ⱦ 5) Ƨ (4 Ⱦ 5) Ƨ (6 Ⱦ 5) Ƨ (5 Ⱦ 5) Ƨ (3 Ⱦ 5) Ƨ (7 Ⱦ 5) Ƨ (5 Ⱦ 5) (xi Ⱦ x–)2 (0)2 Ƨ (Ⱦ1)2 Ƨ (1)2 Ƨ (0)2 Ƨ (Ⱦ2)2 Ƨ(2)2 Ƨ (0)2 n 0Ƨ1Ƨ1Ƨ0Ƨ4Ƨ4 Ƨ 0 ɕ 10 Σ (xi Ⱦ x–)2 10/(7 Ⱦ 1) ɕ 10/6 iɕ1 Σ /n (xi Ⱦ x–)2 n Ⱦ 1 iɕ1 Σ /n 1.67 (Eq. 4-3) s2 ɕ (xi Ⱦ x–)2 n Ⱦ 1 iɕ1 SpreadFIGURE 4-3. The spread is defined by the standard deviation and the coefficient of variation. CHAPTER 4 ■ EVALUATION OF METHODS AND QUALITY MANAGEMENT 91TABLE 4-1 COMPARISON OF SD AND CV FOR TWO DIFFERENT ANALYTESFSH SD ᴺᴸHCG SD CVCONCENTRATION 0.09 CV CONCENTRATION 0 ,09 CV CONCENTRATION 0.09 CV 0.8 8.0 0.25 9.0 10 5.5 5.5 1 0.40 5 .0 100 5.5 5 1.20 4.0 1 000 52.0 5.0 10 3.80 4.8 1 0 000 500,00 4,9 25 3,8 100 000 4897,0 100FSH, follicle stimulating hormone; ᴺᴸhCG, ᴺᴸ-human chorionic gonadotrophin. To calculate the SD (or s), simply take the square root commonly discussed is the gaussy distribution of variance: (also referred to as normal distribution; 4-4). Gauss's distribution describes many continuous labo-ΣΣ /n ratory variables and shares several unique characteristic (☐) ɕ s2 ɕ iɕ1(xi Ⱦ x–)2 n Ⱦ 1 (Eq. 4-4) tics: mean, median and mode are identical; distribution is symmetric-which means that half of the values fallSomething is important to understand how these on the left side of the diameter, and the other half fall domeasures are calculated, many laboratory tools right- and symmetric shape is often referred to asinformation systems, and software packages determine the bell curve. these automatically. SD describes the distribution of alldata points around the average. The total area under the Gaussian curve is 1.0 or 100%. A large part of the area –68,3 % – below the normal curve is another way of expressing the SD in terms of CV between ↑1 SD (☐ ↑ 1☐) (Fig. 4-5A). Most of the area — the CV is calculated by splitting the SD by medium and 95,4 %– in the normal curve it is between ↑2 SD (☐computor by 100 per cent: ↑ 2☐; 4-5B). And almost all areas-99.7%- below the normal curve is between ↑3 SD (☐ ↑ 3☐)CV (%) ɕ 100s (Eq. 4-5) (Fig. 4-5C). x Rule 68–95–99 summarises the above rela-CV simplifies the comparison of SD test results ex-tionships between the area under gaussian distribution-pressed in different units and concentrations. As stated in tion and SD. In other words, due to any gaussianTable 4-1, analytes measured at different concentrations distributed data, Þ68% of data between ↑1 SD may have a significantly different SD, but a comparable CV. Þ95% of the data are among ↑2 the SDsK is used to a large extent to summarize QC data. From the diameter; and Þ99% decrease between ↑3 SDsCV highly accurate analyzers may be less than 1%. from the diameter. Similarly, if you selected a value in the dataset that is gaussian distributed, there is a 0.68Measures shaped chance that lies between the ↑1 SD from the average; aIch when there are hundreds of different shapes- 0.95 probability that lies between the ↑2 SDs; and adistributions-that datasets can show, most Shape FIGURE 4-4. A shape is defined by how data distribution is related to the center. This is an example of data that have a normal or Gaussian distribution.92 PART 1 ■ BASIC PRINCIPLES AND PRACTICE OF CLINICAL CHEMISTRY –3σ –2σ –1σ μ +1σ +2σ +3σA – 3σ –2σ –1σ μ +1σ +2σ +3σB –3σ –2σ –1σ μ +1σ +2σ +3σCFIGURE 4-5. The normal distribution includes (A) Þ68% of results within ↑1 SD (1s or 1☐), (B) 95% results within ↑2s (2☐) and (C) Þ99% results within the ↑3☐.0.99 probability of lying between ↑3 SD. [Note: commonly found in comparison-of-methods (COM) terms coincidence, probability, and probability are experiments. The COM experiment involves measuring pa-synonyms.] samples by both the existing (reference) method and the new (test) method (described in the reference section, interval and assessment of the method at a later stage). Patient data are not normally divided. These obtained from these comparisons consist of two specific data, which can be distorted or exhibit multiple centers (bi-ments for each patient sample). The easiest is to presentmodal, trimodal, etc.) as shown in Figure 4-6. Rendering and summarising data comparing data on paired methods in histograms such as these is an easy way of visibility (Fig. 4-7). By convention, the values obtained by the theatricalization division. However, a mathematical reference method and analyses of values (e.g. normality tests) shall also be plotted on the x-axis to confirm whether the data obtained by the test method are plotted on a given y.a. The importance of recognising whether or not data is commonly distributed is given in Figure 4-7, an agreement between the two methods on how they can be statistically analysed. is estimated from the line that best suits the points. While a visual estimation can be used to draw a line, the aDescriptive Statistics of Groups statistical technique known as linear regression analysis of paired observations provides objective location measures and dispersion for the line. Three factors are generated in linearS although the use of basic descriptive statistics is satisfactory regression-inclination, y-intercept, and correlationfor examining one method, laboratorians often coefficient (r). Pictured (Fig. 4-7), is linearneed to compare the two Methods. This is the most relationship between the two methods in the wholeAPTER 4 ■ METHOD EVALUATION AND QUALITY MANAGEMENT 93 Normal (Gaussian) Distribution Range values. Linear regression is defined by the equation y ɕ mx Ƨ b. The slope of the line is described bevel distribution m, and the value of y-intercept (b) is determined by connecting x ɕ 0 to the equation and the solution for y. The coefficient of the cor-bimodal distribution relationship is a measure of the rela-tionship force between the two methods. Correlation coeffi-FIGURE 4-6. Examples of normal (gaussian), beveled, and bimodally sufficient can have values Ⱦ1 to 1, with an indicat-distribution sign. The type of statistical analysis that is performed on the direction of the relationship between the two variables.alyze data depends on the distribution (shape). A positive r indicates that both variables increase and de-crease together, while negative r indicates that as one variable increases, the other decreases. A value of r 0 in-dicates no relationship, while r ɕ 1.0 indicates a perfect relationship. Although many laboratories equate high positive r values (0.95 or higher) with excellent approval between the test and the comparative methods, most comparisons of clinical chemistry should have correlation co-efficacy values greater than 0.98. if less than 0.99, the re-mixing formula may underestimate the slope and overestimate the intersection. The absolute value of the correlation coefficient may be increased by extending the comparison of the range of sample concentrations. However, if the correlation coefficient remains below 0,99, alternative re-gression statistics should be used to derive more realistic regression estimates, slope and y-intercept.1,2 Linear regression equation (y = 0,89x + 6,0) 90 80 Slope m = 1 Method 60 Standard error 50 estimation Sy/x = 5,0 4 0 30 20 10 Y-intercept Correlation coefficient b = 6,0 r = 0,998 10 20 30 40 50 60 70 80 90 Reference methodFIGURE 4-7. A general example of linear regression. Linear regression extras two tests and provides important information about a systematic andrandoma error. A systematic error is indicated by changes in the intersection (constant error) and slope (proportional error). A random error is indicated by a standard estimation error (Sy/x); Sy/x basically represents the dis-dances of each point from the regression line. Correlation coefficient indi-categorizes the strength of the relationship between tests.94 PART 1 ■ BASIC PRINCIPLES AND PRACTICE OF CLINICAL CHEMISTRY Percentage bias of the analyte +40 Reference method-Test method +30 +20 +10 0 -10 -20 -30 -40 10 20 30 40 50 60 70 80 90 Mean concentration of analytes of the reference and test methods FIGURE 4-8. An example of the difference (Bland-Altman) plot. Different differences charts are a useful tool for visualizing concentration error. An alternative approach to visualizing the paired data is the error is as SD points odifference plot, which is also known as the Bland-Altman regression line (Sy/x). Sy/x basically refers to the average dis-plot (Fig. 4-8). Difference rendering charts either percent dance data from the regression line (Fig. 4-7). Theor absolute bias (difference) between reference and higher Sy/x, the wider the variance and the higher the istest method values in the average range of values. This number of random errors.access makes it easy to compare differences with demanding maximum limits. As seen in Sy/x Standard DeviationFigure 4-8, it is easier to imagine any concentration-de-regression linependent differences than linear regression analysis. In this example, the percentage difference is clearly largest on Sy/x ɕ Σ Add lower concentrations, which is not an apparent finding of Sy/x ɕ Σ(yi Ⱦ Yi) using regression fig (see Fig. 4-7). The distance of each y-value from the regression- the difference between the test- and the reference method of re-zion linesults is called an error. There are two types of errors measured in COM experiments: random and systematic. Random Sy/x ɕ Σ(yi Ⱦ Yi)2 A square of each differenceerror is present in all measurements and can be either to make the sum apositive or negative. As described above, a random positive error number can be the result of many factors, including tools, op-erator, reagent, and environmental variation. Random Sy/x ɕ [Σ(yi Ⱦ Yi)2/n Ⱦ 2] Divide the sum of squares by the number of points and subtract 2 TYPES OF ERRORS IN LABORATORY SY /x ɕ sqrt [Σ(yi Ⱦ Yi)2/n Ⱦ 2] Take the second rootTESTING: PREVIEW THINGSTO COME (Eq. 4-6)1. System error 2. Random error in Figure 4-7, Sy/x is 5.0. If the points were perfectly consistent with linear regression Sy /x would be equal to 0.0Constant proportionally indicating that there would be no random error. Sy/x is also known as standard estimation error, SE. A systematic error affects observations consistently in one direction (higher or lower). Inclination and y-intercept measures provide estimates of systematic error. The systematic error can be further broken down into constantAPTER 4 ■ METHOD EVALUATION AND QUALITY MANAGEMENT 95error and proportional error. Constant systematic error Reference intervals include all data points that de-exist when there is a constant difference between the test fine range of observations (e.g. if the interval is 5-10,method and the comparative values of the method, regardless of the patient result 5 would be considered within the in-concentration. Figure 4-7 shows a constant difference- terval). The upper and lower reference limits are set so that between the values of the test method and com- define a specified percentage (usually 95%) values of the parative method. This constant difference, which is reflected for the population; this means that the percentage (usually in y-intercept is called a constant systematic error. 5%) Z will fall outside the reference intervalProportion error exists if the differences between the absence of any condition or disease. The reference in-test method and the values of the comparative method are prothermals, sometimes erroneously referred to as normal for analyte concentration. Proportional error ranges. While all normal ranges are in fact reference in-is present when slope 1. In the example, the slope of the tervals, not all reference intervals are normal ranges. This0,89 represents a proportional error where samples will be illustrated by a reference interval for therapeutic methods underestimated in concentration-dependent levels of medicinal products. In this case, the normal individual would not be a test method compared to the reference method; have any drug in their system, while the patient's ther-error is proportional because it increases with apy has a defined target range. Reference intervals areanalytical concentration. sometimes referred to as reference ranges; the preferred term is the reference interval because the range means the ab-inferential statistics of the maximum and minimum values. Another level of complexity beyond the paired descriptive theory for the development of reference interval statistics is derived statistics. Derived statistics were the work of the two main committees of experts.3-6 These are used to draw conclusions (conclusions) on committees that have reported the importance of standardising two sets of data. In the research analysis of benchmarks and the estimation of reference studies, statistical collection inference procedures are most frequently found, but can be used in COM studies. intervals and the selection of reference populations. Reference intervals are usually determined by a scientifically relevant aspect for inferential statistics being community or reagent manufacturers and new distribution of data (shape). Distribution of methodologies. The development of reference intervals often has athe data determining what kind of inferential statistics have a financial impact on suppliers and labor-marketing that can be used to analyze data. Normal dis-Tory products. Laboratorians need to be aware these sci-tributed (gaussian) data are usually analyzed using entific and economic forces when examining supplier datawhat known as parametric tests that include and determine the need for reference interval studies. Student's t-test or variance analysis (ANOVA). Data Two main types of reference interval studies that are not normally distributed require non-paramet- examined in this section are (1) the establishment of a reference analysis. Non-parametric tests occur in the interval and (2) the verification of the reference interval.studies of reference intervals, where population data are often distorted. While many software packages are able to implement BOXof definitions either or non-parametric analy-ses, it is important that the user understands that the type of determination of the reference interval: New reference data (shape) dictates which statistical test is appropriate ence interval is determined when there is no ex-for analysis. Inappropriate analysis of sound data relating to the analyte or methodology in a clinical orcan may give the wrong conclusion. the reference laboratory with which the studies to be compared are to be carried out. It's a costly and labor-intensive REFERENCE INTERVAL STUDY study that will include laboratory resources at all levels and may require between 120 and as many as laboratory test data Þ700 be used to make medical diagnoses, study individuals.assess physiological functions, and manage therapy. When interpreting laboratory data, physicians compare meas- Reference interval verification (transmission): This test result from a patient with a reference interval. is carried out to confirm the validity of the existing reference interval for the analyte using the same type of analytical system (method and/or instru-ment) (identi- DEFINITIONS BOX cal). These are the most common reference in-reference interval: a pair of medical decisions of terval studies conducted at clinical laboratory points that cover the limits of the results expected for and may require only 20 study subjects. under that condition. CHAPTER 6 ■ PRINCIPLES OF AUTOMATION OF CLINICAL CHEMISTRY 177FIGURE 6-12. Photooptic system for chemistry analyzer: discarded. For endpoint analyses, the initial absorbance (1) is a source with a reflector, (2) a fixed focus switch, (3) a filter wheel, measured before the components had time to react, (4) a two-brick engine, (5) a fibre optic bundle, (6) a cyvet, (7) a filter usually for several seconds and is considered a blind meas-wheel and (8) a photomultiplier tube. (Courtesy of Dade International.) urement. After sufficient time has elapsed to complete the reaction, further absorbance is subtract.monochrome measurement. Recording optical readings- For speed analyses, the initial absorbance is measured and at different wavelengths the instrument computer is then allowed a delay time (the preset to the instrument can then use this data to correct the reaction mixture in each analysis). For each test, several data points are de-terferences that may occur in adjacent, as well as desirable, termed in a programmed time interval. Instrumental lengths. monitor the absorbence measurements at each data point and calculate the result. Many newer tools use optical fibers as a medium to transmit light signals from remote reading stations back slide technology depends on the reflection spectropho-based central monochromator detector box for tometry analysis, as opposed to traditional pho-throughput signals. Optical cables, or light pipes, such as tometry, provide a quantitative result. A number of them are sometimes called, they are connected from several in the indicator layer is read after light the stations containing the reaction mixtures on the indicator layer shall be reflected from the bottom of the centralised filter disc/detector unit, which, in conjunction with a layer containing pigment (usually spread by computer, sequence and analysed by a large vol-layer) and returned through the indicator layer to aume light signals from multiple reactions (Fig. 6-12). light detector. For colorimetric determinations, the light source is a tungsten halogen filament lamp. The beam focuses on vessels holding the reaction mixture also play a filter wheel holding up to eight interference filters, which is a vital role in the measurement phase. In most discreet, they are separated by dark space. The beam is focusedwet chemistry analyzers, the cyvet used for analysis is at a 45-degree angle to the lower surface of the slide,also a reaction vessel in which the whole procedure has and silicone photodiode detects part of the beam formed. The volume of the reagent, and therefore the sample, which reflects down. For the com-size, the speed of analysis and sensitivity of the measuring putter, three values are read to derive the reflectance density. These three recorded are some aspects influenced by the method of analysis. The signals received are (1) a filter wheel blocking the beam,the beam of light is concentrated through a vessel holding (2) the reflectance of the reference white surface with the reaction mixture. The amount of light released from the gramomed filter in the beam and (3) the reflectance of the vessel is determined primarily by the absorbance of the slide with the selected filter in the beam (Fig. 6-13).the light of the reaction mixture. The output light strikes the aphotodetector, which changes light to electric After the image is read, the shuttle is back in the direction of energy. Filters and components of the focus light allow from which it originates, where the separator doors allow the desired light wavelength to fall to get into the photodetetor. In the garbage bin. If the subtraction was first for the two-photometer continuously sequentime sample photodetec-point speed test, the trap door remains closed, and thetor output voltage and, as is the process in most analyze-slide reenters incubator.ers, compares it with the reference output voltage. Electrical impulses are sent to a reading device, such as a number of fully automated, random access printers, or a storage and retrieval computer. immunoassay systems that use chemiluminescence or electrochemiluminescent technologies for reaction analy-centrifugal measurement analyses occur while sis. In chemiluminescence, the quantification of the anrotor is rotated at a constant rate of approximately the analyte based on light emissions resulting from 1000 rpm. Subsequent sample measurements, chemical reaction shall be taken.9 The principles of chemiluminescent dark current (readings between cuvettes) and ref-immunoactive tests are similar to those of radioimmunoassayerence cuvet. Each syvette light source (RIA) and immunoradiometric test (IRMA), except that every few milliseconds. After all data points have acridinium

ester is used as a tracer and paramagnetikabe determined, centrifugation stops and the results are particles are used as a fixed phase. Sample, tracker and print. Remove the rotor from the analyser and add the paramagnetic particle reagent and incubate in single-use plastic cellurs depending on the proto-col test. After incubation, magnetic separation and particle washing are automatically performed. The yvettes are then transported to a light-sealed luminometer, where suitable agents are added to initiate the chemiluminescent reaction. After injecting reagents into the sample sway, the luminometer system detects a chemiluminescent signal. Luminometers are similar to gamma counters in that they use photomultiplier tube178 PART 1 ■ BASIC PRINCIPLES AND PRACTICE CLINICAL CHEMISTRY White reference area Infrared filter Lamp housing Filter lamp Image read Aperture Station Lens Lens Lens Filter Wheel Mirror Photocell Figure 6-13. Components of the system for the formation of colorimetric determinations with slide technology. (Courtesy of ortho-clinical diagnostics code.) detector; However, unlike gamma counters, luminometers electronically for each run. Other tools are self-required crystal to convert gamma rays to light calibration after analyzing standard solutions.photons. Light photons from the sample are detected by di-rectles, converted to electrical impulses and then counted. The original continuous flow analyzers used six stan-dards tested at the beginning of each run to create a signal calibration curve for processing and manipulating data for a specific batch. Now, contin-uous-flow analyzers use a single-level calibrator calibrator-Since most automated tools print out the results of brate each run with water used to determine the baseline.reportable form, accurate calibration is essential for ob-tainting accurate information. There are many variables the centrifugal analyzer uses standards pipetted to ensure it can enter into the use of calibration standards. The specified cell in each run to analyze endpoints.matrix standards and unknown may vary- After delta absorbance for each sample was ob-ent. Depending on the methodology, it may or may not be tainted, the computer calculates the results based on presence issues. If secondary standards are used to calculate the constant for each standard. The constants of the ibrate should be known methods used to derive derived divisions of the concentration of the basic values of the Standard. (before entering the computer) delta absorbanceStandards containing more than one analyte per vial can and on average constants for each of the standards cause interference problems. Because there are no at-get factor. The concentration of each the amarin standards available for enzymes, either secondary unknown, shall be determined by multiplying the delta ab-standards or calibration factors based on molar ex-sorbance unknown by a factor. If concentration tinction coefficients of product reactions can exceed the range of standards,apply. the result is printed with a flag. Enzyme activity is de-rived linear regression fit delta absorbance ver- Many times, the laboratory will have more than one in-sus time. The inclination of the line produced shall be multiplied by extrusion capable of measuring the component. If the enzyming factor (pre-specified) for activity calculation.different normal ranges are not published for eachmethod, the instruments should be calibrated so that Slide requires more sophisticated calculus results to be comparable. The advantage of calibrating ions to achieve results. Calibration materials re-automated device is a long-term stability quire protein-based matrix due to the need for a prestandard curve that only requires monitoring with calibrators to behave like a serum when responding to checks on a daily basis. Some analyzers use low and different layers of slides. Calibration fluids are high concentration standards at the beginning of each bovine serum and the concentration of each ana-run, and then use pro-calf response absorbances by reference methods. Endpoint tests carried out according to the standards for the establishment of a standard curve require three calibration fluids, tests requiring a blank test require four calibration fluids and enzyme methods require three calibrators. Colorimetric tests use a spline suitable for productionCHAPTER 6 ■ PRINCIPLES OF CLINICAL CHEMISTRY AUTOMATION 179Standardization. In enzymary analysis, the curved flag, clot detection, reaction vessel or chamber under test estimate the change in reflector density to temperature and reagent stocks. Printer canunit time. It is converted to either absorbance or also to show patients' results, as well as various warning-density changes per unit time. Then, quad-already mentioned. Most manufacturers of the toolratic equation converts a change in the transmission of den-menu computer software for preventive maintenance to volume activity (U / L) for each test. plans and algorithms for diagnostic troubleshooting. Some manufacturers also install phone modems on the most automated analyzers to maintain calibration for the analyzer for direct communication connections between in-each batch of a particular method, while the laboratorian strument and their service center for instant trou-programs tool for recalibrating. Apparatus viterisation and problem diagnosis.calibration shall be started or verified by analysing at least three levels of primary standards or, in the case of an en-selection of AUTOMATED ANALYZERSzymes reference samples. The values obtained are with known concentrations using each manufacturer's linear approach to automation is unique.regression, with the x-axis representing the expected val-rated instruments being evaluated accordion and y-axis representing the mean part of the values representing the previously identified needs. One laboratory can do it. Inclination (scale factor) and y-intercept (offset) need a stat analyzer, while another need may be aare adjustable parameters. On older models of this batch analyzer for highly tested volumes. When considering the instrument, the parameters were determined and specified costs, the price of the tool and even more manually to the instrument computer by the operator; the total cost of consumables is considerable. In fact, for later more automated models, the high cost of capital per instrument may be small when divided by the large number of samples to be taken. After calibration and processing. It is also important to calculate the total cost or electrical analysis of the sample either in each test for each tool that is considered.progress or completed, the computer of the device goes in addition, break-even analysis to study the relationshipto data collection and computational mode. The process of fixed costs, variable costs and profits can be useful, it may involve averaging the signal, which can mean hundreds of analyses of the financial justification and economic impact of data pulses per second, as with a centrifuged analyser, on the laboratory. Of course, the method of acquisition, toa blanks and correction formulas for interfernts is, buying, renting, renting, and so on, must also be fac-, which are programmed into the computer to calculate tored into this analysis. Variable cost of consumablesdems. increased when more than one test is carried out or samples are analysed. Ability to use reagents produced more All advanced automated devices have some as a single supplier (open versus closed reagent systems)the method of reporting printed results with reference to a sample can provide a laboratory with the ability to customize identification. In sophisticated demo-testing systems and can also save money. The working-component sample information is given in the instru-also should be evaluated. With a large number of in-ment computer along with the required tests. Then the struments available on the market aim to find out the sample identification is printed with the test results. the correct tool for each situation.10Mome laboratories use barcode labels printed by the Laboratory Oratory Information System (LIS) to identify the sample. Another big concern toward selecting anBar code-marked samples can be loaded focused on the tool's analytical capabilities. What are the instru-analyzer without the need to enter the identification of informa- ment performance for accuracy, preci-tion manually. Microprocessors check tests, zion, linearity, specificity and sensitivity (which may be reagents and timing, verifying the method-dependent barcode), calibration stability and stability of each sample. This is the link between the results of the reagents reported (both on-board and reconstituted)? and the identification of the sample. Even the simplest of the best way to verify these performance characteristicssystems gradually number test results to provide the analyzer before making a decision on the deviceconnection with samples. can see it in use. Ideally, if the manufacturer places the tool in the lab of a potential buyer Because most of the tools now have either built-in on a trial basis, then its analytical performance can be eval-or connected video monitor, sophisticated software made to customer satisfaction with studies to verify programs that come with the tool can be dis-accuracy, accuracy and linearity. At the same time, the labo-played to determine the status of various aspects of the ratory staff can observe such structural elements as the his-test process. Computer monitoring is available- test menu, true, walk-away capabilities, user friend,capable for such parameters as response and tool and space that the device and its consumables oc-linearity, quality control data with different options for cups in their laboratory.statistical display and interpretation, short samples sens-ing with flags on print, abnormal patient results180 PART 1 ■ BASIC PRINCIPLES AND PRACTICE CLINICAL CHEMISTRY Instrumentation clinical chemistry provides speed of open architecture components that provide more flexi- and precision for tests that would otherwise be in the implementation of double-digit automation.13 Example of manual shape. The selected methodologies and TLA system for commercial compliance is shown in Figure 6-14.ence of the test requirements provide accuracy. No one can assume that the result is a cor-Preanalytic Phase (Sample Processing) rect value. Automated methods must be evaluated together as routine before being adopted. It is important that the sample manipulation protocol currently available at all understands how each tool actually works. the main chemistry analysers are the use of the original sample collection tube (primary tube sampling) of any size (afterTOTAL LABORATORY AUTOMATION plasma or serum separation) as a sampling cup on the analyser and the use of a barcode reader, also for ana-pressures of health care reform and controlled lyser care, to identify the sample. The automated process has caused a growing interest in improving productiv- gradually replacing manual handling and presentation of the pre-analysis and post-analysis phase of the analyser sample. Increasing the efficiency of at-work testing.11 As regards the analytical process itself, normal falling costs were The main impetus for laborato-analyzers in clinical chemistry today is having almost all of them begin to integrate some aspects of the overall laboratory treatment they need. A new generation of au-automation to their operation. Conceptually, TLAtomation will replicate the Japanese practice of black refers to automated devices and robots integrated withbox labs, in which the sample goes at one end and existing analyzers perform all phases of the laboratory printed result coming out at the other end.12 Much of the effort it has tested. Most of the attention to date has been paid to de-being spent over the past decade on development-velopment front-end systems that can identify ament automated front-end feeding samples into label samples, spin samples and prepare analytical boxes and computer/automated manage-aliquot parts, and sort and deliver samples to the orment data analyzer that come out at the back end of the box. for storage.14 Back-end systems may include removalThere have been many developments in the three phases of samples from the analyzer and transport to the warehouse, re-from the laboratory test process-that is, preanalytical rubs from storage for re-testing, re-aliquant, or dis-(sample processing), analytics (chemical analysis), and posal, as well as comprehensive datapostanalytic management (data management) as they merge closer from the analyzer and link with lis.into integrated system of total laboratory automation (TLA). Automation vendors are developing Dr. Sasaki and colleagues to install the world's first fully auto-mated clinical laboratory in the Koshi Medical Robotic Analyzer Online AnalyzerInterface (Sysmex) interface (J&J 950) Sample Sorter Recapper Automated Aliquotter Uncapper Loading Station Automated Transport Centrifuge SystemFIGURE 6-14. Diagram of the total laboratory automation system. (Courtesy of the kindly adage of Labotix Automation.) CHAPTER 6 ■ PRINCIPLES OF AUTOMATION OF CLINICAL CHEMISTRY 181School in Japan15; Since then, the concept has gradually, sorting, centrifuging, uncapping, aliquote, and directbut relentlessly, becomes a reality in the United States. Device interface options. The University of Nebraska and the University of Virginia have been pioneers for developing the TLA system. In large part the benefit of TLA is derived from automa-1992, a prototype laboratory automation platform for front step processing. Therefore, several were developed at the University of Nebraska, key manufacturers have developed separate automated components such as conveyor system, bar-coded spec-front processing systems. Genesis FE500 (Tecan) isimens, a computer software package to control samples example of a separate front-end system that can drive and track, and the coordination of robots with centrifucopy, uncap, an aliquot part into a marked casting tube, tools like working cells.16 Some of the first auto- and into the analyser stands. Systems with similar func-mated laboratories in the United States have reported that they are available from Labotix, Motoman, and PVT.their experience with front-end automation with an example of one such system is shown in Figure 6-15.wealth information for others interested in tech-stand-alone automated samples of uncappers and recappersnology.17,18 The first hospital laboratory to install au- are available from PVT and Sarstedt. These latter facilities were university of Virginia hospitals less flexible than the complete stand-alone front-endin Charlottesville in 1995. Their systems of medical automation and require samples to be presented to them at theResearch Center collaborated with Johnson & Johnson Racks, which will work with one analyzer. Some Labor- and Coulter Corporation using Vitros 950 attached to the Tories have taken a modular approach with devices for the Coulter/IDS U streak for direct sampling of speci-only some automated features. Ciba-Corning Clinicalmen conveyor without the use of intervening robotics.19 Laboratories have installed Coulter/IDS robotic systems the first commercially available turnkey system was several regional laboratories.19 Recently, thawing-mix-Hitachi clinical laboratory automation work cell system that is compatible with trackside system v a (CLAS) (Boehringer-Mannheim Diagnostics; now Roche referral lab has been described.25 Bottom lineDiagnostics). This pairs hitachi series of analyzers is that robotics and front-end automation are here conveyor tracked system to provide a completely functional stay. As more and more clinical laboratories reengineersystem with all interfaces.20 for overall laboratory automation, they are building basic laboratories containing all their automated robotics analyzers and leading automation are changing as a necessary first step to easier connection of the different face of the clinical laboratory.21 Much of the tools with benefits to one TLA system.26rivable from TLA can only be realized by automation of the front. Planning, implementation and per- Analytical phase (chemical analysis)formance evaluation of the automated transport and sorting system by a large reference laboratory there have been changes and improvements that are described in detail.22,23 Several tool manufacturing, which are now common to many general chemistry analyzers. Theyers are currently working on or are already on the market–they include ever smaller microsampling and agent dispens-terfacing front-end devices along with software for ing with multiple additions possible from randomly re-their own chemistry analyzers. Johnson & Johnson in-placed reagents; extended on-board and overall test menus, troduced Vitros 950 AT (Automation Technology), in particular medicinal products and hormones; accelerated reaction system in 1995 with open architecture that allows times with chemistry for faster throughput choose from many front-end automation to dwell time; optics with higher resolution with grate mono-systems, rather than be locked into proprietary in-chromators and diode arrays for polychromatic analysis, terface. The Lab-Track interface is now available on improved flow electrodes; improved user dimension RxL (Siemens), which is compatible with the main friendly interactive quality control software, mainte-laboratory automation vendors and allows direct nance and diagnostics; integrated modems for online re-purchase from the track system. Also, technology now solving problems; Press-link data management sys-exists for microcentrifugal separators to be integrated tems; reduced calibration and control frequencies;to clinical chemistry analyzers.24 Several other automated systems for calibration, dilution, rerun and are now on the market, including maintenance of Advia LabCell; ergonomic and physical design (Siemens), which uses a modular approach to improvements for simplicity, uptime and operator omation. Basic processor power system (Beckman reduced maintenance. According to a recent survey by SPPCoulter) conducted sorting, spin and cap re-data, the eight most popular general chemistry analyzersmoval. EnGen Series Automation System (Ortho- are Aeroset (Abbott Diagnostics), Advia (Siemens), AUClinical Diagnostics) provides sorting, spin, systems (Olympus), Dimension (Siemens), Hitachi sys-uncapping, and sample archiving features and interface tems (Roche Diagnostics), Integra Systems (Roche s vitros 950 AT analyzer). Three Automation Diagnostics), Synchron Systems (Beckman Tools) systems are available from Olympus that can perform and vitros (ortho-clinical diagnostics).27 Features a182 PART 1 ■ BASIC PRINCIPLES AND PRACTICE CLINICAL CHEMISTRYSpecimen sorter Automated centrifugationUncapper Transport systemFIGURE 6-15. Diagram of the pre-analysis automation system. (Courtesy of the kindly adage of Labotix Automation.) specifications of these eight systems are summarised in the data. Two-way communication between ana-table 6-1. One of the main advantages of modular chemistry an-lyzer(s) and host computer or LIS has become ab-alyzers is scalability. As the workload increases, another solutely essential reference to the application for tests and enter patientmodules can be added to increase throughput. The demographics that automatically transmit this customized modular analytics system creation scheme to the analyzer (analyzers) as well as the post results in (Roche) are shown in Figure 6-16. This system can ac-patient recording. Evaluation and data management will be returned from one to four modules D, P or E. This has become more flexibility to adapt to the changing workload from the time of analysis to the broadcast. sophisticated and automated with the integration of workChemia and immunoassay testing can be combined, station managers throughout the need to divide the samples. Re-testing can be system.28 Most data management devices are automatically complicated by using the rerun buffer, a propri-holding computer module that holds samples until all testing is complete. ethical software that is interfaced with one or more of their analyzers and host PRESS. They offer automated man-Postanalytic Phase (Data Management) aging quality control data with storage and evalua-Although most of the attention in recent years in the total amount of quality control results against the lab's slaboratory automation concept has been devoted to front-defined quality management circuits with multiple plot-end systems for sample manipulation, several manufacturers ting, imaging and reporting capabilities. Review and have been developing and improving back-end manipulation by editing patient results before verification and trans missions to host are enhanced by user-defined perime-FIGURE 6-16. Schematic drawing of roche modular analytical ters for range limits reporting, limits of panic value, deltasystem. (Courtesy of Roche Diagnostics.) quality control and quality control comparisons for clinical changes, re-testing and algorithm analysis. Reagent inventory and quality control, along with monitoring of instrument functions, are also managed by worksta-tion software. Most LIS dealers have link soft-ware available for all major chemistry analyzers. Most current LIS cannot adequately process some of the data processing needs associated with automation. For example, most current analyzers are able to assess the degree of hemolysis of the sample, ikterus, and lipemia (seeAPTER 6 ■ PRINCIPLES OF CLINICAL CHEMISTRY AUTOMATION 183Stute 6-1). Making this information available Further new tests on the expanded menu however will be devel-and useful to either the doctor or the laboratory in au-oped, with a mixture of measurement techniques used in a manner requiring further manipulation on the analyzers to include multiple immunoapression adata. Ideally, tests ordered on a sample, thresh-PCR-based tests. Spectral mapping or multiple waves old to interference each test with each of the three monitoring lengths, high resolution photometers of inagents, and whether interference is positive or nega-analyzers will be routine for all samples and astive tests need to be determined. In the case of lipaemia, more apparatus shall be constructed with monochrome results for the tests affected until the sampling device is in the path of the light after the cyvette, not earlier,.unclear and the tests are carried out. It is difficult or im-spectral mapping capabilities will allow concurrent possible for current LIS systems to perform this second analysis of multiple chemistry analytes in the same reac-task. A gap must be filled between the container. This will have a huge impact on the instrument and LIS. One company, Data Innovations, and the time of turnover of the test results. Masshas has developed a system called Instrument Manager, Spectrometry and Capillary Electrophoresis to be used, which connects the analyzer with the LIS and provides an abil-more extensive in clinical laboratories for identification for the user to define rules for the release of information and quantification of elements and compounds in ex-to LIS. In addition, flags can be displayed on in-tremely small concentrations. In the coming years, the morestrument operator will perform additional operations, as the system and workflow integration will occur with robot-like sample clarification and re-analysis. The ability to fully ICS and manage data for Labor's more inclusive overall-automated data review using rules-based analysis is key to Tory automation.30 To achieve this, a more companiesfactor is moving towards TLA. They will form alliances to keep their instrumentation prod-ucts in laboratories. The integration of artificial trends in automation into analytical systems will evolve, using both expert systems and neural networks.31,32 This automation of clinical chemistry will continue to evolve on a large scale in the field of robotics technologies, digital pro-fast pace in the 21st, analyzers will continue to perform more cost-effective and efficiently. Finally, technological advances in chip technology andMore integration and miniaturization of biosensor components will accelerate the development of noninva-and systems will persist to accommodate more sofisti-sive, in vivo testing.33-35 Transcutaneous monitoring jecated portable analyzers for successful poc testing already available with some blood gases. True or dye-market. Effective communication between all automation namic values from monitoring in vivo instakeholders components for a given project is key to successful blood and other bodily fluids will revolutionize laboratory implementation.29 Medicine as we know it today. Sounds futuristic, but so did the first AutoAnalyzer 50 years ago.

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Immunoassays CHAPTERSusan Orton 7■ IMMUNOASSAYS CHAPTER OF THE OUTLINE GENERAL CONSIDERATIONS UNMARKED IMMUNOASSAYS ■ LINKS Marked ImmunoassayThat chapter introduces general analytical methods on Ag is referred to as an antigenic determinant or is used in many areas of the clinical laboratory – epitope. In the terminology used, there is some confusion: the binding of antibodies (Ab) to antigen (Ag) for specific some immunologists refer to the immunogen as mole-and sensitive detection of the analyte. In immunoazalky, cule, which induces a biological response and the synthesis of Ag binds to Ab. Ag-Ab interactions can in-Ab, and some use antigen to refer to one that binds this unmarked reactants in a less analytically sensitive Ab. However, they all agree that the antigenic site to which antechnology or labelled reactive agent in more sensitive tech-F(ab) may be epitope.niques. The design, label and detection system are combined to create many different tests that allow meas- The degree of binding is an important factor in the non-completion of a wide range of molecules. immunoaespression. The binding of Ab to Ag is directly related to the affinity and affinity of Ab for the epitope, this chapter examines the concepts binding, de- as well as the concentration of Ab and epitope. Underscribes the nature of the reagents used, and discusses the standard conditions, affinity Ab is measuredbasic determination of the design of selected techniques used in clin-using hapten (Hp) because Hp is a low-molecular-ical laboratory; as such, an overview of the mass of Ag should be considered as only one epitope. Therather as an exhaustive review. affinity for Hp is related to the likelihood of binding or to the degree of complementary nature of each of them. Re-IMMUNOASSAYS versible reaction is summarized in equation 7-1: General considerations Hapten ϩ antibody Ä (Eq. 7-1) hapten Ϫ antibody complexV immunoassay, antibody (Ab) molecule recog-nizes and binds to antigen (Ag). The molecule in-link between Hp and Ab listens to the lawterest can be either Ag or Ab. This binding is a re-mass action and is expressed mathematically inlated to the concentration of each reactive, specificity equation 7-2:Ab for Ag, affinity and dedication to vape, and environmental conditions. Although this Chapter Ka ϭ k1 ϭ [Hp Ϫ Ab] (Eq. 7-2) focuses on immunological tests that use an ab molecule such as k2 [Hp][Ab] binding agent, other tests such as receptor tests and competitive protein binding tests, use the Ka receptor is an affinity or equilibative constant; or transport proteins as a binding agent, reciprocal concentration of free Hp when 50% spectacular. The same principles apply to these tests. Binding posts are occupied. The larger affinity moleculeAb is immunoglobulin with functional do-Hp for Ab, the smaller the concentration of Hpmain known as F(ab); this area of immunoglobulin needed to saturate 50% of the associated Ab sites. Forprotein binds to the place on Ag. Ag is a fairly large example if the affinity constant monoclonal antibody complex and usually has multiple sites that can bind (MAb) is 3 ϫ 1011 L / mol, that is, Hp concentrate-based antibodies (Abs) with different specificities; each location of 3 ϫ 10Ϫ11 mol/l is required to fill half of the binding seats. Typically, the affinity constant Abs used 185186 PART 1 ■ The basic principles and practice of clinical chemical procedures in an immunoa test ranges from 109 to 1011 Conversely, immortal cell lines produce mono-L/mole, while affinity constant for clonal abs transport proteins; each line produces one specific Ab. Toranges from 107 to 108 l/mol and affinity for receptors method brought out as an extension of hybridomaranges 108 to 1011 L/mol. The work published by Kohler and Milstein in 1975.2 The process begins by selecting cells with properties that, as with any chemical (molecular) reaction, will initial allow the synthesis of homogeneous Ab. First, aconcentrations of reactants and products affect the host (usually, the mouse) is immunized with agthe range of complex bindings. In immunoapression, re- (the one to which Ab is desirable); Later, sensitizedaction moves forward (right) (Eq. 7-1) when lymphocytes of the spleen are collected. Secondly, the im-concentration of reactants (Ag and Ab) transcends the con-mortal cell line (usually nonsecretory myelomacentration myelomacentration product (Ag-Ab complex) and when the cell line that is the hypoxantine guanine phosphorus-there is a favorable affinity constant. lack of transferase) it is necessary to ensure that the continued-ous spread in vitro is viable. These cells are then forces that bring antigenic determinant and mixed in the presence of a fusion agent, such as poly-Ab together are noncovalent, reversible bonds that result in ethylene glycol, which promotes the fusion of two cells tofrom the cumulative effects of hydrophobic, hydrophilic, forming a hybridoma. In selective growth, lena hydrogen bonding and van der Waals force. Hybrid cells survive. B cells have a limited nat-most important factor that affects cumulative ral lifespan in vitro and cannot survive, and unfused sticking is goodness (or proximity) fit myeloma cells cannot survive due to their enzyme defi-between Ab and Ag. The power of most of these ciency. If viable mute cells synthesize Ab, then the interactive forces are inversely specificity and isotype Ab are evaluated. MAbtween interactive site. The closer ab and ag can the reagent is commercially produced by increasing the hy-physical approach of one another, the greater the at-bridome in tissue culture or in compatible animals. Towing forces. an important property of the MAb reagent is that Ab is homogeneous (one Ab, not an Abs mixture). After the ag-Ab complex is formed, the probability because it recognizes only one epitope on multiva-separation (which is indirectly related to the tightness of the borrowed Ag and cannot cross-link multivalent Ag.bonding) is referred to as ademsance. Dedication is a value-added phenomenon in which the strength of unmarked immunoactive binding of all ab-epitope pairs exceeds the sum of the sin-gle Ab-epitope binding. In general, the stronger the immune clotting in the affinity and dedication, the greater the possibility in one of the simplest unmarked immunoactive intro-cross-reactivity. to the clinical laboratory, the unmarked Ab was laid on top of the unmarked Ag (both in the fluid phase); The specificity of Ab is most often described during the incubation period, Ab and Ag dispersed and Ag, which induced ab production, homologous presence of precipitation was noted. Precipi-Ag. Ideally, this Ab would only respond with that Ag. tation occurred because each Ab recognized epitopeHowever, Ab can respond with Ag, which is structural and multivalent Ags were cross-linked multiplesimilar to homologous Ag; this is referred to as Abs. If the Ag–Ab complex is of sufficient size, cross-border reactivity. Since antigenic determi-interaction with water is limited so that the complex be-nant can be five or six amino acids or one immun-come unexcessable and clot.odominant sugar, it's no wonder that Ag's similarity is common. The greater the similarity between it was observed that if the concentration of Ag iscross-responsive Ag and homologous Ag, the stronger increased, while the concentration of Ab remains constant,the binding with Ab.1 Reagent Ab production is the amount of clot formed related to the ratio achieved by polyclonal or monoclonal techniques. In Ab on Ag. As shown in Figure 7-1, there is optimal production of ratiopolyclonal Ab, stimulating Ag's in-concentration Ab to ag concentration, which is manifested in an ag-responsive animal; the animal results in maximum precipitation; this is the zone oftects of this foreign Ag and mounts an immune response equivalence. Outside the equivalence zone, the amount to remove Ag. If part of this immune response in-clot is reduced or absent because of the ratio of key strong Ab production, then the blood collected by Ab ag is disproportionate and cross-linking and Ab is collected, characterized, and purified to yield ag is reduced. When Ab exceeds and commercial anti-serum agent. This polyclonal Ab cross-linking is reduced, the test is in prozone.reagent is a mixture of ab specifics. Some Abs respond conversely, when ag concentration is above and cross-with stimulating epitopes and some are endogenous the link is reduced, the test is in postzone. Although to the host. More Abs aimed against the more originally described precipitation reactions, these con-epitopes on ag are present and can cross-link themultivalent ag. Polyclonal Abs are often used as capture abs in a sandwich or indirect immunoassay. CHAPTER 7 ■ IMMUNOASSAYS 187Amount Clots Prozone Postzone Formed Antibody Antigen Excess Zone Equivalency Antigen Concentration IncreasesFIGURE 7-1. Precipitate curve showing the amount of precipitate concentration of ver-sus antigen. The antibody concentration is constant.cept refers to other tests in which the ratio of Ab to Ag of the precipitation band is interpreted. As stated inis critical. Figure 7-2, the precipitate band of an unknown sample is compared with the precipitate band of a sample known to be precipitated in a gel not so often per-containing Ab. The identity pattern confirms the presence in today's clinical laboratory. The gel is diluted with Ab in an unknown sample. Partial iden-agarosis patterns (usually less than 1%) dissolved in aqueous darkness and non-entities are ambiguous. This technique can bebuffer. It provides a semisolid medium through which abs are used to detect associated with autoimmune diseases, soluble Ag and Ab can easily pass through. Precipitated immune both Sm and RNP detected in systemic lupus erythe-complexes are easier to discern in gel versus liquid sus-matosus, SSA and SSB in Sjogren's syndrome, and Scl-70pension. Immune clotting methods in the gel can be clas- in progressive systemic sclerosis. However, most of them are detected as passive methods or in those using Abs electrophoresis are also detected by enzyme immunoacalases and are summarised in Table 7-1. The simplest and least or multiplex barub system (Luminex).sensitive method is double diffusion (Ouchterlonytechnique).3 Agarose is placed on a solid surface and al-lowed solidified. Wells are cut into agarose. The com-mon template is six Ab wells, surrounding one Ag wellin center. Soluble Ag and soluble Ab are added to separate wells and diffusion occurs. Intensity and patternTABLE 7-1 IMMUNE METHODS OF PRECIPITATION FIGURE 7-2. A scheme demonstrating a pattern of identity. The centre contains well the antigen, rabbit thymus extract. No 1Gel is filled with serum known to contain the antibody Sm. Test serums are Passive in wells 2 and 3; identity pattern, smooth continuous lineHomous diffusion (Ouchterlony technique) between three wells confirms the presence of Sm antibody in the Diffusion of Sm (radial immunodiffusion) test series. No 4 is filled with serum known antibody to U1-RNPElectrophoresis. Test sera in wells 5 and 6 also contain U1-RNP antibodies, contraimunoelectrophesis confirmed by an identity pattern between known serum and Immunoelectrophoresis test sera. Immunofixation electrophoresisRockesisOluble PhaseTurbidimetryNephelometry188 PART 1 ■ BASIC PRINCIPLES AND PRACTICE OF CLINICAL CHEMISTRY Single diffusion technique, radial immunodiffusion temperature changes; However, the turnover time of ission (RID) is the immune clotting method used longer compared to the kinetic method.quantitate protein (Ag). In this method, monospe-cific antiser is added to liquefied agarose; then counterimunoelectrophoresis is immune pre-agarose is poured into a plate and cooled. Wells are a cipitation method that uses an electric field to cause a cut into the froze agarose. Multiple standards, one or Ag and Ab migrate to each other. Two parallel multiple quality control samples, and patient samples are lines of wells are cut into agarose; Ab is placed in one lineconscientious to the wells. Ag diffuse from the well in all and Ag is placed in the second. Ab will migrate to the cat-directions, binds to the soluble Ab in agarose, and ode and Ag to the anode; precipitin line forms a complex perceived as a centred precipitin ring, where they meet. This qualitative test is useful for detection (Fig. 7-3). The diameter of the ring is related to con-bacterial antigens in the cerebrosbrostic fluid and other fluidation of Ag, which dissipated from the well. Stan- when a rapid laboratory response is needed.the bold curve is designed to determine concentrations in quality control and patient samples. The applicable range of immunoelectrophoresis (IEP) and immunofixation is between the lowest and highest standards. Electrophoresis (IFE) are two methods used in clin-If the ring is larger than the highest standard, the sam-ical laboratory to characterize the monoclonal proteins inple should be diluted and retested. If the ring is smaller than serum and urine. In 1964 Grabar and Burtin6 published the lowest standard, the sample should be run on low methods for examining serum proteins using an elec-level plate. There are two variants: endpoint (Mancini) trophoresis together with immunochemical reactions of inmethods4 and kinetic (Fahey-McKelvey) method.5 agarose. Serum proteins are electrophopically separatedContinual method requires that all Ag dispersed from and then reagent Ab is placed in the troy running paral-well and the concentration of Ag is related to the lel to the separated proteins. Ab reagent and sepa-square diameter of the cipitin ring; standard dimensioned serum proteins dispersed; when the reagent Ab recog-curve is plotted on a linear graph of paper and is a line of serum protein nizes and the reaction is in the zone that is most capable. To ensure that all Ag are dispersed, in-equivalence, precipitin arc (Fig. 7-4). The diving time is 48 to 72 hours, depending on the mo-agarose plate being stained (typically, with protein spotty weight Ag; for example, IgG quantification, such as Amido black 10), destained, and dried en-required 48 hours, and IgM requires 72 hours. In con-hance readability precipitin arches, especially weaktrast, kinetic method requires that all rings are meas-arches. the size, shape, density and location of the curves held within a fixed period of 18 hours; sample with a greater interpretation of the protein. All interpretation isconcentration will be diffused at a faster pace and will be performed by comparing the arches of the patient sample blackened at a fixed time. Plot the concentration Ag against serum using a semi-logarithmic graph of the sample curves to check the quality of normal human paper. Since IEP is used to evaluate the monoclonaldiameter of the precipitin ring; the line is drawn by a point to a point. For those who perform RID, the endpoint method isfavorized because of its stability and indifference toFIGURE 7-3. Radial immunodiffuous plate for haptoglobin detection. FIGURE 7-4. Immunoelectrophoresis. Wells 1, 3, 5 and 7 contain the diameter of precipitate in a ring is related to the concentration of normal human serum and wells 2, 4 and 6 contain the serum haptoglobin test serum. The anti-serum agent is in troughs: antihuman whole serum (A), antihuman IgG (B), antihuman IgA (C), antihuman IgM (D), antihu-man □ (E) and antihuman □ (F). Arrows at the upper point to an ⁄⁄ a chain that reacts with an anti-IgG reagent. A similar band is shown at the bottom with an anti-□ agent. There is also a pattern of identity with an anti-□ agent that shows the free □ chain in the test serum. CHAPTER 7 ■ IMMUNOASSAYS 189protein, heavy chain class and light chain rocket type shall be proportional to the concentration of Ag present; To evaluate the most common concentration, the following antisera are used based on calibration of monooclonal proteins: curve. A narrow range of linearity may require dilution of the whole serum (which contains a mixture or concentration of an unknown sample. Abs against major serum proteins), antihuman IgG (⁄⁄-chain specific), antihuman IgM (□-chain specific), Detection of Fluid-Phase Antigen-Antibodyantihuman IgA (␣ chain specific), antihuman □ (□-chain Complexesspecific) and antihuman □ (□-chain specific). Test Another strategy for quantifying Ag–Ab complexes is toturnaround time and the mead in interpretation use a tool to detect soluble Ag-Ab com-discourage the use of IEP as the primary method of plexes as they interact with light. When Ag and Abevaluate MAbs. combined, complexes are formed, which act as particles in suspension, and thus can dissipate light. The size of IFE has replaced IEP in many laboratories.7 Serum, particles determine the type of dispersion that will be domi-urine, or a sample of fluid is placed in all six nate when the solution interacts with almost mono-stripes of agarose gel and electrophoresis to separate the chromatic light.10 When particles such as albumin proteins. Cellulose acetate (or some other porous or IgG, is relatively small compared to wave-material) is saturated with Ab reagent and then the applied length of incident light, the particle will dissipate light into one strip of separated protein. If ab reagent symmetrically, forward and backward. Mini-recognizes the protein, an insoluble complex is formed. Mom scattered light is detectable at 90 degrees odPo staining and drying agarose film, interpreting the incident of light. Larger molecules and Ag-Ab com-tion is based on migration and the appearance of belts. plexes have diameters that converge on the wavelengthAs shown in Figure 7-5, monoclonal protein will ap-incident light and light scatter with greater intensitypear than discrete band (with heavy and light in the forward direction. Wavelength of monospecific antiserum ischain, which occurs in the same po- chosen based on its ability to be dispersed in the atrial). Polyclonal proteins appear as a diffusion group. direction and ability of ag-ab complexes toconcentration the patient sample may need to be adjusted- absorb wavelength light.ment to ensure that the reaction is in the zone of equivalence. Clouding measures transmitted light and the latest method of immune clotting in gel discussed nefalometry measures diffuse light. The cloudy technique of the rocket (Laurell technique or electroim- (spectrophotometers or colorimeters) is designed so that the test is not assisted.8,9 In this quantitative technique, reagent Ab measures the light passing through the solution, so that the fos-is mixed with agarose; Ag is located in the well and the elec- todetektor is located at an angle of 180 degrees from the in-trophoresed. As Ag moves through the agarose, it re-cident light. If the absorbance of light is negligible, the clouding acts with the reagent Ab and forms a rocket, s can be expressed as absorbance, which is directly precipitating along the edges. Heightfigure 7-5. Immunofixation electrophoresis. (A) IgG □ monoclonal im-munoglobulin. (B) IgA □ monoclonal immunoglobulin with free □ lightchains. C IgG □ and IgM □ immunoglobulins. (D) Diffusion chain IgAheavy without corresponding light chain is

required to determine the road length concentration.190 PART 1 ■ Basic principles and practice of clinical chemistry related to suspended particle concentration and curve. Nephelometers shall measure light at an angle to known samples.other than 180 degrees from the light at the event; most meas-ure
forward light dispersed to less than 90 degrees because the marked Immunoassaysthe sensitivity is increased (see Chapter 5). The relative concentration of ab reagent and ag is crucial to ensure general considerations that the formed complex is best detected in all designated immunoazals, the reagent
(Ag or Ab) is usu-nephelometer or clouding and that immune re-ally marked by the connection of a particle or molecule that willaction is not in the postzón or prozone. Therefore, it may be better to detect lower concentrations of Ag-Ab complexes.important to test more than one patient concentration
Therefore, the label improves analytical sensitivity. All as-sample, monitor the presence of excess Ab, or add says they have a binding agent that can bind to the Ag oradditional antiserum and monitor the tip. Surplus Ab ligand. If the binding agent is Ab, the test is an im-would indicate that there is little Ag
and that the reaction munoassay. If the binding agent is a receptor (e.g. estro-is underestimated. gen or progesterone receptor), the test is a receptor test. If the binding agent is a transport protein (e.g. for clouding and nehelometry, all thyroxine-binding globulin agents or transcortin), the test and the sera
must be free of particles that could dissipate, they may be called protein binding competitive tests.light. Pretreating serum with polyethylene glycol, immunoassays are used today almost exclusively, snonionic, hydrophilic polymer, increases Ag-Ab in-two notable exceptions: estrogen and progesterone re-
teraction. Since the polymer is more hydrophilic than ceptor tests and the thyroid hormone-binding ratio, Ag or Ab, water is attracted from Ag and Ab to which it uses thyroxine-binding globulin.polyethylene glycol. The result is a faster and greater amount of complex Ag-Ab formation. Immunoanalyses may
be described on the basis of a label marked as reaction, relative concentration and both methods may be carried out at the endpoint or source ab, the method used to separate the non-kinetic regimen. In end-point mode, the measurement is bound reagent, the signal to be measured and taken at the
beginning of the reaction (background of the method used to assign the concentration of the analyte insignal) and one is taken at a specified time later in the react sample. Immunoassay design, therefore, has a lot (platform or end point signal); concentration is a variable to be considered which leads to
different tests.determined using the calibration curve. In kinetic mode, the speed of complex formation is continuously monitored and the maximum speed is determined. Peak The simplest way to identify the test is according to the label used.the speed is directly related to the concentration of Ag, al-Table
7-2 lists commonly used labels and although it is not necessarily linear. This means that the calibration methods used to detect the label. TABLE 7-2 LABELS AND DETECTION METHODSIMMUNOASSAY COMMON METHOD OF DETECTION OF LABELS 3HEIA 125I Liquid scintillation countercla
Chren peroxidaseFIA photometer, fluorometer, alkaline phosphate luminometer Photometer, fluorometer, ᴺL-D-Galactosidase Glucose-6-phosphate fluorometer, luminometer dehydrogenase Photometer, luminometer Isoluminol derivative Luminometer Acridinium esters Luminometer Fluorescein
Fluorometer Europium Fluorometer Phycobiliproteins Fluorometer B Fluorometer Umbelliferone FluorometerCLA, chemiluminescent test; EIA, enzyme immunoa test; FIA, fluorescent immunoa test; RIA, radioimunoassay. CHAPTER 7 ■ IMMUNOASSAYS 191Radial labels measured the effect of the
medicinal product on the hiss reaction. Atoms with unstable nuclei that spontaneously emit radiation- Depending on the substrate used, the product of fo-ation can be radioactive and referred to as radionuclides. Tometric, fluorometric or chemiluminescent. For test-emission is known as radioactive
decomposition and is independ-ple, a typical photometric reaction using HRP-labelled Abent chemical or physical parameters such as tempera- (Ab-HRP) and substrate (peroxide) to generate theterium, pressure, or concentration. Of the three forms of ra-product (oxygen). Oxygen can then oxidise the
decrease in the herd, only beta and gamma-chromogen (reduced orthophenylenediamine [OPD]) tooratory are used in the clinical laboratory. For beta emissions, the core can emit a negative colour compound (oxidised OPD) that is charged with electrons or positively charged particles called
photometer.positrons. The electrons emitted are also known as beta particles. Tritium (3H) is Ƨ radionuclide commonly used by Ab Ɒ HRP and peroxide → Ab ϭ HRP Ƨ O2in cellular immunology tests for diagnosis and research. O2 Ƨ opd → oxidized OPD Ƨ H20 (Eq. 7-3) Gamma emissions are
electromagnetic radiation with very few wavelengths originating from unstable cores. As fluorescent Labelsradionuclide releases energy and becomes more stable, this fluorescent label (fluorochromes or fluoroopholor) disintegrates or disintegrates, releases energy. Specific spec-compounds that absorb
the radiant energy of one wavelength of the energy level is associated with each radionuclide. and emit radiant energy of longer wavelengths in a less standardized unit of radioactivity is becquerel than 10–4 seconds. In general, radiated light (Bq) is detected, which is equal to one decay per second. At an
angle of 90 degrees from the path of the excitation light unit is a curie (Ci) equal to 3,7 ϒ 1010 by means of a fluorometer or modified spectrophotometer. TheBq; 1 □Ci ϭ 37 kBq. The half-life of a radionuclide is the difference between the wavelength of excitation and the emission-time required for 50% of
the radionuclide to disintegrate and the Zion wavelength (Stokes shift) is usually between 20% stable. The longer the half-life, the slower nm and 80 nm for most fluorochromes. Some fluorescent decays, increasing the length of time that can be measured. immunoassays simply replace fluorescent
markings (such as Radioactive substances used in diagnostic tests, it's like fluorescein) to denote an enzyme and quantify that emissions have adequate energy orescence.13 Another approach, a time-resolved level of fluorescence and that the long half-life is relatively long; The 125I sat-cence immunoa
test uses a highly effective fluorescent following requirements and is the most commonly used label, such as europium chelate,14, which fluoresces ap-gamma-emitting radionuclide in a clinical laboratory. proximal 1000 times slower than natural back-ground fluorescence and has a wide Stokes shift.
Delay gamma-emitting nuclides are detected using a crystal allowing a fluorescent label to be detected with a minimalscintillation detector (also known as a gamma counter). interference from background fluorescence. LongSomething released during decomposition excites a fluor such as Stokes shift
makes it easier to measure radiation emissions of tallium-activated sodium iodide. Excited fluor re- without excitation radiation. The resultinglets a photon of visible light, which is amplified and the test is very sensitive and time-resolved, with a mini-detected photomultiplier tube; amplified light mized
background fluorescence.energy is then translated into electricity. Detectable radionuclide decay is expressed as the number of luminescent labels per minute (CPM). Luminescent labels emit a photon of light due to an electrical, biochemical or chemical reaction.15,16 In immunoanalyses, one reaction
substance is radiolabelled. In some organic compounds they excite when oxidized competitive tests, Ag is labeled and called and emits light when he returns to the country state.tracer. The radiolabell must allow the indicator to be fully oxidants include hydrogen peroxide, chlorine, or functional and
compete equally with unlabelled ag oxygen. Sometimes a catalyst is needed, such as peroxi-for binding sites. When the Ab detector is radio-daesh, alkaline phosphatase or metal ions.beled, the site of ag combination must remain biologically active and undisturbed. Luminol, the first chemiluminescent
label used in im-munoassays, is a cyclic dialylhydrazide that emits lightEnzyme Energy labels under alkaline conditions in the presence of per-enzymes commonly used to designate Ag/Hp or oxide and peroxidase. Since peroxidase can serve asAb.11,12 Horseradish Peroxidase (HRP), an alkaline fos-
catalyst, tests can use this enzyme as a label; tefatase (ALP) and glucose-7-phosphate dehydrogenase chemiluminimigenic substrate, luminol, will produce the light used most often. Enzymes are biological catalysts which are directly proportional to the amount of peroxidase increasing the conversion rate
of the substrate to the product and the presence (Eq. 7-4): not consumed by reaction. As such, the enzyme cancatalyze many substrate molecules, amplifying the amount of Luminol Ƨ 2H2O2 Ƨ OHϒ _P_erox_ida_se→of product created. The enzyme activity can be moni-tored directly by measuring the
product formed or 3-aminophthalate Ƨ light (425 nm) (Eq. 7-4)192 PART 1 BASIC PRINCIPLES AND PRACTICE OF CLINICAL CHEMISTRY Popular chemiluminescent label, esters of acridinium, gether to yield bound antigen (Ag*Ab), bound-is triple-ringed organic molecules linked by ester unlabelled
antigen (AgAb), and free-label (Ag*) as shownbond on the organic chain. In the presence of hydrogen in Figure 7-6 and equation 7-6:peroxide and under alkaline conditions, the ester bond is a broken and unstable molecule (N-methyl aceridon) re-Ag* (fixed reagent) Ƨ Ag Ƨ Ab (restricted reagent)
network. Light is radiates as the unstable molecule returns to a more stable state of land. → Ag* Ab Ƨ AgAb Ƨ Ag* (Eq. 7-6) Acridinium ester Ƨ 2H2O2 Ƨ OHϒ → A general, heterogeneous, competitive simultaneous test begins with the pipetteing of the test sample (quality control, N-mylacridon Ƨ CO2 Ƨ
H2O Ƨ light (430 nm) (Eq. 7-5) calibrator, or patient) into tubes. The marked reagents Ag and Ab are added. After incubation and separa-alkaline phosphate, which is commonly conjugated to Ab tion of loosely marked (unbound) Ag, bound Aghas has been used in automated immunoassy analyzers to
measure.produce some of the most sensitive chemiluminescent tests. ALP catalyzes adamantyl 1,2-dioxetane arylfos- Alternatively, a competitive test can be accom-phate substrates (AMPPD) to release light at 477 nm. sequential steps. First, the marked Ag is incubatedThe detection limit is close to 1
zmol, or approximately with the reagent Ab, and then the label Ag is added. After 602 enzyme molecules.17,18 longer incubation period and separation step are measured bound Ag. This approach enhances the Asssay Design analytical sensitivity test. Competitive immunoassaySal immunoassay was
competitive immunoas- Consider the example in Table 7-3. Relatively small,say, in which the radiolabelled antigen (A *; also called still constant, the number of Ab combining sites available totracer) competed with an unlabelled antigen (Ag) for lim-combine with a relatively large, constant amount of
Ag*ited number of binding points (Ab) (Fig. 7-6). Pro- (tracker) and calibrators with known Ag concentrations.part Ag and Ag* link to Ab is related Because the amount of tracker and Ab are constant, the concentration of Ag and Ag* and requires a limited Ab only in the variable in the test system is the
amount of unmarked reaction. In a competitive test, Ag*concen-Ag. As the concentration of unmarked Ag increases, thetration is constant and limited. How the concentration (or percentage) of the free indicator increases. Ag increases, more binds to Ab, which leads to a smaller binding ag *. These limited
reagent tests were very much insensitive with multiple calibrators because low concentrations of unmarked Ag were established. Since the concentration of unmarked Ag in-yielded a large measurable signal from bound-marked folds, the concentration of the tracer that binds to AbAg. If the competitive is
designed to achieve equilibly declining rates. In the example given in Table 7-3, if tetria, the incubation period is often long. the amount of unmarked Ag is zero, the maximum tracer is connected to Ab. If no unmarked Ag is present, the Ag–Ab response can be met in one step the maximum tracer binding
is possible; this is indicated if the antigens (Ag*), unlabelled antigen (Ag) and B0, Bmax, maximum binding or zero antibodies of the standard.reagent (Ab) are simultaneously incubated to- If the quantity of unlabelled Ag is the same as the tracer, each is equally bound to Ab. As the concentration of Ag
increases in a competitive test, the amountab CFIGURE 7-6. Competitive labeled immunoa test. During simultaneous incubation, marked antigen and unlabelled antigen compete for an-tibody-binding sites. The bound label in the precipitate is oftenmeased. CHAPTER 7 ■ IMMUNOASSAYS 193TABLE 7-
3 COMPETITIVE BINDING TEST EXAMPLEAG ⁒. AG* ⁒. AB → AGAB ⁒. AG*AB ⁒. AG Reagent concentration PRODUCTSAG AG AB AGAB AG*0 200 100 0 100 1 0 050 200 100 20 80 120100 200 100 34 66 134200 200 100 50 50 150400 200 100 66 34 166SAMPLE CALCULATIONSSeal [Ag] % B
B/F0 100 ϭ 50 100 ϭ1 200 10050 80 ϭ 40 80 ϭ 67 200 120100 66 ϭ 33 66 ϭ 33 200 134200 50 ϭ 25 50 ϭ 33 200 150400 34 ϭ 17 34 ϭ 20 200 166tracer, which is complexed with a binding reagent, is decreasing. tested when a new test is introduced. Every time a tracker is low molecular weight, a free
tracer is often test is performed, the dose-response curve should be pre-measured. If the tracer has a high molecular weight, it shall be analysed to check the performance of the test. The remembered tracker is measured. The data may show that the relative error for all radioimunoatria (RIA) in one of
three ways: bound/free versus arithmetic dose curve and dose response is minimal when B/B0 ϭ 0,5 and unmarked Ag; percentage bound compared to log crease dose at both high and low concentrations of plot.unmarked Ag; and logit bound/B0 compared to log dose As shown in Figure B/B0 versus logs
Ag concen-unlabeled Ag (Fig. 7-7). (Fig. 7-7), a relatively large change in the expression on both branches of the curve causes a small change the bound fraction can be expressed in several differences in the value of B/B0. Patient values derived from B/B0ent formats. Bound/free (B/F) is the CPM of the
bound fractional value of Ͼ0.9 or Ͻ0.1 should be interpreted with caution.tion compared to the CPM of the free fraction. Percentage When the same data are displayed by using logit-logbound (%B) is the CPM bound fraction of the compared plot, it is easy to overlook the error on both sides with the CPM
of the maximum tracer binding (B0) line.multiplied by 100. Transformation Logit B/B0 is natu-ral log (B/B0)/(1 ϒ B/B0). Non-competitive immunoactive tests Sometimes known as immunometric tests, non-competitive- When using logit-log graph paper on which is an itative immunoalysation to use the
labelled agent Ab for detection on the ordinalyte and log the dose of unmarked Ag. Excess marked Ab is necessary to ensure thatAg is plotted on abscissa, a straight line with a nega-marked Ab reagent does not limit the reaction. Con-tive inclination is produced. More often, the microcomputers center ag
is directly proportional to the recalculate the best straight lines using linear regression; marked ab as shown in Figure 7-8. The values of the relationship and the patient can then calculate the computer ship is linear up to the limit and then is subject to high-use of this relationship. effect with a dose of hook.
It is important to note that the best type in the sandwich test to detect Ag (also known as ancurve-mounting technique is determined by experimentation and ag capture test), immobilized unmarked Ab captures that there is no guarantee that logit-log plot data Ag. After washing to remove unresponsive
molecules,it will always generate straight lines. To determine the best method of plotling data, 276 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL PROCEDURESCREATININE8 PLASMA, SERUM, URINE METHOD ENZYMATIC METHOD Plasma or serumPOPULATION Plasma or serum
0.9-1.3 mg/jL (80-115 □mol/l) 0.6–1.1 mg/jL (55-96 □mol/l L)Adult male plasma or serumAdult Female urine 24-H 0.6–1.1 mg/0L (53–97 □mol/l) 0.5–0.8 mg/0.5 mg/0L (40-66 □ Human urine 24-HAdult Male 0.3-0.7 mg/dL (27-62 □mol/l) 0.0-0.6 mg/0L (0-52 □mol/l)Adult female 800–0.6 mg/ln 2,000
mg/day (7.1-17.7 mmol/day) 600-1800 mg/day (5.3-15.9 mmol/day)Pathophysiology nine is inversely proportional to creatinine clearance. Therefore, when plasma creatinine concentration of el-creatinine is avoided, GFR is reduced, indicating renal impairment. Increased creatinine concentration is
associated with plasma creatinine is a relatively insensitive marker of abnormal renal function, especially since concerns may not be measurably elevated until renal function. Plasma creatini concentration- worsened more than 50 → Ƨ ϒOH%. Positive bias ␣ ketoacids and detection of color formation
timed to prevent cephalosporins; requires automatic interference of necreatinine chromogen devices for precisionJaffe with creatinine in a protein-free filtrate adsorbed on adsorbent improves specificity;adsorbent Fuller's ground (aluminium magnesium silicate); it was then previously considered that the
reference responded with an alkaline pike to the formation of a complex of the colour method Positive bias against ascorbic acid,Creatinine-free Jaffa in a protein-free filtrate reacts with glucose, glutathione, ␣-ketoacidy, uricadsorbent alkaline pike to form color complex acids, and
cephalosporinsENZYMATIC METHODSCreatininase-CK Creatininase Requires a large sample; are not widely used Ƨ H2O → creatine Adapted for use as a dry slide method; potential to replace Jaffe; no ck interference from acetoacetate or creatine Ƨ ATP →← creatine phosphate Ƨ ADP
cephalosporins; some positive bias due to PK on lidocaine phosphoenolpyruvate Ƨ ADP → pyruvate Ƨ ATP Highly specific; accepted reference method LD Pyruvate Ƨ NADH Ƨ HƧ →← lactate Ƨ NADƧCreatininase-H2O2 Creatinine creatinine Ƨ H2O → creatine creatinase Creatine H2O → sarcosine Ƨ
urea Sarcosine oxidase Sarcosine Ƨ → Ƨ <9> O2 Ƨ H2O → glycine Ƨ CH2O Ƨ H2O2 Peroxidase H2O2 Ƨ colourless substrate → colour product Ƨ H2OOTHER METHODS Isotopic dilution Detection of characteristic fragmentsmass spectrometry after ionization; quantification using (IDMS) isotopicly
labelled compoundsCHAPTER 11 ■ NONPROTEIN NITROGEN COMPOUNDS 277 CASE STUDY 11-3A 3-year-old girl was taken with a diagnosis of questionsacute lymphocytic leukemia. Her labour-tory returns are given in the case study table 11-3.1. How would you explain the significant increase
After admission, she was treated with packaged cells, uric acid upon receipt?two units of platelets, IV fluids, and alophrinol. On the second hospital day, chemotherapy began, on March 2, 2015. What two factors are responsible for the normal use of IV vincristine and prednisone and intrathecal levels of
uric acid seen in subsequent taking?injections of methotrexate, prednisone and cytosinearabinozide. She was released into home care 5 3. Which is the most likely cause of abnormal days later. She was continued on prednisone and al-low levels of urea nitrogen observed at 12/6?lopurinol at home.
Received another What additional laboratory result would be useful for chemotherapy a month later (11/1) and again to confirm your suspicion?11/14. At 12/6, she was readmitted because she had stomach ulcers in her mouth and was unable to eat. CASE STUDY TABLE 11-3.1 LABORATORY
RESULTS11/14 12/6 6/1 10/2 10/3 10/4 11/14 12/6 6/20Creatinin, mg/0L 12.0 * 15 4.0 2.0 Uric acid, mg/7WBC, mm3 12.0 9.2 4.0 1.9 2.3 3.1 56 300 3.700 2.800 3.700*Indicates that the test has not been 12.0. Creatine liver in the production of urea. In normal physiologicalV muscular diseases such as
muscular dystrophy, polio-pH, most ammonia in the blood exists as ammonium ionmyelitis, hyperthyroidism, and trauma, both plasma cre- (NH4Ƨ). Figure 11-5 shows the pH-dependent equilibly-atine and urinary creatinine are often elevated. Plasma rium between NH3 and NH4Ƨ. Ammonia is excreted
asceatinine concentrations are usually normal in these pa-ammonium ion kidneys and acts on the urinary buffer.28tients. Creatine kinase measurement is typically used for the diagnosis of muscle disease, since analytical clinical applications of creatine are not readily available in most clinical laboratories.
Plasma creatine concentration is not clinical conditions in which there is an increased concentration of ammonia in the blood in kidney disease.6 tion provides useful information on liver disease, Reye's syndrome, liver syndrome, inherited deficiencies of urea enzymes cycleAMMONIA. Severe liver
disease is the most common cause of impaired ammonia metabolism. Monitoring of Inroduction of blood ammonia can be used to establish the prognosis, although a correlation between the range of hepatic en-ammonia is formed in the deamation of amino acids cephalopathy and plasma ammonia
concentration is not always consistent during protein metabolism.5 It is always consistent. The concentration of arterial ammonia is accumulation and converted into urinary in the liver. Free ammunition-a better indicator of the severity of the disease.46nia is toxic; however, ammonia is present in plasma
at low concentrations. Reye syndrome, occurring most commonly in chil-dren, is a serious disease that can be fatal. Often,the physiology of the disease is preceded by viral infection and ad-ministertion of aspirin. Reye syndrome is acuteAmmonia (NH3) is produced in the catabolism of aminomatic liver
disorder and autopsy findings and bacterial metabolism in lumen intestine.7 Some results of ammonia from anaerobic meta-NH4+ + H2O NH3 + H2O+bolic reactions that occur in skeletal muscle during exer-cise. Ammonia is consumed by parenchyma cells figure 11-5. Interconversion of ammonium ions
and ammonia.278 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL PROCEDURES Show severe fatty infiltration of this organ.7 Blood am-coenzyme, since it is used specifically by the concentration of glutamate de-mo, can be correlated with both hydrogenases; NADH will participate in the
reactions of otherssee diseases and prognosis. Survival reaches endogenous substrates such as pyruvate. Adenosine 100% if plasma NH3 concentration remains below five diphosphates (ADP) is added to the reaction mixture at in-times normal.47 lapse reaction rate and stabilize GLDH. Testing should
be conspiratorial for any newborn with unexplained nausea, vomiting, dry slide automated system uses thin film colori-or neurological deterioration associated with feeding.19 metric test.51 In this method, ammonia reacts with an in-dicator to produce a color compound that is detected the Ammonia test
can be used to monitor hy-spectrophotometrically. Direct measurement by ion therapy has been developed and urine-selective electrode measurement.52 Electrodemonia can be used to confirm the ability of the kidneys to limit the pH of the ammonium solution to the production of ammonium chloride.
Analytical methods for ammonia are summary methods rized in Table 11-7.Accurate laboratory measurement of ammonia in the sample requirement and interferingplasma is complicated by its low concentration, instability, substances and Contamination. Two approaches were taught to measure plasma
ammonia. One is careful handling of samples is an extremely important fortwo-step approach in which ammonia is isolated from plasma ammonia tests. Whole blood ammonia concen-sampled and then tested. The second involves a rapid increase in direct titration after sampling of ammonia
measurement by an enzymatic method or ion due to in vitro deamination of amino acids. Venous blood electrodeactive electrode odor. Tests detect NH3 or NH4Ƨ. should be obtained without trauma and immediately placed on the ice. Heparin and EDTA are suitable anticoagic- One of the first analytical
methods for ammonia, devel-lants. Commercial collection containers should have been evalu-oped by Conway in 1935, taking advantage of the volatility am-ated for ammonia interference before a new batch is given a domonia to separate the compound in microdiffic use. The samples should be
centrifuurated at 0 °C to 4 °C within 4 °C.48 Ammonia gas from the dispersion of the sample within 20 minutes of collection and the plasma or serum is reseparated and absorbed in a solution which is separated. Samples should be refreshed with a pH indicator as soon as possible. The amount of
ammonia has been frozen or frozen. Frozen plasma is stable for several days and determined by titration. ϒ20°C. Erythrocytes contain two to three times as much ammonia as plasma; haemolysis. Ammonia may be measured by an enzymatic method using glutamate dehydrogenase. This method is a
common technique used at present.49 Ammonia contamination is an important source and most common technique. It is recommended that pa-Reduction of absorbance at 340 nm, since nicotinamide tients do not smoke for several hours before isodenin-dinukleotide phosphate (reduced, NADPH) is
taken. NADPH is the preferredtable 11-7 SUMMARY OF ANALYTICAL METHODS-AMMONIACHEMICAL METHODS DIFFUSION NH3 through good accuracy asebral electrodes selective membranes to accuracy; NH4Cl membrane causing a change in pH, stability may be a problemSpectrophotometric,
which is measuredENZYMATIC METHODS potentiometrically the most common onGLDH automated devices; NH3 Ƨ bromophenol blue → → blue dye GLDH NH4Ƨ Ƨ 2-oxoglutarate Ƨ NADPH Ƨ HƧ →← glutamate Ƨ NADPƧ Ƨ H2OCHAPTER 11 ■ NONPROTEIN NITROGEN COMPOUNDS 279 Many
substances affect in vivo ammonium AMMONIUM 8concentration.8,22 Ammonium salts, asparaginase, barbi-turates, diuretics, ethanol, hyperalimentation, narcotics Adult plasma 19-60 □g/dL (11-35 □mol/l)analgesics and some other medicinal products may increase ammonia Plasma 68-136 □g/dL (40-
80 □mol/l) plasma. Diphenhydramine, Lactobacillus acidophilus, Childlactulose, levodopa and several antibiotics reduce val- (10 days–ues. Glucose at concentrations greater than 600 mg/dL for 2 years)(33 mmol/l) interferes with dry slide methods. The results are in conventional units of micro-records of
gram defects per decilitre and can be converted into international units using the molecular weight of ammonia (17 g/mol). Ammonia contamination is a potential problem in measuring ammonia.19 Precautions must be taken to minimise contamination in the Labour-Tory test. Elimination In severe liver
disease in which ammonia contamination is significant, there may be significant im-lateral circulation, or if parenchymal liver cell function is the projection of ammonia test results. Sources of severely affected ammonia not removed from cir-contamination include tobacco smoke, urine, and am-kulation and
increased blood concentrations. High con-monia in detergents, glassware, reagents and water. nh3 are neurotoxic and often associated with encephalopathy. Toxicity may be partly due to the ammonia content of serum-based control material increased extracellular glutamate concentration and sub-is
unstable. Frozen aliquo parts of human serum albumin con-sequent depletion of adenosine triphosphate (ATP) containing known amounts of ammonium chloride or ammo-brain ammunition. Solutions containing known ammonium sulphate are commercially available. Hyperammoemia is associated with
hereditary defi-pricing of urea cycle enzymes.54 Measuring the plasma ammonia reference interval is important in the diagnosis and itoration of these hereditary metabolic disorders (see Values obtained vary slightly depending on the method used. Chapter 34). Higher concentrations were observed in
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Burtis CA, Ashwood ER, Bruns DE, eds. Tietz textbook of clinical chemistry and molecular diagnostics. 4. ed. Philadelphia, Pa.: WB Saunders, 2005:2207.B.:Kamisha Johnson-Davis, Gwen A. McMillin 12CHAPTER WARPST■ GENERAL PROPERTIES AND DEFINITIONS ASPARTATE
AMINOTRANSFERASE■ CLASSIFICATION ENZYME NOMENCLATURE Alanine aminotransferase■ ENZYME KINETICS Alkaline phosphatase Phosphatase Catalytic mechanism of enzymes ⁄⁄-Glutamyltransferase factors that influence enzymatic reactions Amylase Enzyme measurement Activity
Lipase Calculation of enzyme activity Glucose-6-Phosphate Dehydrogenase Measurement of enzyme matter Enzymes metabolising enzymes as agents ■ REFERENCES■ ENZYMES OF CLINICAL IMPORTANCE Creatine kinase Lactate DehydrogenaseEnzymes are specific biological proteins which
catalyse with the resulting twisting of polypeptide chains (secondary biochemical reactions without changing the equilibrium structure), which are then folded (tertiary structure) and rerium point of reaction or consumed or changed in structural cavities. If the enzyme contains a viacin composition. Other
substances in response are more than one polypeptide unit, the quarter structure refers to products. Catalysed reactions are fre- to spatial relations between subunits. Eachquently specific and necessary for physiological functions, the enzyme contains an active site, often a water-free cavity,such as
hydration of carbon dioxide, a nerve conduc-where the substance on which the enzyme acts (sub-tion, muscle contraction, nutrient degradation, and en-loss) interacts with specific charged amino acids use. Found in all body tissues, enzymes often residues. The allosteric site - a cavity other than an
active serum icing after a cellular injury or, some- a site -- can bind the regulator molecules, thereby being sig-fold, in smaller amounts, from degraded cells. Some nificant on the underlying enzyme structures.enzymes, such as those that facilitate coagulation, are specific to plasma and are therefore
present in signifi- Although a particular enzyme maintains the same plasma concentrations. Plasma or serum enzyme catalytic function throughout the body that the enzyme canle are often useful in diagnosing specific dis-exist in different forms within the same individual. Dif-facilitates or physiological
abnormalities. This Chapter may be distinguished from each other on the basis of the general characteristics and principles of enzymes, certain physical characteristics such as electrophoretic mo-aspects relating to the clinical diagnostic significance of bility, solubility or resistance to inactivation. The
terms specific physiological enzymes and test methods for isoenzyme are generally used to discuss such en-enzymes. However, the International Union of Biochemistry (IUB) proposes to limit this concept to several forms of ge-GENERAL CHARACTERISTICS AND DEFINITIONS OF NON-PHYSICAL
ORIGIN. Isoform results when the enzyme undergoes post-transformation changes. Isoenzymes and iso-enzymes catalyse many specific physiological reactions. forms contribute to heterogeneity of properties and func-These reactions are facilitated by the structure of enzymes.and several other factors.
As a protein, each enzyme con-tains a specific amino acid sequence (primary structure), in addition to the underlying enzyme structure, a nonpro-tein molecule, called a cofactor, may be needed for en-zyme activity. Anorganické kofaktory, ako je chlorid alebo 281282 ČASŤ 2 ■ KLINICKÉ KORELÁCIE A
ANALYTICKÉ POSTUPYKLASIFIKÁCIA ČASTO KVANTIFIKOVANÝCH ENZÝMOVTRIEDA ODPORÚČANÝ SPOLOČNÝ ŠTANDARDNÝ KÓD ES SYSTEMATICOxidoreductases SKRATKA NÁZOV SKRATKA Č. NÁZOV LDH LDH 1.1.1.27 Laktát 1.1.1.49 L-laktát:NADƧ dehydrogenáza G-6-PDH G-6-
PD oxidoreduktáza 1.4.1.3 Glukóza-6- GLD GLD D-fosforečnan glukóza-6- fosfát 2.6.1.1:NADPƧ dehydrogenáza 1-oxidoreduktáza 2.6.1.2 Glutamát L-glutamát L-glutamát:NAD(P) dehydrogenáza 2.7.3.2 oxidoreduktáza, 2.3.2.2 deaminaseTransferázy Aspartát amino- GOT (glutamát ASTHydrolases transferáza
oxaloacetát ALT 2.5.1.18 L-aspartát:2- transamináza) 2.4.1.1 oxaloglutarate Alanine amino- aminotransferáza transfer GPaseT (glu 2.7.1.40 transaminease) 3.1.3.1 L-Alanine:2- oxaloglutarate creatine kinase CPK (creatine CK 3.1.3.2 aminotransferase phosphokinase) GGT ⁄⁄-Glutamyl - 3.2.1.1 ATP
:Creatine N- Transferase GGTP 3.1.1.8 Phosphotransferase 3.4.21.1 Glutathione-S-␣-GST GST 3.4.21.36 (5-glutamyl)peptide: transfer GP GP 3.1.36 (5-glutamyl)peptide: GP GP transferase 3.1.36 (5-glutamyl)peptide: GP GP transferase 3.1.1.36 3.5 amino acid-5-3.1.1.3 glutamyltrans Glycogen 3.4.21.4
phosphorous 4.1.2.13 Glutathione transferase Pyruvatekinase PK PK 5.3.1.1 ALP ALP 6.3.2.3 1,4-␣-D-glucan : Alkaline orthophosphate ␣-D-phosphatase glucosyltransferase ACP phosphatase Phosphatase Pyruvatic kinase ␣-Amylase AMY AMS Orthoforforical Pase CHE monoester phosphorus -
Cholinesterase CHY CHE drolasis (alkaline E1 optimal) Chymotrypsin NTP CHY Elastase-1 E1 Orthosophageal 5-nucleotidase TRY NTP monoester phosphorus-ALD drolasis (acid optimal) Triacylglycerol LPS lipase 1,4-D-glucan Trypsin TRY glucohydrolase Aldolase ALD AcylcholineIsomerases
Triosephosphate TPI TPI acylhydrolaseLigase isoMeride GSH-S GSH-S Chymotrypsin Glutathione Synthesise Elastase 5 00-Ribonucleotide Phosphohydrolase Triacylglycerol acylhydrolase Trypsin D-D-Fructose-1 , 6-bisdipophosphate D-glyceraldehyde-3-phosphate-lyase Triose-phosphate-
esomesterase Glutathione synthasePreparation with the approval of Competence Assurance, ASMT. Enzymology, training program. Bethesda, Md.: RMI Corporation, 1980.CHAPTER 12 ■ ENZYMES 283magnesium ions are called activators. Coenzyme is a vent to identify enzymes. A common abbrevia-
organic cofactor, such as nicotinamide adenine dina-ions, previously accepted klootide (NAD). With close binding to the enzyme, the names of enzymes were used until the standard ab-coenzyme is not a prosthetic group. The enzyme por-breviations listed in table.2,3 Thesis (apoenzyme) with appropriate
coenzyme are standard abbreviations used in the United States and are a complete and active holoenzyme system. are later used in this chapter to designate specific enzymes. Some enzymes, mostly digestive enzymes, are origi-ENZYME KINETICSnally excreted from the production organ in the struc-
catalytic mechanism of enzymes in a generally inactive form called proenzyme or zymogen. A chemical reaction can occur spontaneously if looseooth enzymes later change the structure of proenzyme energy or available kinetic energy is higher for reac-making active sites hydrolyzing specific tants than
for products. The reaction then continues with the remnants of amino acid. This mechanism prevents the digestive towards lower energy if a sufficient number of re-enzymes from digesting their place of synthesis. Actuas molecules have enough excess energy to break their chemical bonds and collide
and form new bonds. TheENZYME CLASSIFICATION And excess energy, called activation energy, energy re-NOMENCLATURE is necessary to increase all molecules in 1 mole compound at a certain temperature to a transient state on topIt standardize the enzyme nomenclature, enzyme energy
barrier. In a transitional state, it is equally likely that each moleculecommission (ES) of the IUB adopted a classification in 1961; standards were revised in 1972 and remained unresponsive molecules. Reactants pos-1978. The IUB system assigns enough energy to each sessing to overcome the energy
barrier, define the substrate it acted on, the reaction cat, participate in product formation.and possibly the name of any coenzyme involved in the reaction. Because many systematic names are one way to provide more energy for the response, it is in-lengthy, more usable, trivial, the recommended name is
also to extinguish the temperature, thereby increasing the intermolecularassigned system IUB.1 collision; however, it usually does not occur physio-logically. Enzymes catalyse low physiological reactions- In addition to naming enzymes, the IUB system must achieve four digits separated by decimals to
achieve the activation energy level of the reactants (subtified by each enzyme by the EC code number containing the strates). The first digit Reaction may then occur more easily on the statuses of the enzyme in one of the following six classes: equilibrium in which there is no net ante or reverse reaction,
even if the equilibrium constant 1. Oxidoeductase. Catalyze oxidation-reducing reactions between two substrates FIGURE 12-1. Energy versus energy indicating the energy barrier that the substrate needs to overcome in order to respond with a2. Interchange. Catalyse the transfer of another group without
enzyme catalysis. The enzyme significantly reduces hydrogen-free from one substrate to another energy needed to activate the reaction.3. Hydrolase. Hydrolysis catalysis of various bonds4. Lyase. Removal by catalysis of groups from substrates without hydrolysis; the product contains double bonds5.
Isomerases. Catalyse the conversion of geomet-ric, optical or positional iomers6. Ligases. Catalysing the bonding of two moles of a substrate together with the breakage of a pyrophosphate bond in adenosine triphosphate (ATP) or a similar compound The second and third digit numbers of the EC code
represent the subclass and subclass of the enzyme, re-spectively, divisions which are specific to enzymes in the class. The final number is the serial number specific to each enzyme in the sub-class. Table 12-1 contains ec code numbers as well as systematic and recommended enzyme names rarely
measured in a clinical laboratory. Table 12-1 also lists common and standard abbre-viations for commonly analysed enzymes. Without IUBrecomcomdation, uppercase letters were used as con-284 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL PROCEDURESReaction does not change. The
extent to which the reaction when the product is formed, the resulting free enzyme im-progresses depends on the number of molecules of the substrate mediated with the excess free substrate. Reac-, which go through the energy barrier. is in the kinetics of the zero order and the rate of reaction of de-
pends only to the concentration of enzymes. The general relationship between enzyme, substrate and product can be represented as follows: Michaelis-Menten constant (Km), derived from the theory of Michaelis and Menten, is the constant for AE Ƨ S K ES K E Ƨ P (Eq. 12-1) a specific enzyme and
substrate under defined reaction conditions and is an expression of the relationship to be-where E is the enzyme, S is the substrate, the EC is the enzyme-substrate tween rate of enzymatic reaction and the sub-complex , and P is the product. concentration with loss. The assumptions are that the
equilibrium between E, S, ES and P is rapidly determined by the Es complex being the physical binding of the substrate and that the reaction of E Ƨ P → ec is negligible. Speed-active place of the enzyme. The structural arrangement of the restrictive step is the formation of residues of the product and the
enzyme amino acids in the enzyme makes a threesome from the EC complex. Then the maximum speed of fixed,dimensional active pages is available. Sometimes the binding and reaction rate function is just an enzyme league and drives a regrouping to make an active point. Concentration. As shown in
Figure 12-2, the km transition status for the EC complex has a lower energy-specific substrate concentration at which en-activation is than the transient state of S itself, that zyme brings half the possible maximum speed.the reaction takes place after the complex is created. Actual Therefore Km indicates
the amount of substrateaction required may include several substrates and products. for a specific enzymatic reaction. Different enzymes are specific to substrates in different- Michaelis-Menten hypothesis of relationship ranges or respects. Some enzymes show an absolute between the reaction rate and

the specificity of substrate concentrations, which means that an enzyme that is only combined with can be mathematically represented as follows: one substrate catalyzes only the corresponding response. Other enzymes are group-specific because Vϭ Vmax[S] (Eq. 12-2) is combined with all substrates containing a specific chemical group Km Ƨ [S], such as a phosphate ester. Yet other en-zymes are specific to chemical bonds and thus exhibit where V is measured by reaction rate, Vmax's maxi-bond specificity. mummy rate, [S] is the concentration of the substrate, and Km is the Michaelis-Menten enzyme constant for specific stereoisometric specificity refers to enzymes that pre-substrate.dominantly combine with only one optical isomer of the acertain compound. In addition, the enzyme can bind theoretically, Vmax and then Km could be determined by more than one molecule of the substrate, and it can occur from the plot in Figure 12-2. However, Vmax is difficultin cooperative mode. Binding one substrate mole to determine from a hyperbolic conspiracy and often notcule, therefore, can facilitate the binding of other sub-actually achieved in enzymatic reactions because of the en-loss of molecules. zymy may not function optimally in the presence offactors that affect enzymatic reactions FIGURE 12-2. Michaelis-Menten speed curve versus substrate concentration for enzymatic response. Km is the concentration of the concentrate-substrate substrate at which the reaction rate is half the maximum level. The rate at which an enzymatic reaction occurs and whether a forward or reverse reaction occurs depends on several reaction states. One of the main effects of the naenzymatic reaction is the concentration of the substrate. In 1913, Michaelis and Menten assumed the role of substrate concentration in the formation of the enzyme-substrate (ES) complex. According to their hypothesis, re-sent in Figure 12-2, the substrate is easily bound to freeenzyme at low substrate concentration. For an amount of enzyme exceeding the amount of substrate, the rate of reaction is constantly increased when more substrate is attached. Reaction is after kinetics first order cause, the rate of reaction is directly proportional to the concentration of sub-loss. In the end, however, the substrate concentration is high enough to saute all available en-zyme and the reaction rate reaches its maximum. CHAPTER 12 ■ ENZYMES 285FIGURE 12-3. Lineweaver-Burk transforms Michaelis-cules, the speed at which intermolecular collisions occur, and the energy available for response. This caseMenten curve. Vmax is a reciprocal x-intercept with enzymatic reactions until the temperature is high. Km is a negative reciprocal x-intercept enough to denature the protein composition of an en-same line. zyme. For each temperature increase of 10°, the reaction rate is approximately doubled until, of course, the excess substrate doubles. More accurate and convenient de-protein is denatured.termination of Vmax and Km can be done through the Lineweaver-Burk plot, a double-reciprocal plot of each enzyme works optimally on a particular tem-Michaelis-Menten constant that delivers a straight line perature that is influenced by other reaction variables,(Fig. 12-3). Reciprocal is taken from both substrates, especially the total reaction time. Optimal concentration and rate of enzymatic reaction. the temperature is usually close to the physiological en-equation becomes the vironment of the enzyme; however, some denaturation may occur at a human physiological temperature of 1 ϭ Km 1 Ƨ 1 (Eq. 12-3) 37 °C. The rate of denaturation increases, when tempera-Vmax [S] climax increases and is usually significant at 40° to 50°C.Enzyme concentration Because low temperatures make enzymes impossible by reversibilityAging enzymes catalyse physiological reactions, en-inactive, many serum or plasma samples at enzyme concentration affecting the rate of catalysed remerge are chilled or frozen to avoid active effect. If the substrate concentration exceeds the loss until analysis. Storage procedures may differ from the concentration of the enzyme, the rate of reaction is the enzyme to the enzyme due to individual stability charac proportional to the concentration of the enzyme. Higher territorial. However, repeated freezing and thawing tends to be associated with enzyme levels, the faster the reaction to the prosthesis protein will be and should be avoided.because more enzymes are present that bind to the substrate. Due to their sensitivity to temperature, enzymesspH should be analyzed at strictly controlled temperatureEnzymes are proteins that carry pure molecular charges. Policies. The incubation temperature should be exactChnistments in pH can denature the enzyme or affect its Ϯ0.1 °C. Laboratories usually try to create an ionic state, resulting in structural changes or a change in the temperature of the analysis of routine enzyme measurement on the rest of the amino acids at the active site. 25°, 30°C or 37°C. Attempts to create uni-Therefore each enzyme works within a specific pH range of universal temperature for enzyme analysis were in vain and maximum at a specific pH. Most physiological enzymes- and thus reference ranges for enzyme levels, maymatic reactions occur in the pH range from 7.0 to 8.0, but vary significantly between laboratories. In united enzymes, they are active in wider pH ranges than others. However, 37 °C is most commonly used in the countries. The pH of the reaction is carefully checked at optimum pH using appropriate buffer solutions. Cofactors Cofactors are non-protein entities that must bind to ular enzymes with a temperature of parti temperature before reaction. Common activatorsEales usually increases the speed (inorganic cofactors) are metal (Ca2Ƨ, Fe2Ƨ, Mg2Ƨ,chemical reaction by increasing the movement of moles- Mn2Ƨ, Zn2Ƨ and KƧ) and non-metallic (Brϫ and Cl–). The activator may be necessary for the reaction or may only en-hance the rate of reaction in proportion to the concentration to the point at which the excess activator begins to inhibit the reaction. Activator function by alternating the spatial configuration of the enzyme for proper substrate binding, linking the substrate to the enzyme or coenzyme, or undergoing oxidation or reduction. Some common coenzymes (organic cofactors) are nu-cleotid phosphates and vitamins. Coenzymes serve as second substrates for enzymatic reactions. When they bind firmly to the enzyme, coenzymes are called prosthetic groups. For example, NAD as a cofactor may be reduced to nicotinamide adenine dinucleotide phosphate (NADP) in a reaction in which the primary substrate is oxidised. Increasing coenzyme concentration increases the rate of enzymatic response in a way that is synonymous with increasing substrate concentration.286 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL PROCEDURESIn quantifying an enzyme that requires a certain enzyme binding strategist. The amount of inhibitory isofactor that the cofactor should always be provided in ex- then negligible comparison, and the reaction will be pro-cess so that the extent of reaction does not depend on ceed at a slower pace, but on the same maximum rate of cofactor concentration. in response without inhibitions. Km is a constant for each enzyme and cannot be altered. However, since inhibitors amount of substrate needed to achieve a certain ve-Enzymatic reaction may not proceed normally if par-locity is higher in the presence of a competing inhibitor, a ticular inhibitor, interfering with reac-Km appears to increase when reporting the effect oftion. Competing inhibitors physically bind to an active inhibitor.instead of an enzyme and compete with the substrate for the active site. At a significant concentration of substrate substrate and inhibitor, commonly metallic ion, higher than the inhibitor concentration, the inhi-bind enzyme at the same time in uncompetitivebition is reversible, since the substrate is more likely to inhibit. The inhibitor can inactivate either an ES com-like inhibitor to bind the active site and enzyme plex or just an enzyme by causing structural changes inbola not being destroyed. Enzyme. Although the inhibitor binds competitively and does not activate the enzyme, the presence of an A inhibitor of an uncompetitive inhibitor binds the enzyme to the enzyme slows down the rate of reac-other than the active site and may be reversible in response. Therefore, in the event of uncompetitive inhibition, it is not possible to achieve maximum appropriateness that some naturally present metabolic agents have a reaction rate. Increase in substratecombinin reversible with certain enzymes. levels have no effect on the binding of uncompetitive inhibition of an uncompetitive drug can also be irreversible if the inhibitor, so km is unchanged.the inhibitor destroys part of the enzyme involved in cat-alytic activity. Because the inhibitor binds the enzyme in- Because uncompetitive inhibition requires form-dependent from the substrate, an increase in the EC substrate complex, an increase in the concentration of the concentrate substrate does not reverse inhibition. increases inhibition. Therefore, a maximum rate equal to the rate of uninhibited reaction cannot be inhibition uncompetitive is another type of inhibition achieved and km appears to be decreased.in which binds the inhibitor to the EC complex – the concentration of the substrate in the folds results in more EC com-measurement of the enzyme activityplexes to which the inhibitor binds, thus in-resulting inhibition. Enzyme inhibitor and substrate Because enzymes are usually present in a very small quasi-complex do not deliver the product. in biological fluids and often difficult to isolate themselves from similar compounds, an appropriate method of enzyme Each of the three types of inhibition is unique with re quantification is a measurement of catalytic activity. Activityspect to effects on the Vmax and Km of enzymatic reac- is then related to concentration. Common methods (Fig. 12-4). With competitive inhibition, the effect of photometrically measured increase in the inhibitor product may be counterproached by the addition of excess sub-FIGURE 12-4. Normal Lineweaver-Burk plot (fixed line) compared to each type of enzyme inhibition (dotted line). (A) competitive inhibi-tion Vmax unchanged; Km appears to be increased. (B) Inhibition of Vmax reduced uncompetitively; Km unchanged. C) uncompetitive inhibition of Vmaxdecreased; Km seems to be reduced. CHAPTER 12 ■ ENZYMES 287Concentration, reduction of substrate concentration, and (usually by inactivation of the enzyme with weak acid), reduction of coenzyme concentration or increase and measurement of the amount of reaction Concentration of altered coenzyme. that have occurred. The reaction is assumed to be linear during the reaction period; the greater the reaction, the more if the amount of substrate and any coenzyme in the exenzyme is present.cess in the enzymatic reaction, the amount of substrate or coenzyme used, or the product or altered by coenzyme formed, in continuous monitoring or kinetic tests, the number will depend only on the amount of enzyme present on the measurements, usually changes in absorbence, the reaction is induced. Therefore, enzyme concentrations are carried out in zero order during the reaction either at certain time intervals (usu-are always with the Ally every 30 or 60 seconds) or continuously contrasting in sufficient excess to ensure that no more than a tinuous-record spectrophotometer. These tests are20% of the available substrate is converted to a product. advantageous in methods with a fixed time, because lin-Any coenzymes must also be higher. NADH's coen-earity reaction may be more adequately verified. Ifzyme often measured in the lab. THE AB-ABSORBANCE OF NADH is measured at intervals, several data points absorb light at 340 nm, while NAD does not, and are necessary to increase the accuracy of linearity assessment-change absorbance at 340 nm is easily measured. (a) Priority shall be given to continuous measurements because any deviation from the linearity is easily observable. In specific laboratory methodologies, substances other than substrate or coenzyme are required, which must be the most common cause of deviation from linearity. NAD or NADH is often advisable when the enzyme is so elevated that all substrates react to a joined-enzyme test when neither is used at the beginning of the reaction period. For the rest NAD or NADH is a coenzyme for reaction. In other reactions, the change in speed is minimal, with tests of the impli-coupled-enzyme adding more than one enzyme that the concentration of coenzyme is very low. In excess as a reagent and multiple reactions are cat-continuous monitoring, the laboratorian can observe aalyzed. When an enzymatic enzyme catalyses its spe-sudden decrease in reaction rate (deviation from reaction, the product of this reaction becomes a kinetics of the zero order) of a particular determination and substrate on which the intermediate auxiliary enzyme may repeat the determination using a smaller patient sample. The product of the mean reaction becomes a decrease in the amount of sample the patient acts assubstrate for the final reaction, which is catalyzed by dilution, and the response obtained can be multiplied by the indicator of the enzyme and normally involves the conversion of the dilution factor to obtain the final response. NAD samples to NADH or vice versa. itself is not diluted in such a way that the diluent cannot interfere with the reaction. (Dilution of the sample with solution may be necessary to minimise negative effects in the analysis of caused kinetics when conducting enzyme quantification, inhibitors must be absent and other vari-haemolysis or lipaemia.) Measurements of enzyme activity that may affect the rate of reaction may not be accurate if storage conditions are carefully controlled. Constant pH should be the main tegrit of the protein, if enzyme inhibitors are present, ortained with a suitable buffer solution. If cofactors are not present.the temperature should be constant within Ϯ0,1 °C when the test is overdue at a temperature at which the enzyme is ac-Activitytive calculation (usually 25°, 30°, or 37°C). when, when they are quantified for their activity During the course of the reaction, the period for direct concentration measurement must also be carefully selected. When en-units used to manage enzyme levels are activity units. Thezyme is initially introduced into reactants and the ex-definition of the activity unit must take into account that the substrate of variables is constantly combined with available, which can change the results (e.g. pH, temperature, substrate).enzyme, reaction rate rises. After the enzyme is satu-historically, specific methods developers often estab-rated, the rate of product formation, the release of the enzyme, lished their own units for reporting results and often and recombination with multiple substrates to continue linearly. named units consecutively (i.e. Bodansky aPo time, usually 6 to 8 minutes after the reaction of the initia-king unit). To standardise the quantification reporting system, the response rate decreases because the substrate is de-titrated, the EC has defined an international unit, reverse reaction occurs noticeably and (IU) as the amount of enzyme that catalyzes the reac-product begins to inhibit the reaction. Therefore, en-tion 1 mol substrate per minute under specified quantifications is radiated during linear temperature conditions, pH, substrates and activators.reaction phase. Because specified conditions may vary among Labour-Tories, benchmarks are still often laboratory specific. One of two general methods, which are usually expressed in perth units of the enzymatic response range, may be used to measure enzyme concentration: (1) fixed time and litre (IU/L). Unit of enzyme activity recognised (2) by continuous monitoring or kinetic test. In the fixed-time method, reactants are combined, reactions are stopped for a specified time, the reaction is stopped288 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL PROCEDURES International Unit System (Système International ciency testing for all laboratories). Quality problems'Unités [SI]) is katal (mol/s). Mole's unit for control materials for enzyme testing were signifi-substrate concentrations, and the unit of time is an sec-outweighed problem. Differences between clinical samples and two samples. The enzyme concentration is then expressed as catalytic control serums including the types of origin of the enzyme v per litre (cat/l) (1,0 IU ϭ 17 nkat). tegrity of molecular species, isoenzyme forms, solution matrix, addition of preservatives and lyophilisity- When enzymes are quantified by measuring in-tion processes. Many studies have been conducted to en-excite or reduce NADH at 340 nm, molar ab-sure accurate measurement of enzymes and good quality sorptivity (6.22 ϫ 103 mol/ l) NADH is used for calcium-control materials.4late enzyme activity. Enzymes such as reagentsAddual of enzyme-weight enzymes can be used as reagents to measure many enzyme enzyme conenzymatic components in serum. For example, glu-centers by weight are also available and are commonly used, cholesterol, and uric acid are often quantifiedused to quantify certain enzymes, such as creatine using enzymatic reactions that measure tekinase (CK)-MB. Immunoactive tests may overestimate the active concentration of the analyte due to the specificity of teensome due to possible cross-reactivity with the inac-enzyme. Enzymes are also used as reagents for enzyme methods such as zymogens, inactive isoenzymes, quantifications of analytes, which are substrates for cor-macroenzymes or partially digestible enzymes. Quantification of the enzyme rela-sponding. One example is lactation between enzyme activity and the amount of enzymes dehydrogenase (LDH), may be an agent in lactate or generally linear, but should be determined for each en-pyruvat concentration. For this kind of meth-zyme. Enzymes can also be determined and quantified (ods), the enzyme is added in excess of the amount of suffi-by electrophoretic techniques that provide a resolution sufficient to provide a complete response in a short period of time.isoenzymes and isofores. Immobilized enzymes are chemically bound to adsor-ensuring accuracy of enzyme measurements has bents, such as agarose or certain types of cellulose, after a long time was of interest to laboratories. Clinical azide groups, diazo and triazine. Enzymes act as a relaboratory improvement Change from 1988 (CLIA '88) covering agents. When the substrate passes has established guidelines for quality control and profi-preparation, the product is obtained and analyzed, and the enzyme is present and free to react with multiple sub-CASE STUDIES 12-1 loss. Immobilized enzymes are suitable for dose analyses and are more stable than enzymes in the solution. The 51-year-old, overweight white man visiting his family Enzymes are also commonly used as agents by a compet-doctor with a symptom of 5 days' indigestion and uncompetitive immunoa reagents such as these. He also had bouts of sweating, malaise, which is used to measure human immunodeficiency virus (HIV) and headaches. His blood pressure is 140/105 mm Hg; antibodies, therapeutic drugs and cancer antigens.his family history includes a father with diabetes who commonly used enzymes include horseradish-died at age 62 AMI secondary diabetes mellitus. dáza, alkaline phosphatase, glucose-6-phosphate dehy-Electrocardiogram revealed changes from one per-drug exchange and ᴺL-galactosidase. The enzyme in these as-formed 6 months earlier. The patient's results say it acts as an indicator that reflects either the important work are as follows: the presence or absence of an analyte. CK 129 U/L (30-60) ENZYMES OF CLINICAL IMPORTANCECECK-MB 4% (Ͻ6%)LDH 280 U/L (100-225) Table 12-2 presents commonly analysed enzymes, in-LDH Isoenzymes LDH-1 Ͼ LDH-2, which have been assigned to their systematic names and clinical 35 U/L (5-30) Each enzyme is described in this chapter in terms of tissue source, diagnostic significance, test method, source of error questions and reference range.1. Can this patient be excluded from diagnosis of AMI? Creatine Kinase2. What other heart markers should be operated on Ck's enzyme with the molecular weight of this patient? 82 000, which is generally associated with the re-generation of ATP in contractile or transport systems. His pre-3. Should this patient be admitted to the hospital? dominant physiologic function occurs in muscle cells,CHAPTER 12 ■ ENZYMES 289TABLE 12-2 MAJOR ENZYMES OF CLINICAL SIGNIFICANCEENZYME CLINICAL SIGNIFICANCEAcid phosphatase (ACP)Alanine aminotransferase (ALT) Prostatic carcinomaAldolase (ALD)Alkaline phosphatase (ALP) Hepatic disorderAmylase (AMS) Skeletal muscle disorderAngiotensin-converting enzyme (ACE)Aspartate amino-transferase (AST) Hepatic disorder Bone disorderChymotrypsin (CHY)Creatine kinase (CK) Acute pancreatitisElastase-1 (E1) Blood pressure regulationGlucose-6-phosphate dehydrogenase (G-6-PD)Glutamate dehydrogenase (GLD) Myocardial infarction⁄⁄-Glutamyltransferase (GGT) Hepatic disorderGlutathione-S-transferase (GST) Skeletal muscle disorderGlycogen phosphorylase (GP)Lactate dehydrogenase (LDH) Chronic pancreatitis insufficiencyLipase (LPS) Myocardial infarction5J-Nucleotidase Skeletal muscle disorderPseudocholinesterase (PChE) Chronic pancreatitis insufficiencyPyruvate kinase (PK)Trypsin (TRY) Drug-induced hemolytic anemia Hepatic disorder Hepatic disorder Hepatic disorder Acute myocardial infarction Myocardial infarction Hepatic disorder Hemolysis Carcinoma Acute pancreatitis Hepatic disorder Organophosphate poisoning Genetic variants Hepatic disorder Suxamethonium sensitivity Hemolytic anemia Acute pancreatitiswhere it is involved in the storage of high-energy crea- CK is present in much smaller quantities in other tissuetine phosphate. Each contraction cycle of muscles re-sources, including the bladder, placenta, gastrointestinal outcomes when using creatine phosphate, with the production of tract, thyroid, uterus, kidneys, lungs, prostate, spleen, liver, ATP. This results in relatively constant levels of muscles and pancreas. The reversible reaction catalysed by TA is shown in equation 12-4. Diagnostic significance Due to the high concentrations of TA in muscle yus-Creatine Ƨ ATP CK Creatine phosphate Ƨ ADP sue, CK levels are often elevated in car-diac and skeletal muscle disorders. Ta level is considered a sensitive indicator (Eq. 12-4) of acute myocardial infarction (AMI) and muscular dystrophy, especially duchenneTissue Source. A significant increase in CK occurring in Duchenne-typeCK is widely distributed in tissue, with the highest activity of muscular dystrophy, with values reaching 50 to 100 found in skeletal muscle, heart muscle, and brain tissue. upper limit of normal (ULN). Although a total of 290 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL levels are sensitive indicators of these disorders, they because the increase in the enzyme is found in many dis-are not entirely specific indicators, because CK ele-orders, the separation of the total CK into its various isoen-vation is found in various other abnormalities of cardiac zyme fractions is considered a more specific indicator and skeletal muscle. Ck levels also vary with different muscle disorders than overall levels. Typically, the clinical pile, and therefore may depend on gender, race, degree of relevance of CK activity depends more on isoenzyme physical condition, and age. fractionation than at overall levels. Elevated CK levels are also occasionally seen in central TA occurring as a dimer consisting of two subumer, whichnervous system disorders such as cerebrovascular acci-can be easily separated into three distinct molecular defects, seizures, nerve degeneration, and central nerve forms. Three isoenzymes were described as systemic shock. Damage to the blood-brain barrier must allow CK-BB (a type of brain), CK-MB (hybrid type), and CK-MMoccur to allow the release of enzymes into the peripheral circulation. (type of muscle). At electrophoretic separation, CK-BB migrates fastest toward the anode, and therefore is called other pathophysiological states in which increased CK-1. CK-BB follows CK-MB (CK-2) and finally, CK levels occur are hypothyroidism, malignant hyper-by CK-MM (CK-3), exhibiting the slowest mobility (Fig.pyrexia, and Reye syndrome. Table 12-3 gives the main 12-5). Table 12-3 indicates the localization of tissuedisorders associated with abnormal CK levels. Serum ISOENZYMES CK and the main conditions associated with el-levels and the CK/progesterone ratio were useful in avoiding levels. Separation of TA isoforms may also be vi-diagnosing ectopic pregnancies.5 Total serum TA levels sualized by high-voltage electrophoretic separation.have also been used as an early diagnostic tool to identify Isoforms after splitting carboxyl-termi-patients with Vibrio vulnificus infection 6TABLE 12-3 CREATINE KINASE ISOENZYMES-TISSUE LOCALIZATION AND SOURCES ELEVATIONISOENZYME TISSUE CONDITIONCK-MM HeartCK-MB Skeletal muscle attack Skeletal muscle disorder Skeletal muscleCK-BB Cardiac muscular dystrophy Skeletal musculature Polymyositis Hypothyroidism Cerebral malignant Bladder hyperthermia Physical activity Pulmonary intramuscular injection Myocardial infarction Prostate infarction Colon damage Colon ischaemia Thyroid angina heart Inflammatory heart disease Cardiac cardiac cancer Duchenne-type muscular dystrophy Polymyositis Malignant hyperthermia Reye syndrome Rocky Mountain spotted fever Carbon poisoning Carbon monoxide Shock central nervous system Anoxic encephalopathy Cerebrovascular accident Seizure Placental or uterine trauma Carcinoma Reye syndrome Carbon monoxide poisoning Malignant hyperthermia Acute and chronic renal failureCHAPTER 12 ■ ENZYMES 291FIGURE 12-5. Electrophoretic migration pattern of normal and although brain tissue high concentrations of TA, atypical ISOENZYMES CK. rarely contains CK-BB of brain origin. Due to its molecular size (80,000), its passage through the blood-brain amino acids from the M unit of the serum auto-barrier is defended. However, when extensive damage toboxypeptidase N. Three isoforms were described for the brain occurred, a significant amount of CK-BB canCK-MM and two isoforms for CK-MB; clinical signif-sometimes be detected in serum.icance is not well established. It has been observed that CK-BB can be significantly el-Main isoenzyme in the serums of healthy people avoids patients with various organ cancers. It has MM form. Values for MB isoenzyme range from have been found in conjunction with untreated prostate carci-undetectable monitor (Ͻ6% of total TA). Also, it appears noma and other adenocarcinomas. These findings suggest that CK-BB is present in small amounts of serum, that CK-BB may be a useful tumor-related marker.9healthy people; however, the presence of the CK-BB ad depends on the method of detection. Most tech-most common causes of CK-BB height areniques cannot detect CK-BB in normal serum. damage to the central nervous system, tumors, childbirth, and the presence of macro-CK, enzyme-immunoglobulin CK-MM is the main isoenzyme fraction found in the complex. In most of these cases, the CK-BB level is striped muscle and normal serum. Skeletal musculature con- greater than 5 U/L, usually in the range of 10-50 U/L.tains almost completely CK-MM, with a small number of additional conditions listed in Table 12-3 usually show CK-CK-MB. Most ck activity in the heart muscle is BB activity lower than 10 U/L.10 also attributed to CK-MM, with approximately 20% of the result of CK-MB.7 Normal serum consists of approximately- the CK value of isoenzyme separation can be foundmatematically 94% to 100% CK-MM. Damage to the heart mainly when detecting myocardial damage. Cardiac y yokes- and skeletal muscle accounts for the majority of cases sued containing a significant amount of CK-MB, approximate-CK-MM increase (Table 12-3). Hypothyroidism re-mately 20% of all CK-MB. While CK-MB is found insults at CK-MM altitudes due to the involvement of small amounts in other tissues, myocardium is essentially muscle tissue (increased membrane permeability), the only tissue from which CK-MB enters serum in the sig-effect of thyroid hormone on enzyme activity, and, nificant amount. Demonstrating elevated levels, slower clearance of CK due to CK-MB, greater than or equal to 6% of total TA, is a con-slower metabolism. a good indicator of myocardial damage, particu-larla AMI. Other nonenzyme proteins, called troponins, mild to strenuous activity can contribute to increased ve been found to be even more specific and can increase levels of VOK as well as intramuscular injections. In physical in the absence of CK-MB height. After myocardial activity, the extent of elevation is However, heart attack, CK-MB levels begin to rise to 4 to 8degree exercises in relation to the exercise ability of the clock, peak at 12 to 24 hours, and returning to normal levels is the most important factor in determining within 48 to 72 hours. This timeframe must be considered for altitude level.8 Patients who are physically conditioned to interpret CK-MB levels.well show lower altitude levels than patients who are less air-conditioned. Levels may be ele-CK-MB activity has been observed in other heartvated as long as 48 hours after exercise. (Table 12-3). Therefore, the elevated amounts are not entirely specific to AMI, but probably reflect CK heights are generally less than five times ULN of a certain degree of coronary heart damage. The specificity of offollowing intramuscular injections and usually not ap-CK-MB levels in AMI diagnosis may be increased ifparent after 48 hours, although increases may persist for interpreted in conjunction with LDH isoenzymes and/or 1 week. The predominant isoenzyme is CK-MM. troponins and if measured gradually over a 48-hour period to detect a typical increase and fall in enzyme activity- The amount of CK-BB in the tissue (Table 12-3) is seen in the AMI (Fig. 12-6).usually small. A small amount along with its rela-tively short half-life (1-5 hours) results in CK-BB activi-MB isoenzyme also being detected in seraties, which are generally low and transient, and not usually in patients with noncardial disorders. Ck-MBmeasurable levels when tissue damage occurs. The highest concen- found in these conditions probably represent leaks located in the central nervous system, gas from skeletal muscle, although in duchenne-type mus-trointestinal tract, and uterus during pregnancy. dystrophy, there may also be some heart failure. Ck-MB levels in Reye's syndrome can also cause myocardial damage. Despite the detection of CK-MB levels in disorders other than myocardial infarction, its presence is still re-emined by an important indicator of AMI.11 A typical elevation time of CK-MB after AMI was not found under other conditions.292 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL PROCEDURESFIGURE 12-6. Time activity curves of enzymes in myocardial infarction for AST, CK, CK-MB and LDH. Ck, specifically MB fraction, increases initially, followed by AST and LDH. LDH is the longest increased. All en-zymes usually return to normal within 10 days. Myocardial infarction of nonenzyme (troponin I and troponin T). The incidence of CK-Mi rangeshave was used as a more sensitive and specific marker from 0.8% to 1.7%. To be detected in serum, damage to the exten-myocardium. These proteins are released into tissue tissue tissue damage to occur, causing decay earlier and persisting longer than CK and it's mitochondrion and cell walls. Its presence is not jeizoenzyme CK-MB. Learn more about these proteins with any specific disease status, but signs of AMI appear to be found in Chapters 10 and 25. CK-Mi has been de-tected in cases of malignant tumor and cardiac abnor- Numerous reports have been made describing ap-malities.pearance of unusual CK isoenzyme bands displaying inesecttrophoretic properties that differ from the three due to the indefinite correlation between these major isoenzyme fractions12-16 (Fig. 12-5). These atypical CK forms and the specific condition of the disease, it appears that local forms are generally of two types and are referred to as that their importance mainly concerns the methodsmacro-CK and mitochondrial CK. used to detect CK-MB. In some analytical proce-dures, these atypical forms can be measured as CK-MB, Macro-CK appears to migrate to a position mid-resulting in erroneously high CK-MB levels.tween CK-MM and CK-MB. This type of macro-CK largelycompilates CK-BB complexed with immunoglobulin. In the methods used to measure isoenzymes of TA in most cases, it is an associated immunoglobulin of IgG, al-clude electrophoresis.; ionomemen chromatography.although the IgA complex has also been described. Several immunoagens, including radioimmunoassayterm macro-CK, have also been used to describe complexes (RIA) and immunoinhibition methods. Although the weight of lipoproteins with CK-MM. methods are more sensitive and are preferred in quantifying CK-MB, electrophoresis was the reference incidence of macro-CK in serums ranging from 0.8% method. Electrophoretic properties of isoen-1.6% CK Currently, there is no specific defect associated with the zymes shown in Figure 12-5. In general, tech-with its presence, although it appears to be age and sex nique consists of carrying electrophoresis on sam-related, occurring most often in women older than plu, measuring the reaction using overlay technique.age 50. and then visualizing strips under ultraviolet light. In electrophoresis, the atypical sepa-Mitochondrial CK (CK-Mi) bands can be bound to the rated exterior, allowing their detection in addition to the three main surface membranes of the internal mitochondrial membranes of the mus-strips. Often a strongly fluorescent band appears, which,čl, brain and liver. It migrates to the point of cathodal migrates in close proximity to the CK-BB form. ExactCK-MM and exists as a dimerial molecule of two identities- the nature of this fluorescence is unknown, but it was a subunit. It occurs in serum in dimeria attributed to the binding of a fluorescent medicinal product or bilirubin state and in the form of oligomer aggregates with high molecular weight albumin (350,000). CK-Mi is not present in normal serum and is usually not present poCHAPTER 15 ■ ELECTROLYTES 375 If total Ca2Ƨ is the only reported result, hypocal and loss of protein and albumin that are best suitedcemia may occur with hypoalbuminemia. Common for monitoring the Ca2Ƨ status of ionised Ca2Ƨ are associated with chronic liver disease, ments. Normalization of ionized Ca2Ƨ may have benefi-nephrotic syndrome and malnutrition. In general, with the initial effects on cardiac output, Ca2Ƨ and blood pressure. Typically, blood-ionized Ca2Ƨ con-centers in newborns are high at birth and then rapidly About half of patients with acute pancreatitis decrease by 10%-20% after 1-3 days. After about 1 week, hypocalcaemia develops. The most converging cause of ap-ionized Ca2Ƨ concentrations in newborn atpear is the result of increased intestinal binding of levels slightly higher than in adults.23Ca2Ƨ as there is increased intestinal lipase activity.22 Lack of Vitamin D and malabsorption may cause de-concentration of ionized Ca2Ƨ may reduce the absorption of rap-creased, leads to increased PTH pro-idly in the early neonatal period because of infant mayduction or secondary hyperpathyroidism. Lose Ca2Ƨ quickly and not easily reabsorb it. Several pos-sible etiologies have been suggested: abnormal PTH and patients with kidney disease caused by glomerular failure of vitamin D metabolism, hypercholesterolaemia, hypefos-often altered concentrations of Ca2Ƨ, PO4ϫ, albumin, fatemia, and hypomagnesaemia. Mg2Ƨ and HƧ (pH). In chronic kidney disease, secondary perpathyroidism often develops when the body tries symptoms of hypocalcemia. Neuromuscular irritability to compensate for hypocalcemia caused by either hyper- and cardiac irregularities are the primary groups of symp-phosphaemia (PO4ϫ binds and reduces ionized Ca2Ƨ) or toms that occur with hypocalcemia. Neuromuscular metabolism of vitamin D. Monitoring and control of symptoms include paraesthesia, muscle cramps, tetanus, ionized Ca2Ƨ concentrations can avoid problems due to and seizures. Cardiac symptoms may include arrhythmiaspocaemia such as osteodystrophy, unstable heart or heart blockage. Symptoms usually occur with severe withdrawal or blood pressure, or problems resulting from hyper-hypocalcaemia, in which total levels of Ca2Ƨ are below 1.88calcaemia, such as kidney stones and other calcifications. mmol/l (7.5 mg/dL).22Rhabdomyolysis, as with major crushing injuries and muscle damage, may cause hypocalcaemia due to increased treatment of hypocalcaemia. Oral or parenterally Ca2ƧPO4ϫ release from cells that bind to the Ca2Ƨ ions. Vitamin D can sometimes be pseudohypoparathyroidism is a rare hereditary disorder administered in addition to oral Ca2Ƨ to increase absorption-in, in which the PTH target response of the tissue (end) is reduced. If hypomagnesaemia is a concomitant disorder, Mg2Ƨorgan resistance). Production of PTH responds normally to treatment should also be provided.loss of Ca2Ƨ; however, without a normal response (decosified cAMP [cyclic adenosine 30.5 5 4-phosphate] pro- Ca2Ƨ is lost in the urine or remains in the bone Primary hyperpathathyroidism is the main cause of hyper-storage pool. Patients often have frequent physical fea-calcemia. Matic. The population of patients most attentive to primary hyperpathyroidsis are elderly women.22 Althoughsurgery and intensive care. Since appropriate Ca2Ƨ either total or ionized Ca2Ƨ measurements are increased inconcentrations promote good cardiac output and maintain severe cases, ionized Ca2Ƨ is more often increased insufficient blood pressure, maintaining normal subtle or asymptomatic hyperpathyroidism. Generally,ionized Ca2Ƨ in the blood is beneficial for pa-ionized Ca2Ƨ measurements are increased in 90% to 95% of patients either in surgery or in the intensive care unit. Control Ca2Ƨ cases of hyperpathyroidism, while total Ca2Ƨ's ele-concentration may be critical in open heart surgery when ejected in 80% to 85% of cases.the heart restarts and during liver transplantation may be causing large volumes of citrated blood to be given. The second most common cause of hypercalcemia is associ-ated with different types of malignancy, with hypercal- Because these patients can get large amounts of ecemia sometimes the only biochemical marker forcitrate, HCO3ϫ, Ca2Ƨ salts or fluids, the largest dis-disease.22 Many tumors produce PTH-related peptides between total Ca2Ƨ and ionized concen-(PTH-rP) Ca2Ƨ that binds to normal PTH receptors and ionized Ca2Ƨ concen- (PTH-rP), which binds to normal PTH receptors and rinsings can be observed during major surgical operations. causes an increased Ca2Ƨ level. Tests to measure PTH-rPConsequently, ionized Ca2Ƨ measurements are Ca2Ƨ available because this abnormal protein is not de-measuring the greatest clinical value. most PTH tests. Hypocalcemia occurs commonly in critically ill pa- Due to the proximity of fat totientes, that is, those with sepsis, thermal burns, renal failure- thyroid gland, hyperthyroidism can sometimes cause, or cardiopulmonary insufficiency. These patients rarely have acid-ase regulatory abnormalities376 PART 2 ■ CLINICAL CORRELATION AND ANALYTICAL PROCEDUREShyperparatyroidism. Rare, benign, familial hypocalcaecal Ca2Ƨ or Zn2Ƨ with a small amount of eurie has also been reported. Thiazide diuretics increase heparin dispersed in an inert cloud, which essentially elimi-Ca2Ƨ reabsorption, which leads to hypercalcemia. Prolonged nates interference heparin.immobilization can cause increased bone resorption. Hypercalcaemia associated with immobilization is further for the analysis of Ca2Ƨ in the urine is precisely timed by renal insufficiency. urine collection is preferred. Urine should be acidi-fied with 6 mol/l HCl, with approximately 1 mlSymptoms of hypercalcemia. Mild acid added to each 100 ml of urine. (2.62-3.00 mmol/l [10.5-12 mg/dl]) is often asymptomatic.22 Moderate or severe Ca2Ƨ increase includes neu-Methodsrologic, GI and renal symptoms. Neurological symptoms Two commonly used methods of overall analysis Ca2Ƨ may include mild drowsiness or weakness, depression, the use of ortho-cresolphthalate complex (CPC) or ar-lethargy and coma. GI symptoms may include constipation-senzo III dye to form a complex with Ca2Ƨ. Before the teation, nausea, vomiting, anorexia, and peptic ulcer dis-dye-binding reaction, Ca2Ƨ is released from its protein auto-light. Hypercalcaemia can cause renal symptoms of rier and complexes by acidizing the sample. Tenefolisiasis and nefocalcinosis. Hypercalciuria, the CPC method can use 8-hydroxyquinoline to prevent Mg2Ƨresult in nephrogenic diabetes insipidus, causing interference. AAS remains the reference method for totalpolyuria, which results in hypovolaemia, which is further ag-Ca2Ƨ, although rarely used in the clinical environment.gravates hypercalcemia. Current commercial analysers that measure ionised/ Ca2Ƨ ise for this measurement. These systemsTreated hypercalcemia. Treatment of hypercalcaemia can use membranes impregnated with special molecules dependent on the level of hypercalcemia and cause. Often, they selectively, but reversingly, bind Ca2Ƨ ions. As Ca2Ƨpeople with primary hyperpathyroidse are asympto-binds to these membranes, electrical potential develops smatically. Oestrogen deficiency in postmenopausal women has through a membrane that is proportional to ionizedby being involved in primary hyperpathyroidism in older Ca2Ƨ concentration. Diagram of one such electrode iswomen.22 Often, estrogen replacement therapy decreases shown in Figure 15-6.Ca2Ƨ levels. Parathyresectomy may be required in some patients with hyperpathyroidism. Patients with moderate to se-Reference range of hypercalcaemia are treated to reduce Ca2Ƨ levels. Salt For total Ca2Ƨ, the reference range varies slightly Ca2Ƨ and it is recommended to increase the age of excretion.3 In general, Ca2Ƨ concentrations are higher and dehydration can be prevented, which may multiply hyper-Ca2Ƨ adolescence when bone growth is most active. Ionised/freecalcaemia. Thiazide diuretics should be discontinued. Ca2Ƨ concentrations can change rapidly from day one to dayBiphosphates (a derivative of pyrophosphate) are 3 life. After this, stabilize on the relatively highmain drug class used to reduce Ca2Ƨ levels, reached its levels, with a gradual decline through adolescence; seebinding effect on bone that prevents bone resorpt.22 Table 15-18.Determination of calcium phosphateSpecimen phosphate Physiology Preferred sample for total Ca2Ƨ determination is found everywhere in living cells, phosphate compounds of semia or lithium-heparin plasma collected without participation in many of the most important Stasis. Because of anticoagulants such as EDTA or processes. Genetic materials of deoxyribonucleic acidoxalate bind Ca2Ƨ and bind tightly and interfere with measures- (DNA) and ribonucleic acid (RNA) are complex fos-ment, they are unacceptable for use. phodiesters. Most coenzymes are phosphoric acid or pyrophosphoric acid esters. The most important reservoirs of proper sampling for ionized Ca2Ƨ biochemical energy are ATP, creatine phosphate and medical systems require greater care. Since the loss of CO2 phosphoenolpyruvate. Phosphate deficiency may lead to an increase in pH, samples must be taken anaerobicly. ATP depletion, which is ultimately responsible for manyNext heparinized whole blood is preferred by sam- of the clinical symptoms observed in hypophosphatemia.ple, serum from closed evacuated tubes for blood collection can be used, if clotting and spinning are performed Changes in concentration 2,3-bisphosphfo-fast (Ͻ30 minutes) and at room temperature. No liq-glycerrate (2.3-BPG) in red blood cells affects affinityuid heparin products. Most heparin anti-hemoglobin oxygen, with an increase in facilitating coagulants (NaƧ, lithium) partially bind to Ca2Ƨ and release oxygen in the tissue and reduce the formation of ionized Ca2Ƨ concentrations. Heparin concentrate-oxygen bound hemoglobin less available. By affecting 25 IU/ml, for example, it reduces ionized Ca2Ƨ formation of 2.3-BPG, inorganicabout concentration 3%. Dry heparin products available titrated with phosphate indirectly affects the release of oxygen from hemoglobin. CHAPTER 15 ■ ELECTROLYTES 377 FIGURE 15-6. Diagram of the ionised calcium electrode for the ICA ionised calcium analyser. (Courtesy of Radiometer America, Westlake, Ohio.) Understanding the cause of altered phosphate Disorders of any of these processes can change the concentration of fos in the blood is often difficult, because tran-phate concentration in the blood; however, loss of cellular shifts of phosphates is the main cause of kidney hyenagulation, which will have the deepest hypophosphaemia in the blood. That is, an increased shift in effect. Although other factors such as vitamin D, calciphosphate in cells can deplete phosphate in the blood. tons, growth hormone, and acid-base condition, can affectIf phosphate is taken into the cell, it remains there for renal regulation of phosphate, the most important factor that is used in the synthesis of phosphorylated compounds. Like PTH, which overall reduces blood concentrations these phosphate compounds are metabolized, an inorganic increase in renal excretion.phosphate slowly escapes from cell to blood,where it is regulated mainly by the kidneys. Vitamin D acts to increase phosphate in the blood. Vitamin D increases the absorption of phosphates in the in-regulation of testin and reabsorpation of phosphates in the kidneys. Phosphate in the blood can be absorbed in the intestines from dietary sources, cells into the blood, and growth hormone, which helps regulate skeleton from the bones. In healthy individuals, all these growth processes can affect circulating fos-concentrations are relatively constant and easily regulated renal ex-phate. In cases of excessive secretion or administrationcrementation or reabsorption of phosphate. growth hormone, phosphate concentrations in the blood may increase due to reduced renal excretionTABLE 15-18 REFERENCE RANGES FOR PHOSPHATE. CALCIUM DISTRIBUTION CALCIUM-SERUM, PLASMA Although the concentration of all phosphate compounds in the blood is approximately 12 mg/dl (3.9 mmol/l), most of it is Ͻ12 years organic phosphate 2.20-2.70 mmol/l (8.8-10.8 mg/dl) and only about 3 to 4 mg/dl is inor-ganic phosphate. Phosphate is the predominant intracell-adult 2.15–2.50 mmol/l (8.6–10.0 mg/dl) lular anion, with intracellular concentration varying, depending on the cell type. About 80% of the total CALCIUM-SERUM body pool phosphate is contained in the bones, 20% in soft tissues, and less than 1% is active in serum/plasma. Child 1.20–1.38 mmol/L (4.8–5.5 mg/dL) Clinical ApplicationsAdult 1.16–1.32 mmol/L (4.6–5.3 mg/dL) HypophosphatemiaIONIZED CALCIUM—PLASMA Hypophosphatemia occurs in about 1% to 5% of hospi- talized patients.24 The incidence of hypophosphatemiaAdult 1.03–1.23 mmol/L (4.1–4.9 mg/dL) increases to 20% to 40% in patients with the following disorders: diabetic ketoacidosis, chronic obstructiveIONIZED CALCIUM—WHOLE BLOOD pulmonary disease (COPD), asthma, malignancy, long- term treatment with total parenteral nutrition (TPN),Adult 1.15–1.27 mmol/L (4.6–5.1 mg/dL)TOTAL CALCIUM—

2.50–7.50 mmol/day (100–300 mg/URINE (24-h) day), varies with diet378 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTIC PROCEDURESinflammatory bowel disease, anorexia nervosa, and alco- TABLE 15-19 REFERENCE RANGES FORholism. The incidence increases to 60% to 80% in patients with ICU INORGANIC PHOSPHORUSpatients with sepsis. In addition, hypophosphaemia may also be due to increased renal excretion, as with hy-SERUM 1.45-2.91 mmol/l perpaterhyroidism (4.5-9.0 mg/ln) and reduced intestinal absorption, Newborns 1.07 -1.74 mmol/l (3.3-5.4 mg/l)as for vitamin D deficiency or antacid use.24 Children aged 15 years 0.78 -1.42 mmol/l (2.4-4.4 mg/2 1.42 mmol/l (2.4-4.4 mg/0L) Adult 13-42 mmol/day (0.4-1.3 g/day) Although most cases are mild and rarely cause urinary problems (24-h), severe hypophosphaemia (Ↄ1, 0 g/0L or 0.3 mmol/l) requires monitoring and possible replacement is severely reduced. Pyruvate is the normal end of prod-therapy. There is a 30% mortality rate in those who are revered by glucose metabolism (glycolysis). Conversion eternally hypophosphatus versus 15% rate in those pyruvate to lactate is activated when deficient with normal or mild hypophosphataemia. 24 oxygen leads to the accumulation of excess NADH (Fig. 15-7). Normally, enough oxygen maintains a favorableHyperphosphatemia high ratio of NAD to NADH. Under these conditions, patients the risk of hyperphosphaemia are those pyruvate is morphyl-coenzyme A (CoA), with acute or chronic renal failure.24 Increased intake, which enters the citric acid cycle and produces 38 moles of phosphate or increased release of ATP cell phosphate for each mole of oxidized glucose. However, it can also cause hyperphosphaemia. Since they may, under hypoxic conditions, acetyl-coa formations have not yet developed mature PTH and vitamin D me-occurs and NADH accumulates, favoring conver-tabolism, newborns are particularly susceptible to hyper-zion pyruvate lactate through anaerobic metabo-phosphataemia caused by increased intake than from lism. As a result, only 2 birthmarks of ATP precow milk or laxative are produced. Increased cell decay can make each mole glucose metabolized to lactate, with ex-sometimes leading to hyperphosphataemia, as well as with severe in-cess lactate released into the blood. This release of lactic infectiouss, intense exercise, neoplastic disorders or in-blood is of clinical importance because of accumu-travascular hemolysis. Since immature lymphoblasts lysis of excess lactate in the blood is early, sensitive and has about four times the phosphate content in a mature quantitative indicator of the severity of oxygen-lymphocyte deficiency, patients with lymphoblastic leukemia are tion (Fig. 15-8).especially susceptible to hyperphosphataemia. RegulationInorganic phosphorus determination Since lactate is a product of anaerobic metabolism, it is not specifically regulated, as with KƧ or Ca2Ƨ, for ex-sample sufficient. Since the oxygen supply decreases below the critical plasma of serum or heparin lithium is acceptable for analy-level, lactate concentrations in the blood rise rapidly and indi-sis. Oxaltate, citrate or EDTA anticoagulants should not be categorised for tissue hypoxia rather than pH. The liver is majorused because it interferes with the analytical method. lactate removal by converting lactate back into Haemolysis should be avoided due to higher con-glucose by a process called gluconeogenesis. Circulating lev-els phosphate is subject to circadian rhythm with the highest levels in clinical applications in the morning and lowest in the evening. Urinalys analysis for measuring lactate in the blood are useful for metabolic phosphate requires 24-hour sampling for monitoring in critically ill patients, for indications ofsignificant diural variations. severity of the disease and to objectively determine the pa-tient of the prognosis. MethodsMome of current methods for the determination of phosphorus- There are two types of lactic acidosis. Type A is an association involving the formation of ammonium phosphorus-ifosfo-ciated with hypoxic conditions such as shock, myocar-molybdate complex. This colorless complex can be dialed heart attack, severe congestive heart failure, pulmonary-nausea ultraviolet absorption at 340 nm or may be edema, or severe blood loss. Type B is origin, reduced to a form of molybdenum blue, stable blue chro- such as diabetes mellitus, severe infection, leukemia, mophore, which is read between 600 and 700 nm. liver or kidneys and toxins (poisoning with ethanol, methanol or salicylate). The reference values of the Phosphate range vary by age. Table 15-19 shows the determination of lactic ranges divided into age groups. Handling of samples Particular attention should be practised when collecting and han-dling specimens for lactate analysis. Ideally tourniquetLactate Biochemistry and PhysiologyLactate is a by-product of the emergency mechanism, whichproduces a small amount of ATP when the supply of oxygenCHAPTER 15 ■ ELECTROLYTES 15 379Aerobic metabolism1 mol glucose pyruvate acetyl-CoA citric acid cycle NADƧ NADH Oxidative phosphorylation in mitochondria quickly oxidize NADH back to NADƧ. 38 mol ATP producedAerobic metabolism1 mole glucose pyruvate acetyl-CoA NADH Without oxidative NADƧ phosphorylation, NADH LACTATE accumulates, which prefers to convert pyruvan to lactate. 2 minor ATP produced BYFIGURE 15-7. Aerobic versus anaerobic glucose metabolism. Reduced supply of O2 to tissueOxidative metabolism rate reduces NADH accumulates (NAD decreases)Pyruvate converts to lactate lactate instead of acetyl-coA accumulatesThe less ATP produced (ATP depletion) intracellular ion environment disturbed cell death (increased Ca and Na; decreased K and Mg) FIGURE 15-8. Metabolic effects of hypoxia leading to cell death.380 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL PROCEDURESTABLE 15-20 REFERENCE RANGES FOR LACTIC ENZYMATIC METHOD, PLASMA COLORIMETRIC, WHOLE BLOOD 0.9 -1.7 mmol/l (8.1 - 15.3 mg/dL)Venous 0.5-2.2 mmol/l (4.5-19.8 mg/dL) Ↄ1.3 mmol/l (Ↄ11.3 mg/2 Arterial 0.5-1.6 mmol/l (4.5-14.4 mg/dL)CSF 1.0-2.9 mmol/l (9-26 mg/l) should not be used because venous stasis is increased The most commonly used enzymatic method uses lactate levels. If a turnstile is used, blood should be col-tate oxidase to produce pyruvate and H2O2.lected immediately and the patient should not exercise his hand before or during collection.14 After sample collec-lactate Ƨ O2 _La_cta_te o_xid_as→e pyruvate Ƨ H2O2 (Eq. 15-6)tion, glucose is ed into lactose by anaerobic glycolysis and should be avoided. Heparinized blood One of two pairs of reactions can then be used.can be used, but it must be delivered to ice, and the plasma must be rapidly separated. Iodoacetate or fluoride, which peroxidase can be used to create color glycolysis of chromogeninhibit without affecting coagulation, are usu-ally satisfactory additives, but it is necessary to consult a specific method direc- of H2O2.tions. H2O2 Ƨ H donor Ƨ chromogen _Pe_roxi_das→eMethodsEal lactate is a sensitive indicator of insufficient yew-color Ƨ 2H2O (Eq. 15-7) to sue oxygenation, the use of blood lactate measurements was prevented because older methods were slow and reference ranges. Other means after perfusion or oxy- See table such as dwelling catheters that measure blood flow, pulse oximeters, ANION GAP base excesses and oxygen consumption measurements (VO2). Current enzymatic methods make lactate de-routine electrolyte measurement usually involves lentermation readily available. NaƧ, KƧ, ClӾ and HCO3Ӿ (as total CO2). These values can be used to approximate the anionic gap (AG), which is the difference between an unmeasurable anionic and non-measurable cations. There is never a gap between total cation fees and anion charges. AG is created by the concentration difference between the commonly case study 15-4Chame change the following laboratory results from three • Primary hyperpathyroidismadult patients: • Malignancy • Hypomagnesaemic hypocalcaemiaQuestions1. Which set of laboratory results (Case A, B, or C) is most likely associated with each of the following diagnoses:CASE STUDY TABLE 15-4.1 LABORATORY RESULTS REFERENCE RANGESCase Ion Ca2٪. Total Mg2٪. PO4٪.. Hematocrit IntactA 1.16–1.32 0.63–1.0 0.87–1.45 35–45% ParathyroidB mmol/L mmol/L mmol/L 42 HormoneC 40 13–64 ng/L 1.44 0.90 0.85 30 100 1.08 0.50 0.90 25 1.70 0.98 1.43 12CHAPTER 15 ■ ELECTROLYTES 381 Mgɞ2 HPO4, HSO4ɞ2 Mgɞ2 HPO4, HSO4ɞ2 Caɞ5 Org acidɞ3 Org acidɞ9 Proteinɞ17 Caɞ5 Proteinɞ17 Kɞ4 Kɞ5 HCO3ɞ22 HCO3ɞ28Naɞ142 Clɞ103 Naɞ141 Clɞ103 Normal Lactate Acidosis Anion Gap ɞ 15 Anion Gap ɞ 21(142Ƨ4)Ӿ(103Ƨ28) (141Ƨ5)Ӿ(103Ƨ22)FIGURE 15-9. Demonstration of the anionic gap from the concentrations of anions and anions in the normal state and lactic acidosis.measured NaƧK and commonly measured sum of excretion and preservation of electrolytes in ions (ClƧHCO3), as shown in Figure 15-9. AG is useful in healthy individuals:indicating an increase in one or more non-measured serum and also as a form of quality control 1. Glomerulus: This part of the nephron acts as a fil-for analyzer used to measure these electrolytes. ter, maintaining large protein and protein-bound con-consistently abnormal anionic serum gaps from healthy stituents, while most other plasma components of passersby may indicate a problem with the tool. into the filtrate. Concentrations in filtered There are two commonly used methods for calculating the anionic gap. The first equation is Distal tubuleAg2Ƨ ɞ NaƧ Ӿ (ClӾ Ƨ HCO3Ӿ) (Eq. 15-8) Proximal tubular is equivalent to an unmeasurable anionic minus unmeasurable HƧmeasured of the cations in this way:(PO4Ӿ Ƨ 2SO42Ӿ) Ӿ (KƧ Ƨ 2Ca2Ƨ Ƨ Mg2Ƨ) (Eq. 15-9) NaƧ CO2 Ƨ H2O Collection of ƧHCOӾ3 pipes Reference range for Ag2Ƨ using this calculation H2O osmolis 7–16 mmol/L.3 The second calculation method is osmolAg2Ƨ ɞ (NaƧ Ƨ KƧ) Ӿ (ClӾ Ƨ HCO3Ӿ) (Eq. 15-10) H2O blood with a blood vessel present AVP KƧ or HƧ Has a reference range of 10–20 mmol/l,3 70% NaƧ Ƨ ClӾ with aldosterone present Increased anionic gap may be caused by uremia/renal failure, resulting in PO4Ӿ SO42Ӿ retention; 70% of H2O NaƧ Ƨ H2Oketoacidosis, as seen in cases of starvation or dia-KƧbetes; ethanol, ethylene glycol poisoning, orsalicylate; lactic acidosis; hypernatremia; and the PO43Ӿerror. Low anion gap values are rare, but shypoalbuminiaemia (reduction of unmeasurable anions) or Henlesevere hypercalcemia loop (increase in unmeasurable cations) may be seen. osmolELECTROLYTES AND RENAL FUNCTION FIGURE 15-10. Summary of electrolytes in renal tubules. The kidney is central to the regulation and preservation ofelectrolytes in the body. An overview of kidney structure can be found in Figure 15-10 and Chapter 26. The following is the A382 PART 2 ■ CLINICAL CORRELATION AND ANALYTICAL PROCEDURES CASE STUDY 15-5A A 15-year-old girl in a coma was referred to the Department of Questions by her parents. He has dia-betes and has been insulin dependent for 7 years. 1. What is the diagnosis? Her parents said that in the 2nd and 2nd edi- Calculate the anionic difference. What is the cause of the pain and that their daughter was often too anion gap to result in this patient?busy to give her insulin injections. The results of the laboratories obtained on admission are given in the case of the 3rd Why is chloride and hydrogenone reduced? WhatStudy Table 15-5.1. is the importance of increased potassium value? 4. What is the meaning of plasma osmolality? CASE STUDY TABLE 15-5.1 LABORATORY RESULTS Blood conduction NaƧ REFERENCE RESULT RANGE KƧArterial blood ClӾ 145 mmol/l 136–145 mmol/LUrine HCO3Ӿ 5.8 mmol/l 3.4–5.0 mmol/l Glucose 3.4 -5.0 mmol/l Glucose mmol/l 98-107 mmol/l Urea nitrogen 8 mmol/l 22-29 mmol/l Creatinine 1050 mg/lát 70-110 mg/ol Lactate 35 mg/7-18 mg/lát Osmolality 1.3 mg/0.5 -1.3 mg/ol 5 mmol/l 0.5-2.2 mmol/l pH 385 mOsmol/kg 275-295 mOsmol/kg pO2 pCO2 2 7.7.m 11 7,35–7,45 98 mm Hg 83-100 mm Hg Glucose 20 mm Hg 35-45 mm Hg Ketones Normal negative 4Ƨ Negative 4Ƨ plasma should be approximately equal to ECF with stimulated aldosterone , NaƧ is reabsorb in exchange for KƧ in distal tubules. (HƧ com-out protein. petes with KƧ for this exchange.) E. ClӾ is partially reabsorbed by passive transport in2. Renal tubules: proximal tubules along the concentration of gradi-a. Phosphate reabsorption is inhibited by PTH and walnut-NaƧ. F. KƧ is reabsorbed by two mechanisms: increased by 1,25-dihydroxycholecalciferol. Active reabsorption in the proximal tubule al-PO4 excretion is stimulated by calcitonin. most completely saves KƧ. B. Ca2Ƨ is reabsorbed under the influence of PTH and Exchange with NaƧ is stimulated by aldos-1,25-dihydroxycholecalciferol. Calcitonin stimu-teron. HƧ competes KƧ this exchange. late excretion of Ca2Ƨ. C. Mg2Ƨ reabsorption occurs predominantly in dense as-g. Bicarbonate recovers from a glomerular fil-track and is converted to CO2, HƧ excreted in the centered limb of the Henle loop. Urine. D. Sodium reabsorption may occur through three Henle loops: With normal AVP function, the crea mechanisms: Approximately 70% of the NaƧ in the filtrate is ates osmotic which allows for an increase or decrease in water reabsorbation in proximal tubular isoo-osmotic sorption in response to reabsorption. However, it is limited by the availability of changes in body fluids in osmolality. ability ClӾ maintain electrical neutrality. Collection channels: Even under the influence of AVP, this NaƧ is reabsorbed in exchange for HƧ. This re-effect is associated with HCO3Ӿ and depends on the car where the final water excretion treatment is made. bonic anhydrase. CHAPTER 15 ■ ELECTROLYTES 383REFERENCE 13. Gennari FJ. Hypokalaemia. N Engl J Med 1998;339:451-458. 14 Burtis CA, Ashwood ER, Bruns DE, eds. Tietz basics 1. Rose BD, ed. Clinical physiology of acid-beasic and electrolytes. 5. ed. 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Laessig, JRoohbnerJt.aAAn.cyMartindale CHAPTER WARP■ DEFINITIONS: ACID, BASE, BUFFER ■ MEASUREMENT■ ACID-BASE BALANCE Spectrophotometric (Cooximeter) Determination of oxygen saturation Maintenance of HƧ blood gas analysers: pH, pCO2 HƧ pO2 Damping Systems: PO2 Measurement Control of Acid-Base Balance: Lungs PH and pCO2 and Kidney Types of Electrochemical Sensors■ ACID-BASE HOMEOSTASIS Optical Sensors Two-CarbonAtion Damping System and Calibration of Henderson-Hasselbalch Equation Calculated Acid-Base Parameters Failures : Acidosis and correction of alkalosis to temperature■ OXYGEN AND GAS EXCHANGE Oxygen and carbon dioxide ■ QUALITY ASSURANCE Transport Oxygen Pre-analytical aspects Quantities associated with the evaluation of analytical evaluations of the patient: Quality control and expertise in oxygen status Testing interpretation of haemoglobin and oxygen dissociation Results ■ REFERENCES An important aspect of clinical biochemicals is infor-Acid is a substance, which can produce hydrogen ion mation on the patient's acid-ase balance and blood gas (HƧ) or hydronium ion when dissolved in water. Basehomeostasis. These data are often used to evaluate patients is a substance that can yield hydroxyl ions (OHӾ). In life-threatening situations. Since the test parameters are interconnected, test sites are expected to panels the relative strength of acids and foundations, their ability to dis-of tests often supplemented by calculated parame-ters. Focusing on only one test result can be misleading. sociate in water are described by their dissociation con- This chapter deals with the exchange of gases, carbon tents (also ionization constant value K). Tables can bedioxide and oxygen, along with the mecha-nisms of the body to maintain acid-based balance. Interpretation is found in most biochemical texts. PK, defined asof the data, from the measurement of pH and other blood gases pa-rameters, and techniques and instrumentation used negative log ionization constants, is also described in these measurements. Pre-analysis confusion – sampling and manipulation – that pH is addressed, in which protoned and unprotoned forms significantly affect the quality of test results. Quality assurance approaches to blood gas analysis are also present at the same concentrations. Strong acids are presented. pK values are less than 3.0, while strong bases haveDEFINITIONS: ACID, BASE, BUFFER pK values greater than 9.0. In the case of acids, increasing the debate on the balance between pHA on acid-bead requires a review of the sev-eral basic concepts-acid, base, buffer, pH, and pK-a above pK cause the acid to separate and give principles of equilibrium and the law of mass action HƧ. At bases, lowering the pH below pK will cause the base to OHӾ. Many species have more than one pK, which means they can accept or donate more than one HƧ. A buffer solution, a combination of weak acid or weak base and its salt, is a system that resists pH changes. The effectiveness of the buffer depends on the pK of the buffer system and the pH of the environment in which it is located. In plasma, the bicarbonate-carbonic system384CHAPTER 16 ■ BLOOD GASES, pH and TL SYSTEMS is 385acid, with pK 6.1, one of the main carbonic acids (H2CO3) and its saline or conjugate base, bi-buffer solutions. carbonate (HCO3Ӿ) for a bicarbonate and carbonic acid dampening system. H2CO3 is a weak acid because it is notH2CO3 ↔ HCO3Ӿ Ƨ HƧ (Eq. 16-1) completely separated into HƧ and HCO3Ӿ. (Conversely, bicarbonate of strong acids, such as HCl, completely dissociates into HƧ and ClӾ in solution.) When the acid is added to the bicarbonate acid system, HCO3Ӿ will com- The blood plasma pH reference value is 7.40. bine with HƧ acid to form H2CO3. When aWeisberg gave an example to demonstrate an effective base, H2CO3 is combined with a group of OHӾ blood buffers.1 If a pH of 100 ml is distilled to produce H2O and HCO3Ӿ. In both cases, less water is 7,35 and one drop of 0,05 mol/l HCl is added, the pH change would result from the addition of acid or the pH is changed to 7,00. To change 100 ml of normal base to a non-used solution, blood from a pH of 7,35 to a pH of 7,00 is required, approximately 25 ml of 0.05 mol/l HCl. With 5.5 l of blood Although the bicarbonate acid system has an average body, more than 1300 ml of HCl would have a low damping capacity, there is still an important buffer that is needed for the same pH change. three reasons: (1) H2CO3 is divided into CO2 and H2O, allowing the elimination of CO2 by the lungs and HƧ asACID-BASE BALANCE; (2) changes in CO2 change the ventilation rate (or maintenance of H٪. ratory); and (3) the HCO3Ӿ may be altered by the kidneys. In addition, this imme-normal concentration of HƧ in the extracellular diatela calculates the effects of solid non-volatile acids (HƧAӾ) by binding a disociated hydrogen ion (HƧAӾbody fluid ranges from 36-44 nmol/l (pH, 7.34-7.44); Ƨ HCO3Ӿ ɞ H2CO3 Ƨ AӾ). The resulting H2CO3 then dissociates, and HƧ neutralized bufferinghowever, through metabolism, the body produces a large capacity of hemoglobin. Figure 16-1 shows the mutually larger quantities of HƧ. Through the excellent mechanisms of hemoglobin in red blood cells and HƧ from the buffer system bicarbonates.which include the lungs and kidneys, the body controls and HƧ to preserve the pH of the homeostasis. Other buffers are also important. A phosphateHƧ outside this range causes changes in the damping system (HPO4Ӿ2 Ӿ H2PO4Ӿ) playing a role in plasma and red blood cells and contributes to ex-rates of chemical reactions in the cell and affects the change in the satin ion in the urine filtrate HƧ. Plasma proteins, especially the imidazole groups of histidine, also have metabolic processes of the body and can lead to the development of an important damping system in plasma. Most circu-lating proteins have a pure negative charge and are capa-changes in consciousness, neuromuscular irritability, ble bonds HƧ.tetans, coma, and death. The lungs and kidneys play an important role in the regu-Logarithmic pH scale expressing the HƧ of blood pH. The interrelation of lungs and kidneys in maintaining the pH is shown Henderson-(c is the concentration): Hasselbalch equation (Eq. 16-4). The numerator (HCO3Ӿ) indicates renal function, while the name-pH ɞ log 1 ɞ Ӿlog cHƧ (Eq. 16-2) nator (pCO2, which represents H2CO3) indicates cHƧ function. The lungs regulate the pH by retention or elimination of CO2 by changing the speed and volume the reference value of the arterial pH of the blood is 7.40 and is ventilation. The kidneys regulate the pH by acid excretion, corresponding HƧ a concentration of 40 nmol/l. Because primarily in ammonium ions, and reclamationpH is negative log cHƧ, increasing HƧ con-HCO3Ӿ from glomerular filtrate.centration reduces pH, while reducing HƧ Regulating Acid-Base Balance: Lungsconcentration increases pH. PH under reference and renal range (Ↄ7.34) is referred to as acidosis, while APH above reference range (Ͼ7.44) is referred to as carbon dioxide, the final product of most aerobic meta-bolic processes, easily diffuse from tissue where there is is isolosis. Technically, suffix-osis refers to the process produced and into plasma and red blood cells in sur-rounding capillaries. In plasma, a small amount of CO2 is in the body; suffix -emia refers to the corresponding physically dissolved or in combination with proteins to shape the condition in the blood (-osis is the cause of -emia). Arterial pH is controlled by systems that regulate the production and retention of acids and bases. These include bumpers, respiratory center and lungs and kidneys. Damping Systems: Regulating H٪.The body's first line of defense against extreme changes in HƧ is the dampening systems present in allbody fluids. All buffer solutions consist of weak acid such as 386 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL PROCEDURESLungs Venous/Arterial peripheral kidneys are also able to secrete variable amounts of TissueHCO3Ӿ acid circulation or base, making them an important player in O2 HHb HCO3Ӿ and venous O2 by regulating acid-base balances. The kidney's roleHƧ HƧ maintenance of acid-base homeostasis is to HCO3Ӿ from glomerular filtrate. Without this recla- H2CO3 H2CO3 mation, loss of HCO3Ӿ urine would lead to co2 Ƨ H2O arterial excessive acid gain in the blood. The main place for exhalation of H2O HCO3Ӿ of reclamation are proximal tubules (Fig. 16-2). CO2 Glomerular filtrate contains essentially the same HCO3Ӿ as plasma. This process is not directly generated by the transport HCO3Ӿ through the tubular membrane to from the blood. Instead, sodium (NaƧ) in the glomerular filament line is replaced by a HƧ in a tubular cell. Metabolism HƧ combined with HCO3Ӿ in filtrate to form H2CO3, which is reduced to H2O and CO2 carbonic an-FIGURE 16-1. Correlation between hydrocarbonate and hemoglobin hydrate. CO2 easily dissipates into tubular and sweat systems. responds with H2O to H2CO3 reform and then HCO3Ӿ, which is reabsorbed into the blood together with the compoundsssodium.carbamino. Most CO2 is combined with alkalotic conditions, the kidneys excreted HCO3ӾH2O form of H2CO3, which is rapidly dispersed into HƧ to compensate for increased blood pH. Replacement and HCO3Ӿ (Fig. 16-1). The reaction is accelerated HƧ and NaƧ suggests, in part, why doctors have an enzyme in carbonate anhydrase found in red blood cell meme-brane. Dissociation of H2CO3 causes HCO3Ӿ con-order pH and blood gases together, along with elec-centration to increase in red blood cells and disperse into troy (NaƧ, KƧ, and ClӾ), to assess patient-plasma. To maintain electroneutrality (the same number (reabsorption or reclamation refers to the process of reof positive and negatively charged ions on each side of the blood entry. Secretion or excretion of tubular cell membrane), chloride diffuse into the cell. These cells concentrate or remove substances from the filtrate.is known as chloride displacement. Plasma proteins and plasma These reactions determine the pH of urine, as well as combine it with HƧ to maintain a stable pH. blood pH.) In the lungs, the process is reversed. Inspired by O2 dif-fuses from alveol into the blood and is bound to under normal conditions, the body produces nethemoglobin, forming oxyhemoglobin (O2Hb). The excess HƧ (50-100 mmol/l) of HƧ acid (HƧ) each day, which had to be transmitted to (reduced) haemoglobin in the blood blood ve-nous, is released into the recombinal with HCO3Ӿ to exclude the form by the kidneys. Because the minimum urineH2CO3, which dissociates into H2O and CO2. CO2diffuses into alveol and is eliminated venti-pH is approximately 4.5, the kidney secretes a little non-buffered HƧ. The rest of the urinary tract HƧ for the rest of the urinary tract. The net effect of the interaction of the two systems with diphase phosphate (HPO4ɞ) and HƧ-ling dampening systems (NH3) is minimal change in concentration- and is excreted as dihydrogen phosphate (H2PO4Ӿ) and ammonium (NH4Ƨ). The HPO4ɞ fortion between venous and arterial circulation. When combined with HƧ quite constant; therefore daily excretion of HƧ urine largely depends on the amount of lungs do not remove CO2 at the rate of its production NH4Ƨ form. Since renal tubular cells are able (due to reduced ventilation or disease), it is generated by NH3 from glutamine and other amino acids,accumulating in the blood, causing an increase in HƧ con-concentration of NH3 to increase in response to reduced pH. centre of blood. However, if the removal of CO2 is faster than pro-duction (hyperventilation), the concentration of HƧ will various factors affect the reabsorption of HCO3Ӿ. When blood or plasma HCO3Ӿ is higher than normal. As a result, ventilation affects the pH of 26-30 mmol/l, HCO3Ӿ is excluded. Blood is unlikely. A change in HƧ blood concentration means that plasma exceeds HCO3Ӿ 30 mmol/l unless these excretory abilities fail (e.g. this is the result of non-respiratory disruption, causes renal failure). However, a common exception to this respiratory center to respond by changing the rate out is compensatory retention HCO3Ӿ for chronic hypercarbia, as seen with chronic lung disease.tilation in an effort to restore blood pH to normal. The HCO3Ӿ may increase if excessivefuses, by reacting within seconds, along with the amount of lactate, acetate, or HCO3Ӿ is intravenously infused. It also can increase, if oversupply systems, provide the first line of defense to the loss of chloride without compensation (as there is a change in the acid-based state. CHAPTER 16 ■ BLOOD GASES, pH, A DAMPING SYSTEMS 387 Proximal and distal renal tubes and/or collection of blood vessel cells LumenO2 O2 + substrates CO2 + H2OH2O H2OCO2 PCO2 PC2 PC2 PC2 C-A Na+ + HCO3Ӿ CO2 + H2O H2CO3 Na+ H+ + + HCO3Ӿ + HPO4Ӿ Na+Glutamine Glutamine Na+ + ClӾ For +glutamic acid GlutaminaseHCO3Ӿ + K+ glutamic acid + NH3 + SO4Ӿ Na+ K+ Na+ + HCO3[A] NaHCO3 HCO3Ӿ Na+ + HCO3ӾNa+ + HCO3Ӿ H+ H2CO3 CO2 CO2 CO2 + H2O[B]Na+ + HCO3Ӿ NaHCO3 HCO3Ӿ Na+ + HPO4Ӿ H + Na+ + H2PO4Ӿ[C] 2NaHCO3 2HCO3Ӿ Na + Na + HCO3Ӿ + SO4Ӿ 2H + Na + HCO3Ӿ 2NH3 Na + 2H + 2H + 2N H3 NH4+ + SO4Ӿ NH4+[D] NaHCO3 HCOӾ3 Na+ + ClӾNa+ + HCO3Ӿ K+ K+ + ClӾ5Na+ + 5HCO3Ӿ CO2 + H2O Na+ + H2PO4Ӿ NH4+ + SO4Ӿ NH4+ K+ + ClӾFIGURE 16-2. Reabsorption of bicarbonate by proxial tubule. C–A, carbonic anhydrase.388 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL PROCEDURESSweeping, vomiting or prolonged nasogastral suction) Henderson-Hasselbalch equation expresses acidity because HCO3Ӿ retains tubular based on relationships in the mathematical formula:to preserve electroneutrality. pH ɞ pK啦 Ƨ log cAӾ (Eq. 16-3) Several factors can lead to a decrease in HCO3Ӿ levels. cH 〈Largests of diureli, regardless of the mechanism of action, prefer the excretion of HCO3Ӿ. Reduced reabsorption, in which the AӾ is HCO3Ӿ proton acceptor or base (e.g. HCO3Ӿ), also occurs under conditions in which the excess-HA proton donor or weak acid (e.g. H2CO3) is HCO3Ӿ a zealous loss of cation. In the kidneys (such as pK' is a pH at which there is the same concentration of chronic nephritis or infection), HCO3Ӿ reabsorption of protonated and unprotoned species. Knowing that everyone can be disturbed. of the three variables allows the calculation of the fourth. ASSESSMENT OF HOMEOSTASIS ACIDOBASE In plasma and at body temperature (37°C), the bicarbonate control system pK 1 of the buffer system containing bicarbonate is 6.1. The equilibrium of the Henderson and Hasselbalch equations between H2CO3 and CO2 in plasma is approximately 1:800. The concentration of H2CO3V assessment of homeostasis acidobase, the component is proportional to the partial pressure exerted by the bicarbonate damping system is measured and the calcu-dissolved CO2. In plasma at 37 °C, the value was fortized. Conclusions can be drawn from the data regarding the combination of the solubility constant for pCO2 with other buffers and systems that regulate the pro- and factor for converting mm Hg into millimole perduction, retention and excretion of acids and bases. For the liter is 0,0307 mmol и LӾ1и mm HgӾ1. Temperature bicarbonate damping system, dissolved CO2 and solvent effect on the constant. If any of them (dCO2) is equilibrium with CO2 gas, which may be changes, the solubility constant will also change. They're both burnt out by the lungs. Therefore, the pH of bicarbonates and pCO2 are measured in the analysis of blood gas, the buffering system is referred to as the open system and the pKA is constant; therefore, HCO3Ӿ bedCO2, which is controlled by the lungs, is respiratory calculated:component. The lungs are rapidly involved in regulating blood pH through hypoventilation or hyperventilation. pH ɞ pK′ Ƨ log cHCO3Ӿ (Eq. 16-4)Mainly kidney, non-respiratory or previously 0,0307 ϒ PCO2known as metabolic component, control of bikar-bonat concentration. In health, when the kidneys and lungs function properly, the ratio of 20:1 HCO3Ӿ to H2CO3 will be maintained case study 16-1 (resulting in a pH of 7,40). This is illus- trated by replacing normal values (Table 16-1) for HCO3Ӿ and pCO2 into the previous equation:A 50-year-old man came to the emergency room 24 mmol/l after returning from a foreign trip. Its symptoms (0.0307 mmol/l Ӿ mm Hg) ϒ 40 mm Hgincluded persistent diarrhea (in the last 3 days) arapid breathing (tachypnoea). Blood gases were ɞ 24 ɞ 20 (Eq. 16-5) with the following results: 1,2 1pH 7,21 Addition of logs 20 (1.3) to pK' bicarbonate pCO2 19 mm Hg the system provides a normal pH of 7,40 (7,40 ɞ 6,1 Ƨ 1,3).pO2 96 mm HgHCO3Ӿ 7 mmol/l TABLE 16-1 ARTERIAL BLOOD GAS2 96 % (calculated) (reference range, Ͼ95%) REFERENCE RANGE AT 37°CS pH 7,35 -7,451. What is the patient's acid-based condition? pCO2 (mm Hg) 35-452. Why is HCO3Ӿ so low? HCO3Ӿ (mmol/l) 22-263. Why does the patient breathe quickly? Total CO2 content (mmol/l) 23-27 pO2 (mmol/l) 80-110 SO2 (%) O2Hb (%) Ͼ95 Ͼ95CHAPTER 16 ■ BLOOD GASES, pH and buffer Disease-based disorders: Acidosis and alkalosis CASE STUDY 16-2Acid-base disorders result from various pathological 80-year-old woman fell on ice and broken conditions. When the pH of the blood is less than the reference of her femur. After a few hours when she arrived, it was called acidemia, which reflects excess acid, or in the emergency room, was anxious, HƧ concentration. PH greater than the reference range gasped, and complained of severe chest pain andis called alkalemy, or excess base. A disorder caused by not being able to breathe. Her pulse was rapidventilation dysfunction (change in pCO2, respectively- (tachycardia), as her respiratory rate (tachyp-ratory component) was called primary respiratory acidosis nea). Blood gases were pumped and brought by the theorist alkalosis. A disorder resulting from a change in bi- the following results: the level of carbonate (renal or metabolic function) refers to the term non-respirational disorder. Mixed respiratory and nonres-pH 7,31piratory disorders occasionally arise from more than one pCO2 27 mm Hgpathological process and represent the most serious pO2 62 mm Hgmedical conditions as a substitute for primary dis-HCO3Ӿ 12 mmol / Lorder failures. SO2 78% (calculated) (reference range, Ͼ95%) Since the body's cellular and metabolic activities are QuestionspH dependent, the body tries to restore acid-base home-1. What is the patient's condition based on acid?ostasis whenever an imbalance occurs. This action shall, by 2 December 2006, be taken into account for the Why is HCO3Ӿ so low?the body is called compensation-the body reaches 3. What clinically caused the imbalance of acid and base?it by changing a factor that is not primarily influenced by the pathological process. For example, if the imbalance of the body compensates for non-respiratory acidosisnonrespiratory origin, the body compensates by changing through hyperventilation, which is an increase in ventilation. For respiratory compote disorders- rate or depth of breathing. By blowing off CO2, then, the kidneys compensate for selective excretion or the base-to-acid ratio returns to normal. Secondary reabsorbating anions and cations. The lungs can compen- compensation occurs when the original organ (thesate immediately, but the answer is short-term and often the kidney, in this case) begins to correct the ratio re-incomplete. The kidneys are slower to react (2-4 days), taining bicarbonate.however, but the response is long-term and potentiallycomplete. Fully compensated means that the pH has re-primary respiratory acidosis resulting from a decrease in the inverted to the normal range (the ratio of 20:1 was alveolar ventilation (hypoventilation), causing a decrease in the effidy); partially compensated means that the pH is ap-elimination of CO2 in the lungs: proaching normally. While compensation can successfully reverse the ratio to normal 20:1, the primary abnor-pH κ NcHCO3Ӿ Ͼ 20 (Eq. 16-7)malita is not corrected. ↑(0.0307 ϒ PCO2) 1 Acidosis may be caused by primary Breathing is regulated in the marrow of the brain.abnormalities or primary respiratory problems. In Chemoreceptors present in the aortic arc and carothermal non-respiratory acidosis, bi-sinus response to HƧ (pH), O2 and CO2 levels in carbonate (Ↄ24 mmol/l) decreases, resulting in a decrease in pH of both blood and cerebrosbrosime fluid. There are several situa-result ratios for non-respiratory to respiratory diseases, including many lung diseases in which CO2 is not effectively removed from the blood in the Henderson-Hasselbalch equation. In some patients, who have less than 20:1: with chronic obstructive pulmonary disease (COPD), for example, destructive changes in the airways and alveolarpH κ ♥cHCO3Ӿ Ↄ 20 (Eq. 16-6) walls increase the size of the alveolar air space, while N(0.0307 ϒ PCO2) 1 resulting reduction in the surface area of the lungs available for gas exchange. As a result, CO2 is retained if N is normal and κ indicates proportional. causing chronic hypercarbio (increased pCO2). When non-respiratory acidosis can be caused by direct bronchopneumony, gas exchange is made more difficult due to secretions, white blood cells, bacteria, and fibrinadministration of acid-producing substances, as in alveols. Hypoventilation caused by drugs such as ammonium chloride or calcium chloride, or excessive production of organic acids, as seen in diabetic ketoaci-dosis and starvation. Non-respiratory acidosis was also observed with reduced acid excretion, as in renal tubular aci-dosis and excessive loss of diar-rhea bicarbonate or bile duct drainage, pancreatic or intestinalfistula.390 PART 2 ■ CLINICAL CORRELATIONS AND ANALYTICAL PROCEDURES CASE STUDY 16-3 barbiturates, morphine or alcohol increase blood The 24-year-old postgraduate student was brought to pCO2 levels, as well as mechanical obstruction or asphyxia-emergency department in a coma after tion (strangulation or aspiration). A reduced heart attack found unconscious in his room. A bottle ofsecobarbital was on his bed stall. He did not re-give, as seen with congestive heart failure, alsoresponded to painful stimuli, his breathing was barely perceptible, and his pulse was weak. Blood gases will result in less blood presented to the lungs for gases being pumped, and yielded the following results: exchange, and therefore increased pCO2.pH 7.10 In primary respiratory acidosis, compensation of 70 mm HgpO2 58 mm Hg occurs through non-respiratory processes. 20 mmol/l kidneysHCO3Ӿ excretion HƧ and increase in reclamation-O2Hb by 80% (reference range, Ͼ95%) and HCO3Ӿ. Although renal compensation is gins-gins immediately, it takes days to weeks for maximum compensation issues to occur. When HCO3Ӿ in the blood increases due to action action disease of the kidneys, the1. What is the patient's acid-based condition? the base-acid ratio changes and the pH is returned2. What caused the deep hypoventilation? towards normal.3. As soon as the respiratory component returns to normal, what is expected in the patient As with acidosis, alkalosis can result from nonrespira-acid-base status? respiratory causes. Primary non-respiratory alkaline alkalosis is the result of an increase in HCO3Ӿ, causing an increase in the non-respiratory component and pH: pH κ ↑cHCO3Ӿ Ͼ 20 (Eq. 16-8) N(0.0307 ϒ PCO2) 1 CASE STUDY 16-4 This condition may result from excessive administration-A 24-year-old Himalayan man was admitted to graduate-tion sodium bicarbonate or ingested bicar-uate school in the United States. Before leaving home, he had an extensive physical exam that in-bonate-producing salts such as lactate, citrate, orcluded various blood tests. When medical staff at an American university reviewed his medical records, it was acetate. Excessive loss of acid by vomiting, nasogas-was noted that all test results were normal except for theHCO3Ӿ, which was 15 mmol / l (reference range, tric suction, or long-term use of diutrics by augment22-26 mmol / l). This HCO3Ӿ carried out separately in renal excretion and may HƧ an apparent increase in serum sample. She wasn't part of the blood gas panel. HCO3Ӿ. The body reacts by squeezing the airwaysTo exclude non-respiratory acidosis, university center. The resulting hypoventilation increases the reten-doctor wanted HCO3Ӿ repeat. The repeat value was 24 mmol/l. co2. Primary respiratory alkalosis from increased speed Questions alveolar ventilation causes excessive elimination of CO21. Was the original assumption of a non-respiratory lung: acidosis valid? pH κ NcHCO3Ӿ Ͼ 20 (Eq. 16-9)2. What would be a better description of acid- ♥(0.0307 ϒ PCO2) 1 underlying failure? Causes of respiratory alkalosis include hypox-3. Why, in repeated testing, the HCO3Ӿ return emia; chemical stimulation of the respiratory center to normal? medicinal products such as salicylates; increase in ambient temperature- mental temperature; fever; hysteria (hyperventilation); pulmonary embolism; pulmonary fibrosis. The kidneys compensate by excretion HCO3Ӿ urine and re-HƧ into the blood. Popular treatment of hys-teric hyperventilation, breathing into a paper bag, is self-explaining. OXYGEN AND GAS EXCHANGE Oxygen and carbon dioxide The role of oxygen in metabolism is crucial for life. In cellular mitochondria, electron pairs from NADH and FADH2 oxidation are transferred to molecular oxygen, causing the release of energy used for synthesizing ATP474 PART 3 ■ EVALUATION OF ORGAN SYSTEM FUNCTIONSCate bochromocytoma is repeated in 14% (48% of which were malignant). In patients without relapse (86%),After diagnosis of pheochromocytoma, all patients experienced HTN-free survival was only 74% after 5 years and 45% by surgical candidates after appropriate medical prepa- after 10 years (family history of HTN and increasing age therapy. Removal is high risk The biggest were risk factors). In 90 patients, the 20-year total scabbing series (147 patients with pheochromocytoma) at one cause-specific survival rate was 80% regardless of institution (1975-1997), the overall perioperative mortality of pheochromocytoma was 2.20 Long-term monitoring and morbidity rates were 2.4%. even those who are appar-preoperative HTN, tumors with high secretion, or those untreated.dergoing repeated intervention were at highest risk of complications. Catecholamines fall into normal as part of ADRENAL INCIDENTALOMA 1 week resection. Due to the very frequent use of CT, MRI and ultrasound although perioperative ␣-blockade is widely recom-imaging of the abdomen for reasons not related to adre-mended, fewer perioperative complications were ob-nal glands, many adrenal glands, usually more than 1 served in those not listed ␣-blockers (studies with a diameter of 113 cm, are found by the way, and therefore suggest efemoochocytomy of patients undergoing resection). Incidentalomas. Autopsy studies indicate the frequency of the second regimen proposed by the Cleveland Clinic re-adenomas of the adrenal glands at about 6%, and the prevalence in-sulted in the successful use of calcium channel blocker for folds with age.24,25 Although most of these lesions court pressure control. inoperable and benign, all should be evaluated for ma-lignancy or hypersecretion.26 Surgery is considered if there is cancer and prognosis of the adrenal glands; autonomously secretes cor-ticole, aldosterone or catecholamines; is 4 cm or higher inSurgical removal of focromocytoma is pri-diameter; or growing.27mary therapy; however, excision does not lead to a long-term cure for pheochromocytoma, or Figure 20-19 illustrates a brief preHTN function screen (even in patients with benign tumors). Adrenal patients who assess the function of all adrenal glands with familial pheochromocytomas are more likely to layer and serve as a clinical summary.have relapses. In one series of 114 patients, random evaluation of function Screening tests Negative results Clinical propertiesPheochromocytoma and HTN 24-hour urine metanephrines (paroxysmal) with spells Ↄ 1 g or 5.5 mol/ mg creatine (sweating, HA, or palcking) Cushing's syndrome HTN, obesity (truncal) 1 mg bedtime dexamethasonePrimary aldosteronism weakness 8 am cortisol Ↄ 3.6 g/0L, or 24-hour urine without cortisol normal HTN, hypokaemia, weakness of potassium in serum, if low urine KƧ excretion (Ↄ30 mEq)Adrenocarcinoma Virilization (Ƨ above) Plasma renin:al ratio Ↄ 30 Plasma DHEAS (Ↄ 9.2 mol/l) Urine 17-ketosteroid Ↄ 20 mgFIGURE 20-19. A concise functional screen for the adrenal glands. CHAPTER 20 ■ ADRENAL FUNCTION 475 CASE STUDIESHypertension is common and most often presented as an independent medical condition. Occasionally, hypertension is the result of an underlying disease and requires other treatment. Because adrenal function is crit-ical for (1) blood (2) Potassium and (3) glucose homeostasis, adrenal etiology should be considered in all patients with blood pressure problems accompanied by electrolyte abnormalities, unexplained weight change, inability to thrive, inappropriate virilisation and anxiety periods. Eight different clinical scenarios are listed below. Each presentation is associated with a different diagnosis and treatment. Discussions about adrenal causes, diagnoses, and treatments for everyone can be found in the chapter. Each numbered case study is complemented by the following introductory statement: a 22-year-old woman (previously admitted, currently not taking medication, negative medical history) poses with . . . CASE STUDY 20-1 CASE STUDY 20-4. . . hypertension with weakness and hypokaemia. hypertension, with periods of panic attacks andute the patient also has high urine excrement potassium-hot flashes. She also poses with headaches, hyper-tion without diuretics. glycaemia, hyperthyroidism and gastrointestinal problems. Question 1. What's the diagnosis? Question 1. What's the diagnosis? CASE STUDY 20-2 CASE STUDY 20-5. . . hypertension with weakness and rapid onset . . hypertension, with virilization. This youth. This patient also exhibits central fat pads, a woman posing with irregular menses diagnosed with a buffalo hump, a plethmetic amount, thin skin, purple stretch marks, easily with polycystic ovary syndrome. It has border-bruising, osteoporosis, hyperglycemia/insulin resist-line low cortisol and increased 17-OH progesterone.ance, and recurrent infections. Question Doubt 1. What's the diagnosis?1. What's the diagnosis? CASE STUDY 20-3 CASE STUDY 20-6. . . hypertension, with weakness, irregular menstruation, . . hypertension and hyperkalymia. He has normal and hypokalymia. Her young age, borderline low kidney function (low urine potassiumƧ) and metabolic cortisol, and low androgens are also significant. Acidosis. Question Question 1. What's the diagnosis? 1. What is the diagnosis?476 PART 3 ■

EVALUATION OF ORGAN SYSTEM FUNCTIONS CASE STUDY 20-7 CASE STUDY 20-8. . . hypotension, failing, weight loss, and . . new virilization and hirsutism. Laboratory re weakness. Her lab results show hyper-sults show increased IGF-I (insulin growth factor I), caemia, fasting hypoglycemia, and metabolic DHEA (S), and testosterone levels. She's a healthacidosis. food enthusiast who experiments with nutritional supplements. Question Question 1. What's the diagnosis? 1. What is the diagnosis? REFERENCES 16. Kaplan N.Pheochromocytoma. In: Pine J, ed. Clinical hypertensive. Baltimore, Md.: Lippincott Williams & Wilkins, 1998: 1. Kacsoh D. Adrenal Glands. Dolan J, ed. Endocrin 345-365. Physiology. New York: McGraw-Hill, 2000:360-448. Sowers K. Pheochromocytomas. In: Lavin M, ed. Manual en-2. Orth D. Anatomy and development of the adrenal cortex. docrinology and metabolism. Philadelphia, Pa.: Lippincott Acquired from www.UpToDate.com, online 12.1, 12/03. Williams & Wilkins, 3. Miller W. 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London: McGraw-Hill, 2001:337-341.Gonad function CHAPTERMarissa Grotzke 21CHAPTER OUTLINE■ TESTICLES Menstrual cycle Functional anatomy of male reproductive hormone control ovulation tract Pubertal development in female physiology semen menstrual cycle Abnormalities Sexual development disorders and testicular hirsutism Hypofunctional estrogen Replacement therapy Diagnosis of hypogonadism ■ SUGGESTED READING TESTOSTERONE Replacement therapy ■ LINKS Testosterone monitoring Replacement therapy■ OVARIES Functional ovarian anatomy Hormonal production of ovaries TESTICLES tubuli recti; rete testicles; ductuli efferentes testicles; the head, body and tail of the superseasant; and finally, intoThe testicles are paired, ovoid organs that serve double vas deferens. Various secretory products semi-function (1) sperm production and (2) production of nal vezicles and prostate mix with sperm to form the finale of reproductive steroid hormones.1 In embryonic product: sperm. Seminal secretions of vesicles are rich in vi-stage, dominant male sex hormone, testosterone (T), tamin C and fructose, important for the preservation ofpoument in the development and differentiation of the primordial motility of spermatozoa.gonad. After puberty, throughout adulthood, and to the end in old age, testosterone helps with sperm production Testicular physiology maintains secondary sexual characteristics. SpermatogenesisFunctional anatomy of spermatozoa is formed from stem cells called spermatozoa. Male reproductive tract Spermatogonia undergo mitosis and meiosis; eventually, haploid cells transform into a form of mature sperm.2 The testicles are located outside the body, encapsulated with mus- mature sperm has a head, body and tail, allowing the ocular bag. Blood flow is governed by a complex plexus for swimming in order to form a zygote with tearterial and venous blood flow, which together with con-haploid vova. Some sperm are sweating the division of the dartos muscle in the scrotm, regulating that sperm production is uninterrupted and continuous.the temperature of the testicles at 2 °C below the nucleus of the body of Sertoli's cell are polyfunctional cells that adivate during temperasis. This important function is vital for the non-internal development and maturation of spermatozoa.intermittent sperm production. Also encapsulated in the muscular sheath is a spermatic cord that has the ability to re-hormonogenesistract the testicles into the inuital canal in cases of testosterone, the predominant hormone secreted by an endangered injury. The testicles themselves consist of testicles, primarily by two pituitary hormones: two anatomical units: a network of tubulars, known as follicle stimulating hormone (FSH) and luteinizing hor-seminiferous tubes, and interstitium. Tubules mone (LH).3,4 Since these hormones were first de-containing germ cells and Sertoli cells and are responsible for written in women, they are named in reference to the production of tesperm. Sperm move gradually through the menstrual cycle. Both hormones are produced by one 477478 PART 3 ■ EVALUATION OF THE ORGAN SYSTEM FUNCTIONGroup of cells in the pituitary gland called gonadotrophs. FSH is rich in 5␣-reductase, which converts testosterone toacts primarily into germ stem cells and LH acts classy-DHT. Fetal exposure to drugs that block this hormone rily on Leydig cells-located in testicular inster-leads to feminization of male fetus.stitium-that synthesize testosterone. Postnatal developmentHormonal control of testicular function Testicular function is reactivated during puberty after the hypothalamus, located in the brain, creates a hor-period quiescence to produce testosterone, which resultsmone the so-called gonadotropin-releasing hormone (GnRH) in the development of secondary sex hair (face, chest, axilla, pulsating fashion. GnRH is released into the portal hy- and pubis), increased linear skeletal growth, developmental physial system, which in turn determines the production of internal and external genitalia, increased upper body LH and FSH from the pituitary gland. Impaired pulse musculature and the development of larynx and vocal cord generation GnRH leads to insufficient production of LH with deepening of the voice.6-8 Possible mood swings and FSH, leading to hypogonadism.4,5 First, and aggression are side effects that can occurremetrally, a step in testicular steroidogenesis is during puberty. Linear effects of testosterone growth conversion of cholesterol on pregnenolone. This cholera is final, with epiphysal closure, when the genetically deterrent terol is either captured by endocytosis from the blood or reaches a busy height. Hypogonadism during pubertysyntesized in Leydig cells. LH binds to leads to inaccurate closure of growth plates, resulting in an ex-glycoprotein receptor in the cell wall and induces intra-cessive height, long limbs, and inadequate upper and cellular cyclic AMP production, which in turn activates pro-lower body segments. Male secondary sexual characterized by theinkinase A, which catalyses protein phosphorylation. tics can be staged system development designedThose second step induces testosterone synthesis. Testic-Marshall and Tanner (Table 21-1).ular steroidogenesis pathway is similar to the pathway in the adrenal cortex and have the same enzymatic sys-effect on spermatogenesistems. Testosterone is the main androgenic hormone in stimulating Leydig cells induces the production of tes-blood. It is largely bound, with 2%-3% free. About tosterone. acts with FSH, has paracrine50% testosterone is bound to albumin and about 45% is affecting seminal and Sertoli cells inducingbound to sex hormone-binding globulin (SHBG). Spermatogenesis. Exogenous overuse or abuse of testos-concentration binding proteins determines the level of teron, as occurs in some athletes, reduced thetetal testosterone, but not the free testosterone levels of major- high intrathesicular testosterone concentration, leading to a laboratory estimate. Testosterone and inhibin are used to reduce sperm production.two hormones secreted by the testicles that provide control of the hypothalamus and pituitary gary. The effect on secondary sexual effects of testosterone has growth-promoting effects on various tar-testosterone concentrations fluctuating in circadian get tissues. Secondary sexual characteristics that developfashion, reflecting the parallel rhythms of LH and FSHlevels. This fact should be taken into account when interpreting Table 21-1 TANNER staging GENITALserum testosterone levels: the highest level is found at and pubic hair development in men at 8am and correlates with most laboratory normalranges, and the lowest level is found at about 8 p.m. stages of genital developmentThe cellular mechanism of testosterone action 1 PrepubertalTestosterone enters the cell and converts to dihydrotestos-terone (DHT). DHT complexes with intracellular 2 enlargement of the scrotm and testesreceptor proteins and this complex binds to the nuclear receptor, which affects protein synthesis and cell growth. 3 Increased length of the penis, further enlargement of the testiclesPhysiological action of testosteronePrenatal development 4 Enlargement of the testicles, searly penis, and the penis sEarly in development, embryos have a primordial compote-growth of acorns; darkening of the scrotm skin remains of the genitalorgans of both sexes. Primitivegonads will distinguish about the seventh 5 mature genitalia week embryonic stage. Both chorionic gonadotrophins and foetal LH stimulate the production of testosterone by the phases of pubic hair that develop Leydig cells. Exposure to testosterone onwolffian ducts leads to differentiation of various com-1 Lanugo-type hair (prepubertal) ponents of the male genital tract. Sertoli cells produce a regression factor that helps in the regression of 2 dark trailing hair at the base of the female primary genital tract penis. The scnodling skin is 3 Darker trailing hair spreading through the intersection of pubes 4 Terminal hair covering the pubic region, no spread to the medial thighs 5 Mature stage with horizontal distribution of the final hair on the inner thighsAPTER 21 ■ GONADAL FUNCTION 479 during puberty are maintained until late adulthood box 21-1. CAUSES DELAYEDtestosterone.8 The prostate gland gradually increases during puberty, while exposure to the scalp results in regression-zion of the hair follicles (time recession of the hairline). • Delayed puberty or hypogonadism, sLoss secondary sexual should lead to an increased assessment of gonadotrophins (FSH and/or LH) of hypogonadism, as, inter alia, ef-• Klinefelter syndromes lead low testosterone levels to bone loss and • Bilateral gonad failure in the development of osteoporosis in men of all ages. • Primary testicular failure • AnorchiaDisorders of sexual development • Disappearing testicles and testicular hypofunction • Chemotherapy agents • IrradiationPuberal development may be premature (premature) • Traumaor delayed, although development is normal at birth.9,10 • Infection (mumps orchitis)Detailed descriptions of the sequence of hormonal pu-bertal abnormalities of hair, genitals and breasts are • Delayed puberty with normal or low FSHbeyondom range of this text. Differential diagnosis and/or hypogonadism LHof includes a diverse group of disorders • Constitutionally delayed puberty51.52, which affects the testicles and hypothalamus-pituitary reg- • Hypothalamus dysfunction of the testicles listed in box 21-1. Some im- • Disorders important for malnutrition are explained in the following section. • Chronic systemic disease • Severe obesityHypergonadotropic hypogonadism • Central nervous system tumoursHypergonadotropic hypogonadism includes group • Hypopituitarism of disorders characterised by low testosterone, increased • PanhypopituitarismFSH or LH and impaired sperm production. • Kallmann syndrome (anosmia, cleft palate and decreased levels of FSH and LH)Klinefelter syndrome • Isolated GH deficiencyKlinefelter syndrome occurs in about 1 in 400 men and • Hyperprolaccitemia (prolactinoma is caused by the presence of another chromosome. Or the drug induced)the most common karyotype is 47.XY.11 Men with this disor- • Hypothyroidism have small (Ↄ2.5 cm) solid testicles. Gynecomastia (enlargement of the male breast) may also be present at • various 52-year-olds of diagnosis. Due to reduced testicular production • Prader-Willi syndrome, levels of FSH and LH are elevated. These men also • Laurence-Moon syndromes have azoospermia and the resulting sterility. Men with mo- • LEOPARD syndromesaicism can produce some sperm and pregnancy have • Bloom syndromes have been reported in these men. Elevated levels of FSH and • Germ cell neoplasiaLH induced increased aromatase activity, leading to oestrogen levels in pseudohermafrodited men. Men with Klinefelter syndrome • Ataxia-telangiectasiamama may have reduced bone density and breast cancer. • Steroidogenic enzyme defectsThetesicular feminization syndrome 5␣-Reductase DeficiencyTesicular feminization syndrome is the most serious genotype in 5␣-reductase deficiency is XY. The reducing form of androgen resistance syndrome, which leads to a lack of enzyme 5␣-reductase levels leads to testosterone in the target tissue. Due to reduced testosterone levels. Physical development is a testosterone deficient effect, a physical development similar to a female phenotype until puberty, when cleaned phenotype, with fully duced residual enzyme activity sufficiently converts testos-breast and female fat and hair distribution. Most of the terone on dihydrotestosterone, which leads to the development present for the evaluation of primary amenorrhea, on the male phenotype.which becomes a fact of the lack of female internal genitalia. The testicles are often unseeded, and myotonic dystrophyfailure immediately remove these organs results in the malig-myotonic dystrophy being hereditary in autosomal domi-nant transformation. Biochemical evaluation reveals nant fashion and poses with hypogonadism, muscle normal testosterone levels with increased FSH and LH weakness, frontal baldness, diabetes, and muscle dysto-levels. There is no tool or response to the filing. Testicular failure usually accounts for fourth exogenous testosterone. decade of life.480 PART 3 ■ EVALUATION OF ORGAN SYSTEM FUNCTIONSThematic injury and damage to infection, autoimmune pituitary gentritis or granulomatous or post-pubular mumps infection may result in mumps or metastatic disease. Hemochromatosis is a rare cause ofchitis and permanent injury to the testicles. Dysfunction of the pituitary gland damaged the testicles.due to viral orchitis and HIV infection was also transmitted again. Radiation and chemotherapy for cancer can also diagnose Hypogonadismresult in long-term damage. Both clinical and biochemical properties must be metSertoli cell-only syndrome (Fig. 21-1) in order to make a diagnosis of hypogonadism. Sertoli cell-only syndrome is characterized by a lack of testosterone levels having circadian rhythm and timegerm cells. Men present with small testicles, high levels of FSH should be considered. Multiple estimates ofzoospermia, and normal testosterone levels. Testicular free and bound testosterone levels need to be done onbiopsia is the only procedure to confirm this diagnosis. different days before the diagnosis of hypogonadism.18 The difference between primary (disease orhypogonadotropic hypogonadism destruction of the testicles) versus secondary (disease orSymbron disorders hypogonadotropic hy- pit destruction) is relatively simple make.pogonadism is the incidence of low levels of FSH testosterone and/or LH19 are elevated at primary hypogo-levels along with low or inappropriately normal FSH nadism and are inappropriately normal or low with sec- or LH levels. ondary etiology. Pituitary MRI should be performed in sec-ondary hypogonadism in juveniles. Older Indi-Kallmann syndrome viduals often have secondary or tertiary (hypothalamus) Kallmann syndrome is the result of hereditary, X-linked dysfunction due to reduced hypothalamus pulse properties, which manifests itself as hypogonadism during generator frequency, resulting in low or inappropriate symptoms. The frequency of this syndrome is 1 in 10,000 normal levels of FSH and/or LH. Clinical signs and symp-men. Related errors such as anosmia (inability to hypogonadism (e.g. loss of secondary sexualto smell) and midline defects (cleft palate and lips), characteristics, osteoporosis) should be confirmed should alert the doctor to suspect this disorder.12,13 with low testosterone levels, especially when the testicles-Some men also have reddish-green color blindness, con-terelon replacement therapy is considered.genital deafness, or brain dysfunction. Testosterone Replacement TherapyHyperprolactemiaProlactine increase resulting from any cause (drug- Currently available methods of testosterone administrationis-induced or prolactin-producing pituitary tumors) tration in the United States are as follows:may result in hypogonadotrophic hypogonadism.14,15 1. Parenteral testosterone. This is the most widely-utilized Age capable and cost-effective method of administration. TheThere is a gradual reduction in testosterone after 30 years, cypionate and enanthate testosterone esters are with an average decrease of 110 ng/dL every decade. available for intramuscular injection. The maximum level is baltimore longitudinal aging study revealed re-achieved in 72 hours and the effect lasts for a duration of reduced total testosterone levels of 19% at age 60.28% to 1-2 weeks. Weekly administration provides a reduction of 70, and 49% at age 80.16.17 with free lion-peak testosterone and minor fluctuations in normal ranges much lower in these men. Age is also associated with testosterone levels. The usual weights are a 50-100 mg weekly increase in SHBG of approximately 1% per year. Total testicles- or 200–250 mg once every 2 weeks. Testosterone doseterone levels may be normal in aging men, but free should be based on lean body weight, not on the body's (unbound) testosterone levels being more reliable indi-weight, and is best achieved by administering normalcators biochemical reduction. Related dose testosterone function with a small dose of escalation based on reduced secondary sex hair growth, muscle loss on serum testosterone levels measured in the center of be-volume and strength, and loss of bone density are confirmed by two injections. The goal is to maintain this mid-rative evidence suggesting a lack of tissue effects of dose levels in the middle of normal ranges.testosterone. Testosterone deficiency is a constellation of clinical properties of hypogonadism combined with low 2. Transdermal testosterone therapy. This method of ad-serum testosterone levels. The combination of biochem-ministertion provides more physiological levels of testicle-ical and clinical evidence testosterone should prompt the terrine. The patch's permeability is increased to help prevent testosterone replacement in older men. absorption of testosterone through normal skin. Local skin irritation may occur and the use of the patch may be restricted. Pituitary gland DiseaseHeded hypogonadism can follow after pitu-3 injury. Testosterone gel. This hydroalcholic gel preparation isitary due to tumors, surgical trauma, blood vessels applied to non-genital skin once a day. Absorption is a gradual provides blood testosterone levels inCHAPTER 21 ■ GONADAL FUNCTION 481 Poor seminal properties Measurement of testosterone Reduced Normal Testosterone PRL Measurement measures FSH, LH FSH, LHIncreased Normal Increased FSH NormalLH, FSH LH, FSH Normal LH LH, FSH Evaluate hCG Stimulation Not Increased Rate Testosterone Testosterone Seminiferous Congenital Abnormality,Increased TubularLH, FSH Ducal Obstruction Failure Perform Spermatogenic GnRH Stimulation Failure Increased Not Increased LH , FSH LH, FSH Pituitary hypothalamus failureFIGURE 21-1. Clinical diagnostic evaluation of male hypogonadism. normal range for 24 hours. The main concern with a concentration of testosterone that is many times higher than this preparation is the potential transfer to female serum concentrations. If an increase in PSA is observed after partners or children in close contact with the skin. testosterone replacement, prostate evaluation with possi-4. Buccal testosterone. This plastic tablet is placed along the ble biopsy is recommended. Active prostate cancer is a line of gums twice a day. Local discomfort and contraindication to testosterone replacement. need twice a day to tap sometimes to limit use. Ovarian Complications of Testosterone Replacement Are Poly-Cytemia, enlargement of the prostate gland, possible growth- The ovaries are paired organs that, like male genital glands, promote the effect on undiagnosed prostate cancer, perform dual functions gamete (ovum) and steroidworsening obstructive sleep apnea, peripheral edema, hormone production.20-22 Unlike men, during-and gynecomastia. mordial reproductive cells in females typically pre-duce lonely gamete. Ovarian and menstrual events aremonitoring testosterone carefully synchronized complex interplay of hor-replacement therapy mones between the hypothalamus, pituitary gland and ovaries to prepare the uterus for embryo implantation. InProstate-specific antigen (PSA), blood count, and lipid absence of implantation, uterine lining is shed, levels should be controlled 3-6 months after initiation resulting in menses. Routine clinical evaluation of leg edema, deterioration of the length of the menstrual cycle is the time to be-sleep apnea, and prostate enlargement is also recom-tween no two consecutive cycles. Typical duration is named. Pharmacological use of testosterone can also take 28 (↑3) days with average menstrual flow asincreased sperm count by reducing intrathesicular 2-4 days.23,2 4482 PART 3 ■ EVALUATION OF ORGAN SYSTEM FUNCTIONFunctional anatomy ovarian deficiency results in irregular and incomplete devel-opment endometrium.21,29 Ovaries are oval organs, which lie in the pelvic fossa,formed by the posterior and lateral pelvic walls and at-Progesteronetach on the posterior surface of a wide ligament by progesterone is a carbon-21 compound within the teperitoneal fold , otherwise known as steroid family and is produced by the corpus luteum. They are located near the fimbral end of the fallopian tube Progesterone induces secretive activity of these en-tubes, which are associated with the uterine cavity. Dometrial glands that were directed by estrogen, adult ovaries, diameter 2-5 cm in length, weigh the average rate of endometrium for implantation of embryos.14 grams and usually contain 2-4 million primordial follicles- Other effects include thickening of cervical mucus, dial follicles.21,25 These primordial follicles are present in the reduction of uterine contractions and thermogenic childbirth; however, maturation is blocked until puberty. effect, in which basal body temperature rises after the onset of puberty, each ovarian cycle is ovulation. This effect is clinically relevant in the labelling of several primary follicles for the occurrence of ovulation. Progesterone is the dominantmatuation. Typically, all but one of these follicles will be the hormone responsible for the luteal phase, and defi-then atrophy, in a process called the follicular phase. ciency results in failure of implantation em-bryo.21,23–25,27 The only remaining follicle – known as Graafianfollicle – consists of the outer and inner layers (Androgenstheca externa and theca internala, respectively) lining the ovaries produce androgens androstenedione, dehy-central fluid-filled cavities and a layer of cells known as droandrostenedione, testosterone, and dihydrotestos-granulosa layer.21,24-26 Grains of ovum bind teron , all of which are carbon-19 compounds. Excess inside the follicle through cells derived from granu-production of ovarian androgens in women leads to ex-losa cells, called cumulus cells. During the luteal phase of cess hair growth (hirsutism), the loss of a female-characterized ovarian cycle, the Graafian follicle releases its ovarian flows, and-in severe cases—the development of a nasal malein response to ovarian LH stimulation. When secondary sexual features (masculinization or viriliza-ovum extruded, graafian follicle passes plague-tion).30-33 Unlike estrogen, which is not produced in theological change with hypertrophy theca and gran-ovaries after menopause, ovarian androgen synthesis con-ulose cells to become the corpus luteum. This process is well into advanced age.called luteinization. Corpus luteum is rich in choles-terol and acts as a substrate for the continued production of other progesterone and estrogen, preserving endo-inhibins A and B that are produced by the ovaries, aremetrium for conception. If conception or implantation hormones appear that inhibit the production of FSH.25,34 Activin is deficient, the endometrium is a shed and hormone corpus that increases FSH secretion and induces atrophy of the atretic follicle. steroidogenesis. Folliculostatin, relaxin, follicle regula-tory protein, oocyte maturation factor, and meiosis-hormone production of ovaries inducing are hormones that seem to have im-portant, but not clearly characterized, functions. As in the adrenal glands and testicles, steroidogenic pathways and synthetic enzymes are present in menstrual cycleovaries. Cholesterol is either synthesized from acetate oractively transported from low density lipoprotein by convention, the menstrual cycle is considered to be the beginning (LDL) of particles in the blood and then used as a substrate for the first day of menstruation (day 1). The menstrual cycle ofhormonal production.23,24,27,28 consists of two phases of parallel events occurring in the ovaries and endometrium. In the ovaries, these Estrogen events are known as follicular and luteal phases,Naturally synthesized estrogens are carbon-18 com-while concomitant endometrial events are known aspounds. The main estrogen produced in the ovary is the proliferative and secretory phases.20,21,23–25,34,35estradiol. Estrone and estriol are primarily metabolites of intraovarial and extraglandular conversion. Follicular phaseEstrogens promote breast, uterus, and vaginal development- The follicular phase begins with the onset of mensesment and also affects the skin, vascular smooth muscles, and ends on the day of LH increase. At the beginning of follicular cells and the central nervous system.23,24,27,29 Phase, the ovary secretes very little estrogen or proges-oestrogen deficiency, which occurs naturally with the onset of the terone. However, the increase in FSH stimulates the estrogen promenade leading to atrophic changes in these organs. duction. Estrogen secreted by the developing follicle-During the reproductive period, it is estrogen that is re-cle in the ovary stimulates the epithelial cells of the uterus,sponsible for changes in the follicular phase in the uterus, with the growth of blood vessels, and endometrial glands develop-CHAPTER 21 ■ GONADAL FUNCTION 483ment to increase the thickness of the endometrium. CASE STUDY 21-1Intensive secretion capacity of uterine glandscrew secretion, which helps implant a 39-year-old woman presented with hot flashes. irregular menstrual cycles for 6 months. The clinical examination did not reveal an abnormality. Luteal phase laboratory evaluation reveals the following: CBC, estrogen levels peak 1 day before ovulation, at which normal; glucose, 89 mg/dL; TSH, 1.5 IU;a point of positive feedback system leads to an increase in LH. FSH, 128 IU; and LH, 30 IU. The beginning of the luteal phase is marked by extrusion of the owl approximately 36 hours after this increase in LH This clinical situation is consistent with one of the subsequent luteinization of graafian follicle to the following: formed corpus luteum. Corpus luteum secretes progesterone to assist in implantation of the embryo. 1. PCOSV absence of fertilization, gradual decrease in the production of progresteron and estrogen corpus 2. Prolactinomaluteum is the loss of endometrial blood supply; these endometrial spillover results approximately The pituitary gland tumor 14 days after ovulation occurred. The typical duration of menstrual bleeding is 3–5 days, with blood loss aver-4. Menopause 50 ml. The onset of menses means the end of the luteal phase. 5. Deficiency of the hypothalamus hormoneHormonary control of ovulation in years, FSH levels are increased at the beginning of the follicle-lar phase. Midcycle increase in LH production stimu-Central control of FSH and LH secretion lies in a late series of events that culminate in ovulation, GnRH pulse generator arcuate core and i-s FSH levels decrease after this event. Any injury to the preotic yaludia hypothalamus.20,21,23–25,34,35 hypothalamus or the presence of psychoso-positive and negative feedback reactions exists between cial or physical stressors leading to changes in these oestrogen, progresthron, LH, and FSH production. It is hormonal stimuli and results in anovulation and for lack of estrogen after menopause that amenorrhea.29,36,37na fsh and LH levels rise.20,34,35 During reproduction- Pubertal development in femaleTABLE 21-2 TANNER Staging BREASTAND PUBIC HAIR DEVELOPMENT IN WOMEN, puberty in women consists of a sequence of hormonally mediated events that result in the development of stages of breast development of secondary sexual characteristics and the achievement of the final height of an adult. Thelarche (breast development1 Prepubertal tissue) is usually the earliest symptom of sexual development, followed by the development of pubic hair. Menarche,2 Increase in breasts and papillae, areolar or initiation of menses, occurs on average 2-3 years enlargement after the onset of puberty.3 Increase in breast tissue and papillae Tanner staging designed by Marshall and Tanner as a way how to determine4 Increase in areola and papillae in the secondary mound of pubertal staging, Tanner staging system, outlined above breast level Table 21-2 , is used to monitor and evaluate the stages of growth of breast and pubic hair.285 Mature stage : recession areols to the breast with projection of the papillae only menstrual abnormalitiesDIAGES OF PUBIC HAIR DEVELOPMENT Menstrual cycles range from 25 to 35 days with an average 28-day duration. The average age of menopause1 Lanugo-hair type (prepubertal) in the United States is between 45 and 55 years with a median age of 53.20,21.23-25.3 4 352 Dark love on the poysm majora Amenorrhea is defined as the absence of menstruation menses.3 Terminal hair covering the poysum major and spreading primary amenorrhea describes when a woman has never had mons pubis menstruated, while secondary amenorrhea is used to write off a woman, which had at least one menstruation4 Trailing hair fully covering the labia majora and mons pubis5 Trailing hair covering the labia majora , mons pubis and inner thighs484 PART 3 ■ EVALUATION OF ORGAN SYSTEM FUNCTIONS TABLE 21-3 ETIOLOGIES AMENORRHEA is delayed or phased out, resulting in implantation failure. Multiple causes and female infertility areHypotalamus PRIMARY SECONDARY shown in Table 21-4.Pituitary glessness 27% 38% Polycystic Ovaries 2% 15% Principles, which are the basis of evaluation of disor-syndrome 7% 30% ders normal menstrual functions, as shown in the ovary of the World Health Organization, are the same for the ovaries andUterus / outflow 43% 12% pituitary dysfunction (Box 21-2). Diagnostic approach 19% 7% to secondary amenorrhea is shown in Figure 21-2.cycle followed by absence of menza for minimal Hypogonadotrophic Hypogonadism3-6 months.26,29,36-39 Frequency of various etiolo-Hypogonadotrophic hypogonadism, or gonadotropingies for amenorrhea, both primary and secondary, is (FSH and LH) deficiency leads to a decrease in sexlisted in Table 21-3. steroid production is a common cause of secondary amenorrhea. There are many physiological and patho-Oligomenorrhea refers to the rare irregular male-logical causes of hypogonadotrophic hypogonadism, in-strual bleeding, with a cycle length of more than 35-40 cluding to weight loss as associated with anorexia nervosa. Uterine bleeding exceeding 7 days is a dysfunctional or various diseases, intense physical exercise and is called menorrhagia. In a patient with infertility (commonly referred to as runner amenorrhea), and pituitary gland diagnosis of insufficient luteal phase, when tumors that interfere with the secretion of FSH or LH.18,36,37,39-41lut phase is less than 10 days or when the production of endometrial Prolactin prolactin prolactinomas may have a similar biopsy, indicates the progression of the effects of endometrial changes.29,36 Any secondary cause of chronic hypogo-nadism may cause pathological bone loss, lead to osteophosis or, if severe, osteoporosis. TABUĽKA 21-4 PRÍČINY NEPLODNOSTI CIEĽ VÝSLEDOK PRÍČINAFEMALEHypothalamus Znížená GnRH Lieky Zvýšený stresPituitary Znížená FSH, LH Dietovaries Znížená estradiol alebo progesterón Deštruktívny nádor alebo vezikulárna lézia Zlyhanie orgánovFallopian rúrky Nedostatočná dysgenéza orgánov endometria a maternice Tubal zjazvenie a uzavretie Antiovariká protilátkyPochyb znížená cervikálny hlien Podvýživa, veľmi nízka hmotnosť, metabolické ochorenieMALE Imobilizácia a deštrukcia Nízky progesterón výstupHypotalamus spermií Zápalové ochorenia panvy a hypofýzy Cervikálne infekcieTesty Oligospermia na azoosperm protilátky (bez spermií)Prostata Primárne chyby v hypotalamu alebo hypofýzyUretrogeniálny trakt Oligospermia na azoospermiu Exgénne androgény Oneskorené alebo nedostatočné pohlavné zrelosti Testikulárna dysfunkcia Znížená testosterón orchitída Testikulárne infekcie (mumps) Znížená semenná tekutina Alkoholizmus / zneužívanie návykových látok Retrográdna alebo chýbajúca ejakulácia Chromozomálne chyby Infekcie prostaty alebo semenných vezikul fyzických abnormalít Chronická cukrovkaCHAPTER 21 ■ GONADAL FUNKCIA 485 BOX 21-2. KLASIFIKÁCIA PRÍPADOVEJ ŠTÚDIE 21-2 AMENOREA SVETOVOU ZDRAVOTNÍCKOU ORGANIZÁCIOU (WHO) 49-ročná žena so zvýšeným rastom vlasov GUIDELINES39 ,40 for 6 months, which began suddenly; she has a male pattern of hair loss as well. For examina-• Type 1. Hypothalamus hypogonadism tion, she has time loss of hair and clitorimegals. (low or normal FSH and/or LH) Laboratory evaluation shows the following: testicles- • Hypothalamus amenorrhea (anorexia nervosa, teron, 360 ng/dL; FSH, 12. LH, 9 IU; and non-iopathic, induced by exercise). prolactin levels. • Kallmann syndrome • Isolated gonadotrophin deficiency The next step in the assessment is one of the following steps:• Type 2. Euestrogen chronic anovulation (normal FSH and LH) 1. DHEAS • Polycystic ovary syndrome (LH Ͼ FSH in some patients 2:1 or more) 2. Repeat levels of FSH and LH • Hyperthecosis 3. High blood sugar levels• Type 3. Hyperthalamic hypogonadism (increased FSH and/or LSH) 4. High-end lipid levels • Premature ovarian failure • Turner syndrome 5. Computed tomography of the adrenal glands and ovaries Get in ᴺL-hCG positive negativepregnancy Get: Height / weight Serum prolactin (PRL), FSH, and if indicated, testosterone or TSH abnormally increased increased increased Historyheight / weight PRL FSH testosterone D&C predObese excessively exclude ovaries Ↄ200 Ͼ200 amenorrhea, thin hypothyroidism, ng/dL ng/dL failure normal PRL and FSH Investigate medicinal products, Oestrogen/ Pelvic possible renal failure progestin ultrasound Test of dietary/ surrogate and adrenal oestrogen/ nutritional MRI scan of progestin hypothalamus CT to stimulate manipulations; and pituitary discontinuation evaluates ovarian bleeding Treatment of adrenal hyperandrogenism of hyper-dopamine Hysteroscopyandrogenism and agonist Suppress hysterosalpingogram of ovarian androgenic Asherman secretion syndrome with oral contraceptivesFIGURE 21-2. Diagnostic approach to secondary amenorrhea.486 PART 3 ■ EVALUATION OF ORGAN FUNCTIONHypergonadotropic hypogonadism due to congenital chromosomal abnormality (e.g., Hypergonadotropic hypogonadism is characterized by Turner syndrome) or premature menopause.36,37,39,43ovarian failure leading to an increase in fsh concen- Patients with Turner syndrome do not complain of tetration, with or without an increase in LH. Ovarian failure of the same hot flashes experience in patients with secondary fluids naturally between 45 and 55 years in hypergonadotropic hypogonasis. Premature menopauseAmerationAmerial women. When exhaustion of oocytes and can occur in isolation or in conjunction with other en-follicles occurs at the expected time, it is called docrine gland failure, such as hypopathyroidism, hy-menopause. Menopause is a natural, inevitable event that has pothyroidism or hypoadrenalism.25,36,37,39,41 results at altitude FSH and LH levels, with low levels of estrogen.20,25,35,42 Polycystic ovary syndrome This common disorder can be present in many ways: infertile- Premature ovarian failure is defined as primary hysteria, hirsutism, chronic anovulation, glucose intolerance, pogonadism in women 40 years of age and case study 21-3A 21-3A may be a man presented with a history of hay Questionsfever, who was treated with antihistamines. It is associated with a reduced smell. He continues to grow 1. What is the level of defect?slowly and pubertal development has been slow. Theman denies erection or night time emissions. 2. What is the meaning of body measurements? Management 3. What are diagnostic considerations? Testosterone Replacement TherapySexual Maturity 4. What tests could be obtained to salater the desired diagnosis of fertility? GnRH pulsating therapy produced a normal sperm count of 5. What constitutes normal fertility? about 4 months and the man's wife became pregnant. 6. How will you advise this man when he asks if he can have children? EUNUCHOIDAL MAN, APPEAR GnRH STIMULATION YOUNGER THAN AGEPE 72 INCH TIME (MINUTES) LH NORMALHeight 75 inch Pre-, Ↄ5Arm span 180 lb 0,GnRH Ↄ2 mU/ml adult, 3–18Weight 130/82 Post-, 2.5 timesBlood pressure Replacement face, axillary Ↄ2 pubic hair 15 Ↄ2 basic onhair penis, 3.1 cm (small) 30 3 some pointGenitalia Soft, 1 cm ϒ 1.5 cm ϒ 1.5 cm 45 Ↄ2Testes (normal, Ͼ4.5 ϒ 3 cm ϒ 3 cm) 60 3 NORMAL Pre-, Ↄ5 after GnRH priming adults, 3-18 LH riseLABORATORY VALUES TIME (MINUTES) LH more than 2.5 timesTestosterone 157 ng / dL (normal, prepuberal Ↄ100; 0 Ↄ2 mU/ml baseline adult, 300-1000)LH Ↄ2 mU/ml (normal, prepuberal Ↄ5; 15 10 adults, 3-18) 30 12 45 16Prolactin 6 ng/ml (normal , 5–25) 60 12TSH 1.2 mU/ml (normal, 0.3 -5.0)CHAPTER 21 ■ GONÁD FUNCTION 487 BOX 21-3. CLASSIFICATION TABLE 21-5 CAUSES HIRSUTISM HIRSUTISM20,21,23,44 COMMON Functional (normal levels of androgens with excessive hair growth) or true androgen excess (increased idiopathic andro genes) Polycystic ovary syndrome (LH mediated) or adrenal glands (ACTH mediated) UNCOMMON Peripheral conversion of androgens (obesity) Drugs: oral contraceptives with androgenic tumor hyperandrogenism (ovaries, adrenal glands) progestins Chorionic gonadotropin mediated congenital adrenal hyperplasiahyperlipidemia or dyslipidaemia, and hypertension.20,22,23The beginning is often perimenarchial, chronic, and notable for hyperprolactinemia of its slow progression. Investigation of this disorder in-volve estimate of free testosterone, SHBG, FSH, LH, fast-cushing's syndrome of glucose, insulin and lipids. Ovarian ultrasounds show more cysts in many patients (about 30% of pa-adrenal tumors do not have ovarian cysts). Most patients with this purpose are obese; however, patients with polycystic ovarian syndrome (PCOS) of East Asian or South American origin are of normal weight. Most oga symptoms (60% of cases) with PCOS the next most common and laboratory abnormalities are reversed with the cause of weight loss (35%) (box 21-3). Typical causes of hirsutism and increased physical activity. The drug Glucophag, shown in Table 21-5.20,21,23,24 (metformin), commonly used for the treatment of dia-betas, is useful in this condition, even in the absence of di-hirsutism, contextual bets. Although not the U.S. Food and Drug Administration of female ethnicity. Women of Italian, Eastern approved for this use, it supposedly normalizes menstrual European, Eastern Indian and Irish origins and improves conception rates. more androgen-sensitive terminal hair than most Northern European women, making the careful invocation of Hirosutism of ethnic origin important before starting an extensive laboratory evaluation in a woman born in Hirosutism is an abnormal, abundant, androgen-sensitive ter-United States. It is estimated that about 5%-10% of minesome hair growth in areas in which trailing hair By American women have hirsutism, which can be quang-follicles are sparsely distributed or are not commonly found in cleavage using a measuring technique known as women. Most commonly, hirsutism is idiopathic in the Ferriman-Gallwey etiol scale, which identifies nine areas (lips, chin, sideburn region, neck, chest, abdomen, upper and lower back, and thighs) for evaluation and rationing points on a scale of 1-4 based on hair thickness and pigmentation. Scores higher than 8 is consistent with the diagnosis of hirsutism.20,21,23,44 Hormonal ab-normalities associated with hirsutism are summarized in Table 21-6.TABLE 21-6 LEVELS OF ANDROGENS IN HIRSUTISM AND VIRILIZATION ANALYTECONDITION TOTAL TESTOSTERONE FREE TESTOSTERONE DEHYDRATION ABOUTEPIANDROSTERONEIdiopathic hirsutism ↑ ↑↑↑ SULFATE (DHEAS)Polycystic ovary syndrome ↑ ↑↑ ↑↑ ↑↑ ↑Congenital adrenal hyperplasia ↑↑ ↑↑ ↑↑ ↑ ↑↑Ovarian VIRILIZING TUMOURSAdrenal ↑↑↑ ↑↑ ↑↑↑↑Modified with the consent of Demers LM. Hirsutism and virilization, news and opinions. Washington, DC: American Association for Clinical Chemistry, 1989.488 PART 3 ■ EVALUATION OF THE ORGAN SYSTEM FUNCTIONSCASE STUDY 21-4A A 17-year-old boy presented. School physical exami-PE: 73 inches showed less pubic and axillary hair than those height of 74 inches peers, penis and bow, which are smaller, breast yew-arm range 148 pounds from age 13, no erections or night emissions, Weight 110/70 mm Hgand no teen growth spurt. Sleeve and trousers BP 69 bpmlengths increase every 4-6 months. Pulse Disappearing axory and pubic hair Moderate gynecomastia, 3 cmQueries Breast Penis, 4.5 cm Genitals 1.5 ϒ 1 ϒ 1 cm, bilateral1. At what level is the error in this case? Testicles (normal, Ͼ4,5 ϒ 2,5 ϒ 2,5 cm)2. What diagnostic options would explain laboratory 115 ng/ln (normal, 300-1000) endocrine data? Testosterone, total 42 mU/ml (normal, adult, 3-18) LH 47,XXY3. What treatments would be available: Karyotype a. Treat his lack of androgens? B. Let him father children if he wanted fertility?4. Its appearance shows that there was an error: a. Before birth (during the life of the foetus) b. After birth (postnatal) ratio of estrogen replacement therapy, 1.26) and formation of venous clots and without the benefit of cognitive decline or coronary artery disease. Estrogen replacement remains a contentious issue. Conversely, a decrease in bone Colon Polyp Form-Women's Health Initiative study enrolled 16,608 post-tion, and menopausal symptoms (hot flashes, vaginal menopausal women who were placed on conventional dryness) were noted. Currently, estrogen replacementhormone replacement combinations. The study showed that it remains a treatment option for selected women after caring for an increased incidence of invasive breast cancer (risk advice. Sultan C, Lumbroso S, Paris F, et al. Androgen disorders action. Semin Reprod Med 2002;20:217-228. 1. Dewing P, Bernard P, Vilain E. Gonády Development Disorders. Semin Reprod Med 2002;20:189–198. Styne DM. Puberty and its disorders in boys. Endocrinol Metab Clin North Am 1991;20:43–69. 2. Sharpe RM, McKinnell C, Kivlin C, Fisher JS. Proliferation and func-tional maturation of Sertoli cells, and their relevance to disorders of the 12th Seminara SB, Hayes FJ, Crowley WF Jr. Gonadotropin-releasing testicle function in adulthood. 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Stanford JL, Hartge P, Brinton LA, et al. Factors influencing age in natural menopause. J Chronic Dis 1987;40:995-1002.CHAPTER 26 ■ RENAL FUNCTION 573a water leads to a significant increase in extracellular Disease. The National Kidney Foundation has a volume of fluids, which leads to peripheral edema, hypertension, formulated guidelines for early diagnosis, treatment and congestive heart failure. However, the most important is the prevention of further progression of the disease. See Table 26-3 is the onset of uremic syndrome or ESRD, in which for five stages of CKD. GFR and evidence of renal elevated SERUM BUN and creatinine values are observed by measuring proteinuria or others together with previous symptoms. The result of these markers forms the basis of classification.23Disclosement is either recovery or, in case of irreversible damage, progression to chronic renal failure.5,8 Conditions, which may precipitating acute renal failure may also lead to chronic renal failure.5,16 Several other causes of chronic renal failure (chronic kidney disease) are shown in Table 26-4.Chronic kidney disease (CKD) is a clinical syndrome that arises when there is a gradual decrease in kidney function Increasing incidence of chronic kidney disease over time (Fig. 26-7). According to the 2007 U.S. Renal There is a growing incidence of CKD in the UnitedData System (USRDS) Annual Data Report, one in nine states due to an increase in diabetes, aging poached-U.S. adults have CKD and 20 million more are at risk.22 tion, obesity, and metabolic syndrome. Diabetes melli-Early detection and treatment are necessary to prevent pre-tus can have a profound effect on renal system.gression on ESRD and complications such as coronary vas- Patients with type 1 diabetes have an insulin deficiency. Approximately 45% of patients with type 1 diabetes willpancreatitis Colitis ThrombocytopathyPerikitis Not known Mechanisms of bleeding tendency Severe coma Polyuria anemia Encephalopathy Loss of water Loss of balance functionerytropoetine Retention of endogenous toxins Production of acidosisLos endocrine Rhinical Chronic failure of ion function homeostasisHypertension Phosphate failure to convert retention Pulmonary vitamin D edemia Hypocalcemia Sodium retention Hyperpathathyroidism Peripheral bone reconsidation edemFIGURE 26-7. Pathophysiology of chronic kidney disease.574 PART 3 ■ ASSESSMENT OF ORGAN SYSTEMIC FUNCTION TABLE 26-3 SYSTEMATIC CLASSIFICATION OF STAGES OF CHRONIC KIDNEY DISEASE DESCRIPTION GFR (ML/MIN TO 1.73 m2) With increased risk* 90 (with risk factors)Renal impairment in normal or GFR Ꞓ902 Renal impairment with normal or ♥ GFR 60-893 Moderate ♥ GFR 30-594 Severe ♥ GFR 15–295 Renal failure Ↄ15*Patients at risk should be screened. Stages 1 to 5 illustrate the progression of CKD. Source: National KidneyFoundation. K/DOQI clinical practice guidelines for chronic kidney disease: summary. New York:National Kidney Foundation, 2002:16.develop progressive deterioration of renal function prevention of high blood pressure can prolong onset (diabetic nephropathy) to 15-20 years after diag- chronic renal failure.nosis. Less people with dia-betes type 2 will also develop this condition. The effects are in addition to hypertension and diabetes, age is key especially glomerular, but can affect all kidney predictor CDK. Due to a decline in fertility and in-structure as well and being theorized to be caused by folds in average lifespan, the percentage of supernormal hyperglycemic environments that constantly populations aged 65 or older are projected to increase the vascular system.5.8 from 12.4% in 2000 to 19.6% in 2030, according to the U.S. Census Bureau. This constant increase, despite previ-typically, diabetes affects the kidneys by causing ous decades and those that come, contributing significantly to becoming glucosuric, polyuric, and nocture. increasing incidence of CKD. These conditions are caused by severe kidney requirements on the diurese hyperosmotic urine. In addition, epidemiological evidence links obesity to CKD amild proteinuria (microalbuminuria) often developed by be-ESRD. However, diabetes mellitus and hypertension are 10 and 15 years after the original diagnosis (see have potentially confusing tasks because obesity ismicroalbumin earlier). Hypertension often manifests itself as a risk factor for diabetes and hypertension, the two mostpreliant, further exacerbating kidney damage. Finally, common causes of CKD and ESRD. Recent studies have shown chronic renal insufficiency or nephrotic syndrome may be that obesity alone increases the risk of kidney damage.24evolve, and each can be identified by their characteristics as individual weight gains, nephron number re-symptoms and laboratory findings. Timely treatment di-network is the same; However, GFR increases to meet, which focuses on strict blood glucose control and higher metabolic demands, resulting in kidney damage. TABLE 26-4 AETIOLOGY OF CHRONIC RENAL FAILURE Metabolic syndrome, characterized by pres-ence at least three of the following risk factors-ETIOLOGY EXAMPLES of abdominal obesity, hypertension, low high densityRenal circulatory diseases of lipoprotein cholesterol, hypertriglyceridaemia, or renal vein thrombosis, hyperglycemia-is the predominant disorder in the United Primary Glomerular Malignant Hypertension States. In a population study of representative samplesdiscoverment of the U.S. general population, the risk of CKD and mi-renal consequences of SLE, chronic croalbuminuria gradually increases with greater metabolic disease glomerulonephritis the number of components of metabolic syndrome.25Flatter disease Individuals with metabolic syndrome had 2.6-fold in-Gout, diabetes mellitus, warped risk of developing CKD compared to individ-renal obstructions amyloidosis ual without metabolic syndrome.25 Interventions that focus on the biochemical components of metabolic syndromeCongenital renal tuberculosis , chronic may reduce the risk of CDK.deformity pyelonephritisMicellular conditions Enlargement of the prostate of renal hypertension, Renal hypertension may be caused by calculus of either reduced perfusion to all or part of the kidney (ischaemia). Deficiency of perfusion can be caused by trauma- polycystic kidneys, matrix injury or narrowing of the artery or intrarenal renal hypoplasia Radiation nephritisCHAPTER 26 ■ RENAL FUNCTION 575 CASE STUDY 26-2A 45-year-old man presented to the hospital became agitated and requires an increase in the dose of alcohol withdrawal. After drinking beer brandy benzodiazepines, along with physical limitations daily for the last 5-6 years, he decided to stop controlling behavior. The next morning he was trans-drinking four days ago. He experienced concussions and was in the intensive care unit where he was then visually and auditory hallucinating. Acute renal failure is evaluated upon arrival. The patient was in the hospital, was diaphoretic and tachycardiaic, rehydrated and had arthritis and antidepressant with a pulse rate of 102. His chemistry results are drugs have been withheld. The results of laboratory tests are given below. are as follows:NaƧ 130 mmol/l Total protein content 7,1 g/0L NaƧ 139 mmol/l Creatinine 1,4 mg/dLKƧ 3,7 mmol/l albumin 1 3.7 g/ln KƧ 3.5 mmol/l CK 1626 U/LClɤ 90 mmol/l ALP 63 U/L Clɤ 107 mmol/l CK-MB 3 .4 ng/mLCO2 20 mmol/l AST 42 U/L CO2 23 mmol/l Relative index 0,2BUN 81 mg/dL ALT 16 U/L BUN 16 mg/2LnCreat 4.0 mg/l GGT 131 U/LMagnesium 1.4 mg/dL CK 591 U/L QuestionsAlcohol Negative total bilirubin 0.5 mg/ln 1. Is the patient still in acute renal failure? 2. What was the cause of his acute kidney failure? The patient's medical history included arthritis, 3. Why has the patient's electrolyte state improved?hypertension, depression and alcoholism. He was 4. Why is his CK highly elevated?was he taking anti-inflammatory drugs for arthrosis and antidepressant. Overnight, hearterioles. Chronic ischaemia of any kind leads to circulation and dialysate discarded. Diffusion of adrenaline dysfunction and possible necrosis. The result- low-molecular weight (Ↄ500 Da) to changes in blood and body fluid volumes in dialysate is favored by this process, but medium-molecular-kidneys provoke activation of renin-angiotensin-weight (500-2000 Da) are insufficiently cleared.aldosterone system, set off vasoconstriction, which is creatinine clearance is about 150-160 ml / min.manifested as persistent hypertension. In peritoneal dialysis the peritoneal wall acts as renal hypertension can be evaluated by monitoring the dialysate membrane, and gravity is used to introduce levels of andserum aldosterone, NaƧ and retinal. As a result of the removal of the dialysate. Two variants of this form are available-effect aldosterone, there will be increased serum capable, continuous outpatient peritoneal dialysis (CAPD)NaƧ, reduced serum KƧ, and an increase in urine KƧ. (a) continuous cycling peritoneal dialysis; However, the process is continuous in both, is carried out 24 hourstreating acute renal failure daily, 7 days a week. This method is not as a traditional method of dialysis. Small squanders (e.g. potassium) have patients with acute renal failure, symptoms of urinary disease, significantly lower clearance rates compared to tra-controlled hyperkalymemia, and acidosis have traditionally been a ditional method, but more large solutes are erased and natural indications that the kidneys are unable to secrete equilibable blood analyte levels are maintained.bodily waste products and a replacement method in the form of dialysis was necessary. Dialysis was often introduced before continuous arteriovenous hemofiltration (ultrafilter-this phase, however. There are several forms of dialysis; tion of blood), continuous venovenous hemofiltration,however, all use a semi-permeable membrane of sur-continuous arteriovenous hemodialysis, and continuous around the dialysate bath. venovenous hemodialysis together form a slow continuous renal replacement therapy developed in traditional hemodialysis (removal of waste from the treatment of acute renal failure in critically ill patients in inten-blood), the membrane is synthetic and outside the body. settings. In these methods, semipermeableArterial blood and dialysate are pumped at high rates the membrane is again outside the body. up to 5000 (150-250 ml/min and 500 ml/min. in da (pore size of membranes) and water are slowlypositing directions. Blood is returned to venous (10 ml/min) and continuously filtered from the blood in 576 PARTS 3 ■ EVALUATION OF ORGAN SYSTEM FUNCTIONS CASE STUDY 26-3 the first two methods that cause minimal changes in plasma osmolality. Loss of volume can be replaced by vA 78-year-old woman with a history of hypertension, a form of parenteral nutrition and intravenous medical-aortic thoratic graft, and reflux disease of the esophagus tions. The last two methods are similar to filtration accompanied by fever (100 °C) and weakness. She had methods, but a continuous trickle of dialysis fluid was treated 3 weeks earlier in the hospital on a uri-pumped around the dialysis membrane, leading to infection of the contin-nary tract. She was admitted to hospital for urea diffusion and doubling clearance močoviny.na diagnostic examination and transfusion. Her Labour-Tory results are as follows: Therapy for end-stage kidney disease In patients with irreversible kidney failure, dialysis andNaƧ 129 mmol/l Hct 25.6% transplantation are just two therapeutic options. KƧ 3.7 mmol/l Hgb 8.5 g/5 g/l Initiation of treatment occurs when GFR fallsClɤ 97 mmol/l WBC 9,700 to 5 ml/min (10–15 ml/min in patients with diabetic NEFRO2 19 mmol/l nephropathy). BUN 52 mg/lCreatinine 3.2 mg/ln Dialysis Traditional haemodialysis or its more recent, high urinary culture was positive for Citrobacter. the effectiveness of the form, as well as peritoneal dialysis suurinalysis results are given: available methods. The clinical laboratory used in conjunction with the haemodialysis equipment must be able to adequately monitor haemorrhage/yellow efficiency in wide-range specific gravity of 1,015 ethos areas. Renal dialysis has basic goals, and specificpH 5 laboratory tests should be performed to evaluateBlood Great achievement of each goal. Protein 2Glucose Negative TransplantationKetones Negative Most Effective Hemodialysis Techniques Provide OnlyNitrates Negative 10%-12% Small Reproduction Removal of Two NormalRBC Ꞓ25 Kidneys and Significantly Less Removal of Larger Squid. WBC 1-4 Even patients who are well dialysed have physicalCasts granular, 1-4 disabilities and reduced quality of life. Renal trans-plantation offers the best chance of a complete return to the patient's kidney function to continue to decline, healthy, productive life. However, this option is limited and she has been put on hemodialysis. The kidney biopsy was due to a significant shortage of donor organs. For ESRD, which has been shown in end-stage crescent patients, waiting for organ donation may vary fromlonephritis. Two days later, the patient suffered a few months to several years.a perforated duodenal ulcer that required surgery and a blood transfusion. Subsequently, she developed a kidney transplant from a compatible donor to acogulopathy and liver failure. Her condition is contin-recipient suffering from irreversible kidney failure. Theued deteriorate in the coming days, and she died organ may be from a corpse or a living individual (80% apo removing life support. 20%, respectively, from all kidney transplants in the United States). For this procedure to be successful, the body questions the immune response to the transplanted organ must be vulture-pressed. Therefore, the donor and recipient are carefully1. Looking at the urine analysis, what is the importance projected for the blood group ABO, human leukocyte antigen results 2Ƨ and Ꞓ25 RBC? (HLA) compatibility and pre-formed HLA antibodies. The HLA system is the main inhibitor of transplantation.2. What is the most likely cause of glomeru loneliness? Although kidney transplants have the ability to function for decades, the average half-life is cadaveric3. Why was the patient put on hemodialysis? transplantation is approximately 7 years. Mortality is not significantly different from hemodialysis. Three-year graft survival data range from 65% to 85%, with live grafts doing better. It has been reported that there is no difference in patient survival between hemodialysis, CAPD, and catatherianic kidney transplantation. Live re-lated-donor transplantation is associated with better patient survival than other therapeutic ESRD options. CHAPTER 26 ■ RENAL FUNCTION 577REFERENCES 13. Laboratory Tests Online. Cystatin C at a glance. Obtained from Article 1(1)(http:// www.labtestsonline.org/understanding/analytes/cystatin_c/ of Regulation (EC) No 1 Vander A, et al. Human Physiology: mechanisms of the body func-glance.html. (a) 7. ed. New York: McGraw-Hill, 1998:503, 508, 519. 14 Fraser CG. Biological deviation: from principles to practice. 2. Kaplan A, et al. Clinical interpretations and techniques. Washington, D.C.: AACC Press, 2001. 4. ed. 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Clinical Chemistry: Theory, Analysis, Curr Opin Not Played Hypertens 2007;16:237–241. and Correlation. 4. ed. St. Louis, Mo.: CV Mosby, 2003:462-476. Chen J, Muntner P, Hamm LL, et al. Metabolic syndrome a12. Laboratory Tests Online: GFR and EGFR at a glance. Acquired from chronic kidney disease in adults in the U.S. Ann Intern Med 2004;140: . Funkcia pankreasu a funkcia CHAPTERGastrointestinal 27Edward P. FODYCHAPTER OUTLINE■ FYZIOLÓGIA FUNKCIE PANKREASU Meranie žalúdočnej kyseliny■ OCHORENIA PANKREASu Plazmový gastrín■ TESTY FUNKCIE PANKREASU ■ ČREVNÁ FYZIOLÓGIA ■ CLINICOPATHOLOGIC ASPEKTY ČREVNÉHO sekretínu/Cholecystokinin Testovacia funkcia Analýza fekálneho tuku ■ Testy črevnej funkcie Pot elektroly Stanovenie laktózy Tolerancia Test sérových enzýmov D-Xylóza Absorpčný test■ FYZIOLÓGIA A BIOCHÉMIA SEKRÉCIE ŽALÚDOČNÝCH Sérových karotenoidov Iné testy intestinálnej malbsorpcie■ KLINICKÉ ASPEKTY ŽALÚDOČNEJ ANALÝZY ■ ODPORÚČANÉ HODNOTY■ TESTY ŽALÚDOČNEJ FUNKCIE ■ REFERENCIE Meranie žalúdočnej kyseliny v bazálnych a maximálnych sekrečných testochTehorálny (GI) systém sa skladá z peritoneálnej dutiny v hornej časti brucha približne v ústach, pažerák, žalúdka, tenkého čreva, a úroveň prvého a druhého bedrového stavca , oveľké črevo. Digestion, which is primarily a function of 1-2 inches above the umbilical cord. Located in the current small intestine, is the process by which starches, the curve of the duodenum (Fig. 27-1). Pancreatic proteins, lipids, nucleic acids and other complex mole-consists of two morphologically and functionally degraded into simple components (molecules) for different tissues: endocrine tissue and exocrine tissue.absorption and use in the body. This chapter deals with the endocrine (hormone-releasing) component is far from physiology and biochemistry of gastric secretion, in- the smaller of the two and consists of fasting intestinal physiology, pathological aspects of intestinal func- Langerhans, which are well-defined, spherical or ovate, and tests of gastric and intestinal function. clusters composed of at least four different cell types. Soot cells secrete at least four hormones into the pancreas is a large gland that is involved in di-blood: insulin, glucagon, gastrin, and somatostatin. Thegestive process, but located outside the GI system. There is a larger, exocrine pancreatic component (secreting enzymes) composed of both endocrine and exocrine tissue. Secretes about 1.5-2 l/day fluid, which is rich in diges-liver is another major external gland that is involved in tive enzymes, into channels that eventually empty into the digestive process, and this is included in Chapter 24. Duodenum. Endocrine functions of the pancreas include the production of insulin and glucagon; both hormones are in- This digestive fluid is produced by acinarvolved in carbohydrate metabolism. Exocrine functional cells (grapelike clusters) that line the pancreas and insert the production of many enzymes used in di-linked small channels. These small channels empty the suggestive process. This chapter deals with the physiology of successively larger channels, which eventually form one majorpancreatic function, pancreatic disease and tests of the pancreas duct and smaller accessory duct. The main function of the pancreas. pancreatic canal and common bile duct open into the duodenum on the main duodenal papillae. Normal, pro-physiology pancreatic function of tein-rich, pancreatic fluid is clear, colorless, and watery, with an alkaline pH that can reach up to 8.3. This alkaline-like digestive gland, the pancreas is only the second in the size of a linite is caused by a high concentration of sodium liver, weighing about 70-105 g. It is located za578CHAPTER 27 ■ PANCREATIC FUNCTION AND GASTROINTESTINAL FUNCTION 579FIGURE 27-1. Abdominal structures of the digestive tract.bicarbonate present in pancreatic fluid, which is used atic fluid, which protects the lining of the intestine from time to neutralize hydrochloric acid in gastric damage. Secretin is synthesized in response to acidfluid from the stomach when it enters the duodenum. The contents of the stomach reached the duodenum. Concentrations of cannicarbonate and chlorides vary recipro- they also affect the activity of gastrin in the stomach. This pancreas-cally so that a total of about 150 mmol/l. atic fluid contains several digestive enzymes. CCK, in the presence of fats or amino acids in the duodenum, is a pre-pancreatic fluid has approximately the same concentrations of duced cells of the intestinal mucosa and is re-potassium and sodium as serum. Digestive enzymes, sponsible for the release of enzymes from acinar cells byor their proenzymes secreted by the pancreas, are capa-pancreas into pancreatic fluid.ble digestion of three main classes of food sub-positions (proteins, carbohydrates and fats) and include diseases of the pancreas [1] proteolytic enzymes trypsin, chymotrypsin, elas-tase, collagenase, leucine aminopeptidase, and some auto-other than trauma, only three diseases cause more thanboxypeptidase; (2) enzymes digestive lipids, especially 95% of medical care given to the pancreas. Aclipase and lecitesasis; (3) Carbohydrate-splitting pans affect the endocrine function of the pancreas, these creatic amylases; and (4) several nucleus (ribonucleal diseases may result in altered digestion and me-ase nutrients) that separate the bases containing nitrogen from tabolism. The role of the pancreas in diabetes mellitus is their sugar-phosphate strands. chapter 13. Pancreatic activity is also under the nerve of en-cystic fibrosis (known by various other terms, such as control of cancer. Branches of the vagus nerve can cause fibrocystic diseases of the pancreas and mucoscidosis) is a small amount of fluid secretion, when food is inherited by autosomal recessive disorders characterized by bysmelled or seen, and these secretions can increase as dysfunction of the mucous membranes and exocrine glands throughoutbolus food reaches the stomach. Most pancreas- body. The disease is quite common and occurs with an inatic effect, however, is under the hormonal control of about 1 in 1600 live births. It has various manifestations of sessecretin and cholecystokinin (CCK; previously called and may initially be present in such widely different ways as in-pancreasymine). Secretary is responsible for the production of test-intestinal obstruction of the newborn, excessive pulmonary treatment with abundant bicarbonate, and therefore alkaline pancreatic infections in childhood, or less often than pancreas-580 PART 3 ■ EVALUATION OF ORGAN FUNCTIONSynous malbsorption in adults. The disease causes small pancreatitis or inflammation of the pancreas, there are ul-a large ducts, and acini are dilated and turned into timately caused by autostrade of the pancreas as small cysts filled with mucus, which eventually leads to the reflux of bile or duodenal contents into pancreatic secretions reaching the dude-creatic canal. Pathological changes may include acute edem,num or, depending on the age of the patient, a plug that accumulates with a large amount of fluid in retroperi-blocking lumen of the intestine, which leads to obstruction. As tonal space and associated reduction of effective circu-disease progresses, there is increased destruction and lating of blood volume; cellular infiltration, resulting in necro-fibrous scarring of the pancreas and corresponding de-sis acinar cells, with bleeding as a possible resultcrease in function. Cystic fibrosis is transmitted as necrotic blood vessels; intrahepatic and extrahep-autosomal recessive disorder with a high degree of fat pene-atic necrosis. Pancreatitis is generally classi-trance. It occurs primarily in persons of northern fied as acute (no permanent damage to the pancreas), of European origin. The cystic fibrosis gene known as chronic (irreversible injury) or relapsing/relapsing, whichCFTR occurs on chromosome 7 and more than 900 mu- can also be acute or chronic. It commonly occurs intations causing this disorder have been identified; as middle life. Painful episodes can occur intermittently, usuallyvery, some occur more often than others. In areas that reach a maximum in minutes or hours, a permanent frequency, such as Brittany in western France, can increase by several days or weeks, and often accompanied by more than 10% of the population can carry cystic fibrosis, nausea and vomiting. Pancreatitis is often associated with intation, and 1 in 3,000 infants may be affected, which is alcohol abuse or diseases of the bile ducts, such as gallstones,the most common genetic disorder in these populations. but patients with hyperlipoproteaemia and patients with hy-genetic screening are currently largely perpathyroidism are also at a significantly increased risk of this disease. Pancreatic cancer is the fourth most common form of deadly cancer and causes about 27,000 deaths Other etiological factors associated with acute pancreas-each year in the United States, representing about atitis include mumps, obstruction caused by the bile ducts5% of all deaths from malignant neoplasms. Disease disease, gallstones, tumors of the pancreas, tissue damage, ath-is slightly more common in men than in women and in erosclerotic diseases, shock, pregnancy, hypercalcemia, African Americans than whites. Hereditary pancreatitis with 5 years of survival, associated immunological factors are less than 5% and more than 90% of patients die from postrenal transplantation and hypersensitivity. Most pancreatic tumors arise Symptoms of acute pancreatitis include severe abdominal adenocarcinomas of the ductal epithelium. Since pain that is generalized or in the upper quadrants and pancreas has a rich supply of nerves, pain is often radiating backwards or downwards to the right or leftprominent feature of the disease. If a tumor arises in the side. The etiology of chronic pancreatitis is similar to the body or tail of the pancreas, detection is not acute pancreatitis, but chronic excessive alcoholoften occur up to the advanced stage of the disease-consumption seems to be the most common predisation-cause of its central location and associated vague ing factor.symptoms. Cancer of the head of the pancreas is usuallydiscovered earlier, since its proximity to common laboratory findings include increased amylase, lipase, bile duct. Symptoms of these tumors are jaundice, weight triglycerides, and hypercalcemia, which is often associated with loss, anorexia, and nausea. Jaundice is associated with underlying hyperpathyroiditis. Hypocalcemia maysigns posthepatic hyperbilirubinaemia (intrahepatic to be found and has been attributed to sudden removal of cholestase) and low levels of fecal bilirubin, resulting in large amounts of calcium from extracellular fluid be-in clay-colored stools. However, the findings are not a spe-cause of impaired mobilization or due to calciumcific for pancreatic tumors, and other causes of ob-fixation of fatty acids liberated by the increased action structure of lipase must be excluded. triglycerides. Hypoproteinemia is mainly attributable to significant plasma loss to retroperitoneal tumors of the islet cells of the pancreas affecting the endocrine space. Shift of arterial blood flow from the inflamed pancreas. If the tumor occurs in beta creatic cells on less affected or normal cells causes oxygen cells, hyperinsulinism is often seen, which leads to low lack of blood and hypoxia of tissue in the area of damage, glucose levels, sometimes followed by a hypoglycemic stalk of surrounding organs and tissue.shock. Pancreatic cell tumours, which over-produce gas trin, are called cause Zöllinger-Ellison All three conditions can result in severely reduced syndrome and may have duodenal origin. These tumors have pancreatic exocrine function, which can be significantly associated with watery diarrhea, recurrent peptic compromise digestion and absorption of nu-ulcer ingestion, and significant gastric hypersecretion and hyper-trients. This is the essence of general malabsorptionacidity. Tumors secreting glucagon from pancreatic cells are a syndrome that embodies abdominal bloating and dis-rare; hypersecretion of glucagon is associated with comfort; frequent passage of bulky, malodorous feces;diabetes mellitus. and weight loss. The inability to digest or absorb fats, known as steatorrhea, makes feces look greasy (moreCHAPTER 27 ■ PANCREATIC FUNCTION AND GASTROINTESTINAL FUNCTION 581than 5 g of faecal fat in 24 hours). A malabsorption case study of 27-1syndrome usually involves abnormal digestion or absorption of proteins, polysaccharides, carbohydrates, a 38-year-old man stepped into emergency departure-and other complex molecules, as well as lipids. Severe ment with a complaint of severe, moderate abdominal absorption and electrolyte metabolism, pain 6 hours' duration. A friend who drove water, vitamins (especially fat-soluble vitamins A, D, E, took him to the hospital, said the patient fainted and K) and minerals can also occur. Malbsorption can three times as he has been helped into auto-engaging one substance, such as vitamin B12, which re-bile. The patient had a 15-year history of alcoholism in megaloblastic anemia (malignant anemia) or drank 1–2 liters of whiskey every day. He had had a hadlactosis caused by lactase deficiency. In addition to pan- last was hospitalized for acute alcoholism 3 monthscreatic exocrine deficiency, malbsorption syndrome years ago, at that time had a relatively small abnormal-may be caused by biliary obstruction, which deprives the properties of liver function. On this admission, its bloodyall intestine emulsifying effect of bile, and var- pressure was 80/40 mm Hg; pulse, 110 beats/minute diseases of the small intestine that inhibit absorption and threaded; and breathing, 24 breaths per minute digestible products. and shallow. The results of clinical laboratory tests are presented in the case study table 27-1.1.PANCREATIC FUNCTION TESTS Questions Depending on the aetiology and clinical picture, pancreatic function may be suspected if in-1 is studied. What is the probable disease?crumpled amylase and lipase.4-7 The reader is referred toChapter 10 for in-depth discussion of these enzymes. 2. What is the reason for the low serum calcium content? Other laboratory tests of pancreatic function include those used to detect malbsorption (e.g. examination 3. What is the cause of increased ureastool blood for excess fat, D-xylose test, and fecal fat analysis), nitrogen?tests measuring other exocrine functions (e.g. secretions, CCK, fecal fat, and chymotrypsin), tests evaluating the case study table 27-1.1changes associated with extrahepatic obstruction (e.g. LABORATORY RESULTSSbilirubin) and endocrine-related tests (e.g. gastrin, in-sulin and glucose) reflecting changes in endocrine serum seating cells of 640 units (3.5-260 units) of pancreatic cells. Sodium serum 133 mEq/l (135-145 mEq/l) Direct evaluation of pancreatic fluid may include measurement of total pancreatic fluid and potassium volume of 3,4 mEq/l (3,8-5,5 mEq/l) of the amount or concentration of bicarbonate and enzymes, stimulation of the pancreas. Stimulation can reach 4.0 mEq/l of calcium (4.5-5.5 mEq/l) using pre-written food or administration of secretion, which allows volume and bicar-nitrogen urea in the blood 32 mg/dL (8–25 mg/dL)bonate evaluation, or secretion stimulation followed by stimulation of CCK, which adds enzymes to the pancreas White blood cell count 16,500fluid rating. The advantage of these tests, which require intubation of the patient, is that chemist-Hemoglobin 12 g/lnical and cytological examinations are carried out on real pancreatic secretions. Cytological examination of the volvement fluid, but when used together with the clinical picture can often determine the presence, or at least suspicion, at the time of testing, can provide an important diagnosis of malignant neoplasms, although accurate localisza-information. The following pancreatic function tests of the basic organ of involvement (i.e. pancreas, examined briefly: secretions/CCK test, analysis of fat in feces, bile system, ampoule of Vatera or duodenum) are not pot chloride determination and amylase and lipase are held duodenal aspirations. Interpretation. Due to advances in imaging techniques, these Secretin/Cholecystokinin Teststimulation tests are used less frequently; none of them has been shown to be particularly useful in diagnosing mild or acute pancreas The secretions/CCK test is a direct determination of the disease in which the acute phase has subsided. Most of the exocrine secretory capacity of the pancreas. Tests have found clinical usefulness in excluding the pelvis- it involves intubation of the duodenum without contamina-creas from diagnosis. The sweat test, which is used for gastric fluid screening to neutralise any bikar-cystic fibrosis, is not specific to the assessment of the pancreas in-bonate. The test shall be carried out after 6 hours or overnight fasting. Secretion of the pancreas is stimulated by intravenously administered secretions in a dose ranging from 2-3 U/ kg 582 PART 3 ■ EVALUATION OF THE ORGAN SYSTEM FUNCTIONSCASE STUDY 27-2 Fecal fat AnalysisA 56-year-old man who is an alcoholic gifts with fecal lipids are derived from four sources: unabsorbeda 2-week history of moderate abdominal pain. He also ingested lipids, lipids excreted in the intestine (pre-described by the pound-colored stool, mild ikterus, nausea, dominant in bile), cells shed into the intestine, andvomiting, and 10-lb weight loss. Laboratory metabolism Bacteria. Patients on lipid-free definition are listed in the case study table 27-2.1. diet still excludes 1-4 g of lipids in feces in 24 hours. Even with lipid-rich diets, fecal fat notching usually exceeds about 7 grams in a 24-hour period. Normal fecal lipids consist of about 60% fatty acids; 30%1. What authority is primarily involved? sterols, higher alcohols and carotenoids; 10% triglyc-erides; and a small amount of cholesterol and phosphorus-2. What are the main diagnostic considerations? Lipid. Although significantly increased fecal fat can be caused by biliary obstruction, severe steatorrhea is usu-3. What do laboratory results mean? What allies associated with exocrine pancreatic insufficiency or other laboratory tests would be useful in small intestine disease. Diagnosis? Qualitative screening test for faecal Fat4. What other studies or procedures could various screening tests have been designed to detect required? steatorrhea. These tests commonly use fat-soluble spots (e.g. Sudan III, Sudan IV, Red O oil or Ilio blueCASE STUDY TABLE 27-2.1 sulphate) that dissolve and dyed lipid droplets. Oflaboratory RESULTS of greater importance than a particular technical proce-dure is the level of experience and reliabilityTEST RESULT REFERENCE RANGE of the clinical laboratory conducting the test. 4,2 mg/0,3 -1,0 mg/lnSerum bilirubin 625 IU/L 0–200 IU/l Sudan Colouring of neutral fats of faecal fats (triglycerides) and many other lipid spotsSerum lactate 76 IU/L 0-46 IU/L yellow-orange to red with Sudan III, because d Ye isdehydrogenase much soluble in lipids than in water or ethanol.8,9 462 IU/L 0-80 IU/L Free fatty acids are not significantly stained until the speck serum of alanine men is heated in the presence of a stain with 36 % aceminotransferase acid 80 IU/L 0–85 IU/L. The slides may be examined hot or cold and a negative number 3Ƨ of fat droplets may be evaluated. As the slide cools,Serum alkaline fatty acids crystallize in the long, colorless, needle-like phosphatase of the vagina. Detection of the meat fibre is carried out by the third aliquo part of the faecal sample mixed on the slide with 10% alco-serum amylase hol and a solution of eosine stained for 3 minutes. The meat fibre should be stained as rectangular striped fibres. Pe močbilirubin Sample distribution and detection of neutral fats, fatty acids and undigested meat fibers can provide diagnostic infor-body weight, followed by CCK administration. If there is a simple mania. Increase in fat and undigested meat fibers are in-secretin test is desirable, a higher dose of secretory isdicative in patients with steatorrhea pancreatic origin. Agiven himself. a representative faecal sample is used for analysis. For normal feces there was no single protocol that could have up to 40 or 50 small (1–5 mm) test. Pancreatic secretions are collected differently for 30, neutral lipid droplets on a high-performance microscope array.60, or 80 minutes Administration of stimulants, Steatorrhea is characterized by an increase in the number of ether as a 10-minute sample or as a single, pooled col- and size of stainable droplets, often with some fat globuleslection. PH, secretory velocity, enzyme activity (e.g. in the range of 50- to 100 mm. Evaluation of fatty acids greatertrypsin, amylase, or lipase), and the amount of bicarbonate are more than 100 stained small droplets, along with the presence ofsecured. The average amount of bicarbonate excreted from meat fibers is expected in patients with steatorrhea.per hour is about 15 mmol / l for men and 12 mmol / l for women, with an average flow rate of 2 ml / kg. In view of the total volume output, an assessment of the quantitative faecal fat analysis of enzymes must be carried out. The ultimate test for steatorrhea is quantitative pancreatic flow with fecal deficiency is associated with the determination of pancreatic fat, usually at 72-hour stool collection, obstruction and increased enzyme concentrations. Low although the harvest period can be increased until the concentration of bicarbonate and enzymes are associated with cystic fibrosis, chronic pancreatitis, cysts of the pancreas, calcification and edem of the pancreas. CHAPTER 27 ■ PANCREATIC FUNCTION AND GASTROINTESTINAL FUNCTION 5835 DAYS. Traditional methods for the determination of fecal fat The reference range for faecal lipids in adults is 1-7 gare gravimetric and titrimetric methods. Newer meth- in 24 hours.ods include the use of infrared and nuclear magnetic res-onance spectroscopy.10,11 In the gravimetric method, Pot Electrolyte Determination of persuasive acid soaps (mainly calcium and magnesium fatty acids) are converted to free fatty acids, fol-Measurement of sodium and chloride concentration inlowed by extraction of most lipids into organic sweat is the most useful test for the diagnosis of cysticsolvent, which is then evaporated so that lipid residue fibrosis.12–14 Significantly elevated concentrations of both can be weighed. With titrimetric methods, lipids occur in more than 99% of affected patients. Liquefied with hydroxide and fatty acid salts are doubled to a five-fold increase in sodium sweat and chlorides into free fatty acids using acid. Free fatty acids together are diagnostic cystic fibrosis in children. Even in adults,with various unsatisfyed lipids, they are then extracted without other conditions causing an increase in sweat chloride and organic solvent, and fatty acids are titrated hy-sodium above 80 mmol / l. Pot potassium is also in-potassium after evaporation of the solvent and redissolving shrugged, but less significantly so, and does not generally rely on the rest in ethanol. Titration method of course for diagnosis. Contrary to some claims, pot elec-

measured only saloonable fatty acids, and therefore, trolyte determination do not distinguish heterozygoterender results about 20% lower than in gravi-carrier cystic fibrosis from homozygotes.metric methods. Another caveat is that titrimetricmethods use the assumed average molecular weight for older sweat sampling methods that require fat acid to convert moles of fatty acids into grams of lipids. qualified technologists who frequently performed the test. Induction of sweat included the use of plastic bags or packaging- At one time it was common to measure the amount of ping of a patient in blankets that was full of seri-free fatty acids as a percentage of total lipids at ous risks of dehydration, electrolyte disturbance, and the assumption that a high percentage of free fatty acids in-hyperpyrexia. In 1959, pilocrebin administration with ions indicates adequate activity of pancreatic lipase. This tophoresis method has been reported as an effective method of sweat is no longer considered reliable because of disruptive re-collection and stimulation. Iontophoresis uses electrical results, mainly caused by lipase produced by intes- current, which causes the migration of pilobararin into a limitedtinal bacterium. area of the skin, usually inside the forearm, towards the negative electrode from the moistened support to positive It is necessary that patients are placed on a lipid-rich electrode. The collecting vessel is then applied to the skin.diet for at least 2 days before the start of fecal collec-Pot is then analyzed for chloride. To confirm that, I'm not going to be The diet must contain at least 50 g and preferably the test should be repeated. Commercially available sur-100 g, lipids every day. Fecal collections should expand the face electrodes that analyze sweat chloride are easily after 3 or more consecutive days. Available. See Chapter 28 for details. There are different ways to express feces fecal lipids-It is widely accepted that the concentration of sweat chloride. Expression of lipid excretion as a percentage of wet or greater than 60 mmol/l are diagnostic cystic fibrosis of fecal mass indry is open to a serious challenge for children. Concentrations of sweat sodium and chloride in fe-wide differences in fecal water content even in dry male patients undergo fluctuations with menstrual intake due to food intake. The most widely ac-cycle and peak 5-10 days before the onset of the male-cepted approach is the management of grams of fat feces ex-struation, but do not overlap with the ranges associated in a 24-hour period. cystic fibrosis. Gravimetric method for the determination of faecal fats Serum enzymesAll faecal sample is emulsified with water. Analiquot acids to turn all fatty acid soaps into free Amylase is the serum enzyme on which blunt acids are most often relied, which are then extracted by others soluble to detect pancreatic disease.15,16 However, it is not lipids for petroleum ether and ethanol. After evapora- functional tests. Amylase is particularly useful in the di-tion of organic solvents, lipid residues are weighed. agnosis of acute pancreatitis, in which in-All feces for 3 days are collected in tar con-folds in serum concentrations occur in about 75% oftainers. Containers must not have a wax coating. Patients. Typically, serum amylase increases within the aspecimen to be kept cool. several hours from the onset of the disease, reaches a peak in about 24 hours, and because of its clearance in a child- Total lipids do not change significantly during 5 days' neys, returning to normal within 3-5 days, often makingstorage samples at refrigerator temperatures. urine amylase a more sensitive indicator of acute pan-patients must not swallow castor oil, mineral oil, or other creatitis. The size of the enzyme increase can-fatty laxative and must not use rectal suppositories con- must not be correlated with the severity of the disease.taining oil or lipids for 2 days before the test and during the test.58 4 PART 3 ■ EVALUATION OF THE FUNCTIONS OF THE ORGAN SYSTEM CASE STUDY 27-3Parenti brought their 7-year-old son to the pediatrician Questions with a complaint about frequent fevers and grinding failure. The child had three bouts of pneumonia during Day 1. What is the most likely disease?in the last 2 years and was bothered by chronic bronchi-yis, which caused to cough up large amounts of 2. What clinical laboratory test would be the most sick, yellow, mucous sputum. Despite the great appetite, informative, and what results would be expected? He has gained just 1-2 pounds in the past 2 years and has been a short, fragile stature. He particularly liked salty foods. 3. What other clinical laboratory tests would likely beOn usually had three or four voluminous, foul-smelling abnormal?bowel movements a day. The 9-year-old sister was in a healthy state. Greaterperitoneal cavityLesserperitoneal cavity Liver Lesser omentum Stomach Mesocolon PancreasGreater omentumTransverse colon Duodenum Small intestine Retroperitoneal space Uterus Mesentery Bladder Cul-de-sac Rektumfigure 27-3.1. Peritoneum and mezenteries. The peasant peritoneum lines of the abdominal cavity, and the visceral peritoneum covers the abdominal organs. Retroperitoneal organs are covered with parietal peritoneus. Mesenter-ies are membranes that connect the abdominal organs to each other and to the wall of the body. (Reprinted with permission of Thompson JS, Akesson EJ, eds. Thompson's basic anatomy textbook. 2. ed. Philadelphia, Pa.: J.P.Lippincott, 1990:115.) CHAPTER 27 ■ PANCREATIC FUNCTION AND GASTROINTESTINAL FUNCTION 585 CASE STUDY 27-3 (CONTINUED) TABLE 27-3 D-XYLOSIS RESULTS FOR PAEDIATRIC PATIENTS2.4 AGE REFERENCE RANGE YOUNGER THAN 6 MONTHS 11%-33% 6 -6-1 20 %-32 % 1-3 years 20 %-42 % 3-10 years 25 %-45 % Over 10 years 25 -50 % 27-3,2. Diagram of the pancreas and its relationship to theduodenum. The determination of renal clearance of amylase increases with bone fractures and in conjunction with fat in the detection of a smaller or intermittent increase in the concentration of this enzyme in the embolism.serum. To repair the di-minished glomerular function, useful expression-physiology and biochemistry OFsion is the ratio of amylase clearance to clear GASTRIC SECRETIONance creatinine, as follows: Gastric secretion occurs in response to various stimuli17:% Amylase clearance ϭ 100 ϒ US ϒ SC (Eq. 27-1) Creatinine SA UC ■ Neurogenic impulses from the brain transmitted by vagal nerves (eg, reaction to sight where UA is urine amyla , SA is serum amylase, SC is the smell or food expectations) serum creatinine, and UC is urine creatinine. ■ Stomach dissatisfaction with food or fluid Normal values are less than 3.1%. Significantly in-■ Contact of protein breakdown products, called curved values, on average about 8% or 9%, occur in acute pancreatitis, but can also occur in other conditions, such as secretagogues, with burns of the gastric mucosa, sepsis and diabetic ketoacidosis. ■ The hormone gastrin is the strongest stimulus for the use of serum lipase in the clinical detection of pan-gastric secretion; it is secreted specialized G cells incremental disease has been compromised in the past tech-gastric mucosa and duodenum in response to tonal problems associated with various analytical methods. stimulation and contact with sequoiophes. Improved analytical methods appear to indicate that the increase in serum lipase approximately as soon as the amylase in acute paninhibition effects involves high gastric acidity,creatitis, and that elevated levels persist for slightly longer, which reduces the release of gastrin through the stomach as amylase. As a result, some doctors G cells. Gastric inhibitory polypeptide is secreted byconsider lipase more sensitive than amylase as an indicator of K cells in the middle and distal duodenum and proxi-acute pancreatitis or other causes of pancreatic necrosis. in response to food products such as fats, glucose and amino acids. Both vasoactive intestinal poly-amylase and lipase may be signifyingly elevated, produced by H cells in the intestinal mucosa, in serum under many other conditions (e.g. opiate adminis- directly inhibits gastric secretion, gastrin release, andtation, pancreatic carcinoma, intestinal infarction, gastric thility or perforation, and pancreatic cancer). Amylaselevels are also often elevated in mumps, cholecysti- Gastric fluid is high in hydrochloric acid, yus, hepatitis, cirrhosis, ruptured ectopic pregnancy, and pepsin, and mucus. Hydrochloric acid is excreted in antimacroamylaemia, which is a benign condition in which a hydrogen gradient ion as large as 1 million times teamylase binds to an immunoglobin molecule, causing plasma concentration (i.e. gastric fluid may achieve a pHchronic increase in serum amylase levels, but normally 1,2-1,3 under conditions of augmented or maximum serum amylase. Lipase levels are often significantly stimulating). Pepsin refers to a group of relatively weak proteolytic enzymes with an optimal pH of 1.6 to 3.6 that catalyse all proteins other than mucus. The586 PART 3 ■ EVALUATION OF ORGAN SYSTEM FUNCTION The most important component of gastric secretion in terms (6 g/kg subcutaneously).20.21 Test results reveal that the body's broad physiology is an internal factor that overlaps significantly between healthy individuals and sick patients, cilitates the absorption of vitamin B12 in the ileum. with the exception of anaemia (e.g. malignant anaemia) and extreme hypersecretion, met the syn-clinical aspects of Zöllinger-Ellison gastric analysis. Gastric peptic ulcer is usually associated with normal secretory volume and acidic output. DuodenalGastric analysis is used in clinical medicine mainly for peptic ulcer is usually associated with increased secre-following purposes18.19: tory volume in both basal and maximum secretory tests; nevertheless, there is considerable overlap, with gastric analysis once widely used in the clinical normal range. icin, but has now been largely replaced by fiberoptic endoscopy and better radiological procedures. Gastric acid measurement■ Gastric analysis is clinically used mainly to detect hy-In samples stimulated secretion is the ability of persecretion characteristic of the stomach Zöllinger-Ellison to excrete against the gradient of hydrogen ions de-syndrome. This syndrome includes gastrin-excretion termed by pH measurement. The total acid output in the neoplasm, which is usually found on pancreatic principals, and the time interval is determined from titrated acidi exceptionally high plasma gastrin concentrations. bindings and sample volumes of components. After basal 1-hour secretion, it usually exceeds 10 mEq and tubation is absorbed and stored. the ratio of basal 1-hour to maximum secretion usually secretion for the next 10-30 minutes is discarded exceeds 60% (i.e. the stomach is not really in order to allow adjustment of the patient to the intubation of the basal state, but is pathologically stimulated by the procedure. Samples are usually obtained as 15-high plasma gastrin levels). minute collections for 1 hour. Gastric analysis is also occasionally used to evaluate gastrino response to intravenous secretion stimulation of malignant anaemia in adults. Gastric atrophy can be used to examine patients with mild ele- in this condition, and the stomach cannot excrete serum gastrin levels. In this test, the pure porcinetrinsic factor, which binds to vitamin B12 to prevent its secretions, is administered intravenously and gastrin levels are treated with gastric acid. ph of gastric fluid at 5-minute intervals over the next 30 minutes. This condition usually does not fall below 6, even in patients with Zöllinger-Ellison syndrome, the maximum stimulation of gastrin levels. Rarely, gastric analysis can help increase by at least 100 pg/ml above basal level. Patients, in determining the type of surgery required for normal peptic ulceration, or others to treat ulcers. a slight decrease in gastrin concentration. Previously, various substances were used to stimulate volume, pH and titrable acids and gastric calculation secretion (e.g. caffeine, alcohol and test dishes), the acid production from each sample is reported as submaximial stimuli and obsolete. Since 1953, the total volume and production of acid has been used for each trial period until the end of the 1970s. There is a considered-maximum stimulus for gastric secretion. Due to ad-able changes in gastric acid output between healthy sub-verse effects, some of them severe, histamine now has jects in both basal and maximum secretory tests.has been replaced by pentagastrine, which is a synthetic pen-Yet, in the basal test, most healthy individuals have a se-tapeptide composed of four C-terminal amino acids crete 0-6 mEq acid in a total volume of 10-100 ml. Ingastrin associated with a substitute derivative of alanine. maximum 1-hour test, using histamine or pentagastrine as a stimulus, most men secrete 1-40 mEq of acid in normal gastric fluid is translucent, light gray, and a total volume of 40-350 ml. Women and the elderlylightly viscous and often has a weak apride odor. usually secrete slightly less acid than young men. The residual volume should not exceed 75 ml. Residues occasionally contain blood stains or are plasma Gastringreen, brown or yellow from bile reflux during the inibubation procedure. The presence of food particles is the measurement of plasma gastrin levels is invaluable inabnormal and indicates obstruction. diagnosis of Zöllinger-Ellison syndrome, in which fast levels usually exceed 1000 pg/ml and can achieve gastric function tests of 400,000 pg/ml compared to the normal range of 50-150 pg/ml.24.25 Gastrin is usually not increased when measuring stomach acid in basal and maximum simple peptic disease. Elevated plasma gastrineReeal tests levels occur in most malignant patients with anemia, but decrease toward normal when hydrochloric acid is arti-after a night fast, gastric analysis is usually per-ficially instilled in the stomach.formed as a 1-hour basal test, followed by a 1-hour stim-ulated test after administration of pentagastrineCHAPTER 27 ■ PANCREATIC FUNCTION AND GASTROINTESTINAL FUNCTION 587INTESTINURAL PHYSIOLOGY CASE STUDY 27-4Digestion, predominantly the function of small intes- 34-year-old man was admitted for diagnosis is a process, in which starches, proteins, lipids, evaluation with a complaint of epigastric pain of nuclear acid and other complex molecules are de-2 years duration, which has been variously described asgraded to monosaccharides, amino acids and oligopep-chewing or burning. 18 months ago he was diagnosed with atids, fatty acids, purines, pyrimidines and another sim-duodenal peptic ulcer; at that time, sheet metal components. For most large molecules, treatment of antacids and dietary revisions providedit will make for absorption to occur. Every day, duod- a significant relief of symptoms. Recently, num gets about 7-10 L of ingestion of water and food pain has become more permanent and awakened and secretion from the salivary glands, stomach, pan-patient 4-6 times every night. Radiological studiescreas, and bile ducts. The materials then enter them, revealing a 2.5cm ulcer crater in the first part of the ileum, where another 1-1.5L of secretion is the duodenum and the 0.5cm ulcer in the antrumadded. Eventually, however, only about 1.5 l of gastric fluid. Serum electrolytes were normal.the material got into the cecum, which is the first part of the hemoglobin was 8.3 g/dl with normal red blood cells of the colon or colon. This considerable absorp-indices. The number of white blood cells was 13 100. Gastric ability is possible, because analysis of the small intestine revealed 640 ml of secretion in the basal (about 20 feet long) has numerous mucous folds, an hour, with an acidic output of 38 mEq, and 780 ml of minute protrusions from the surface of the luminal called secretion in a 1-hour pentagastric stimulation test,villi and microscopic protrusions on mucous membranes with an acid output of 48 mEq.called microvilli, all of which significantly increase the secre-tory and absorption surface to an estimated 200 m2. Absorption issues are carried out by passive diffusion for somebusinesses and active transport for others. In addi- 1. What is the probable disease?tion, the small intestine actively secrete electrolytes and other metabolic products. Large intestine (about 2. Given the existing data, what other test would be five feet long) has two main functions: water resorption, virtually diagnostic for this disease?, in which 1.5 l of fluid received by cecum is reduced to about 100-300 ml of feces, and storage of 3. What is the explanation for reducing hemoglobinfeces before defecation. Abdominal structures that and increased white blood cell counts?represent the digestive tract are shown in diagrammati-cally in Figure 27-1. TESTS OF INTESTINAL FUNCTIONCLINICOPATOLOGICAL ASPECTS Lactose tolerance Test of intestinal function Disaccharides, lactase (which breaks down lactose into clinical chemical testing of intestinal function focuses glucose and galactose) and saucrase (which breaks down sucrose almost entirely to evaluate absorption and its on glucose and fructose), are produced by mucous anments in various conditions of the disease. As is said in the cells of the small intestine.24,25 Congenital deficiencies of part of the function of the pancreas in this chapter, diseases of these enzymes are rare, but acquired lactase deficiencies of the exocrine pancreas and biliary tract can normally also be found in adults. Affected patients experi-cause malabsorption. Bowel diseases that can cause abdominal discomfort, cramps and diarrhoea following malbsorption syndrome are (v) their gestational milk or milk products. About 10%-20% whiteetiology, pathogenesis, and severity. These gut Americans and 75% of African-Americans are affected.Diseases and disorders include tropical and nontropicalor celiac spruce, Whipple's disease, Crohn's disease, pri-lactose tolerance testing has been used to determine thismarin intestinal lymphoma, small intestinal resection, diagnosis, but the test is subject to many false-positive intestinal lymphangectasis, ischaemia, amyloidosis, and false-negative results. This test was largely a re-30. In addition to malbsorption syndrome, located by hydrogen breath testing.which usually causes impaired absorption of fats, proteins, carbohydrates and other substances, there are also specific states of absorption of D-Xylose Testmalbsorption (e.g. obtained defi-ciency lactase, which prevents the normal absorption of D-Xylose, is pentosal sugar, which is usually not present vlaktosis, and Hartnup syndrome, a genetic disorder that blood in any significant amount.26,27 As with other involves insufficient intestinal transport of phenylalanine monosaccharides, pentosal sugars are absorbed unchanged and leucine). in the proximal small intestine and do not require intervention of pancreatic lytic enzymes. Therefore, 588 PART 3 ■ EVALUATION OF THE ORGAN SYSTEM FUNCTIONThe ability to absorb D-xylose has value in distinguishing mal- they are synthesized by many plants and pass on the absorption of intestinal etiology from exocrine pan-yellow color to some vegetables and fruits. Great creative inadequacy. Since only about half of the oral carotenoids in the human serum are lycopene, xanthophenyl,administered D-xylose is metabolized or loses smell, and beta carotene, the main precursor to the intestinal bacteria of vitamin A, a significant amount is excreted in un-humans. Since they are fat soluble, carotenoids are absorbed in the urine. Some protocols use meas- in the small intestine in conjunction with lipids.urement only D-xylosis excreted in the urine during malbsorption of lipids usually leads to serum con-5 hours after ingestion of a 25-g dose of fasting carotenoids lower than referenceadult (0.5 g/ kg in a child). Even in the normal renal func-range of 50-250 mg / 0L. Starvation, dietary idiosyncrasis-tion, false positive and false negative results often crasies, and fever also cause serum concen-lowering to occur. Blood levels were measured one or more times after in-trations. The test distinguishes between differentgestion D-xylose (e.g. after 30 minutes, 1 hour, or 2 hours) of malbsorption etiology.significantly improve the diagnostic reliability of the test. Some protocols use smaller doses of D-xylose to prevent ab-other tests of intestinal malabsorptiondominal convulsions, intestinal hypermotility, and osmotic di-arrhea, which often accompany the 25-g dose. Deficiencies of many analytes can occur with the association of Malabsorption. Measurement of D-Xylose Test these analytes is usually a value, not so much in con-after ingestion of a specified solution of D-xylose, blood firming diagnosis of malabsorption as in determin-samples are obtained and urine is collected for a 5-hour ing range of nutritional deficiency, and thus a period to determine the extent of absorption of D-xylose. The need for replacement therapy. Decreased appetite and concentration of D-xylose is determined by heating pro-dietary intake are usually more severe in patients whotein-free supernates of urine and plasma convert xylose have little absorption with intestinal etiology. Bodyto furfural, which is then reacted with p-bromoaniline to waste or cachexia can be difficult. Often, becausetheform of the pink product, the absorbance of which is measured by the loss of albumin to the intestinal lumen and dimin-at 520 nm. Tiourea is added as an antioxidant to prevent protein intake in the indous, which accompany the formation of interferurómes. After ished absorption of oligopeptides and amino acids, overnight quickly, the patient cavities and drinks D-xylose so-negative nitrogen balance occurs along with de-lution: 25 g of D-xylose in 250 ml of adult water and crumpled serum total protein and albumin. Serum al-0.5 g/kg for children or a dose other than that determined. Pa-bumin smaller than 2.5 g/dl is a much more characteristic drink with the same amount of water during another intestinal disease as pancreatic disease. In about an hour. No other foods or liquids should be taken until a treatment with severe small bowel disease, defitest, has been completed. Urine is collected 5 hours after fat-soluble vitamins A, D, E and K appear. Ingestion of D-xylose. A blood sample is taken in potas- Vitamin K deficiency, in turn, causes deficiencies of oxal cesia after 2 hours (usually, For vitamin K-dependent coagulation factors II (prothrom-children), 1 hour is chosen, VII (proconversity), IX (a component of thromboplastin in plasma) and X (Stuart-Prower factor), which are normal blood concentrations of D-xylose in association, which are reflected in abnormal prothrombin and a partial portion with reduced urinary excretion indicate a worsening of thromboplastin time tests. Aspirin therapy reduces the excretion of D-xylose by the kidneys, while in severe small intestinal diseases, such as tropical orindomethacin, it reduces intestinal absorption. After spruce, malabsorption of folate and vitamin B12 cangesion at a dose of 25 g of D-xylose should occur, healthy adults should occur, and megaloblastic anemia is quite common and at least 4 g will multiply within 5 hours. For infants and has some benefit in distinguishing the intestine from pan-children, excretion after a dose of 0.5 g/ kg for creatic disease. Iron absorption is usually reduced, different ages expressed as a percentage of the dose ingested and a tendency to serum iron levels may be shown in Table 26-1. Blood levels in healthy adults exacerbated by intestinal blood loss. Intestinal absorptionavary widely, but the concentration of calcium in the blood is often reduced due to calcium25 mg / dl after 2 hours should be considered an abnormal binding of unbsorbed fatty acids and concomitant vi-after 25-g doses. At a dose of 0.5 g/kg, infants should be deficient in tamin D and reduced serum magnesium.less than 6 months of age should have a blood concentration because sodium, potassium, water absorption and me-at least 15 mg/dL after 1 hour, infants over 6 months of age may also be severely deranged, serum sodium and children should reach levels of at least 30 mg/0L. and potassium levels are reduced and oc-curs dehydration. Impaired absorption of carbohydrates in intestinal diseases serum carotenoids, such as spruce, results in a decrease to flat blood concentration curves in glucose, lactose and sucrose tol-carotenoids are various yellow to orange or purple erance tests.pigments that are widely distributed in animal tissue; CHAPTER 27 ■ PANCREAS FUNCTION AND GASTROINTESTINAL FUNCTION 589KASA 27-5A A 26-year-old woman appeared in an outpatient clinic Fecal examination did not reveal any eggs or parasites and with a complaint of abdominal discomfort; diarrhoea; bacteriological culture has not revealed any pathogens.and 18-lb, unintentional weight loss during the last 2-3 years. She related a similar period of 5 or 6 years to issues of abdominal suffering and diarrhea in childhood, but this 1. What is the process of the disease?basically disappeared when she was about 12-13 2. What is the likely etiology in this case?years. Now she had three to five guts 3. What is the cause of abnormal coagulation tests?movements daily, which have been described as voluminous, mal-4. What is the likely main cause of anemia, scented, and floating. She weighed 106 pounds and was 67 inches tall. She's never had surgery. Physical and what are the other possible contributing causes? The examination revealed poor skin turgor, general pallor and a protuberant abdomen. Abnormal clinical labor-tory values included in case study table 27-5.1.CASE STUDIES TABLE 27-5.1 LABORATORY RESULTSANALYT RESULTHemoglobin 8.1 g/LHematocrit 8.1 g/LHematocrit 30%RBC 4.1 ϒ 106/LSerum 134 mEq/LPotassium 3.4 mEq/LSerum carotenoids 3.4 mEq/LSerum carotenoids 4.4 m 14 g/LFecal fat 22 g/24 hD-Xylosis absorption test (25-g dose) 5-hour excretion 1.3 g and blood level after 2 hours 8 mg/LNProthrombin time 15.8 seconds (12-14 seconds)Activated partial thromboplastin time 56 seconds (30-45 seconds)RECOMMENDED VALUES 5. Lankish PG. Chronic pancreatitis. 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Acta Anaesthesiol Scand 1985;29:65. 1982;28:1448.Clinical chemistry CHAPTERand geriatric patient 33Betty WhiteCHAPTER OUTLINE■ EFFECT OF GERIATRIC PATIENTS ON CARDIOVASCULAR AND LIPID CHANGES CLINICAL LABORATORY CHANGES ENZYMES ■ CLINICAL CHEMISTRY AND AGEING RESULTS■ AGEING THEORIES SETTING REFERENCE INTERVALS FOR OLDER PEOPLE■ BIOCHEMICAL AND PHYSIOLOGICAL CHANGES Pre-analytical variables, Elderly, and Chemistry Results of Aging Therapeutic Drug Monitoring in Older Endocrine Functions Changes Effects of Exercise and Nutrition on Older Diabetes Mellitus and Insulin Resistance and Chemistry Results of Renal Function Changes ■ LINKS Liver Function Changes in Pulmonary Function and Electrolyte ChangesA ging, simply put, means getting older. The number will increase. Geriatrics, according to Taber's medical physiological changes occurring as people age, causing the dictionary, is a branch of health care dealing with graduate deterioration resulting from time-dependent, ir-care age, including physiological, pathological,reversible changes in this individual. These changes will have psychological, economic and sociological problems. reflected in the results of clinical laboratory tests. Since gerontology is a study of the aging process.there is variability in the age of onset, speed, and coursestructic and functional changes, determining the demand for health care system geriatriclaboratory benchmarks is complex. patients is different from the rest of the poula-tion. Healthcare will have to shift its focus to satisfying the impact of geriatric patients on the needs of the chronically ill. As the population ages, the clinical lab increases chronic diseases such as cancer, arthritis, hypertension, and diabetes, as well as other diseases, as the U.S. population ages and is in the midst of being expected. This will pose a major challenge to the on-longevity revolution. Life expectancy has increased over the past century from 47 years for a person to a clinical laboratory. This will mean more doctors in Officeborn in the 1900s to 77 years for those born in 2001.1.2 visits, hospital stays, and laboratory tests. This gain was largely due to improved volumes of sanitation- the test will increase as a result of our ageing, medical care and increased use of the preventive population and the rapid development of new tests.of health services. Setting up Medicare benefits at age 65 Since 2012, nearly 10,000 Americans will turn 65 was based on an estimate that 1% of the population every day. By 2030, when the last baby boomers would be at age 65, when benefits were needed that were mesmerized by age 65, 20% of all Americans would be at age 65 with no impact on the economy. That view has changed.or older. For people who are older than 65 are called older people, Medicare accounts for 17% of health care dollars. Toseniors, and geriatric patients. It is expected that the number of older percent will increase over the next few yearsAmericans are expected to reach 71 million by this time. deficits in the Medicare programme. However,from 2030 to 2050, the growth rate is projected to be in-only about 3% of total Medicare spending is to re-lapse another 14%, bringing the population to 86.7 million-lated for laboratory services. Reduced refunds, lion. Due to the increase in percentage and percentage cost increases and the shortage of laboratory staff of the elderly, the need for geriatric medical care threatens the quality of laboratory services and reduces the evaluation of testing. 673674 PART 4 ■ SPECIAL AREAS OF CLINICAL CHEMISTRY Clinical laboratory experts must familiarise proteins with glycated end products and associate them with problems that are unique or especially com-protein molecules. These modified proteins can eventu-mon in the geriatric population. Geriatric patients may accumulate and interfere with cellular structure and may be exposed to loss of vision, hearing, mobility and loss of function. This process can then result in a variety of prob-independence, all of which can affect their emotional lems characteristic of older people (e.g. stiffness or lossy states. During interaction with the geriatric patient, the clinical laboratory worker must explain the process clearly and treat the patient with dignity and respect. Developmental theories of aging include im-they must be aware of specific considerations of re-mune and neuroendocrine systems, none of which nichgarding blood sampling development provides a plausible explanation for aging. Immune sys-reference intervals, the effect of drugs on chemistry tem ability decreases with age.3 Thymic atrophy oc-results, and diagnosis of diseases in the elderly. Most im-curs at the beginning of the aging process. Reducing important T-cells, however, must thoroughly understand a population with associated B cell loss leading to aging aeffects at laboratory values. reduced response to new antigens. However, ex-posure on neoantigens is highest at an early age. Intheories of aging addition, misfolding abnormalities that occur in boiling forms of amyloidosis, are not the only associated theories of aging they describe as (genetic) with chronic infection and autoimmune disorders.and external (environmental) factors that are aso-familial amyloid polyneuropathy associated with poly-ikemia with structure change and cell damage, com-merization transthyretin molecules (55 mutants) bination that can be attributed to aging is an example of this disorder limited to community inprocesses. Table 33-1 contains (a) random genetic barrier - Portugal, Brazil, Japan and Sweden. Toto and en-age, b glycation, c) developmental processes involving the docrine model are not sufficient to explain the variousiminite and neuroendocrine systems, d) genetic infectious diseases and disorders (e.g. autoimmune deprogramming and (e) free radical damage.3,4 commands, lymphocytic leukaemia and cancer) in eld-erly.3,6,7 Aging theory involving neuroendocrine Theory involving damaged DNA is not accidental , the system focuses on the hypothalamus-pituitary system to address damage or changes in genetic materials and its target glands. The most serious changes in involv-mutagens such as background radiation (ultraviolet) and decrease in endocrine function occur in post-acting chromosome or DNA damage.3 This damage is menopausal to women and involve loss of estrogen andcumulative, but failure associated with aging is bone calcium. In men, plasma testosterone levels de-reduced the ability to repair damaged DNA.3.5 fold with age.1 Error disaster theory requires posttranslational mod-ification proteins, which lead to genetic abnormalities and genetically programmed aging theories indicates the last death of the cell.3 The loss of the insignificant that genes play a role in the aging process and that every amino acid in the protein, as with ck-mm isoforms, is one being programmed by their genes to live with nonfunctional changes. Glycation theory claims that the number of years.3 Support for this theory is based on the non-enzymatic interaction of glucose with numerous general observations of life span in families and various aging syndromes such as progeria, Werner syndrome, TABLE 33-1 SOME CURRENT THEORIES AND DOWN SYNDROME.3 There is a more convincing work inOF AGING basic science that has opened up a large range of studies of cell signaling and cell death. Genes carry the instructions notRANDOM GENETIC DAMAGE not only for growth and development, but also for cellular destruction, causing the decline of the body and the ultimate death of mutagen or damage to background radiation. Reprogrammed cell death is called apoptosis, from the Greek term for falling out. Apoptosis wasErrors in chromosome translation or transcription first described in 1972 as a process of cellular development and aging different from necrosis.8 While necroticGlycation protein cells swell, apoptotic cells usually shrink and separate from surrounding parenchymal cells. At the same time, the volume of cellDEVELOPMENTAL decreases and chromatin condenses at the edge of the nucleus. Apoptotic cells die design, whileimmune system decreases necrotic cells die by accident and fatal injuries.8 In-vestigation apoptosis was driven by its observation inNeuroendocrine nematode Caenorhabditis elegans, followed by the identification of death gene homologues in other organ-genetically programmed isms.9 Aberrant control of apoptosis contributes to preprogrammed cell death (apoptosis)Free radical damage (O2иϪ e.g. laboratory medicine and ageing process). Chicago: American Society of Clinical Pathologists, 1996.CHAPTER 33 ■ CLINICAL CHEMISTRY AND GERIATRIC PATIENT 675well-known pathologies such as autoimmune diseases, some typical age-related diseases, as well as deterrent carcinoma and viral infections.10 During apoptosis, cellular oration of physiological processes (e.g. re-immune system is killed by a class of proteases called cascades. Some sponsitivity and glucose metabolism).4 Reasons for prekaspasy (i.e. i.e. cascades 8 and 10) are involved in ini- these effects appear to be associated exclusively with caloric recreational tilation of apoptosis and others (cascades 3, 6 and 7) and not with a reduction in one dietary factor,to execute a death order by destroying essential proteins such as fat intake or a dietary supplement such as vitamins in a cell. The apoptotic process can be summarized or antioxidants. Unfortunately, effect of caloric re-as follows: stricture on aging in humans is still unknown.4Activation of the initiation of cascades with specific signals BIOCHEMICAL AND PHYSIOLOGICALActivation of cascades by initiation of cascades, CHANGES IN AGING that can cleave inactive cascades at specific sites Aging theory have intersections and areDegradation of basic cell proteins by protease, which is not mutually exclusive. Aging is a complex phenoma that involves biochemical and physiological adjustment of caspas activity. In general, aging is associated with decreasingMitochondria have a central role in the mechanism of effectiveness in adapting stress. Aging con-tinue systems function adequately if they are not sub-apoptosis. excessive physiological stress. The body's ability to successfully cope with stress decreases with advertising- Changes also occur from the action age of reactive oxygen and vary between individuals. The ranges created and erased incompletely through- and the rate at which the ability to adjust the decline depends on the growth of the cell cycle, affecting between cellular interactions through a number of factors, including heredity, lifestyle, and nutri-changes in the intercellular matrix, intercellular tion; therefore, it is difficult to generalize andinversion of trophic factors, the release of inflammatory complex aging process. There is aging-associ-cytokine mediators, and other effects. The basis of etc. reduction of total body water, muscle mass, in-free radical theory is that oxygen radicals cause pro-crumpled bone density with rebuilding (and reduced aggressive, accidental damage to cellular components. Free mass with osteoporosis); an increase in lipids (e.g. cho-radical is an atom or molecule with one or more un-lesterol, high density lipoprotein [HDL] cholesterol, paired electrons; therefore free radicals have odd and triglycerides); and a gradual decrease in respiratory,number of electrons, resulting in open binding, or half cardiovascular, kidney, liver, gastrointestinal, immune, bond, which is highly reactive. Free radical can be neurological, and the functions of the endocrine system.1,4,6,12,13deproceded by the upper dot, which means not paired electron, for example, H2O ϭ OHϪϩ Hϩ Hy- Differentiation between age associated decline and ions (OHϪ) is highly reactive free radicals and pathological conditions can be difficult in older pa-possibly one of the most harmful cells. Free radicals tient, because the disease presentation can be atypical withare also electrophilic and attack sites increased elec-nonspecific complaints and no classic symptoms. When the density of this throne (eg DNA, RNA, proteins, membranes). diagnosis and treatment may be delayed. Finally, damage to cellular components by free radicals causes cell death.3,5,11 Aging is also typically associated with the development of several diseases and disorders.4,14 Table 33-2 shows Superoxide (O2Ϫ) is another free radical generated in some common diseases and disorders associated with the body by several reactions, including oxidative fos-forylation and cytoplasmic reactions. Fortunately, TABLE 33-2 DISEASES AND DISORDERS Are the body has a way to handle most of these free radicals. COMMONLY ASSOCIATED WITH AGING3,11Coming superoxide dismutase, an enzyme present in all body cells, is 100 mg/gas (e.g. myocardial infarction, renalide). Other enzymes (e.g. glutathione peroxidase and disease, stroke) catalysis) then inactivate hydrogen peroxide. Hydroxyl radicals are neutralized by nonenzymatic cancer squads such as vitamins C and E and provitamin Aand beta-carotene (antioxidants).3,6,11 Recently, diabetes mellitustam has been a great support and research on this particular theory of aging.11 Hyperpathathyroidism Although many aging theories have been suggested, Hyperthyroidismno one mechanism has been fully supported by research. In fact, in studies of several species, a single interven-hypothyroidism is known that the delay in aging is a caloric restriction. For example, in rodents, caloric restriction increased the average life of ex-monoclonal gamopathy (e.g. multiple myeloma)pectancy and maximum life span and delayed onset of Osteoporosis676 PART 4 ■ SPECIAL AREAS OF CLINICAL CHEMISTRY 33-3 TOP TEN MAJOR CAUSES Laboratorians should explore the possibility of setting-OF DEATH (AGE 65 and older) ing age-adjusted reference intervals based on analy valte-ues healthy, elderly adults. Many doctors on 1 May 2004 were not in a coma Heart disease accepts that the greatest benefit derived from laboratory 2. Cancer data come from the monitoring of a particular individual test 3. Thrust values over time. 4. Chronic diseases of the lower respiratory tract 5. Accidental death due to inadvertent injury Change in endocrine function 6. Diabetes 7. Alzheimer's disease has long been known to endocrine-related abnor-8. Influenza or malite pneumonia are common in the elderly and tend to increase by 9. Nephritis, nephrotic syndrome or nephrosis in frequency during the aging process. Not only are they there10. Septicaemia apparent changes in the production of hormones of the genital organs, there are also changes in the thyroid, pituitary gland, cdc, national center for health statistics, national vital and adrenal function. The most valuable changes relate to the witness statistics for 2004. gonád and thyroid hormones.the aging process. The main causes of death in people aged 65 years Various significant and complex hormonal years and older are shown in Table 33-3. changes in gonád function occur in both men and women, including a decrease

in the formation of gonády- Specific biochemical and physiological changes in the tion of estrogen in women (menopause) and testos-aging process, as they relate to the clinical chemistry of the terrine in men (andropause); adrenal production tests are discussed in the following sections. Table 33-4 dehydroepiandrosterone (DHEA) and DHEA sulfatesummarizes changes in chemistry of associated analytes (DHEAS) (adrenopause); and reduce activity with the ageing process. Changes in analytes are those of growth hormone (GH)–insulin-like growth factor (IGF) generally cited in the literature.4,13 Whenever possible, axes (somatopause).15,16 As a result, hormone replacement-ment regimens are developed as a strategy to delay or prevent certain consequences of aging. INTERMEDIATE 33-4 CHANGES IN SELECTED CLINICAL CHEMICAL ANALYTES WITH AGE1,3,4,6,18-20,25,28 INCREASE DECREASE UNCHANGEDGGT Albumin Chloridealkalaline phosphatase, women Aldosterone Cortisol␣1-Antitrypsin Bilirubin Free T4Amylase Creatinine Clearance HaptoglobinAST DHEA insulin, pCO_2 growth hormone pCO_2, or a slight increase inCreatin kinase, mild pO_2 pH, or slight decrease⫽-Globulin, mild T3 sodium glucose, fasting Total protein T4 or slight decreasehdl Transferrin Globulin binding thyroid (TBG)Inorganic phosphateLakt dehydrogenase (LDH)pCO_2Potassium , slightTotal cholesterolTriglyceridesTSH, mild changes in uric acid in analytes are those generally cited in the literature. Some variability in the results of aging studies and laboratory results may exist (i.e. one author may not report any significant change for the analyte; another may report a slight decrease or increase in the same analyte). CHAPTER 33 ■ CLINICAL CHEMISTRY AND GERIATRIC PATIENT AGED 67760 AND 80 YEARS, ratio of testosterone to estra- between hypovitaminosis D and secondary hyperparaty-diol from 12:1 to 2:1.1,4 Decrease in testosterone roidism (increased alkaline phosphatase and osteoporoticis associated primarily with reduced testicular function.1,17 changes) in the elderly.21,22 This would likely re-in women, changes in the endocrine system are primarily re-lated for food intake, lack of exposure to sunlight, and tolated for menopause when stopping the ovaries is reduced by converting 25-hydroxy vitamin D_3 into 1.25-estrogen production. Menopause is a permanent ces-form of kidney.sation menstruation caused by a decrease in ovarian follicular activity; it usually occurs between 35 and the thyroid gland is central to the regulation of meta-58 years. The process of apoptosis, non-aumatic Bolivian processes (i.e. regulation of metabolic rate). Reporting and non-inflamed cell death, it balances proli-cell conflict with age-related changes in structure and function, and maintains homeostasis. Aging disrupts the thyroid gland. Increased fibrosis and nodularitaorderly neuroendocrine feedback regulation of secre-glands are generally agreed upon. Although there is little treatment for GH, luteinising hormone (LH), follicle stimulating evidence of changes in thyroid function in elderly patients, hormone (FSH) and the incidence of both adrenocorticotropin hypothyroidism and hyperthyroidism (ACTH).17 Specific gene products either promote (Bax) increase.1.3 4 Prevalence or against (Bcl-2) controlled cell death via mitochondrial hypothyroidism in elderly patients has been reported to be between 0.5% and 4.4% effects. Dysregulation of apoptosis has been implicated, while the prevalence of hyperthyroidism is among the development of diseases that predominate in 0.5% and 3.23 Hypothyroidism, although more often individuals, such as cancer and neurodegenerative in the elderly, are often more difficult to diagnose.24disorders (e.g. Alzheimer's and Parkinson's disease). Typically, symptoms of hypothyroidismThe main consequences of oestrogen deficiency are os- (e.g. dry skin, sparse hair, constipation, forgetfulness) theoporosis and coronary artery disease (CHD).18.19 can be easily misinterpreted as just old age. For example, in subclinical hypothyroidism, patients have little or no osteoporosis in older men as well as clinical signs, but have normal thyroxine (T4) levels, but especially in postmenopausal women (with elevated thyroid stimulating hormone (TSH) at approximately 50 years of age). It is estimated that about 30% of the level.24 Whether these patients should be treated with older women and 20% of older men have osteoporo-roid hormone or followed with regular thyroid tests issis.18,19 Osteoporosis includes gradual bone loss; Controversial. Inappropriate treatment of the thyroid gland's haz-skeleton becomes weak and less dense due to in-intensive in the elderly, as this can exacerbate the manifestations of incremental bone resorptive unbalanced remodeling of bones. coronary artery disease. Interpretation of thyroid functionAreal cell maintains bone homeostasis. Re- in hospitalized and very sick patients is also difficult, sincesorption and bone formation are performed in an orderly se-effect of non-thyroid-related diseases on common patients throughout the life of osteoclasts and osteoblasts. Tests of thyroid function.24 The disease can depress serum con-osteoclasts to remove bone matrix at a rate of 150 □m percentrations of both triythyronine (T3) and T4, while today; refilling is carried out by osteoblasts at a □ and the TSH remains normal or even decreases. Changes per day. The filling of bones increases the strength of bone protein binding, metabolism of thyroid hormones and vulture-by structure of the osteon. Partially driven muscle compression of the pituitary gland releasing TSH can lead to sedation, the filling is affected by estrogen. Uncompensated these findings in non-thyroid-related diseases. In general, loss of skeletal matter can lead to microfractures and pain. these patients are considered eutyreoids (i.e. they have comginal clinical chemical values in osteoporosis being normal thyroid function) and no hormone supplements generally normal, including serum calcium, phosphorus, tion is prescribed.23 Many early studies have attributed changes in magnesium, alkaline phosphatase and parathyroid status in thyroid function to the natural aging process. More re-a thyroid hormones,18,19, because bone loss is not an aso-cent study, however, suggest that abnormal thyroid glands with massive, rapid removal of minerals, such as for hy-function is probably secondary to some underlying or as-perparatyroidism (parathyroid hypercalcemia) or Paget's sociated disorder, not only in old age.3 Since the thyroid gland (uncontrolled bone turnover). After diagnosing disorders can be presented gently and are often severe di-osteoporosis has been performed, laboratory tests to assess the diagnosis, laboratory evaluation becomes important. Metabolism of the spine (turnover) are useful in following its healthy elderly, there is basically no change in T4, progress in response to treatment. The main risk factors include free T4, thyroid-binding globulin and reverse T3 lion diet, inactive lifestyle, genetic predisposition, smoking, els.6,23,24 However, some studies have shown significant endocrine disorders, and medications.20 The biggest decrease in T3 levels after 50 years of age, with mild in-problem secondary osteoporosis is a hip fracture that crease in TSH levels, perhaps as a normal response to shutdown and associated with nonhealing in older low T3 levels. It is still unclear whether these changes will be popular. Osteoporosis is therefore an important cause or result of the underlying disease.6,23lem in the elderly, leading to increased morbidity and health care costs. There is also a significant association There are few morphological changes in the pancreas in elderly patients, as a certain degree of atrophy and an678 PART 4 ■ SPECIAL AREAS OF CLINICAL CHEMISTRY CASE STUDY 33-1 RISK. This syndrome is characterized by varying degrees of glucose intolerance, abnormal cholesterol and/or a 65-year-old woman hospitalized for levels of triglyceride pneumonia, high blood pressure and upper body and uncontrolled diabetes had an obesity thyroid test, all independent risk factors for heart disease.the results presented in the case study table 33-1.1. PROCAM (Prospective Cardiovascular Munster) study, which examined the relationship between differentDecloths heart risk factors and the incidence of heart attack in 2754 men aged 40-65 years over a 4-year period,1. Based on the patient's condition and laboratory test, it turned out that the presence of diabetes or high blood pres-results, how could thyroid data be explained? same alone increased the risk of heart attack 2.5 times. When diabetes and high blood pressure were both present, risk2. Should further testing be carried out? increased 8 times. Abnormal lipid profile increased the risk 16 times; when abnormal lipid levels were pres-CASE STUDIES TABLE 33-1.1 ent with high blood pressure and/or diabetes, the risk results were 20 times higher. These abnormalities represent insulin resistance syndrome. Result of insulin resistance syn-TEST RESULT REFERENCE RANGE drome was first described in 1988 when it was suggested that the defect was related to insulin □ □. U/ml estimates the impact of 70-80 million Americans.30 Since resistance usually develops long before these diseases AP-Total T4 3.8 □g/dL 4.5-12 □g/dl pear, identification and treatment of insulin-resistant patientsTotal T3 55 ng/dL 60-220 ng/dL has a potentially high preventative value. This condition is common in people with obesity (defined as a physically increased incidence of tumours.25 Although other aspects of the weight index [BMI] are 30 kg/m^2). The pattern of obesity endocrine functions, such as the hypothalamus-anesthesia, is also very important. There is a strong relationship-pituitary system and adrenal glands, show no change in the vessel between abdominal obesity and the degree of insulin recontizol production, aldosterone and decrease in DHEA, regardless of total body weight.31 Degree with age.26,27 Prevalence of both hypertension and in-abdominal obesity can be estimated using cir folds of the waist with age, with about 60% of people older than cumference or waist-hip ratio. The waist is usually 60 years old with the condition.27 Causes include increased measured at the narrowest point and hips in the warmest peripheral resistance due to atherosclerosis, a chronic renal point around the butto offal. Waist-hip ratio to major and endocrine disorders and multiple medications. In men as 1.0 in men or women, the efficacy of homeo-abdominal obesity and insulin resistance and constention control also decreases.4.27 fers increase associated diseases. Advanced glycation end products (AGEs) are thought to play a key role in diabetic nephropathy (DN) and other diabetic complications. These have pronounced effects on endothelial tolerance of glucosis decreases with age, in the elderly cells, monocytes, macrophages, and bind to AGE recep-with slightly higher fasting serum glucose than tors (RAGEs). AGEs are formed by the binding of aldosesyounger adults. Fasting glucose increases by about 1-2 per free NH_2 protein group. Ages tomg/dl binding (0.11 mmol/l) over a decade over a lifetime.6,28 Rages activates endothelial cells, monocytes, areal threshold (the point at which glucose spills in macrophages, which after activation produce cytokinesis) also increases with age; in- and express adhesion of molecules and tissue factors is also available. Thesesulin response to glucose.1,3 Diabetes mellitus is com- they have a role in increased oxidative stress and mi-mon problem in the elderly, with a prevalence of about crovascular lesions in diabetes.32 There is also a complex18.4% in people aged 65 years and older. The above relationship between fatty matter, tumor necrosis factor about expected values for healthy ␣ (TNF-␣) and insulin resistance; however, belly-can be misleading. The definition of diabetes and predia-nism (predia-nism) by which TNF-␣ induces insulin resistance has been revised to glucose values above 126 mg/dL. TNF␣-induced genes include tran- and 100-126 mg/0L. Hyperglycemia, the scripting factors involved in the preadipocyte gene expression—even if it is mild, is not a benign state and appears zion or nuclear factor-□B activation, cytokines and accelerate the aging process. There has been an alarm-cytokine-induced proteins, growth factors, enzymes, and an increase in obesity with increased type 2 diabetes and signaling molecules.33 Based on this, type 2 diabetes is associated with an increase in hypertension and cardiovascular more suspected than chronic inflammatory disease than as pancreatic endocrine dysfunction. CHAPTER 33 ■ CLINICAL CHEMISTRY AND GERIATRIC PATIENT 679Chaining of real function CASE STUDY 33-2All aspects of renal function are affected by aging An otherwise healthy 70-year-old man entered the process; it is the age of onset, specific changes, and conse- hospital for abdominal surgery. Preoperative functions that differ in the elderly.34 The results of kidney function chemistry tests are presented in a case study that begins to decrease after 30 years and, by age 60, Table 33-2.1.is is further halved. What is the BUN/creatinine ratio for this patient?dence of pathological processes (e.g. atherosclerosis).34 2. What do these data suggest? Renal function can be evaluated at any age clinical trials, including urine volume, analysis 3. Which test results support this conclusion?components and concentration, urea nitrogen in the blood (BUN), uric acid and several clearance tests.34 Creatinine 4. How common is this condition in the elderly?clearance, glomerular filtration rate (GFR) and renal reaction flow decrease with age. Analytes, BUN, uric acid and phosphate inor-ganic were found to be reflecting GFR increase.6 REFERENCE RANGE OF TEST RESULTS In general, older people have reduced albumin capacity of 53 g/l 35-50 g/L to preserve water through the kidneys and significantly lower thirst.35 This, of course, can lead to a BUN of 40 mg/dl 8–26 mg/dLdehydration, a common and undervalued problem in elderly patients. Dehydration is not only a common finding in patients with creatinine 1.6 mg/1.5 mg/1.5 mg/l, this may also be serious, leading to an increase in the rate of plague-tality.3 Clinical laboratory tests suggestive of deha-serum osmolality of 330 mOsm/kg 275-295 mOsm/kg increased BUN/creatinneratio, increased serum osmolality and increased sodium sodium intake of 150 mmol/l 135–145mmol/Lspecific gravity.3 in the outpatient elderly population cannot be discounted except for the above mentioned renal. In the population of nursing homes, protein-changing aging, there is an increased incidence of renal ergy malnutrition rates are as high as 40%-50%. If jedisease and reduced ability to handle the excretion of malnutrition rate was 10% and outpatient age drugs. Problems affecting renal function are related to people having albumin 2.8 g/dL, damage to serum albumins by infections or drugs (drugs), hyper concentration per sample of 10,000 people at settlement or disorders such as diabetes mellitus, tuberca-3.5 g/dL would be reduced to 3.4 g/dL. In addition, typelosis and nephritis.34 2 diabetes may be associated with nonalcoholic steatosis and steatohepatitis (fatty liver), which would lead to de-liver changes in creased albumin and transferin function and increased alkaline phosphatase. ⫽-Globulin and ␣1-antitrypsin, however, in general, atrophy and decreased liver weight, as well as a slight increase with age, while haptoglobin remains a decrease in liver function, they are common in the elderly.3,36 are essentially the same as in young adults.28 Similarly, im-although the liver performs many functions (see chapter munoglobulins are reduced in part of the population.24), three important functions—synthetic, excretion areticum, and detoxification and metabolism of drugs—some enzymes are also altered. Alkaline phosphatasesóded in this section in relation to the aging process. (ALP) and lactate dehydrogenase (LDH), for example an increase in both men and women, but synthetic liver function is unlikely to be age-related changes. Synthesis of urea, a process using concentrations of plasma proteins. Tietz, which occurs in hepatocytes, and bilirubin metabolism al.28 reported a slight decrease in total protein in fit as a decrease with age.3.36 liver detoxifying oraging people. Albumin and transferin show a decrease as the drug's metabolism function is important when considering well. This observation should be taken with some reser-increased number of medications that are usually vation. Homogeneity of the population of appropriate aging prescribed by the elderly. Although people aged 65ple need to be taken with a reservation. Declines in age and older make up about 12% of American pop albumin and transferin are likely attributable to ulation, receiving about one-third of all prescribed malnutrition and liver disease in drugs.3 In addition to possible drug interactions, the population studied. The existence of malnutrition, alcoholic liver disease, depression, and bad nutrients680 PART 4 ■ SPECIAL AREAS OF CLINICAL CHEMISTRY Edrog toxicity is a potential problem. As mentioned previ-states, and its prevalence increases with advancing age.38ously, there is some liver atrophy, as well as risk factors for CHD include age, gender, genetic predis-reduced liver blood flow with the aging process. This, location, obesity, hypertension, poor fitness, diabetes in turn, can lead to the accumulation of certain drugs mellitus, cigarette smoking, and hyperlipidemia.3 (eg lidocaine and morphine) and the possibility of tox-icity.3 Medications and the elderly are discussed in Lipids shown to play a major role in atherosclerotic detail later. process and risk of CHD are HDL and low-density lipoprotein (LDL) cholesterol, total cholesterol, apulmonary function and electrolyte triglyceride changes. A study of Tietz et al.28 fit seniors, total cholesterol, HDL cholesterol and triglycerides found several known anatomical and physiological changes occur as part of the aging process.28 HDL choles-in cardiopulmonary function during the aging process. however, terol, or good cholesterol, is considered to be a human function that actually begins to decrease after an important inverse risk factor for CHD, with values less than 25 due to changes in the lungs, rib cage, as about 35 mg/ dL indicating high risk and respiratory muscle values, and respiratory centers at a price of more than 35 mg/ dL indicates a low risk.3tral nervous system.37 Of course, pulmonary function is, perhaps, more affected by personal habits such as smok-enzyme changes, and environmental pollution.37 Changes in enzyme levels during the aging process have more frequently described changes related to being studied extensively; are diverse and complex.pulmonary function in elderly patients includes pO_2 concentration and enzymes and synthesis are below geneticpCO_2 values. These changes reduced vital control and affected by hormones, substrates and (pulmonary) capacity found in most elderly people.37 Arterial other factors. Enzymes do not appear to follow any par-pO_2, for example, decreases during the aging process, a tic age-related pattern; may increase, decrease, or where a slight increase in pCO_2 is reported or remain the same during the ageing process. However, the same.4,37 An increase in bicarbonate was found to mean the ability to initiate adaptive changes in en-sate activity to increase pCO_2 and maintain blood pH with increasing age.3, which are reportedly relatively constant or are declared only slightly.6,37 Enzymes reported to change in healthy elderly people include aspartate aminotransferase (AST), alanine amino-electrolytes sodium, potassium and chloride all transferases (ALT) , ALP, ⫽-glutamyltransferase (GGT), show little change in healthy elderly people from creatine kinase (CK), LDH, and amylase.28 AST, GGT, seen in younger adults.27 Sodium remains a fairly constant LD, and amylase show increases in both men and women.from young adulthood to older age. Chloride levels are ALT levels showing only a marginal increase in men, while also relatively constant, but moderate values in women were found to show no change. The ALP, however, is showing a high in people over the age of 90. Potassium, as a significant increase in women, while men show no in-ever, slightly increases from the age of 60 to 90.27 folds up to the age of 90. Ck values in men increase slightly between the ages of 60 and 69; however, in the elderly aged 90 years, diseases related to 70 and respiratory diseases predominate, values decrease. In women, CK values and people account for 25% of all deaths in the elderly also increase slightly from 60 to 70 years, but de-as-as 85.37 Respiratory diseases of older people fall extincate in those over 70 years of age. Lipase increases lenchronic bronchitis, chronic obstructive pulmonary dis-mild, if at all, in those aged 60-90 years, but definitely cool (COPD), neoplasia and lung infections, especially in patients older than 90.28tuberculosis and pneumonia.37 CLINICAL CHEMISTRY RESULTS AND CHANGES IN AGINGCardiovascular and lipids In addition to knowledge of basic biochemical and cardiovascular diseases, physiological changes in aging remain important, clinical laboratories that have died in both men and women in old age. must understand other factors that may affect clinicalAtherosclerosis, a type of arteriosclerosis, is the main chemical outcome in the elderly. For example: the cause of death from cardiovascular disease in the process associated aging affects the results of laboratory tests sufficientlyStates.3 Atherosclerosis, a progressive process of the disease that require separate reference intervals for the elderly?, which begins at the beginning of life, includes vascular changes What preanalytical variables (e.g. diet, posture, fatty accumulations in vascular ions) associated with the elderly affect clinical chemistry.3 Atherosclerosis develops slowly over the years. Results? How does the aging process affect the interpretationthe results of atherosclerosis include hypertension, hemorrhage- drug levels in the elderly? What are the effects of ex-rheumatism, thrombosis, stroke, and coronary heart disease ercise and nutrition on older people and chemistry results? (CHD). CHD, also called coronary artery disease, continues to be the leading cause of disability and death in UnitedCHAPTER 33 ■ CLINICAL CHEMISTRY AND GERIATRIC PATIENT 681TABLE 33-5 FACTORS TO CONSIDER IN OFTEN, ABNORMAL TEST RESULTS IN ELDERLY PATIENTS AREINTERPRETATION OF THE CLINICAL LABORATORY INTERPRETED AS NORMAL FOR THE AGE OF THE INDIVIDUAL AND NOTRESULTS FOR THE ELDERLY3,22,33 SIGNS OF DISORDER OR DISEASE.11 Research now shows- ing- , However, that in many cases, abnormal Labor-EXERCISE Tory outcomes in so-called healthy individuals may in fact be associated with unknown subclinical disorders or duration with other secondary conditions.3,11 The fact that refer-ence intervals are not easily determined, even for the type of younger healthy adult population, adds to the problem. Reference intervals are often poorly defined andmedications are not always determined by a single process.39 The es-tablishment of reference intervals includes a well-defined polypharmaceutical protocol, including careful selection of reference subjects, control of preanalytical factors, list of analytical interferences comprehension and mobility, careful and consistent collection of samples for a given analyte, analysis of motion samples under well-defined conditions and identification of data errors.39 It is clear that the determination of holding reference intervals for the elderly may be problematic because a large percentage of older people have a certain subclin-NUTRITIONAL STATUS or an apparent pathological abnormality. The National Committee on Clinical Laboratory Standards (NCCLS)PERSONAL HABITS has published important guidance (C28-A) on the determination of the applicable reference intervals for a titration clinical laboratory test for the use of quasi-alcohol40; however, the guidance is not sufficient to validate or establish smoking reference intervals due to underperformance, sample bias and non-reinstallation in confusing conditions in populations with multiple chronic or subclinical samples. FAULTS Although the ageing process affects some selected INTERVALSREFERENCE THE VALIDITY OF ANALYTES, the relationship between ageing and changes in other analytes is less understood. In general, physiciansSPECIMEN COLLECTION VARIABLES and laboratories agree that separate reference intervals for older people are needed to prevent falsely abnormal re-site sults. Knight3 recommends that until we have a better understanding of the relationships between actual aging, trauma age-related disorders, and various laboratory outcomes, and laboratories should maintain normal volume reference intervals established for healthy, younger adults. It is also useful to compare the current val-table 33-5 of a person, which shows that some factors should be associated with the values obtained during his adult life.sider in interpreting laboratory values for the elderly. In any case, physicians and clinical laboratory scientists should bear in mind all factors influencing the interpretation of reference intervals of laboratory test results for the elderly.for older preanalytical variables, the elderly, the interpretation of test results and reference intervals for and the results of chemistry older people can be confusing and complex. The uniqueness of geriatric clinical laboratory results was many preanalytical variables can influence interpretation, which in the past was lacking due to shorter lifespan, resulting in results of chemistry; however, preanalytical variables may cause geriatric samples to be tested. How people live has a much greater impact on older people. Already pre-analyzing criteria for age-related analysis and patient-related interterms include diet, gender, post-treatment geriatric laboratory tests, which become more impor-ture (e.g. sitting or lying), personal habits (e.g. smokers. However, there appears to be some confusion and alcohol consumption), body composition, physically theme of reference intervals for older people between ex-activity and prescribed medications.41 Any of these mayperts in the field of clinical chemistry and ageing. As described above, many reference intervals foranalyte differ from those of younger ults. Some analytes, such as levels of male and fe-male hormones, are an undeniable result of aging or gans. However, for other analytes, the case may not be clear.3 A certain variation in analyte levels may be the result of various secondary conditions (e.g. subclinical discount medicines, inactivity, nutrition) and not the ageing process itself;–for example, the relationship between insulin-dependent diabetes mellitus and an increase in serum glucose levels.682 PART 4 ■ SPECIAL AREAS OF CLINICAL CHEMISTRY affect the concentration of different analytes; For example, therapeutic monitoring of drugs in the elderly body composition changes with age. In healthy people, body fat increases and muscle mass decreases in the elderly, involving 13% of the U.S. population, age. Due to the increase in type 2 diabetes, body fat and inherent health risks, the link to healthy people who use 34% of the prescription and 30% of all non-prescriptions may be unfounded. Body weight and height also reduce medications and are often on several medications.43po approximately 60.41 These changes in turn can affect the levels of various analytes (e.g. creatinine). Unfortunately, overdose and adverse drug reactions Other preanalytical variables affecting laboratory outcomes (from multiple medicines) are problematic in the include sampling for analysis.42 Several physical and physiological changes in the erm. With the normal aging process, there is a lota concern can affect the collection and quality of sample changes in how the body handles drugs.43 Absorption,and phebotomy challenge for phebotomist. Since elderly patients may have diseases such as artri-distribution, metabolism, and excretion of the drug are alltis, malnutrition, or dehydration, in addition to aging alone, muscle tone reduction and aging may be affected. (For multi-level elasticity (i.e. flaccid skins), see Chapter 29). Veins can be difficult to find or unsuitable for use, making phebotomy dif-information on the pharmacokinetics of drugs.) For ex-ficult and causing hemolysis and increased likelihood of increased levels of plasma hemoglobin and LDH.42 Sufficient gastric emptying time can be prolonged in the drugs of elderly patients, it can also provide poor blood senior, causing a delay in the absorption of the drug.44,45flow, resulting in less than adequate sample for some laboratory tests. Although he in the liver may have been disturbed due to age-related being confused, he collaborated and responded to reduced liver mass and blood flow.44physician questions. During the interview, it turned out that the patient did not remember the most serious pharmacokinetic difference in when or when he took digoxin. A decision has been taken to carry out random digoxin levels on the elderly associated with the elimination of medicinal products by means of a limit of the present amount. Kidney. Renal mass and blood flow as a decrease in the number of advancing age.44 Serum albumin may also be reduced1. Based on these results, what are the two options because of the malnutrition of protein energy that affects that may explain the results? medicinal products that are transported bound to albumin. GFR (as2. Further testing ruled out problems with the kidneys, liver or circulation. What is the most likely cause of measured creatinine clearance) also decreases linearly this increased level of digoxin? with age.4,44 For medicinal products which are mainly excreted in the urine,3. What could interfere with the digoxin test? caution should be exercised when treating to avoid overdose or toxic effects Ϫ ϭ.33-1) (72) (serum creatinine in mg/dL)CASE STUDY TABLE 33-3.1 Multiply by 0.85 in women. The ideal weight, rather than the usual weight results, corrects weight in obese individuals. As serum creatinine lower than 0.9 provides a high creatinine clearance, creatinine should be Psychosocial factors common in older people, such as depression, dementia, poverty and loneliness, are also factors that contribute to drug problems. For example, elderly patients may be less compliant (e.g. due to poverty) or unable to follow treatment guidelines, leading to a problem.44 As the effects of medicines are more exaggerated in the elderly, laboratories should be familiar with the principles of monitoring therapeutic medicinal products and the impact of ageing on the monitoring of therapeutic medicinal products. To provide the best quality of care for the elderly, frequent monitoring of medicines and development of therapeutic ranges for the elderly may be necessary.45Digoxine 2.6 ng/ml 0.5–1.5 ng/ml Effects of exercise and nutrition (therapeutic) on the elderly and chemistry results Ͼ 2.0 ng/ml (toxic) Many studies suggest that exercise is an excellent way for older people to maintain health and increase longevity. CHAPTER 33 ■ CLINICAL CHEMISTRY AND GERIATRIC PATIENT 683Rowe and Kahn,35 in a post-exercise study of more than 40,000 patients in patients with type 2 diabetes. menopausal women over a 7-year period reported that with a transient increase in glucose levels of about 1 hour who exercised regularly, they were 20% less likely to die after exercise.47 than those who were sedentary. Shephard46 concluded that exercise can be beneficial for sedentary older popul- Nutritional problems are common in elderly patients.tion (75-80 years) by improving overall health, There is a growing body of information about nutritionally increasing social contacts, and improving brain func-needs of the older population. Older people are attion. Obesity due to metabolic changes reduces the increased risk of poor nutritional status (e.g. protein activity and age-related muscle changes, calorie malnutrition) compared to younger adults, such as the amon problem in the elderly, which is also helped due to both physiological and psychological factors, in-exercise.47,48 Other studies have shown that a) more indications of age-related changes in taste and smell; malabsorp-frequent exercise, the greater the advantage, b) mod-tion caused by drugs or changes in acidity of the stomach; erate exercise (e.g. walking) is almost as beneficial as vig- and mobility, disability, depression and poverty.46,49,50orous exercise, and (c) exercise can negate the adverse incidence of malnutrition in the elderly ineffective other risk factors, such as high blood pressure long-term medical facilities is reported in the range and hyperglycemia.27 Health benefits from exercise 50%-85%; in acute care hospitals, the percentage may include reduced cardiovascular risk, weight control, ranging from 17%-65%.51crete functional capacity, better nutrient intake and better sleep.3,47,48 Relationships between nutrition, overall health, ageing and diseases are important and need to be invested. people are encour-gated further. Protein-calorie deficiency can lead to de-age to participate in exercise programs, laboratorians shrugged off infection and lymphopenia.Will need a better understanding of how exercise affects excessive calories resulting in obesity and the likelihood of test results for these individuals; such as type 2 diabetes exercise. Deficiencies in vitamins A, C and E (effects of lipids by reducing triglycerides and increasing HDL antioxidants) can lead to atherosclerosis and increasedolesterol, insulin decreases and GH increases.47 the risk of cancer.8 Low fiber diets can lead to diverticulitis and colon cancer.3 It is also necessary to consider the type of exer-cise, timing of sampling and prescribed through nutritional evaluation, tokedications may be possible. For example, glucose and insulin do not prevent and identify nutritional deficiencies in the elderly that are consistent with different types of exer-people, thus preventing many chronic diseases of aging and inflammation. Both essentially remain stable during isometric support for better health.38,52,53 In a clinical laboratory exercise with large muscle groups, while insulin de-nutrition evaluation includes measurement of var-folds during dynamic exercise of moderate to high proteins (e.g. albumin, prealbumin, transferin, andtensivity.47 Timing of sampling is an important retinol binding protein) and levels. In detail, since glucagon secretion is stimulated after intensive discussion of nutrition evaluation and vitamins can be found in Chapter 32.

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Clinical Chemistry and CHAPTERthe Pediatric Patient 34Dean C. Carlow, Michael J. BennettCHAPTER OUTLINE■ DEVELOPMENTAL CHANGES FROM NEWBORN Nitrogen end products as markers for adult renal function Respiratory and circulatory liver function Growth tests ■ calcium and bone metabolismMus IN PEDIATRICS Organ development Hypocalcaemia and hypercalcaemia Problems of prematurity and immaturity ■ ENDOCRINE FUNCTION IN PEDIATRICS Hormone Secretion■ FLEBOTOMY AND HYPOTHAL SELECTION pituitary-thyroid system instrumentation for hypothalamus-pituitary-adrenal cortex system PEDIATRIC SAMPLES Growth factors Phlebotomy Endocrine control sexual maturation Preanalytical concerns ■ EVOLUTION OF THE IMMUNE SYSTEM Selection of the analyzer Basic concepts of immunity Components of the immune system■ POINT-OF-CARE ANALYSIS IN PEDIATRICS NEONATAL AND INFANT Anti-body formation■ REGULATION OF BLOOD GASES AND immunity disorders pH ■ GENETIC DISEASES IN NEWBORNS AND INFANTS Cystic fibrosis Measurement of blood gas and acid-base Newborn screening for the entire population■ REGULATION OF ELECTROLYTES AND Diagnosis of metabolic diseases in WATER : RENAL FUNCTION Clinical adjustment disorders affecting electrolytes ■ DRUG METABOLISM AND PHARMACOKINETICS AND THERAPEUTIC MONITORING OF WATER BALANCE MEDICINAL PRODUCTS■ EVOLUTION OF LIVER FUNCTION Toxicological problems in paediatric clinical chemistry Physiological jaundice ■ REFERENCES Energy metabolism Nitrogen metabolism Developmental changes from those in adult situations. In addition, the nature of newborns to adult body size, and therefore the available volume of blood, creates various problems for the analyst with the selection of pediatric laboratory medicine, providing a number of unique operating devices and test menu.portunities to study how homeostatic and physiological mechanical methods that control normal human development The biggest childhood challenge is the birth of evolution. With these opportunities to study the development of the child. There is currently a requirement for a whole new set of challenges, many based on adaptation from intrauterine life, in which homeostasis is the failure of certain components of normal development maintained by maternal and placental remedies, to self-process and resulting diseases. With this scenario in mind, maintenance needed to adapt to extrauterine life. It is clear that the environment for the specialist associated with this adaptation is further complicated by a preddediate laboratory doctor, which differs from the maturity of the environment or intrauterine growth deceleration (IGR), when it is found in practice for adults, in which many organ physiological systems have not reached sufficient maturity development, is not a major issue. Diseases that allow the newborn to adapt to the necessary changes in pediatric practice therefore vary considerably at the time of birth.1 685686 PART 4 ■ SPECIAL AREAS OF CLINICAL CHEMISTRYRespirational and circulation constitutional development of secondary sexual characteristics of adults and possibly for adults. At birth, the normal baby adapts quickly by starting active breathing. Stimuli for this process include problems of prematurity and immaturitylamping pimples, cutting off the mother's oxygen supply, and the baby's first breath. Initiation of breath- Intrauterine development is programmed for normalization requires normal expression of the surfactant in 38- to 40-week gestation.1 Many organs are not completely lungs. Surfactants are essential for normal expansion prepared to deal with extrauterine life prior to this agreement. Toa contraction alveol and allows gaseous exchange of organs immaturity leads to many clinical prob-act. lems that we see associated with prematurity by in-clude respiratory distress (pulmonary immaturity), electrolyte initiation of breathing and expansion of lung volume and water imbalance (immaturity of the kidneys), and excessive attachments increased lung blood flow and decreased jaundice (immaturity of the liver). Infants born before their great pressure. This, in turn, leads to the conclusion of the due date represent a great burden for laboratory.ductus arteriosus and shift in blood flow through they not only have abnormal biochemical parameters of the heart, which allows newly oxygenated blood from the lungs, which require frequent blood collection, they also have to be directed through the left side of the heart to the volumes of blood from which to draw.body. Blood flow now goes from the right side of the heart to the lungs for oxygenation. The closure of phlebotomy ductus and CHOICEarteriosus is essential for this process. INSTRUMENTATION FOR PAEDIATRIC

SAMPLESGrowth PhlebotomyA normal child delivered in term weighs about 3.2 kg. Ababa weighing less than 2.5 kg in term is considered to be blood collection from infants and young children is small due to gestational age (SGA), which is usually a result associated with patient size and often IGR ability. Babies birth weight born before the deadline is a patient who communicates with a phebotomist.considered premature. In the first days of life, the weight of a small volume of blood of small patients dictated by bothloss is the result of insensitive loss of water through the skin. the number of tests that can be safely performed onThing is generally compensated by weight gain of 6 g / kg per day of the patient and the number of times that blood can safely beas feeding is initiated. The infant's body weight will be stretched for repeated analysis.2 Table 34-1 shows the per-ble in 4–6 months. Premature babies tend to grow in centering total body blood, which is drawn from the indi-slower pace and often still weighs less than the term baby on the vidual with a 10-ml blood draw. This volume is standard equal to the term. in laboratory medicine for adults, but the table clearly shows that this amount of blood accounts for about 5% of the total volume of blood development in preterm newborns. It is clear that frequent blood donations of this kind quickly lead to anemiaMly organs are not fully rolled at birth. Glomerular and the need for blood transfusions. Table 34-2 shows the rate of renal filtration and renal tubular function during the first year of life, at which point labo-TABLE 34-1 CONSEQUENCES of 10-mLratory markers of renal function approximate adult val-BLOOD DRAW IN INFANT POPULATIONUES. Liver function can take 2-3 months to fully mature. Motor function and visual acuity develop during age weight (kg) TOTAL BLOODfirst year of life. This development is accompanied by a 26-week pregnancy of 0.9 VOLUME (%) changes in the electroencephalogram until a normal 32-week picture of pregnancy of 1.6 adults is shown. There are dramatic changes in 34 weeks' gestation 2.1 9.0hematopoiesis as the transition from fetal hemoglobin to the term 3.4 5.5adult hemoglobin takes place. This coincides with sig-3 months 5.7 4.0nificant hyperbilirubinemia as fetal hemoglobin is bro-6 months 7.6 2.5ken down, coinciding with immature liver pathways 12 months 2.0 bilirubin metabolism. Bone growth in the rapid 24 months of the 10, 1 1, 6growth phase in the first few years of life and in puberty 12.6 1.4 results in cyclic changes in bone growth markers. 1. Sexual maturation results in significant changes in the endocrine system, especially the hypothalamus-pituitary-gonade hormone pathway, which eventually lead to THECHAPTER 34 ■ CLINICAL CHEMISTRY AND PAEDIATRIC PATIENT 687TABLE 34-2 RECOMMENDED BLOOD DRAW BONES. A stab in the bone can result in osteomyelitis. VOLUMES FOR PEDIATRIC PATIENTS Excessive squeezing or milking at the site of the lancet can re-sult in both hemolysis and factitious hyperkalysis from the mass MAXIMUM WEIGHT OF MAXIMUM TISSUE FLUID LEAKAGE. (kg) VOLUME FOR (kg) VOLUME FOR ONE BLOOD ONE BLOOD Pre-analytical concerns 2 DRAW (ml) 30 DRAW (ml) 4 32.5 There is a growing trend towards complete front-end au-6 4 35 60 tomation in clinical chemistry laboratories. 8 8 37.5 65 advantage with automation the processing of personal data is subject to 10 407 5 critical data and additions to some tips on how to choose to roam in 2019 10 42 5 75 Per turn or a few days. Several issues were retarded by the introduction of 17.5 25 47.5 85 full-scale automation in pediatrics. A typical pediatric 20 30 50 90 chemical laboratory receives samples in tubes of many 22.5 35 52.5 95 different sizes, differ from standard adult tubes to 25 40 55 100 small peditubes. While several large chemistry 27.5 45 105 analyzers can direct samples from small pediatric tubes, 50 110 since then, no manufacturer of laboratory equipment 55 has developed a fully automated system that can handle this range of tubes. Courtesy of Dr. David Friedman, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania. However, there have been reports in the literature from individual laboratories that have modified existing lines for blood volume collection developed on robotic instrumentation to complete automation of Children's Hospital of Philadelphia. It is necessary to test the process using pediatric tubes.4,5occasion advise the doctor that a particular set of oforders can result in excessive blood exhaustion and trans- The second important question concerns the requirement of evaporation evaporation. samples from open tubes. Most automated sampling systems require open tubes for processing. Infants and children have smaller veins than adults; with large volumes of samples, the effect of evaporation is to ensure that small veins do not collapse, narrow gauge at least. With small volumes that have relatively large coincidences are generally used for venituncture. Smaller nee- surface areas on the total volume, evaporation can be signifi-dles increase the risk of hemolysis and hyperkalysis. can affect results by up to 10%. Often, good access to veins is impossible in pe- Choice analyzerdiatric patient with intravenous and central lines in place. Capillary samples are often collected when suit- careful inspection and selection of analytical systems to remain veins are not available. However, capillary blood is crucial for handling paediatric samples. Until recently, tainted by skin puncture is usually contaminated, at least a few analyzers were able to perform more to some extent interstal fluid and tissue impurities. Protein concentration (and protein-bound con-TABLE 34-3 DIFFERENCES IN COMPOSITENStituents) is approximately three times lower in intersti-capillary and venous serumial fluid than in plasma. Table 34-3 highlights the main differences in analyte composition between venous capillary value NO DIFFERENCE CAPILLARY VALUEserum and capillary serum. Lower concentrations greater than between less THANprotein, bilirubin, and calcium in the capillary sample of VENOUS CAPILLARY VENOUSlikely reflect mixing (and dilution) with interstitial fluid. VALUE (%) A VENOUS VALUE (%)Capillary samples with either heel or stick, valuesbe should be collected by flebotomists with pediatric expertise. Glucose 1,4 Bilirubin 5,0Eeth should be included and well perfusion on arteri-phosphorous capillaries. This can be achieved by gentle rubbing- Potassium 0.9 Calcium 4.6bing area or immersion in warm water. Lancet urea should be in the heel area from 1.8 sodium chloride 2.3 Total Protein 3.3 From Burtis CA, Ashwood ER, eds. Tietz basics of clinical chemistry. 5. ed. Philadelphia, Pa.: WB Saunders, 2001.