ERYTHROMYCIN

A MEDICAL DICTIONARY, BIBLIOGRAPHY,
AND ANNOTATED RESEARCH GUIDE TO
INTERNET REFERENCES

JAMES N. PARKER, M.D.
AND PHILIP M. PARKER, PH.D., EDITORS

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The collective knowledge generated from academic and applied research summarized in various references has been critical in the creation of this book which is best viewed as a comprehensive compilation and collection of information prepared by various official agencies which produce publications on erythromycin. Books in this series draw from various agencies and institutions associated with the United States Department of Health and Human Services, and in particular, the Office of the Secretary of Health and Human Services (OS), the Administration for Children and Families (ACF), the Administration on Aging (AOA), the Agency for Healthcare Research and Quality (AHRQ), the Agency for Toxic Substances and Disease Registry (ATSDR), the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the Healthcare Financing Administration (HCFA), the Health Resources and Services Administration (HRSA), the Indian Health Service (IHS), the institutions of the National Institutes of Health (NIH), the Program Support Center (PSC), and the Substance Abuse and Mental Health Services Administration (SAMHSA). In addition to these sources, information gathered from the National Library of Medicine, the United States Patent Office, the European Union, and their related organizations has been invaluable in the creation of this book. Some of the work represented was financially supported by the Research and Development Committee at INSEAD. This support is gratefully acknowledged. Finally, special thanks are owed to Tiffany Freeman for her excellent editorial support.

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FORWARD

In March 2001, the National Institutes of Health issued the following warning: "The number of Web sites offering health-related resources grows every day. Many sites provide valuable information, while others may have information that is unreliable or misleading."[1] Furthermore, because of the rapid increase in Internet-based information, many hours can be wasted searching, selecting, and printing. Since only the smallest fraction of information dealing with erythromycin is indexed in search engines, such as **www.google.com** or others, a non-systematic approach to Internet research can be not only time consuming, but also incomplete. This book was created for medical professionals, students, and members of the general public who want to know as much as possible about erythromycin, using the most advanced research tools available and spending the least amount of time doing so.

In addition to offering a structured and comprehensive bibliography, the pages that follow will tell you where and how to find reliable information covering virtually all topics related to erythromycin, from the essentials to the most advanced areas of research. Public, academic, government, and peer-reviewed research studies are emphasized. Various abstracts are reproduced to give you some of the latest official information available to date on erythromycin. Abundant guidance is given on how to obtain free-of-charge primary research results via the Internet. **While this book focuses on the field of medicine, when some sources provide access to non-medical information relating to erythromycin, these are noted in the text.**

E-book and electronic versions of this book are fully interactive with each of the Internet sites mentioned (clicking on a hyperlink automatically opens your browser to the site indicated). If you are using the hard copy version of this book, you can access a cited Web site by typing the provided Web address directly into your Internet browser. You may find it useful to refer to synonyms or related terms when accessing these Internet databases. **NOTE:** At the time of publication, the Web addresses were functional. However, some links may fail due to URL address changes, which is a common occurrence on the Internet.

For readers unfamiliar with the Internet, detailed instructions are offered on how to access electronic resources. For readers unfamiliar with medical terminology, a comprehensive glossary is provided. For readers without access to Internet resources, a directory of medical libraries, that have or can locate references cited here, is given. We hope these resources will prove useful to the widest possible audience seeking information on erythromycin.

The Editors

[1] From the NIH, National Cancer Institute (NCI): **http://www.cancer.gov/cancerinfo/ten-things-to-know**.

CHAPTER 1. STUDIES ON ERYTHROMYCIN

Overview

In this chapter, we will show you how to locate peer-reviewed references and studies on erythromycin.

The Combined Health Information Database

The Combined Health Information Database summarizes studies across numerous federal agencies. To limit your investigation to research studies and erythromycin, you will need to use the advanced search options. First, go to **http://chid.nih.gov/index.html**. From there, select the "Detailed Search" option (or go directly to that page with the following hyperlink: **http://chid.nih.gov/detail/detail.html**). The trick in extracting studies is found in the drop boxes at the bottom of the search page where "You may refine your search by." Select the dates and language you prefer, and the format option "Journal Article." At the top of the search form, select the number of records you would like to see (we recommend 100) and check the box to display "whole records." We recommend that you type "erythromycin" (or synonyms) into the "For these words:" box. Consider using the option "anywhere in record" to make your search as broad as possible. If you want to limit the search to only a particular field, such as the title of the journal, then select this option in the "Search in these fields" drop box. The following is what you can expect from this type of search:

- **Erythromycin and Amoxicillin?**

  Source: Journal of the Tennessee Dental Association. 81(1): 34-36. Winter 2001.

  Contact: Available from Journal of the Tennessee Dental Association. 2104 Sunset Place, Nashville, TN 37212. E-mail: tda@tenndental.org.

  Summary: A large number of patients with odontogenic (arising in the teeth) infections are referred to the graduate and undergraduate oral surgery clinics at the University of Tennessee, College of Dentistry. These patients have often been placed on antibiotics by the referring dentist. Two of the more commonly prescribed antibiotics are erythromycin and amoxicillin. This article provides a brief review of the antibiotics most commonly used to treat odontogenic infections, and illustrates why erythromycin and amoxicillin may not be the best choice. Other drugs discussed include penicillin,

cephalosporins, clindamycin, and metronidazole. The author concludes that two drugs that are effective alternatives in the penicillin allergic patient are cephalexin and clindamycin. They are bactericidal and effective against the oral streptococci and oral anaerobes that cause most odontogenic infections. 5 references.

- **Octreotide Enhances the Accelerating Effect of Erythromycin on Gastric Emptying in Healthy Subjects**

  Source: Alimentary Pharmacology and Therapeutics. 16(8): 1563-1570. August 2002.

  Contact: Available from Alimentary Pharmacology and Therapeutics. Blackwell Science Ltd., Osney Mead, Oxford OX2 OEL, UK. +44(0)1865 206206. Fax +44(0)1865 721205. E-mail: journals.cs@blacksci.co.uk. Website: www.blackwell-science.com.

  Summary: Erythromycin exhibits gastrokinetic properties through cholinergic pathways. Reports regarding the action of octreotide on gastric emptying are conflicting. This article reports on a study undertaken to assess the hypothesis that serotonin receptors are involved in the accelerating effect of erythromycin on gastric (stomach) emptying; and any modification of the gastrokinetic action of erythromycin induced by octreotide. Gastric emptying of a standard meal was estimated in 20 healthy subjects by scintigraphy on three different occasions in 3 conditions: after placebo, after 200 milligrams of intravenous erythromycin, and after 200 milligrams of intravenous erythromycin following pretreatment with either 4 milligrams of intravenous ondansetron or 50 micrograms octreotide. Erythromycin significantly accelerated gastric emptying in all subjects by abolishing the lag phase. Pretreatment with ondansetron abolished the accelerating effect of erythromycin by restoring the emptying times to placebo levels. Octreotide significantly enhanced the accelerating effect of erythromycin by reducing both the lag and post-lag phases of gastric emptying. 7 figures. 1 table. 41 references.

Federally Funded Research on Erythromycin

The U.S. Government supports a variety of research studies relating to erythromycin. These studies are tracked by the Office of Extramural Research at the National Institutes of Health.[2] CRISP (Computerized Retrieval of Information on Scientific Projects) is a searchable database of federally funded biomedical research projects conducted at universities, hospitals, and other institutions.

Search the CRISP Web site at **http://crisp.cit.nih.gov/crisp/crisp_query.generate_screen**. You will have the option to perform targeted searches by various criteria, including geography, date, and topics related to erythromycin.

For most of the studies, the agencies reporting into CRISP provide summaries or abstracts. As opposed to clinical trial research using patients, many federally funded studies use animals or simulated models to explore erythromycin. The following is typical of the type of information found when searching the CRISP database for erythromycin:

[2] Healthcare projects are funded by the National Institutes of Health (NIH), Substance Abuse and Mental Health Services (SAMHSA), Health Resources and Services Administration (HRSA), Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDCP), Agency for Healthcare Research and Quality (AHRQ), and Office of Assistant Secretary of Health (OASH).

- **Project Title: 13C 13C CORRELATION SPECTROSCOPY OF U 13C ERYTHROMYCIN**

  Principal Investigator & Institution: Rienstra, Chad M.; Massachusetts Institute of Technology Room E19-750 Cambridge, Ma 02139

  Timing: Fiscal Year 2002

  Summary: In addition to the developments described below, we have recently demonstrated highly precise internuclear distance measurements with the CMR7 pulse sequence. Homonuclear couplings can be measured to a precision of better than 5 Hz with this approach, implying internuclear distance measurements of better than 0. 1-0.2 A precision out to 4-5 'A. We have developed a pulse sequence for efficient double-quantum dipolar recoupling in multiple spin systems under magic-angle-spinning NMR, based on the C7 sequence of Levitt and co-workers. For two-spin systems, the C7 sequence offers higher overall polarization transfer and double-quantum filtration (DQF) efficiency (-73%) than the MELODRAMA sequence (-52%), because the dependence of the recoupled interaction on rotor phase is eliminated. Experimentally, however, DQF efficiency with the C7 sequence depends significantly on the errors that arise from cross terms between chemical shifts and radiofrequency (rf) field inhomogeneity. Many applications require the excitation of DQ coherence in multi-spin systems, which display a wide range of isotropic and anisotropic chemical shifts. Also, the experimental reliability of the sequence is crucial for successful implementation under conditions of low sensitivity and temperature. To meet these requirements, we have constructed a pulse sequenc e that recouples dipolar interactions independent of chemical shifts, by combining Cn elements of various symmetries. The error terms inherent to the C7 sequence are removed by composite MLEV-type rotations; therefore, we refer to the new sequence as CMR7 (Combined MLEV-Refocusing with C7). We have demonstrated the utility of this approach with double-quantum filtration of U-"Clabeled amino acids and "C-"C chemical shift correlation spectroscopy of the U-"C-labeled antibiotic, **erythromycin** A. With 73% polarization transfer, the ratio of crosspeak to diagonal intensity is expected to be almost 3: 1, and in two-spin cases such as U- "C, "N-Gly, we have observed better than 2: 1 relative intensities in 2D spectra. However, the full theoretical DQF efficiency is usually not realized in multi-spin systems and similar behavior is observed in correlation spectra with respect to crosspeak intensities. Nevertheless, in many cases crosspeak intensities exceed the diagonal peaks, and in favorable instances the ratio of intensities is greater than 2: 1, even in the multi-spin limit. An illustrative example of these effects, and the improvement in resolution observed upon extending to a second 13C chemical shift dimension, is provided by the U-"C-labeled macrolide antibiotic **erythromycin** A (EA). EA inhibits protein synthesis by binding to a bacterial ribosome. Actual structural information on the erythromycin-ribosome complex has been inaccessible due to the paucity of available crystal s and poor resolution of the solution NMR spectra due to the slow reorientational motion of the ribosome. As a result, the erythromycin-ribosome complex exhibits a solid-state NMR spectrum even in solution. Therefore, in order to facilitate structural studies of EA in both its free and complexed forms, it is necessary to make unambiguous chemical shifts assignments in the solid state. We have accomplished the chemical shift assignments using the CMR7 method and are pursuing further structural studies based upon the CMR7 method.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: 13C ERYTHROMYCIN BREATH TEST TO DETERMINE DRUG DOSAGES**

  Principal Investigator & Institution: Watkins, Paul B.; Professor of Medicine; University of Michigan at Ann Arbor 3003 South State, Room 1040 Ann Arbor, Mi 481091274

  Timing: Fiscal Year 2002; Project Start 01-MAR-2002; Project End 28-FEB-2003

  Summary: This abstract is not available.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: A NEW LEWIS ACID CATALYZED CLAISEN REARRANGEMENT**

  Principal Investigator & Institution: Macmillan, David W.; None; California Institute of Technology Mail Code 201-15 Pasadena, Ca 91125

  Timing: Fiscal Year 2002; Project Start 01-JUN-2000; Project End 31-MAY-2005

  Summary: (Principal Investigator's Abstract) The objective of this research proposal is to invent catalytic synthetic methods that allow enantioselective access to structural and stereochemical motifs, which although common among anti-viral, anti-cancer, anti-bacterial and anti-inflammatory medicinal agents, connot be readily accessed using conventional methods. In this endeavor, we target processes that are readily applied within the related discipline of enantioselective catalysis and therefore will have a direct and immediate impact on the production of single enantiomer drugs with established biological importance. Our intent is to develop synthetic methods of broad utility and function that will ultimately provide new chemical tools for the diverse range of biomedical researchers that utilize molecule construction. As a consequence, this core research will prove valuable to a number of wide-ranging therapeutical areas. One of the most powerful tools for carbon-carbon bond formation in organic synthesis is the Claisen (3,3)-sigmatropic rearrangement. Remarkably, however, an enantioselective catalytic variant of this reaction has yet to be developed. This proposal outlines a new Lewis acid catalyzed Claisen rearrangement that is amenable to enantioselective catalysis and therefore the construction and modification of a diverse range of biologically important molecules and targets. The strategy is predicated on a new Lewis acid catalyzed Claisen rearrangement recently developed in our laboratory. We have already successfully demonstrated that this catalytic methodology is applicable to the construction of an unusually diverse spectrum of structural motifs. A major goal of this research is to utilize this powerful carbon-carbon bond forming methodology to expedite the synthesis of complex targets with important biological activity. One such example is the proposed general strategy towards the total syntheses of the briaranes, a marine metabolite family with extensive medicinal potential that have yet to be accessed through synthetic construction. This proposal outlines a new Lewis acid catalyzed tandem acyl-Claisen rearrangement that is broadly useful for the rapid construction of molecular complexity from simple reagents. This work will develop an innovative strategy for the one-step synthesis of stereochemically complex acyclic frameworks based upon a new tandem-Claisen reaction sequence. Having demonstrated the feasibility of this transformation, we hope to determine the scope and limitations of this catalytic tandem reaction methodology for the production of a range of functional, stereochemical and structural motifs. This methodology will be used in conjunction with our acyl-Claisen reaction for the highly expeditious synthesis of erythronolide B; a member of the **erythromycin** antibiotic class. This new chemical tool should prove valuable for the rapid construction of erythronolide analogues; an important area of research for treatment of resistant bacterial strains.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: ANTIBIOTIC HYPERSUSCEPTIBILITY MUTATIONS IN BACTERIA**

  Principal Investigator & Institution: Neyfakh, Alex A.; Associate Professor; Medicinal Chem & Pharmacognosy; University of Illinois at Chicago 1737 West Polk Street Chicago, Il 60612

  Timing: Fiscal Year 2002; Project Start 15-FEB-2002; Project End 31-JAN-2006

  Summary: (Adapted from the Applicant's Abstract): The escalating problem of bacterial resistance to antibiotics calls for radical changes in the existing antibacterial therapies. One of the most promising approaches is the use of antibiotic potentiators, compounds that make bacterial cells hypersusceptible to antibiotics. The goal of the project is to identify multiple novel molecular targets for potentiators. This will be accomplished by isolating antibiotic hypersusceptibility mutations of Gram-negative bacteria, Acinetobacter and/or Escherichia coli. These mutations will specify bacterial proteins whose inhibition is likely to potentiate antimicrobial action of antibiotics. Antibiotic hypersusceptibility is a very difficult phenotype to select, and only few such mutations are known. We have designed and tested a novel genetic strategy for selection of hypersusceptibility mutations, termed SDR. Application of this strategy will identify multiple mutations increasing bacterial susceptibility to beta-lactams (ampicillin, ceftazidime, imipenem), translational inhibitors (erythromycin, linezolid, tetracycline, and chloramphenicol) and fluoroquinolone antibiotics (ciprofloxacin). The molecular mechanisms underlying the effects of the most interesting of these mutations will be analyzed. In addition to identifying promising targets for potentiators, the project will help unravel new aspects of the mechanism of action of antibiotics and new features of bacterial physiology.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: ANTIBIOTIC INHIBITION OF BACTERIAL RIBOSOME FORMATION**

  Principal Investigator & Institution: Champney, William S.; Biochem and Molecular Biology; East Tennessee State University Box 70565 Johnson City, Tn 37601

  Timing: Fiscal Year 2002; Project Start 01-JUL-1998; Project End 28-FEB-2005

  Summary: (provided by applicant): The current resurgence of antibiotic-resistant organisms underscores the importance of gaining a better understanding of antibiotic mechanisms, resistance modes and the structural features necessary for optimal effectiveness. The overall objective of this proposal is to learn how five structurally different antibiotics inhibit the process of bacterial cell growth. This investigation will explore the new observation that macrolide antibiotics as well as the ketolides, lincosamides, streptogramin B compounds and oxazolidinones can all inhibit the assembly of the large ribosomal subunit in bacterial cells. Ribosome formation will be analyzed in Staphylococcus aureus and Escherichia coli cells to define the inhibitory features of these compounds. The mechanism of subunit assembly inhibition will be tested by examining the components of the subunit precursor particles which accumulate in the presence of the antibiotic. Aspects of the breakdown of the inhibited assembly intermediate will also be studied. Ribosomal subunits will be reconstituted from component RNAs and proteins to define the molecules involved as targets for assembly inhibition. An investigation of this assembly-sensitive site and the mode of inhibition of assembly will reveal how certain antibiotics can have two inhibitory activities. The findings from this work will help in assessing the effectiveness of existing antibiotics and in developing new compounds as antimicrobial agents.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: ANTIBIOTIC REGULATORY GENES AND METABOLIC ENGINEERING**

  Principal Investigator & Institution: Reeves, Andrew R.; Fermalogic, Inc. Chicago Technology Park Chicago, Il 60612

  Timing: Fiscal Year 2002; Project Start 15-AUG-1999; Project End 31-AUG-2003

  Summary: (provided by applicant): Strain improvement of commercial fermentations helps to reduce the cost of production of existing pharmaceuticals and helps industry to meet the growing demands for desperately needed new products, such as antibiotics, that can be produced in large enough quantities and at prices the public can afford. This project focuses on strain improvement in a bacterium that generates a widely used antibiotic, **erythromycin.** This bacterium, Saccharopolyspora erythraea, is a member of the Actinomycete family and is widely used in academic research and industry, making it an excellent model system for this work. The objective of this project is to identify and manipulate genes responsible for controlling **erythromycin** yield during fermentation. In Phase I a mutagenic plasmid insertion library was created in Sac. erythraea and four classes of morphological and pigmentation mutants were found using a simple visual screen. Three classes of mutants were found that showed significant increases in **erythromycin** production. An efficient plasmid rescue technique allowed recovery of the integrated plasmid and DNA sequence analysis of the plasmid insert. In Phase II the screening and mutant analysis will continue. The strain improvement genes found could have general application to strain improvement programs for other drugs. PROPOSED COMMERCIAL APPLICATION: Not Available

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: ANTI-INFECTIVES WITH MULTI-DOMAIN RIBOSOMAL BINDING**

  Principal Investigator & Institution: Katz, Leonard; Vice Present of Biological Sciences; Kosan Biosciences 3832 Bay Center Pl Hayward, Ca 94545

  Timing: Fiscal Year 2002; Project Start 15-JUN-2001; Project End 31-MAY-2003

  Summary: Resistance to macrolide antibiotics has increased at alarming rates in recent years, driving the need to develop new and more effective antibiotics. The long term objective of this proposal is to develop a novel 16-membered macrolide antibiotic that is active against erythromycin-resistant Streptococcus pneumoniae and other Gram positive pathogens and which can be produced at reasonable cost. The proposed compound is designed to exhibit its potency through the novel mechanism of synergistic multi-domain ribosomal binding. Consequently, the compound should not induce macrolide resistance and evade all known efflux mechanisms that confer macrolide resistance. Phase I is a proof of principle project to produce a small series of derivatives of a 16- membered macrolide that is a readily available fermentation product, and determine whether the derivatives bind to domain II of the ribosomes and exhibit increased potency against macrolide-resistant strains. Phase II Specific Aims will be to optimize the derivatives to achieve oral bioavailability. Lead compounds will be examined in vitro and in animals for efficacy, toxicity and pharmacokinetics with the intent of advancing one or more to clinical development. PROPOSED COMMERCIAL APPLICATION: Clinical development candidates could be commericialized as anti-infective agents only after approval by the appropriate regulatory authorities.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: BIOSYNTHESIS OF MICROBIAL POLYKETIDES**

  Principal Investigator & Institution: Cane, David E.; Vernon K. Krieble Professor of Chemistry; Chemistry; Brown University Providence, Ri 02912

  Timing: Fiscal Year 2002; Project Start 01-AUG-1977; Project End 31-JUL-2005

  Summary: Ongoing studies of the ensymology of complex polyketide natural product biosynthesis will be continued and extended, with focus on the macrolide antibiotics **erythromycin** (1), methymycin (2), and tylosin (3), as well as the antitumor metabolite epothilone (4). Each of these metabolites is assembled by exceptionably large, multifunctional, modular proteins known as polyketide synthases (PKSs) that are closely related to fatty acid synthases, both biochemically and genetically. In addition, epothilone synthase contains additional catalytic activities belonging to the class of non-ribosomal peptide synthetases (NRPSs). A combination of chemical, enzymological, and molecular genetic techniques this being used to elucidate the molecular basis for the programming of the complex series of reactions responsible for polyketide chain elongation. The emphasis in this work is on the elucidation of the mechanisms of multi-step, enzyme-catalyzed transformations leading to formation of biologically important metabolites. It is expected that the results of these studies will be broadly applicable not only to the understanding of polyketide and other natural product biosynthetic processes in general, but will provide fundamental insights into how catalysis and molecular recognition control both product specificity and molecular diversity in Nature. 1) Deoxyerythronolide B synthase (DEBS) is a modular PKS that catalyzes the formation of 6-deoxyerythronolide B (5), the parent aglycone of **erythromycin** A. Individual modules of the DEBS protein, responsible for catalysis of a single round of polyketide chain elongation and functional group modification, can be expressed in E. coli. These modules will be used to study the biochemical basis for the specificity and selectivity of individual catalytic domains, particularly the ketosynthase (KS) domains that mediate the key polyketide chain-building decarboxylative condensation reaction. 2) The methymycin and tylosin PKSs have intriguing similarities and differences to the well-studied DEBS system. Individual modules of the methymycin/picromycin and tylactone PKSs will be expressed in E. coli in order to investigate their biochemical function and substrate specificity. 3) The EpoA protein, the loading module for the epothilone hybrid PKS/NRPS, will be expressed in E. coli in order to study the EpoA-catalyzed conversion of malonyl-CoA to acetyl-S-EpoA, the substrate for the NRPS module EpoB.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: COMPARE GASTRIC ENTERAL FEEDINGS W ERYTHROMYCIN VS TRANSPYLORIC IN CRITICALLY ILL**

  Principal Investigator & Institution: Boivin, Michel; University of New Mexico Albuquerque Controller's Office Albuquerque, Nm 87131

  Timing: Fiscal Year 2002

  Summary: This abstract is not available.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: EFFECT OF CIRRHOSIS AND SHUNTS ON DRUG DISPOSITION**

  Principal Investigator & Institution: Gorski, J. Christopher.; Associate Professor of Medicine; Medicine; Indiana Univ-Purdue Univ at Indianapolis 620 Union Drive, Room 618 Indianapolis, in 462025167

Timing: Fiscal Year 2002; Project Start 15-AUG-2002; Project End 31-JUL-2007

Summary: (provided by applicant): It is well established that hepatic cirrhosis results in reduced clearance of drugs that are highly metabolized and an enhanced sensitivity to the pharmacological and adverse actions of drugs. Chronic alcohol consumption and hepatitis C are the two most common causes of cirrhosis in the United States with an incidence of 3.1 per 1000 people. The development of portal hypertension is the primary mechanism behind several major complications of cirrhosis such as bleeding from gastroesophageal varices, hepatic encephalopathy, and ascites. Transjugular intrahepatic portosystemic shunts (TIPS) and other surgical shunts are performed to manage these complications of portal hypertension. We have demonstrated that in addition to a reduction in hepatic clearance, cirrhotic patients with TIPS experience an increase in intestinal availability of midazolam, a selective cytochrome P450 3A (CYP3A) substrate. This increased bioavailability primarily reflects a functional lack of intestinal wall first-pass metabolism relative to cirrhotics without TIPS and healthy volunteers. The mechanism for this lack of intestinal wall metabolism is unknown. We propose to characterize the mechanism and consequences of this loss of intestinal wall CYP3A activity in cirrhotics with TIPS by directly examining the CYP3A protein and mRNA levels, intestinal permeability, and in vivo hepatic and intestinal CYP3A activity before, immediately after, and I month after TIPS placement. Cirrhotic patients with TIPS, and potentially other types of portosystemic shunts, are expected to be at risk for excessive pharmacological effects or suffer from an increased incidence of adverse reactions following CYP3A substrate administration. We will examine the susceptibility of these individuals to adverse drug reactions and drug-drug interaction by examining the ability of **erythromycin** to prolong the QT interval and clarithromycin to inhibit metabolism of buspirone, a CYP3A substrate. Finally, the expression of other enzymes such as UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs) and p-glycoprotein may also be altered in cirrhosis. We will characterize the changes in these enzymes using the partial clearance of acetaminophen to glucuronide (UGT) and sulfate (SULT) conjugates and the disposition of fexofenadine in cirrhotics with and without TIPS and healthy volunteers.

Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: ENZYMATIC DETERMINANTS OF ERYTHROMYCIN STEREOCHEMISTRY**

  Principal Investigator & Institution: Summers, Richard G.; Chemistry; University of the South Sewanee, Tn 37375

  Timing: Fiscal Year 2000; Project Start 01-SEP-2001; Project End 30-APR-2004

  Summary: (adapted from applicant's abstract): The proposed research focuses on the biosynthesis of the clinically important antibiotic **erythromycin.** It is the long-term goal of this project to produce novel **erythromycin** derivatives that cannot be prepared by traditional chemical syntheses. New antibiotic derivatives such as these are urgently needed, particularly in light of the increased threat posed by newly emergent antibiotic resistant bacteria. In specific, this work seeks to determine the enzymatic domains that dictate the stereochemistry of the **erythromycin** macrolactone ring and then use this knowledge to genetically engineer the antibiotic producing bacteria, Saccharopolyspora erythraea, to produce new **erythromycin** derivatives. Currently, the genes for the **erythromycin** synthase have been cloned, and much is known about the biosynthesis of this chemically complex antibiotic. Yet, the enzymatic domains responsible for the stereochemical configuration of ten distinct sites in the **erythromycin** macrolactone ring are unknown. Since it has already been shown that the **erythromycin** synthase can be

altered to produce new **erythromycin** derivatives through genetic engineering, knowledge of the determinants of **erythromycin** stereochemistry should enable the production of entirely new series of antibiotic derivatives, many of which may be biologically active. Indeed, just through alterations in stereochemistry, over a hundred new erythromycins are theoretically accessible. The approach to be taken here centers initially on the in vitro construction of genetic chimeras encoding altered **erythromycin** synthases using standard recombinant DNA techniques. These altered synthases will feature enzymatic domain interchanges focusing on those domains most likely involved in the determination of **erythromycin** stereochemistry (i.e. a domain thought to produce one stereochemical outcome will be replaced with an analogous domain thought to produce the opposite stereochemical outcome). Once the genetic chimeras have been constructed in vitro, the wild type genes of the natural erythromycin-producing organism will be replaced (via a two step gene replacement protocol) and the **erythromycin** derivatives produced by the mutant organisms will be isolated and characterized by NMR. Importantly, most of the work proposed here will be conducted by undergraduate chemistry and biology majors, consequently this research project will also provide an ideal training opportunity for students interested in medical biotechnology and genetic engineering.

Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: GENETIC ANALYSIS OF RICKETTSIA PROWAZEKII**

  Principal Investigator & Institution: Wood, David O.; Professor; Microbiology and Immunology; University of South Alabama Mobile, Al 366880002

  Timing: Fiscal Year 2002; Project Start 01-JUL-1983; Project End 30-JUN-2005

  Summary: (Adapted from the Applicant's Abstract): Members of the genus Rickettsia are the etiologic agents of rocky mountain and other spotted fevers and endemic, scrub and epidemic typhus, diseases that pose a pernicious health threat worldwide. Rickettsia prowazekii, the etiologic agent of epidemic typhus is an obligate intracellular parasitic bacterium that can grow only within the cytoplasm of a eucaryotic host cell. The ability of rickettsiae to exploit this intracellular niche in animals as diverse as arthropods and humans and to subsequently cause serious human disease provides the impetus for this study. This proposal focuses on the development and application of genetic techniques to address questions regarding the pathogenic bacterium R. prowazekii and its obligate intracytoplasmic existence. It exploits the availability of the R. prowazekii genome sequence and the development of rickettsial genetic technologies to test hypotheses related to rickettsial gene function, DNA replication, and pathogenic mechanisms. In Specific Aim 1 the PI's goal is to capitalize on a rickettsial transformation system and identification of a selectable antibiotic resistance gene that can be expressed in R. prowazekii to discriminate, via knockouts, essential function at the level of single genes. Specifically targeted genes include those that encode products with homology to known virulence genes of other bacteria, genes hypothesized to be expressed only in the arthropod vector, genes hypothesized to be non-functional and part of the process of rickettsial reductive evolution, and finally, genes with homologs within the R. prowazekii genome. In addition, a transposon-based approach will be used to generate random insertion mutants. In Specific Aim 2,the PI's goal is to isolate the functional origin of replication. One approach will attempt to generate a rickettsial mini-chromosome by linking putative origin fragments with the selectable erythromycin-resistant gene, ereB. An alternate method will identify the origin by binding of rickettsial DnaA. Specific Aim 3 will continue the PI's characterization of transcription termination and identification of rickettsial transcriptional changes that

occur just prior to lysis of the host cell. Using ribonuclease protection studies, the PI will determine whether these changes reflect a general property of the rickettsiae by examining additional non-intrinsic termination sites and the effect of cell number on termination at these sites. Modulation of Rho and its correlation to these changes will be addressed.

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- **Project Title: GENETIC AND OTHER DETERMINANTS OF IN VIVO CYP3A ACTIVITY**

  Principal Investigator & Institution: Wilkinson, Grant R.; Professor of Pharmacology; Vanderbilt University 3319 West End Ave. Nashville, Tn 372036917

  Timing: Fiscal Year 2002; Project Start 01-DEC-1982; Project End 30-JUN-2007

  Summary: (provided by applicant): CYP3A is the most abundant of the human cytochrome P450 enzymes in both the intestine and liver. As a result, it is involved in the metabolism of over 50 percent of drugs and is an important determinant of first-pass metabolism following oral drug administration. Despite being metabolized by CYP3A, however, different substrates appear to interact with the enzyme in different ways, so that the metabolic clearance of one does not correlate with that of another. One hypothesis to account for this lack of correlation is that it reflects, in part, the different relative contributions of intestinal and hepatic CYP3A, and, thus, the route of drug administration. Drugs with different metabolic characteristics and routes of administration (midazolam, triazolam and alprazolam) will be used to test this hypothesis. A second possibility that will be investigated is that the CYP3A substrate-active site interaction is substrate-dependent, accordingly, drugs may be characterized into different "groups." Correlation within "groups" will, therefore, be present to a far greater extent than between "groups." In addition to the noted benzodiazepines, this hypothesis will be tested with other CYP3A substrates, such as cyclosporine-A, **erythromycin** and nifedipine, which are postulated to belong to other "groups." An important characteristic of CYP3A is marked interindividual variability in activity (10- to more than 40-fold), which significantly contributes to differences in drug responsiveness between subjects. A genetic determinant(s) is considered to be important in this regard but has never been formally defined and may, in fact, be different according to the tissue localization of CYP3A. Accordingly, the inheritability of CYP3A activity will be determined in monozygotic and dizygotic twins to test the hypothesis that a genetic factor is more important in regulating basal CYP3A-mediated metabolism in the liver than that in the intestine, and also in the enzyme?s inducibility at these two sites. Studies are also proposed which will establish the in vivo functional consequences of the allelic variants CYP3A4*1B and CYP3A5*3, and other known single nucleotide polymorphisms (SNPs). Finally, investigations in European-, African-American, and Japanese populations will be undertaken in order to identify SNPs associated with the interindividual variability in CYP3A activity.

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- **Project Title: GENETIC APPROACHES TO VIRULENCE IN B. BURGDORFERI**

  Principal Investigator & Institution: Cabello, Felipe C.; Professor; Microbiology and Immunology; New York Medical College Valhalla, Ny 10595

  Timing: Fiscal Year 2002; Project Start 01-FEB-2001; Project End 31-JAN-2006

  Summary: (adapted from the applicant's abstract): Borrelia burgdorferi is an in vitro culturable bacterium that is the cause of Lyme disease. Its small genome contain