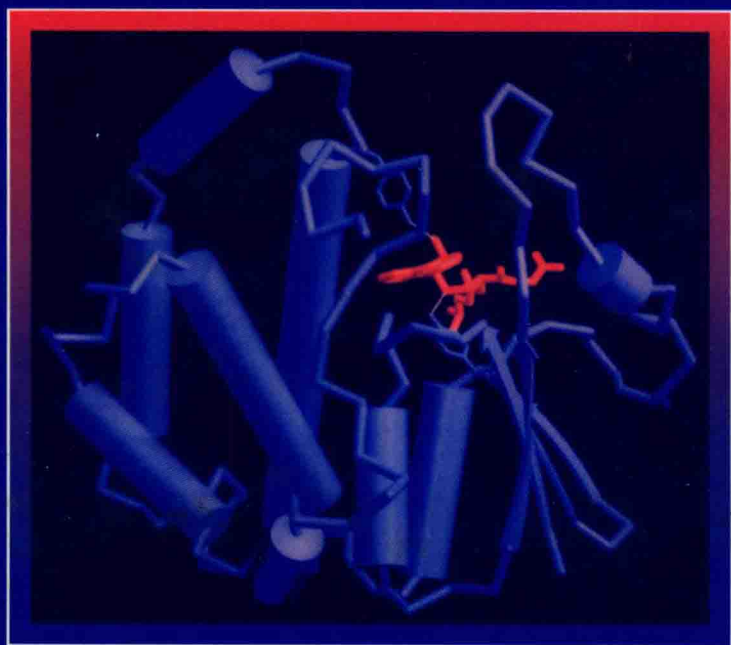

STRUCTURE *and* FUNCTION

of

GLUTATHIONE TRANSFERASES



J. D. Tew • C. B. Pickett • T. J. Mantle
B. Mannervik • J. D. Hayes

STRUCTURE *and* FUNCTION

of

GLUTATHIONE TRANSFERASES

Edited by

Kenneth D. Tew, Ph.D.

*Fox Chase Cancer Center
Philadelphia, Pennsylvania*

Cecil B. Pickett, Ph.D.

*Merck Frosst Centre for Therapeutic Research
Kirkland, Quebec
Canada*

Timothy J. Mantle, Ph.D.

*Trinity College
Dublin, Ireland*

Bengt Mannervik, Ph.D.

*Uppsala University
Uppsala, Sweden*

John D. Hayes, Ph.D.

*Ninewells Hospital and Medical School
Dundee, Scotland*



CRC Press

Boca Raton Ann Arbor London Tokyo

On the Cover: *RASTER3D representation of the three-dimensional structure of the type 3 subunit of a class mu glutathione S-transferase, courtesy of Xinhua Ji, Gary Gilliland, and Richard Armstrong, University of Maryland, College Park, MD, and the Center for Advanced Research in Biotechnology. The diagram was generated from the 1.8 Å structure of isoenzyme 3-3 in complex with 9-(S-glutathionyl)-10-hydroxy-9,10-dihydrophenanthrene, a product of the reaction of glutathione with phenanthrene 9,10-oxide. The α -helices are illustrated as cylinders and the β -strands as arrows. The product is shown in red with hydrogen bonding interactions (dashed lines) to the side-chains of two active site tyrosine residues (tyrosines 6 and 115) involved in catalysis.*

Library of Congress Cataloging-in-Publication Data

Structure and function of glutathione transferases / edited by Kenneth D. Tew...[et al.].

p. cm.

Includes bibliographical references and index.

ISBN 0-8493-4582-0

1. Glutathione transferase—Congresses. I. Tew, Kenneth D.

[DNLM: 1. Glutathione Transferases—congresses. 2. Drug Resistance—immunology—congresses. 3. Neoplasms—chemically induced—congresses. QU 141 S927 1993]

QP606.G59S77 1993

599'.019254—dc 20

DNLM/DLC

for Library of Congress

93-8303

CIP

This book represents information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Every reasonable effort has been made to give reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use.

Neither this book nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming, and recording, or by any information storage and retrieval system, without permission in writing from the publisher.

All rights reserved. Authorization to photocopy items for internal or personal use, or the personal or internal use of specific clients, is granted by CRC Press, Inc., provided that \$.50 per page photocopied is paid directly to Copyright Clearance Center, 27 Congress Street, Salem, MA 01970 USA. The fee code for users of the Transactional Reporting Service is ISBN 0-8493-4582-0/93/\$0.00+\$.50. The fee is subject to change without notice. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

The copyright owner's consent does not extend to copying for general distribution, for promotion, for creating new works, or for resale. Specific permission must be obtained from CRC Press for such copying.

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

© 1993 by CRC Press, Inc.

International Standard Book Number 0-8493-4582-0

Library of Congress Card Number 93-8303

Printed in the United States of America 1 2 3 4 5 6 7 8 9 0

Printed on acid-free paper

To provide this information as quickly as possible,
this book was produced directly from the authors' submitted manuscripts.

STRUCTURE *and* FUNCTION

of

GLUTATHIONE
TRANSFERASES

Dedication

The Asilomar meeting and these proceedings are dedicated to the memory of Dr. Kiyomi Sato, Professor and Chairman of the Second Department of Biochemistry, Hirosaki University School of Medicine, Hirosaki, Japan, who passed away on November 4, 1992, after a year and a half struggle with rectal cancer.

Professor Sato was born on August 6, 1931, in Odate, Japan. In 1959, he received his M.D. from Tohoku University in Sendai, and after a year in internship, he entered the Biochemistry Division, Institute for Tuberculosis, Leprosy, and Cancer, Tohoku University. He started to work on isoenzyme changes of glycolysis, gluconeogenesis, and glycogen metabolism in rat primary and transplantable hepatomas. He worked hard, very often until midnight, obtained a Ph.D. in 1965, and was appointed as a research associate in the Division. Three years later, he found the muscle-type isoenzyme of fructose diphosphatase in rat hepatoma. This discovery provided him an opportunity to spend time in Dr. Sidney Weinhouse's laboratory at the Fels Institute in Philadelphia.

During his stay in Dr. Weinhouse's laboratory (1971–1972), he found the fetal type isoenzyme of glycogen phosphorylase in rat hepatoma. Later, Dr. Sato told his staff of an episode from its discovery. He obtained “leftover” samples that somebody else had used to assay for other enzymes and found the isoenzyme in them. He often said “there is luck in the last helping.” This discovery provided him with a new and life-long position in Hirosaki. During his stay at Fels, he received a paper on glutathione S-transferase (GST) published in *Nature* from Dr. Litwack, and was much impressed by the paper, later leading to his own study on GSTs in Hirosaki.

During the next five years or so, he continued to work with his staff in Hirosaki on isoenzyme changes of sugar-metabolizing enzymes in rat hepatoma. From the late 1970s, his interest gradually shifted to drug-metabolizing enzymes such as γ -glutamyltranspeptidase and GSTs. In 1984, he discovered that the placental form of GST (GST-P) was an extremely useful marker for rat hepatic preneoplastic and neoplastic lesions. He also found the expression of GST- π in human colon, uterine cervix, and other cancers. In a series of studies he described the importance of pi class GSTs in carcinogenesis and malignancy. He organized with Dr. Henry C. Pitot a conference on GST and carcinogenesis as a U.S.–Japan Cooperative Cancer Research Program in 1988 and stimulated many investigators to study GSTs. His reputable reviews on GSTs (*Jpn. J. Cancer Res.*, 79, 556, 1988; *Adv. Cancer Res.*, 52, 205, 1989;



Crit. Rev. Biochem. Mol. Biol., 27, 337, 1992) have emphasized that he was a true scholar with a broad view of science. His outstanding work has been introduced by Dr. Weinhouse on the cover of the September issue of *Jpn. J. Cancer Res.* 82(9) in 1991 with a photograph of GST-P-positive single hepatocytes, putative initiated cells. Besides the pi class GSTs in carcinogenesis, he also contributed to the field by the discovery of several forms with interesting functions, such as GST-Yn₁Yn₁ of rat brain and GST-M₁M₂ of human aorta.

During his career he has obtained many grants for cancer research, including one from the Princess Takamatsu Cancer Research Fund in 1986. As one of the established investigators and a leader in the field of cancer research, he has organized groups to study gene expression of marker enzymes in preneoplastic hepatocytes since 1987 under the support of the Ministry of Education, Science, and Culture of Japan.

Besides being a devoted scientist, Dr. Sato was a warm and friendly person by nature. Everyone who knew him deeply respected and admired him, not just because of his accomplishments, but because of his friendly and outgoing personality. Dr. Sato's dedication to GST has been, and will be, an inspiration for all of us who have had the privilege and the pleasure of knowing him.

Preface

These Proceedings present a series of up-to-date articles by investigators who have focused on structural and functional studies of the glutathione transferase isozyme family. The chapters in this volume resulted from a meeting held in January, 1993, which brought together some of the top investigators in the area of glutathione transferase enzymology. The glutathione transferases are involved in many endogenous and exogenous functions in cells. For the first time, a series of x-ray crystallographic studies have been brought together in one volume and show detailed information on the structural aspects of these proteins. New information on the forces that play a role in glutathione and substrate binding sites are included. The recent success of the crystallography has provided an important platform for continued investigation of structure/function relationships. A number of molecular studies are also represented; these focus on the regulation of the glutathione transferases by identifying important response elements that control gene expression. Metabolism of drugs and carcinogens is also covered in some detail. The role these enzymes play in determining drug resistance at both the preclinical and clinical levels is discussed. Strategies for modulating drug resistance in tissue culture, animals, and in clinical studies is also well represented. In addition, the design and synthesis of novel inhibitors of glutathione transferases may provide an experimentally and (eventually) clinically useful route for the modulation of intracellular functions governed by the glutathione transferases. Thus, this book should provide a broad range of subject matter for biochemists, pharmacologists, medical oncologists, experimental therapeutic specialists, and structural biologists interested in isozyme families exemplified by the glutathione transferases.

Kenneth D. Tew

Acknowledgments

The ultimate success of this meeting was due in large part to the speakers and participants, Donna Bunch for her organizational skills, and to the record-breaking rainstorms of January 1993, which miraculously subsided during the course of the meeting.

The organizers would particularly like to thank Terrapin Technologies, Inc. for their enthusiastic support of the meeting, including generous financing of the speakers' expenses, without which it would not have been possible to bring together this international roster of leading GST researchers.

STRUCTURE *and* FUNCTION

of

GLUTATHIONE
TRANSFERASES

Contents

Session 1 — Functions of GST

Role of Glutathione S-Transferase and Aldehyde Reductase in Resistance to Aflatoxin B ₁	3
<i>J. D. Hayes, D. J. Judah, E. M. Ellis, L. I. McLellan, and G. E. Neal</i>	
Some Functions of Glutathione Transferases	15
<i>B. Ketterer, J. Taylor, D. Meyer, S. Pemble, B. Coles, X.-C. Lin, and S. Spencer</i>	
Structural and Functional Characterization of the Binding Sites for Glutathione (G-Site) and the Hydrophobic Electrophilic Substrate (H-Site) in Glutathione Transferases	29
<i>B. Mannervik, K. Berhane, R. Björnstedt, P. G. Board, T. A. Jones, R. H. Kolm, B. Olin, I. Sinning, G. E. Sroga, G. Stenberg, S. Tardioli, and M. Widersten</i>	
The GST D Genes: A New Family of the Glutathione S-Transferase Gene Superfamily	39
<i>Y.-P. S. T'oung, T.-S. Hsieh, and C.-P. D. Tu</i>	
Leukotriene C ₄ Synthase	47
<i>D. W. Nicholson</i>	

Session 2 — Molecular Enzymology

The Three-Dimensional Structure of Class π Glutathione S-Transferase	65
<i>P. Reinemer, H. W. Dirr, and R. Huber</i>	
The Active Site in Class Alpha Glutathione Transferases	75
<i>I. Sinning, G. J. Kleywegt, B. Mannervik, P. G. Board, and T. A. Jones</i>	
Crystallographic and Mechanistic Studies of Class μ Glutathione S-Transferases	87
<i>R. N. Armstrong, G. L. Gilliland, X. Ji, W. W. Johnson, and S. Liu</i>	
Site-Directed Mutagenesis of Rat Glutathione S-Transferase YaYa: Structure and Function Studies	99
<i>A. Y. H. Lu, R. W. Wang, D. J. Newton, S.-E. W. Huskey, and C. B. Pickett</i>	
Rat Liver Microsomal Glutathione Transferase: Studies on Structure and Function	109
<i>C. Andersson, E. Mosialou, R. Weinander, H. Hebert, and R. Morgenstern</i>	

Session 3 — Gene Expression

ARE and XRE Mediated Induction of the Glutathione S-Transferase Ya Subunit Gene: Induction by Planar Aromatic Compounds and Phenolic Antioxidants	119
<i>T. H. Rushmore, T. Nguyen, and C. B. Pickett</i>	
The Role of AP-1 Transcription Factor in the Regulation of Glutathione S-Transferase Ya Subunit Gene Expression by Chemical Agents	129
<i>V. Daniel, S. Bergelson, and R. Pinkus</i>	
Polymorphic Expression of Human Glutathione Transferases	137
<i>P. G. Board, V. L. Ross, M. Coggan, and T. Suzuki</i>	
Molecular Genetics of the Human Mu Class GST Multigene Family	147
<i>S. Zhong, J. D. Hayes, N. K. Spurr, and C. R. Wolf</i>	

Session 4 — Carcinogenesis and Cancer Incidence

Evolution of GST Genes	163
<i>J. Taylor, S. Pemble, J. Harris, D. Meyer, S. Spencer, C.-L. Xia, and B. Ketterer</i>	
The Glutathione S-Transferase GSTM1 0 Locus and Cancer Susceptibility	175
<i>R. C. Strange</i>	
GST-Mediated Protection against Carcinogens: Aflatoxin B ₁ Detoxification as an Example	187
<i>D. L. Eaton, K. Van Ness, and T. M. Buetler</i>	
High Capacity Binding by Glutathione S-Transferases and Glucocorticoid Resistance	199
<i>I. Listowsky</i>	
Glutathione S-Conjugate Export Pump	211
<i>T. Ishikawa</i>	
Functions of Pi-Class Glutathione S-Transferases, Roles in Carcinogenesis and Suppression by Oxidative Stress	223
<i>S. Tsuchida, K. Sato, K. Satoh, I. Hatayama, Y. Yokoyama, Y. Yamada, H. Shen, S. Nishimura, S. Suzuki, and H. Nakano</i>	

Session 5 — Resistance to Drugs and Oxidative Stress

Mechanisms through which Glutathione S-Transferase-Mediated Resistance to Alkylating Molecules Can be Augmented	237
<i>W. E. Fahl, A. M. Gulick, T. H. Manoharan, W. W. Wasserman, J. C. Gallo, and M. L. Brady</i>	

Principles of Drug Modulation Applied to Glutathione S-Transferases	249
<i>S. Ranganathan, P. J. Ciaccio, and K. D. Tew</i>	
Pharmaceutical Targeting of GST Isozymes	257
<i>L. M. Kauvar</i>	
Glutathione Peroxidase and Resistance to Oxidative Stress	269
<i>J. H. Doroshov, R. S. Esworthy, F. F. Chu, and S. Akman</i>	
The Organization of Human Class-Mu Glutathione Transferase Genes <i>GSTM1–GSTM5</i> on Chromosome 1p13	279
<i>W. R. Pearson, S.-J. Xu, W. R. Vorachek, and D. Patterson</i>	
Mechanism of Specific Activation of Glutathione Transferase P Gene during Rat Hepatocarcinogenesis — A Transgene Study	297
<i>M. Muramatsu, S. Morimura, T. Suzuki, M. Imagawa, and T. Kitagawa</i>	
Index	309

SESSION ONE

FUNCTIONS OF GST

ROLE OF GLUTATHIONE S-TRANSFERASE AND ALDEHYDE REDUCTASE IN RESISTANCE TO AFLATOXIN B₁

John D Hayes¹, David J Judah², Elizabeth M Ellis³
Lesley I McLellan¹ and Gordon E Neal²

¹University Department of Clinical Biochemistry
The Royal Infirmary
Edinburgh EH3 9YW
Scotland
U.K.

²Toxicology Unit
MRC Laboratories
Woodmansterne Road
Carshalton
Surrey SM5 4EF
U.K.

³Biomedical Research Centre
Ninewells Hospital and Medical School
Dundee DD1 9SY
Scotland
U.K.

INTRODUCTION

Aflatoxin B₁ (AFB₁), produced by the mould *Aspergillus flavus*, is a potent hepatocarcinogen. It is often found to contaminate grain and nut crops in regions of the world that experience high humidity. Epidemiological studies suggest that AFB₁ is partly responsible for the high incidence of liver cancer in Asia and Africa.

The carcinogenic effects of AFB₁ result from its metabolism by cytochrome P-450 to AFB₁-8,9-epoxide. Recent studies have shown that cytochrome P-450s produce two epoxide isomers of AFB₁, namely an *endo* and an *exo* AFB₁-8,9-epoxide (Raney *et al.*, 1992a). The *exo* 8,9-epoxide is thought to represent the ultimate carcinogen and binds to DNA primarily through the N⁷ atom of guanine (Essigmann *et al.*, 1977). Formation of such DNA adducts can result in activation of members of the *ras* gene family (Sinha *et al.*, 1988) and may lead to mutation of the tumour suppressor gene *p53* (Bressac *et al.*, 1991; Hsu *et al.*, 1991).

Selective toxicity of AFB₁

Aflatoxin B₁ displays selective toxicity. Species such as mouse and hamster are resistant to AFB₁ whilst the rat, and probably man, are sensitive to this mycotoxin (Newberne & Butler, 1969). Aflatoxin is metabolised extensively in the liver and its toxicity is determined by the relative levels of enzymes responsible for the activation of AFB₁ and those involved in its detoxification (for a review, see Hayes *et al.*, 1991a). Several research groups (Neal & Green 1993; Coles *et al.*, 1985) have shown that the glutathione S-transferases, through their ability to catalyse the conjugation of AFB₁-8,9-epoxide with glutathione (GSH), can provide an important mechanism of resistance to this mycotoxin. Raney *et al.* (1992b) have shown that in the rat the alpha-class GST have the capacity to detoxify the *exo* 8,9-epoxide whilst the mu-class GST are active towards the *endo* 8,9-epoxide (see Figure 1). Since the formation of 8,9-dihydro-8-(N⁷-guanyl)-9-hydroxy-AFB₁ would appear to arise primarily from a reaction between DNA and AFB₁-*exo*-epoxide, rather than between DNA and AFB₁-*endo*-epoxide, protection against AFB₁ carcinogenesis is likely to be conferred by alpha-class GST rather than mu-class GST.

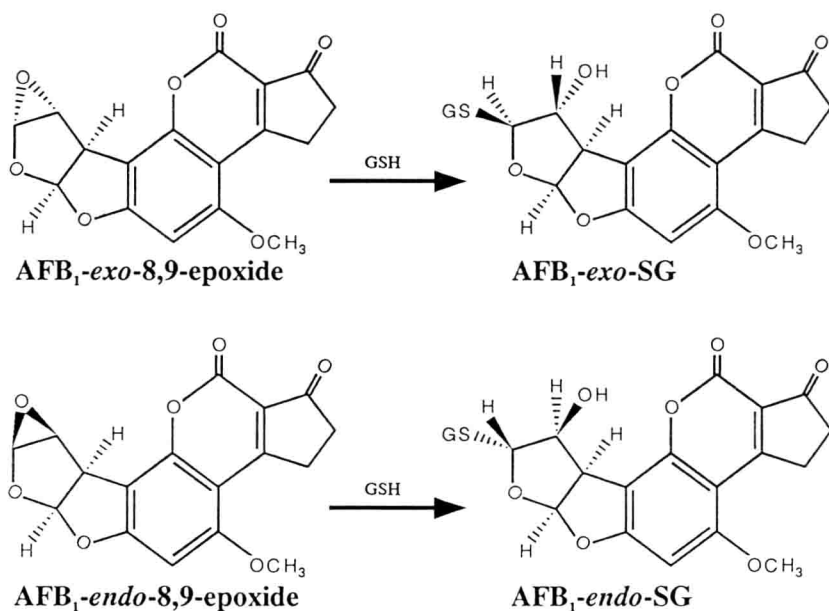


Figure 1. Conjugation of glutathione with aflatoxin B₁ exo- and endo-epoxides

The intrinsic resistance of the mouse to AFB₁ cannot be attributed to failure to activate the mycotoxin as Monroe & Eaton (1987) have shown that murine liver microsomes possess 4-fold greater capacity to form the 8,9-epoxide of AFB₁ than rat liver microsomes. However, mouse liver cytosol exhibits at least 20-fold greater AFB₁-GSH-conjugating activity than does rat liver cytosol suggesting that GST is responsible for the resistant phenotype; this conclusion is supported by the fact that treatment with buthionine-S-sulfoximine and diethyl maleate, which deplete hepatic GSH levels, can produce a marked increase in the sensitivity of the mouse to AFB₁-DNA adduct formation (Monroe & Eaton, 1988) and presumably AFB₁ hepatocarcinogenesis. The high level of AFB₁-GSH-conjugating activity has been attributed to the constitutive expression of a GST Yc subunit in mouse liver (Quinn *et al.*, 1990; Ramsdell & Eaton, 1990) and recently the cDNA encoding this murine GST has been cloned (Buetler & Eaton, 1992; Hayes *et al.*, 1992).

Chemoprotection

Although the male Fischer 344 rat is sensitive to AFB₁, and will develop liver cancer following exposure to AFB₁, resistance to this carcinogen can be induced by the administration of chemoprotectors. Drugs which serve as chemoprotectors (see Figure 2) include the antioxidants, ethoxyquin (Cabral & Neal, 1983), butylated hydroxyanisole (Kensler *et al.*, 1986; Jhee *et al.*, 1988) and oltipraz (Kensler *et al.*, 1987) as well as the enzyme inducer phenobarbital (Lotlikar *et al.*, 1989). The ability of these chemicals to reduce the sensitivity of the rat to AFB₁ is associated with increased hepatic detoxification capacity for AFB₁ and its metabolites.

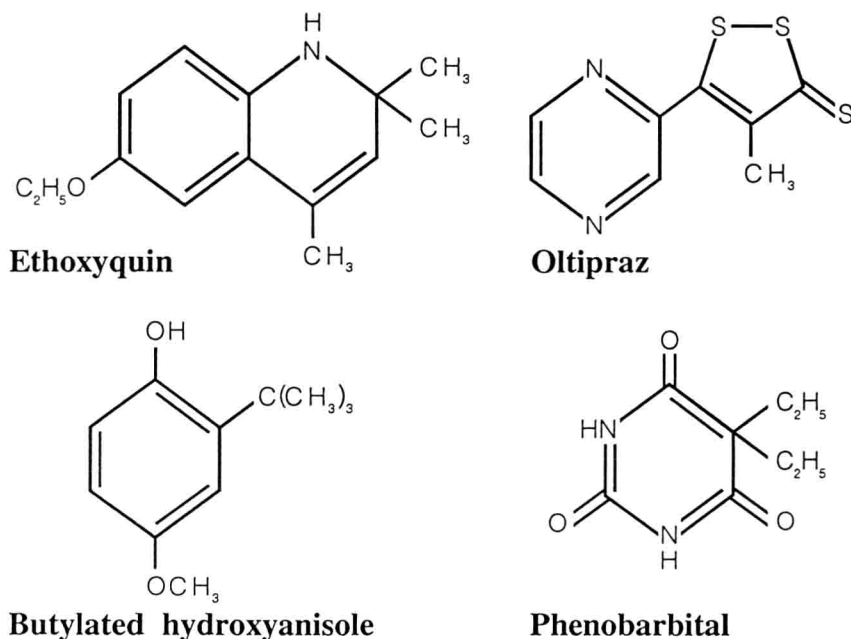


Figure 2. Structure of chemoprotectors against AFB₁ carcinogenesis

Our laboratories have focussed on chemoprotection conferred by ethoxyquin (EQ) in the rat and we have shown that dietary administration of this antioxidant increases several detoxification enzymes. For example, EQ induces the cytochrome P-450s involved in the hydroxylation of AFB₁, to form AFQ₁ and AFM₁ (Mandel *et al.*, 1987), it induces GST responsible for the conjugation of the 8,9-epoxide with GSH (Hayes *et al.*, 1991b) and it induces an aldehyde reductase which reduces the dialdehydic form of AFB₁-8,9-dihydrodiol (Hayes *et al.*, 1993).

Glutathione S-transferase Yc₂

To allow the identification of the rat GST isoenzymes that are responsible for the EQ-inducible conjugation of AFB₁-8,9-epoxide with GSH, the hepatic transferases were resolved by cation-exchange chromatography on CM-cellulose. Figure 3 shows the CM-cellulose elution profile of hepatic GST from Fischer 344 rats that have been fed diets containing EQ. It is important to note that the AFB₁-GSH-conjugating activity from CM-cellulose does not co-elute with either GST activity towards 1-chloro-2,4-dinitrobenzene or peroxidase activity towards cumene hydroperoxide, indicating that the major isoenzymes responsible for detoxifying AFB₁ are distinct from the most thoroughly characterized rat GST, namely transferases F, L, C, B, A and AA.

Following the CM-cellulose step, we purified the transferases which metabolise AFB₁-8,9-epoxide by elution from hydroxyapatite followed by chromatography on a Waters Protein PAK SP-5PW cation exchanger. The enzymes isolated by this procedure, which are active with the 8,9-epoxide, were found to comprise either Yc₁Yc₂ or Yc₁Yc₂ subunits