



National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Pretranslational Depression of Hepatic Cytochrome P450
During Listeria Monocytogenes Infection

by

Steven Armstrong

©

Submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

at

Dalhousie University

Halifax, Nova Scotia

February, 1992



National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-76699-9

Canada

TABLE OF CONTENTS

Table of Contents	iv
Index of Figures	vii
Index of Tables	xi
Abstract	xii
Abbreviations and Symbols	xiii
Acknowledgements	xvii
INTRODUCTION	1
Cytochrome P450 - Background and Nomenclature.....	1
Regulation of Cytochrome P450.....	3
Cytochrome P450 and the Immune System	6
Interferon and Cytochrome P450.....	7
The Influence of the Reticuloendothelial System on Cytochrome P450.....	11
Endotoxin, Cytokines, and Cytochrome P450.....	16
Mechanism for the Suppression of Cytochrome P450 by Interferon.....	21
Mechanism of the Effect of Endotoxin on Cytochrome P450.....	27
<u>Listeria Monocytogenes</u> - An Overview	31
Formulation of the Problem	40
Objectives	41
MATERIALS AND METHODS	42
Chemicals and Reagents	42
Animals and Treatment	45
Bacteria	46
Preparation of Hepatic Microsomes.....	48
Protein Determination	48
Cytochrome P450 Determination.....	49
EROD, BROD, PRGD Determinations	49
Microsomal Aminopyrine N-demethylase Activity	52
<i>In Vitro</i> Incubation of Microsomes with 15U <i>Listeria</i>	53

Determination of Bacterial Liver Burden.....	53
Histological Examination of Liver Sections	54
Isolation of Mouse Hepatocytes	54
Cell Suspension Experiments	56
Adherent Cell Culture Experiments.....	58
Hepatic RNA Isolation.....	61
RNA Gel Electrophoresis	62
Transfer of RNA from the Agarose Gel to Genescreen <i>Plus</i> Membranes.....	64
Slot Blot Analysis of RNA	64
Hybridization of Northern Blots.....	65
Hybridization of Slot Blots.....	66
Nucleotide Sequences of the Hybridization Probes	66
5'-End-Labeling of cDNA Probes.....	67
Autoradiography.....	68
Densitometry	68
Western Blot Analysis of Microsomal Protein	69
Statistical Methods.....	75
RESULTS.....	76
Time Course of cytochrome P450 loss during 15U listeria infection and comparison with infection with the M3D strain.....	76
The Effect of <i>in vivo</i> 15U listeria infection on body weight, liver weight, and liver histology	81
The effect of 15U listeria infection on cytochrome P450-mediated metabolism when incubated directly with hepatic microsomes.....	88
The effect of 15U listeria infection on hepatocytes maintained in suspension.....	88
The effect of 15U and M3D listeria infection on hepatocytes cultured in the presence and absence of nonparenchymal cells	95
The <i>in vivo</i> effect of 15U listeria infection on cytochrome P450IA mRNA levels	101
The effect of 48 hours <i>in vivo</i> listeria infection on cytochrome P450IA1 apoprotein levels after BNF induction	109

Time Course of the effect of <i>in vivo</i> listeria infection on cytochrome P450IA1 mRNA levels, apoprotein content, and EROD activity after BNF induction	117
The effect of 48 hours <i>in vivo</i> listeria infection on cytochrome P450IIC11 mRNA and aminopyrine N-demethylase activity	121
Time Course of the effect of <i>in vivo</i> Listeria infection on cytochrome P450IIC11 mRNA levels	128
DISCUSSION	140
CONCLUSIONS	156
PROPOSALS FOR FUTURE WORK	157
REFERENCES	158

INDEX OF FIGURES

FIGURE	PAGE
1 METHOD FOR PREPARATION OF ADHERENT LIVER CELL CULTURES	59
2 TIME COURSE OF THE EFFECT OF <i>IN VIVO</i> INFECTION WITH 1 X 10 ⁶ CFU OF 15U LISTERIA ..	77
3 EFFECT OF 48 HOURS OF <i>IN VIVO</i> INFECTION WITH 1 X 10 ⁶ CFU OF M3D AND 15U LISTERIA	78
4 EFFECT OF 48 HOURS OF <i>IN VIVO</i> INFECTION WITH 1 X 10 ⁶ CFU OF 15U LISTERIA ON SUBSTRATE METABOLISM CAPACITY	79
5 EFFECT OF 48 HOURS OF <i>IN VIVO</i> INFECTION WITH 1 X 10 ⁶ CFU OF M3D LISTERIA ON SUBSTRATE METABOLISM CAPACITY	80
6 EFFECT OF 48 HOURS OF <i>IN VIVO</i> INFECTION WITH 1 X 10 ⁵ CFU OF 15U LISTERIA AND 1 X 10 ⁸ CFU OF M3D LISTERIA	82
7 EFFECT OF 48 HOURS <i>IN VIVO</i> INFECTION WITH 1 x 10 ⁶ CFU OF 15U LISTERIA ON BODY WEIGHT	83
8 EFFECT OF 48 HOURS <i>IN VIVO</i> INFECTION WITH 1 x 10 ⁶ CFU OF 15U LISTERIA ON LIVER WEIGHT	84
9 EFFECT OF 65 HOURS OF <i>IN VIVO</i> INFECTION WITH 1 X 10 ⁶ CFU OF 15U LISTERIA ON LIVER ULTRASTRUCTURE	85
10 EFFECT OF A 24 HOUR <i>IN VITRO</i> INCUBATION OF 15U LISTERIA WITH HEPATIC MICROSOMES	89
11 EFFECT OF INCUBATION TEMPERATURE ON CYTOCHROME P450 LOSS IN CONTROL HEPATOCYTES MAINTAINED IN SUSPENSION	90
12 EFFECT OF <i>IN VITRO</i> INCUBATION TIME WITH 15U LISTERIA ON CYTOCHROME P450 LEVELS IN HEPATOCYTES MAINTAINED IN SUSPENSION	91

FIGURE	PAGE
13 EFFECT OF 24 HOURS OF <i>IN VITRO</i> INCUBATION WITH 15U LISTERIA ON CYTOCHROME P450, EROD, AND BROD IN HEPATOCYTES MAINTAINED IN SUSPENSION	92
14 EFFECT OF 24 HOURS OF <i>IN VITRO</i> INCUBATION WITH 15U LISTERIA ON EROD ACTIVITIES IN ADHERENT HEPATOCYTE CULTURES AT 37 ⁰ C	96
15 EFFECT OF 24 HOURS OF <i>IN VITRO</i> INCUBATION WITH M3D LISTERIA ON EROD ACTIVITIES IN ADHERENT HEPATOCYTE CULTURES AT 37 ⁰ C	96
16 EFFECT OF 24 HOURS OF <i>IN VITRO</i> INCUBATION WITH 15U LISTERIA ON BROD ACTIVITIES IN ADHERENT HEPATOCYTE CULTURES AT 37 ⁰ C	98
17 EFFECT OF 24 HOURS OF <i>IN VITRO</i> INCUBATION WITH M3D LISTERIA ON BROD ACTIVITIES IN ADHERENT HEPATOCYTE CULTURES AT 37 ⁰ C	98
18 EFFECT OF 24 HOURS OF <i>IN VITRO</i> INCUBATION WITH 15U LISTERIA ON THE VIABILITY AND TOTAL CELLULAR PROTEIN CONTENT OF ADHERENT HEPATOCYTE CULTURES AT 37 ⁰ C	100
19 EFFECT OF 48 HOURS OF <i>IN VIVO</i> INFECTION WITH 15U LISTERIA ON CYTOCHROME P450 AND EROD ACTIVITY AFTER β NF INDUCTION	102
20 EFFECT OF 48 HOURS OF <i>IN VIVO</i> INFECTION WITH 15U LISTERIA ON TOTAL LIVER PROTEIN AFTER β NF INDUCTION	103
21 EFFECT OF 48 HOURS OF <i>IN VIVO</i> INFECTION WITH 15U LISTERIA ON MICROSOMAL PROTEIN AFTER β NF INDUCTION	103
22 AGAROSE FORMALDEHYDE GEL - EFFECT OF 48 HOURS <i>IN VIVO</i> LISTERIA INFECTION ON CYTOCHROME P450IA mRNA LEVELS AFTER β NF INDUCTION	105
23 NORTHERN BLOT - EFFECT OF 48 HOURS <i>IN VIVO</i> LISTERIA INFECTION ON CYTOCHROME P450IA mRNA LEVELS AFTER β NF INDUCTION	107

FIGURE	PAGE
24 SLCT BLOT - EFFECT OF 48 HOURS <i>IN VIVO</i> LISTERIA INFECTION ON CYTOCHROME P450IA mRNA LEVELS AFTER β NF INDUCTION	110
25 SLOT BLOT - EFFECT OF 48 HOURS <i>IN VIVO</i> LISTERIA INFECTION ON TOTAL mRNA LEVELS AFTER β NF INDUCTION	112
26 EFFECT OF 48 HOURS OF <i>IN VIVO</i> INFECTION WITH 15U LISTERIA ON CYTOCHROME P450, EROD, AND CYTOCHROME P450IA mRNA LEVELS AFTER β NF INDUCTION	114
27 WESTERN BLOT - EFFECT OF 48 HOURS OF <i>IN VIVO</i> INFECTION WITH 15U LISTERIA ON CYTOCHROME P450IA1 APOPROTEIN LEVELS AFTER β NF INDUCTION	115
28 TIME COURSE OF THE EFFECT OF <i>IN VIVO</i> INFECTION WITH 15U LISTERIA ON CYTOCHROME P450IA mRNA LEVELS AND EROD ACTIVITY AFTER β NF INDUCTION	118
29 WESTERN BLOT - TIME COURSE OF THE EFFECT OF <i>IN VIVO</i> INFECTION WITH 15U LISTERIA ON CYTOCHROME P450IA1 APOPROTEIN LEVELS AFTER β NF INDUCTION	119
30 EFFECT OF 12 HOURS <i>IN VIVO</i> LISTERIA INFECTION AFTER β NF INDUCTION ON CYTOCHROME P450IA mRNA, EROD, P450, BROD, AND PROD ACTIVITIES	122
31 EFFECT OF 48 HOURS <i>IN VIVO</i> LISTERIA INFECTION AFTER β NF INDUCTION ON CYTOCHROME P450IA mRNA, EROD, P450, BROD, AND PROD ACTIVITIES	122
32 TIME COURSE OF BACTERIAL LIVER LOAD DURING INFECTION WITH 15U LISTERIA AFTER β NF INDUCTION	124
33 TIME COURSE OF THE EFFECT OF <i>IN VIVO</i> INFECTION WITH 15U LISTERIA AFTER β NF INDUCTION ON CYTOCHROME P450IA mRNA LEVELS EXPRESSED WITH AND WITHOUT CORRECTION FOR OLIGO(dT) ₁₈ BINDING	125
34 NORTHERN BLOT - EFFECT OF 48 HOURS <i>IN VIVO</i> LISTERIA INFECTION ON CYTOCHROME P450IIC11 mRNA LEVELS	126
35 SLOT BLOT - EFFECT OF 48 HOURS <i>IN VIVO</i> LISTERIA INFECTION ON CYTOCHROME P450IIC11 mRNA LEVELS	129

FIGURE	PAGE
36 SLOT BLOT - EFFECT OF 48 HOURS <i>IN VIVO</i> LISTERIA INFECTION ON TOTAL mRNA LEVELS	131
37 EFFECT OF 48 HOURS <i>IN VIVO</i> INFECTION WITH 15U LISTERIA ON CYTOCHROME P450, AMINOPYRINE N-DEMETHYLASE AND CYTOCHROME P450IIC11 mRNA LEVELS	133
38 TIME COURSE OF THE EFFECT OF <i>IN VIVO</i> LISTERIA INFECTION ON CYTOCHROME P450IIC11 mRNA LEVELS AND AMINOPYRINE N-DEMETHYLASE ACTIVITIES	134
39 TIME COURSE OF THE EFFECT OF 15U LISTERIA ON CYTOCHROME P450IIC11 AND TOTAL mRNA	136
40 TIME COURSE OF BACTERIAL LIVER LOAD DURING INFECTION WITH 15U LISTERIA	138
41 TIME COURSE OF THE EFFECT OF <i>IN VIVO</i> INFECTION WITH 15U LISTERIA ON CYTOCHROME P450IIC11 mRNA LEVELS EXPRESSED WITH AND WITHOUT CORRECTION FOR OLIGO(dT) ₁₈ BINDING	139
42 PATHWAYS FOR THE MECHANISM OF LISTERIA-MEDIATED DEPRESSION OF HEPATIC CYTOCHROME P450	141

INDEX OF TABLES

TABLE	PAGE
1 SELECTED HEPATIC FORMS OF CYTOCHROME P450	2
2 SELECTED HUMAN CYTOCHROME P450 ISOZYMES	4
3 IMMUNOACTIVE AGENTS AND THEIR EFFECT ON CYTOCHROME P450	8
4 BNF INDUCTION PROTOCOL	47

ABSTRACT

In the past 15 years there has been remarkable interest in the interaction between the system that protects us from infectious disease and that which allows us to inactivate therapeutic agents and environmental chemicals. There have been numerous reports of significant alterations in drug metabolism during viral infections in humans that in some cases have had severe clinical consequences. It is now reasonably well established that this effect is the result of virus-induced interferon production and subsequent depression of hepatic cytochrome P450 levels.

To date, there have been only five reports regarding the effect of active bacterial infections on cytochrome P450. In none of these reports was the mechanism involved determined. One of the bacteria that was shown to depress cytochrome P450 was Listeria monocytogenes and the focus of this study was to determine the mechanism by which this effect occurred. The primary objectives were to establish if the effect was direct on the hepatocyte or involved other cell types, to determine if the mechanism involved an inhibition of synthesis or a degradation of cytochrome P450 apoprotein, and to determine the role of the hemolysin (secreted by listeria) in the effect.

The activity of numerous cytochrome P450 isozymes were reversibly depressed in hepatic microsomes isolated from mice infected *in vivo* with Listeria monocytogenes (strain 15U). The levels of two specific isozymes (cytochrome P450IA1 and P450IIC11) were shown to be depressed by a pretranslational mechanism that involved a selective loss of mRNA coding for the specific cytochrome P450 apoproteins. Listeria had no effect on total hepatic mRNA levels and livers isolated from infected mice did not show any significant histological change.

Listeria significantly depressed hepatocyte ethoxyresorufin-O-dealkylase (EROD) and benzyloxyresorufin-O-dealkylase (BROD) activities after 24 hour incubations with liver cell cultures containing hepatocytes and nonparenchymal cells, demonstrating that the effect of listeria infection on cytochrome P450 is predominantly due to an interaction within the liver and does not require the influence of extrahepatic cells or factors. This depression of cytochrome P450-mediated metabolism in hepatocytes was the result of both a direct effect on the hepatocyte and an interaction of listeria with hepatic nonparenchymal cells. An avirulent strain of listeria (M3D) that does not secrete hemolysin, had no effect on cytochrome P450-mediated metabolism and suggested that the presence of the hemolysin is an essential component of the mechanism.

The incidence of Listeria monocytogenes infection in the general population is low but, if the capacity of listeria to depress cytochrome P450 proves to be an effect that is common to many different types of bacterial infections, then more clinical reports in this regard are likely in the future. This study serves to emphasize the warning that drugs with narrow therapeutic indices should be administered with caution during infectious disease caused by bacteria as well as viruses.

ABBREVIATIONS AND SYMBOLS

AHH	aryl hydrocarbon hydroxylase
ALS	antilymphocyte serum
APR	acute phase response
B. pertussis	<u>Bordetella pertussis</u>
BCG	<u>Bacillus Calmette-Guerin</u>
BHI	brain heart infusion broth
BROD	benzyloxyresorufin-O-dealkylase
C. parvum	<u>Corynebacterium parvum</u>
CFU	colony forming units
DEPC	diethylpyrocarbonate
DMEM	Dulbecco's Modification of Eagle's Medium
DMSO	dimethylsulfoxide
E. coli	<u>Escherichia coli</u>
EDTA	ethylenediaminetetraacetic acid
EMC	encephalomyocarditis virus
ER	endoplasmic reticulum
EROD	ethoxyresorufin-O-dealkylase
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
i.p.	intraperitoneal
IFN- β	interferon- β
IFN- γ	interferon- γ
IFN- α	interferon- α
IL-1	interleukin-1

IL-6	interleukin-6
kb	kilobase
kd	kilodalton
L-15	Leibovitz's medium
LEIF-AD	molecular hybrid of recombinant human leukocyte interferon
LPS	lipopolysaccharide
3-MC	3-methylcholanthrene
mRNA	messenger ribonucleic acid
NADPH	β -nicotinamide adenine dinucleotide phosphate
BNF	β -naphthoflavone
NDV	Newcastle Disease Virus
NK	natural killer cells
OD	optical density
PBS	phosphate-buffered saline
PM	poor metabolizer phenotype
poly rI•rC	polyribinosinic•polyribocytidylic acid
PROD	pentoxyresorufin-O-dealkylase
RES	reticuloendothelial system
SSC	sodium chloride / sodium citrate
SSPE	sodium chloride / sodium phosphate(monobasic) / EDTA
rpm	revolutions per minute
T cell	T lymphocyte
TEMED	N,N,N',N'-tetramethyl ethylenediamine
TNE	tris base / sodium chloride / EDTA
TNF	tumor necrosis factor

TSA	tryptic soy agar
U	units
UV	ultraviolet

There is another way to the truth: by the minute examination of the facts. That is the way of the scientist: a hard and noble and thankless way.

Shakespeare and Spiritual Life

John Masfield

ACKNOWLEDGEMENTS

My sincere thanks are offered to Dr. Ken Renton for his committed supervision and patient guidance. Ken commanded trust and respect while at the same time being a friend, extending an open-door policy and a willingness to meet to discuss any issue even on short notice.

I am grateful to the Department of Pharmacology and to the Faculty of Graduate Studies for financial support with special thanks to Janet Murphy, Luisa Vaughan, Karen Machan, and Sandi Leaf for their assistance with numerous administrative matters including last minute submission of abstracts and scholarship applications. The office staff's willingness to help in a friendly manner most certainly did not go unnoticed. I would also like to thank George Evans for expediting shipments, for his help with the student labs and for his assistance with the maintenance of Departmental equipment.

I have been lucky enough to make some long lasting friendships in the Department and would like in particular to thank Shaun Black and Connie Craig for their companionship. I also wish to acknowledge the technical support and genuine cooperation of Elaine Wright and Sandy Dibb.

One of the prime ways of learning the strengths and weaknesses of your experimental approach is to discuss your ideas with individuals that are willing to take the time to offer constructive criticism and helpful suggestions. I was lucky to receive this type of support from many members of the department including Dr. Rebecca Boyd and Dr. Allistair Cribb. Rebecca and Allistair were both tremendous assets to our lab and I sincerely thank them for lending an open ear on numerous occasions. I offer a special thanks to Allistair for carrying out the Western blots of this thesis.

The unselfish support and eternal optimism of my wife Linda inspired me to stay focused throughout the program. Without her unwavering patience and understanding the completion of this thesis might not have been possible. I was also blessed with the encouragement and support of my family who were always there when I needed them most.

INTRODUCTION

CYTOCHROME P450 - BACKGROUND AND NOMENCLATURE

The name cytochrome P450 was developed around 1960 from the work of several investigators. Cooper and Estabrook discovered that adrenocortical microsomes contained a carbon monoxide binding pigment that had an absorption maximum at 450 nm and was involved in steroid hydroxylation (Cooper et al, 1963; Cooper et al, 1965). At about the same time, the pigment was discovered by Omura and Sato in liver microsomes (Omura and Sato, 1964). Since its discovery, there has been an explosion of research directed at obtaining a better understanding of this complex enzyme system.

Cytochrome P450 is found in plants and microorganisms and exists in mammals as a series of isozymes that reside predominantly in the endoplasmic reticulum of liver cells, although it also exists in other organelles and tissues such as the lung, kidney, adrenal gland, testes, and brain. It is a heme containing enzyme that acts as the terminal oxidase of an electron transfer system responsible for the oxidation of many drugs, alcohols, carcinogens, pesticides, environmental contaminants and endogenous compounds including steroids and eicosanoids (Porter and Coon, 1991). It is an exceptionally versatile enzyme and it is now recognized that several hundred P450 isoforms exist in nature (Juchau, 1990), carrying out reactions such as dealkylation, hydroxylation, and deamination. There are 27 currently recognized families of P450 cytochromes 10 of which are found in mammals and comprise 18 subfamilies (Nebert et al, 1991). Some of the isozymes, the agents known to induce their levels, and the principal reactions that they catalyze are shown in Table 1 (adapted from Murray and Reidy, 1990). The nomenclature used in this thesis conforms to that recommended by Nebert et al (1991).

FAMILY	SUBFAMILY	SYNONYM(S)	INDUCER	PRINCIPAL REACTIONS
				CATALYZED
IA	IA1	mouse P ₁ rat P450c	βNF, TCDD, 3MC	ethoxyresorufin-O-dealkylation
	IA2	mouse P ₂ , P ₃ rat P450d	Isosafarole, βNF TCDD, 3MC	isosafarole metabolism, acetanilide 4-hydroxylation
IIB	IIB1	rat P450b	PB	peroxyresorufin-O-dealkylation
IIC	IIC11	rat P450h	male-constitutive	testosterone 16-α hydroxylation
	IIC12	rat P450i	female constitutive	steroid hydroxylation
IID	IID6	P450db1	constitutive	debrisoquine 4-hydroxylation
IIIA	IIIA2	PB-1	PB	testosterone 6β-hydroxylation
IVA	IVA1	P452	clofibrate	erythromycin N-demethylation

ABBREVIATIONS: βNF - β-naphthoflavone; 3-MC - 3-methylcholanthrene; TCDD 2,3,7,8-tetrachlorodibenzo-p-dioxin;

PB - phenobarbital

TABLE 1 - SELECTED HEPATIC FORMS OF CYTOCHROME P450

There are many factors that play a part in the extreme inter-individual variability in the pharmacological response that results from a fixed dose of drug. This can be the result of differences in absorption, distribution, excretion, receptor status, as well as many other factors. For those drugs metabolized by the cytochrome P450 system, one of the key factors that determines the intensity and duration of action is the rate of metabolism. This biotransformation renders the compounds more water soluble and therefore increases their rate of excretion in the urine and bile, and in most cases produces metabolites with less or no pharmacological activity.

REGULATION OF CYTOCHROME P450

For drugs that undergo only phase I oxidative metabolism catalyzed by the cytochrome P450 system, some are metabolized by only one isozyme and others are metabolized by several. The level of each isozyme is influenced by genetic factors, age, gender, diet, the presence of disease, concurrent use of other drugs (inducers and inhibitors), exposure to aromatic hydrocarbons such as those found in cigarette smoke and charbroiled meats, and numerous other factors. The capacity of an individual to metabolize a given drug is determined by the balance of the various isozymes present in the liver at that time and as a result, there is great inter- and intra-individual variability in rates of drug metabolism (Breimer and Schellens, 1990). The human isozymes responsible for the metabolism of some common drugs are shown in Table 2 (adapted from Guengerich and Turvy, 1991). For drugs with narrow therapeutic indices, this large variability between individual patients often demands that physicians individualize dosage regimens.

There has been extensive research carried out in an attempt to determine the mechanisms by which genetic factors control the levels of various cytochrome P450 isozymes. One of the original studies showed that female rats injected with hexobarbital

ISOZYME	DRUG(S) METABOLIZED
IA2	caffeine, methylxanthine, acetaminophen, phenacetin
IID6	debrisoquine
IIC8, IIC9, IIC10	tolbutamide
IIE1	acetaminophen, chloroxazone
IIIA	warfarin, quinidine, erythromycin, lidocaine, lovastatin, midazolam, triazolam, dapsone
IIIA4	nifedepine, lidocaine

TABLE 2 - SELECTED HUMAN CYTOCHROME P450 ISOZYMES

sleep longer than males (Quinn et al, 1958). It has since been established in rats that males metabolize certain drugs more rapidly than females. The most extensively studied genetic polymorphism in humans is that of debrisoquine hydroxylation. Certain individuals exhibit an exaggerated hypotensive response to the drug due to the expression of a poor metabolizer (PM) phenotype of the isozyme responsible for the oxidation of debrisoquine (P450IID6). The frequency of the PM phenotype is approximately 5-10% in caucasians and renders these individuals more susceptible to certain adverse drug reactions involving not only debrisoquine but potentially involving many other drugs that are metabolised by the same isozyme such as encainide, alprenolol, and imipramine (Eichelbaum, 1988).

The use of drugs known to be inducers of cytochrome P450 can have significant clinical consequences if used concurrently with a drug that has a narrow therapeutic index. For example, the use of phenobarbital (a classical cytochrome P450-inducer) in patients receiving warfarin therapy may require an increase in the dose of warfarin in order to maintain adequate therapeutic levels. If phenobarbital is discontinued at this point (resulting in a return to 'normal' cytochrome P450 levels) without lowering the maintenance dose of the warfarin back to the pre-phenobarbital level, an increase in plasma warfarin levels will occur that will increase the risk of fatal hemorrhage. In women taking phenobarbital and oral contraceptives, there have been reports of pregnancies that resulted from an increase in the metabolism of the oral contraceptive caused by the phenobarbital-mediated induction of cytochrome P450. These two specific examples are taken from a long list of documented interactions between known cytochrome P450 inducers and other concurrently administered therapeutic agents (Park and Breckenridge, 1981).

The therapeutic problems resulting from the use of agents known to cause cytochrome P450 inhibition have also received much attention. There are numerous examples cited in the literature for interactions such as that seen with concurrent

administration of cimetidine (a cytochrome P450 inhibitor) and warfarin, where it has been observed that cimetidine can enhance the anticoagulant action of warfarin. Similarly, hypoglycemic crises have ensued from the co-administration of chloramphenicol (another cytochrome P450 inhibitor) and tolbutamide (Park and Breckenridge, 1981).

Many disease states are known to effect drug metabolism. In a study of human liver samples it was shown that hepatic cirrhosis resulted in significant decrease in the levels of cytochromes P450IA2 and IIE1 as determined by immunoblotting (Guengerich and Turvy, 1991); furthermore, the metabolism of chlordiazepoxide is slower in cirrhotic patients as is the oxidation of propranolol, theophylline, and tolbutamide. The metabolism of methadone and benzphetamine is reduced in hepatoma tissue and surrounding unaffected liver tissue of hepatoma-bearing rats (Gibson and Skett, 1986). Similarly, in mice bearing the S-180 sarcoma the levels of ethoxycoumarin deethylase in liver homogenates were depressed (Bertini and Ghezzi, 1988). Hepatocytes isolated from adjuvant-induced arthritic rats had lower levels of aminopyrine N-demethylase activity than hepatocytes isolated from control rats (Ishizaki et al, 1983). All of these studies serve to illustrate the diversity of the numerous endogenous and exogenous factors known to influence the isozymes that comprise the cytochrome P450 enzyme system.

CYTOCHROME P450 AND THE IMMUNE SYSTEM

In the past 15 years there has been remarkable interest in the interaction between the system that protects us from infectious disease and that which allows us to inactivate therapeutic agents and environmental chemicals. In 1976, Renton and Mannering (1976a and 1976b) showed that many different interferon inducers such as tilorone, quinacrine, polyriboinosinic•polyribocytidylic acid (poly rI•rC), and Escherichia coli (E. coli) endotoxin caused a significant suppression of hepatic microsomal cytochrome P450 content following

their administration to rats. After establishing the temporal relationship between interferon production and the loss of mixed function oxidase activity after treatment with encephalomyocarditis (EMC) virus or the administration of poly I:C in mice, Renton and Mannering developed the "interferon hypothesis" (Renton, 1981) that stated that the ability to depress cytochrome P450-dependent monooxygenase is a property of all interferon inducing agents and may be related to the production of interferon itself.

In 1978, Chang and co-workers reported that plasma theophylline half-life was significantly longer than normal during serologically confirmed upper-respiratory tract infections with influenza A and adenovirus in five asthmatic children. During an outbreak of influenza in Seattle, Washington in 1980, 11 children who had been taking theophylline to control their asthma developed a sudden decrease in drug clearance during this febrile viral illness, and were admitted to hospital with mild to moderate methylxanthine toxicities ranging from headaches to seizures (Kraemer et al 1982). The mechanism involved with the changes in blood levels of the drugs in these two cases was not known at the time, but based on the hypothesis of Renton (1978a) it was likely that endogenous interferon, known to be released in response to viral infections, may have played a central role. As work in this area continued, many different 'immune stimulants' were found to be capable of depressing cytochrome P450-mediated metabolism after their administration to rodents and it became evident that several different types of infection and various vaccines were also capable of impairing cytochrome P450-mediated metabolism in humans. Some examples of these reported responses are listed in Table 3.

INTERFERON AND CYTOCHROME P450

It was known that many different interferon-inducing agents were capable of causing a significant decrease in cytochrome P450-mediated metabolism (Renton and

AGENT	SPECIES	EFFECT	REFERENCE
malaria	rats	↓ aniline hydroxylase ↓ ethylmorphine N-demethylase ↓ hexobarbital metabolism	McCarthy, 1970
Bacillus Calmette-Guérin (BCG) treatment	rats	↓ aniline hydroxylase, ↓ total P450	Farquhar, 1976
Mengo virus	rats	↓ ethylmorphine N-demethylase ↓ aniline hydroxylase	Renton, 1976b
Poly rI·rC	rats	↓ ethylmorphine N-demethylase ↓ aniline hydroxylase ↓ total P450	Renton, 1976b
E. coli endotoxin	rats	↓ ethylmorphine N-demethylase ↓ total P450	Renton, 1976b
B. pertussis vaccine	mice	↓ ethylmorphine N-demethylase ↓ aniline hydroxylase ↓ aminopyrine N-demethylase ↓ total P450	Williams, 1977
B. pertussis vaccine	rats	↓ microsomal metabolism of phenytoin and ↓ in vivo clearance	Renton, 1979
Corynebacterium parvum	mice	↓ aminopyrine N-demethylase ↓ aniline hydroxylase	Soyka, 1979
Newcastle Disease Virus	mice	↓ aminopyrine N-demethylase ↓ aniline hydroxylase	Singh, 1981
Listeria monocytogenes	mice	↓ aminopyrine N-demethylase ↓ total P450 ↓ benzo(a)pyrene hydroxylase	Azri, 1987
mouse hepatitis virus	mice	↓ hexobarbital metabolism	Kato, 1963
influenza A, adenovirus	man	↑ half-life of theophylline	Chang, 1978
influenza vaccine	man	↓ clearance of theophylline	Renton, 1980
herpes simplex infection	man	↓ clearance of theophylline	Anolik, 1982
influenza B	man	↑ serum theophylline concentrations	Kraemer, 1982
BCG vaccine	man	↓ clearance of antipyrine	Gray, 1983
pneumonia	man	↓ clearance of antipyrine	Sonne, 1985

TABLE 3 - IMMUNOACTIVE AGENTS AND THEIR EFFECT ON CYTOCHROME P450

Mannerling, 1976; Leeson et al, 1976) but a causal relationship between these two factors was not established until a number of years later. Singh and Renton (1981) compared the effect of Newcastle Disease Virus (NDV) on cytochrome P450 in two different strains of mice. The first strain carried the high interferon production allele ($I\!f\text{-}1^h$), and the second the low production allele ($I\!f\text{-}1^l$). NDV caused a significant decrease in hepatic microsomal cytochrome P450 and aminopyrine N-demethylase only in mice containing the h-allele at $I\!f\text{-}1$. This autosomal locus determines a 10-fold difference in serum interferon levels that are achieved after infection with NDV (DeMaeyer and DeMaeyer-Guignard, 1979). Poly rI•rC, which induces interferon at loci other than $I\!f\text{-}1$, significantly depressed hepatic microsomal cytochrome P450 and aminopyrine N-demethylase in both strains. The conclusion from this experiment was that interferon, or the process that produces interferon, can depress the level of cytochrome P450 in liver (Singh and Renton, 1981).

In 1982, a highly purified homogeneous human interferon produced in *E. coli* from cloned genes was shown to significantly depress hepatic microsomal cytochrome P450, aminopyrine N-demethylase, and benzo(a)pyrene hydroxylase in male mice (Singh et al, 1982). This interferon (LEIF-AD) was a molecular hybrid formed between two of the human leukocyte interferon subtypes and provided the first conclusive direct evidence to support the hypothesis that the production of interferon is a contributing factor in the depression of cytochrome P450 that occurs during infection or following the administration of interferon-inducing agents. Parkinson et al (1982) also observed a significant decrease in hepatic microsomal cytochrome P450 after the administration of LEIF-AD to female mice. A previous report (Sonnenfeld et al, 1980) had shown that a crude preparation of mouse type II interferon (interferon- γ) was capable of causing a significant depression of cytochrome P450 in hepatic microsomes, and subsequent studies (Franklin and Finkle, 1985; Bertini et al, 1988) with recombinant mouse interferon- γ confirmed that this type of

interferon caused a significant depression of cytochrome P450. A crude preparation of mouse IFN- β was also shown to have the same effect (Singh et al, 1982) but to date, there have been no reports of the effect of a recombinant interferon- β on cytochrome P450-mediated metabolism. These experiments suggested that all three subtypes of interferon (α , β and γ) are capable of depressing the cytochrome P450 enzyme system.

Many of the agents listed in Table 3 are known to be interferon inducers. An attractive hypothesis is that interferon-inducing agents decrease cytochrome P450 only by an interferon-mediated mechanism. However, many of these agents are also more general immune stimulants and have many other effects on host defence mechanisms in addition to the induction of interferon. Some agents such as Listeria monocytogenes can cause a decrease in cytochrome P450-mediated metabolism without causing a detectable increase in serum interferon levels (Azri and Renton, 1987). Endotoxin, the lipopolysaccharide component of the cell wall of gram negative organisms (LPS), is a weak inducer of interferon but is one of the more potent agents that depress the hepatic mixed-function oxidase system (Williams, 1985). Endotoxin also causes the release of cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF) (Flohe et al, 1991), all of which are capable of suppressing cytochrome P450 after *in vivo* administration to rodents as will be discussed in the next section.

Independent of the ultimate mechanisms involved, it has been established that many different types of immune stimulation can result in a suppression of the capacity for oxidative drug metabolism. In addition to the clinical examples listed in Table 3, there have been several other documented examples of compromised drug metabolism in infected humans (Fleetham et al, 1978; Clark and Boyd, 1979; Forsyth et al, 1982; Koren and Greenwald, 1985; Greenwald and Koren, 1990). These examples serve to illustrate the point that drugs with narrow therapeutic indices should be administered with caution during

periods of infectious disease. The advent of recombinant DNA technology has allowed purified interferon of the various subtypes to be synthesized making them available for clinical use. As of early 1991, there were five FDA approved indications for interferon- α and one for interferon- γ , with approximately 25 other applications under review for disease states such as hepatitis, hairy-cell leukemia, basal cell carcinoma, multiple myeloma, AIDS, lupus, and others (Baron et al, 1991). There have been 3 reports of altered drug clearance during the clinical use of recombinant interferon- α (Williams and Farrell, 1986; Williams et al, 1987; Jonkman et al, 1989). A recent report by Okuno and co-workers (1990) examined drug metabolism in microsomes prepared from liver biopsy samples obtained from patients treated with interferon for chronic active hepatitis. This was the first report that directly demonstrated that interferon can depress hepatic cytochrome P450-dependent metabolism in the liver of humans. As the use of interferon in the clinical arena becomes more frequent, there will undoubtedly be an increase in the number of adverse drug reactions that result due to impaired drug metabolism capacity. Obviously, the most likely drug interactions will occur with drugs that have a narrow therapeutic index and it is worth noting that the magnitude of the effect will likely be variable, as was the case in the article of Okuno and co-workers (1990).

THE INFLUENCE OF THE RETICULOENDOTHELIAL SYSTEM ON HEPATIC CYTOCHROME P450

The mammalian liver is composed of several different cell types including parenchymal cells, which constitute about 90% of the total cell mass, and nonparenchymal cells which include Kupffer cells, endothelial cells, fat-storing cells, bile duct cells, vascular smooth muscle cells and other minor cell populations. The nonparenchymal cells represent approximately 35% of total liver cell number but only 10% of the total cell mass (Steinberg

et al, 1987). It is established that most of the drug metabolizing capacity of the liver occurs in the hepatocyte. The nonparenchymal cells of the liver do contain some aryl hydrocarbon hydroxylase (AHH) activity (a cytochrome P450-dependent monooxygenase) but at a level less than 10% of that of hepatocytes (Cantrell and Bresnick, 1972). These cells have also been shown to contain detectable aminopyrine N-demethylase and ethoxyresorufin-O-deethylase but their activities were much less than those contained in hepatocytes (Steinberg et al, 1987). A recent report showed that an ethanol-inducible isozyme of cytochrome P450 (P450III₁) exists in Kupffer cells (Koop et al, 1991) but once again at very low levels relative to hepatocytes. In contrast, the phase II conjugation reaction involving acetylation catalyzed by acetyltransferase occurs mainly in Kupffer cells (Gibson and Skett, 1986).

The reticuloendothelial system (RES) is an old term used to describe a collection of cells that are united by their common property of phagocytosis. This includes circulating monocytes, tissue macrophages such as the Kupffer cells of the liver and granulocytic phagocytes such as neutrophils. In spite of a lack of significant drug metabolism capacity in these cells, the status of the hepatic and extra-hepatic 'immune' cells can influence drug biotransformation in the hepatocyte (Wooles and Munson, 1971). Pyran copolymer is known to produce a biphasic response of the phagocytic activity of the RES (Munson et al, 1968). After the administration of pyran copolymer to mice, hexobarbital sleeping time was significantly prolonged and serum concentrations of hexobarbital were significantly increased during both the depression and stimulation phases of RES activity (Wooles and Munson, 1971). When SKF 525A was given one hour prior to hexobarbital, it markedly prolonged the duration of sleep without affecting the activity of the RES. When SKF 525A was given via a protocol that induced barbiturate metabolism and decreased hexobarbital sleep time, it also had no effect on the RES. The authors concluded that although drugs that influence the metabolism of other drugs can do so without affecting RES activity, drugs that

produce marked alterations in RES activity depress barbiturate metabolism. Since the rate of clearance of hexobarbital is dependent on the rate of hepatic metabolism of the drug, the authors suggested that pyran copolymer produced an inhibition of the rate of hexobarbital metabolism and confirmed this finding in liver homogenates (Wooles and Munson, 1971).

It is evident that many other immunoactive agents (in addition to those listed in Table 3), are capable of depressing hepatic cytochrome P450-dependent drug biotransformation including methyl palmitate, zymosan, colloidal carbon, dextran sulfate, and latex particles (Williams and Szentivanyi, 1985). The ability of these agents to cause this effect was only seen after *in vivo* administration as they had no effect when added *in vitro* to microsomal preparations (Renton, 1983). Furthermore, the *in vivo* effects appeared to involve another cell type and an indirect action on hepatocytes. The effect of Corynebacterium parvum (C. parvum) on aminopyrine N-demethylase activity was blocked by splenectomy, if performed 2 or 48 hours prior to the administration of C. parvum or concurrently with its administration. Similarly, the effect of C. parvum was blocked by whole body irradiation (WBI) if performed immediately before or 2 hours after the administration of C. parvum (Soyka et al, 1979). These authors concluded that macrophages proliferating in response to C. parvum are critical to its effect on cytochrome P450.

Williams et al (1981) examined the effect of splenectomy and the role of the thymus in the depression of cytochrome P450 and ethylmorphine N-demethylase activity that results from the administration of Bordetella pertussis (B. pertussis) vaccine. Splenectomy did not ablate the effect of B. pertussis on P450-mediated metabolism if performed two days prior to the administration of the vaccine. In this study, B. pertussis vaccine produced a significant decrease in hepatic microsomal P450 content and ethylmorphine N-demethylase within 24 hours of administration and this suppression was maintained for at

least 7 days. When the vaccine was administered to athymic mice (nu/nu), hepatic cytochrome P450 content had returned to normal levels by day 7 of the experiment. The authors concluded that it is likely that T cells are involved in the response to B. pertussis that results in a decrease in cytochrome P450 levels, and that the ultimate mechanism involved may be different than that observed with C. parvum.

Peterson & Renton (1984) have shown that cytochrome P450 and aryl hydrocarbon hydroxylase (AHH) were depressed in microsomes prepared from the livers of mice treated with the immune modulating drug dextran sulfate. This effect occurred in the absence of a detectable increase in serum interferon levels. They also showed that cell-free supernatants from the incubation of Kupffer cells with dextran sulfate were capable of depressing cytochrome P450 and AHH activity when incubated with hepatocytes. It was also shown that when dextran sulfate was added to a chamber of a Marbrook vessel containing Kupffer cells, an unidentified factor crossed the semipermeable membrane (molecular weight cutoff at approximately 12,000) and depressed cytochrome P450 levels in the hepatocytes of the other chamber. Dextran sulfate had no effect when incubated directly with isolated hepatocytes. A subsequent paper by Peterson and Renton (1986a) showed that *in vivo* administration of latex particles resulted in a significant decrease in hepatic microsomal P450 content and aminopyrine N-demethylase activity. In a similar fashion to the experiments of the previous article (Peterson and Renton, 1984), when latex particles were added to one chamber of a Marbrook vessel containing Kupffer cells, a factor was released and crossed the semipermeable membrane and depressed cytochrome P450 and benzo(a)pyrene hydroxylase activity in the hepatocytes contained in the other chamber (Peterson and Renton, 1986a). Latex particles had no effect when incubated directly with isolated hepatocytes and *in vivo* administration did not produce a detectable change in serum interferon levels. From these experiments, the authors concluded that dextran sulfate

and latex particles stimulate the release of a factor from Kupffer cells that can decrease cytochrome P450 in adjacent hepatocytes. Preincubation of dextran sulfate with peritoneal macrophages for 30 minutes prior to the addition of the mixture to isolated hepatocytes for an additional 30 minutes, also resulted in a significant depression of cytochrome P450 content (Peterson and Renton, 1984). This experiment illustrated that extra-hepatic macrophages were also capable of suppressing cytochrome P450 levels and suggested that other immune cells, in addition to Kupffer cells, may be involved in the depressive actions of some immunoactive substances on drug metabolism enzymes.

In order to elucidate what role, if any, that lymphocytes played in the depressant effect of dextran sulfate on P450-mediated metabolism, Peterson and Renton (1986b) conducted a study in which mice were pre-treated with antilymphocyte serum (ALS) one day prior to and concurrently with dextran sulfate. This treatment protocol with ALS alone (in the absence of dextran sulfate) resulted in a significant decrease in blood lymphocyte count but did not produce a significant change in the number of monocytes or granulocytes. However, this change in lymphocyte count did not prevent the depressive actions of dextran sulfate on cytochrome P450 and aminopyrine N-demethylase activity, nor did lymphocyte depletion using pre-treatment with cyclophosphamide. The authors concluded that lymphocytes are not involved in the mechanism by which dextran sulfate depresses cytochrome P450.

This series of studies demonstrated that Kupffer cells and certain extra-hepatic immunocompetent cells are capable of mediating a depressant effect on cytochrome P450 in hepatocytes following an interaction with many different 'RES-active' agents.

ENDOTOXIN, CYTOKINES, AND CYTOCHROME P450

Endotoxin, a component of the cell wall of gram-negative bacteria also known as lipopolysaccharide (LPS), is one of the most extensively studied agents known to effect cytochrome P450. It produces a multitude of effects after its administration to experimental animals including macrophage activation, induction of prostaglandin synthesis, induction of interferon, production of a febrile state and platelet aggregation (Westphal, 1975). The liver plays a major role in the clearance and inactivation of endotoxin and studies with radiolabeled endotoxin have demonstrated its localization in Kupffer cells as well as hepatocytes (Utili et al, 1977).

Some early studies demonstrated changes to drug pharmacokinetics caused by the administration of LPS to experimental animals; however, these studies could not conclude that the effect was due to changes in the metabolism of the drugs (Williams and Szentivanyi, 1985). In 1976, Gorodischer and co-workers showed that the administration of E. coli endotoxin to rats *in vivo* was in fact capable of decreasing hepatic microsomal cytochrome P450 content and aniline hydroxylase activity. This study instigated a series of subsequent studies that were designed to gain insights into the mediators and cell types involved in the effect, and raised concerns about the therapeutic management of patients with endotoxemia.

Lipopolysaccharides consist of two main components, a polysaccharide and a lipid (lipid A). For many years there was controversy over which part of the molecule was responsible for toxicity but it is now accepted that the lipid A fraction accounts for most of the effects of endotoxins in animals or on cultured cells (Raetz et al, 1991). More specifically, it was shown that the lipid A portion is responsible for the effect on drug metabolizing enzymes (Egawa and Kasai, 1979). This study compared the effect of the glycolipid portion (a preparation that lacks the O-specific polysaccharide portion) of the LPS from *Salmonella minnesota* R595 and *Selenomonas ruminantium*, as well as the lipid

A degradation products thereof. They found that the acid hydrolysis derived lipid A was equipotent relative to the intact glycolipid in terms of its ability to cause a significant depression of hepatic microsomal aminopyrine N-demethylase and aniline hydroxylase after *in vivo* administration to mice. A significant suppression was observed approximately 12 hours after administration and this suppression was maintained for approximately 12 hours; the enzyme activities had returned to normal levels by 48 hours. Even the direct incubation of very high concentrations of glycolipid from Salmonella with hepatic microsomes did not result in a significant change in aminopyrine N-demethylase activity (Egawa and Kasai, 1979). The incubation of isolated rat hepatocytes with LPS for 8 hours did not result in a significant change in aniline hydroxylase activity or cytochrome P450 levels (Williams and Szentivanyi, 1985). This has since been confirmed by Ghezzi et al (1986b) and Bertini et al (1989). The serum obtained from mice after the administration of a glycolipid preparation of Salmonella minnesota R595 caused a significant decrease in hepatic microsomal cytochrome P450 levels when injected into control animals (Egawa et al, 1981). These studies were consistent with an indirect mechanism for endotoxin-induced decreases in drug metabolism capacity but the mediator of the effect was not known.

Endotoxin is a weak inducer of interferon (Williams and Szentivanyi, 1985) and endotoxin-induced interferon is reported to be heat-labile (Egawa et al, 1981). The serum from mice pre-treated with endotoxin is capable of suppressing cytochrome P450-mediated metabolism after administration to control mice even after heating to 56°C. This suggested that interferon is not involved in the effect of endotoxin on cytochrome P450.

Two different strains of mice have been used to elucidate the mechanism by which endotoxin decreases cytochrome P450-mediated metabolism; 'normal' responsive mice such as the C3H/HeN strain and non-responsive C3H/HeJ mice. Endotoxin stimulates the *in vitro* release of IL-1, and TNF from macrophages of responsive mice and increases serum

interferon levels after *in vivo* administration (Rosenstreich et al, 1978; Beulter et al, 1986; Apte et al, 1977). The C3H/HeJ strain do not produce interferon in response to LPS (Apte et al, 1977). Macrophages from C3H/HeJ mice do not release IL-1 in response to endotoxin (Rosenstreich et al, 1978) nor do they release TNF (Beulter et al, 1986). Many investigators have used this strain, along with responsive strains, in order to determine which mediators are involved in the endotoxin-mediated loss of cytochrome P450. LPS from E. coli significantly decreased ethoxycoumarin deethylase activity in C3H/HeN mice after 24 hours but had no effect in C3H/HeJ mice (Ghezzi et al, 1986b). The serum from the LPS-treated C3H/HeN mice, when injected into C3H/HeJ mice, did produce a significant reduction in ethoxycoumarin deethylase activity in these mice. Peritoneal exudate cells, consisting mainly of macrophages and lymphocytes, obtained from C3H/HeN mice, were injected (i.p.) into C3H/HeJ mice and rendered them responsive to the suppression of ethoxycoumarin deethylase activity after subsequent challenge with LPS. When the supernatants of cultures of human monocytes and LPS were incubated with rat hepatocytes, a significant reduction in ethoxycoumarin deethylase activity resulted (Ghezzi et al, 1986b). LPS was shown to have no effect when incubated directly with isolated rat hepatocytes.

Monocytes and Kupffer cells have been shown to synthesize and release IL-1 in response to endotoxin (Dinarello, 1984) and IL-1 receptors have been demonstrated on rat hepatocytes (Sujita et al, 1990). Human and murine recombinant IL-1- α and murine IL-1- β have been shown to significantly depress hepatic microsomal P450-mediated metabolism after *in vivo* administration to both C3H/HeN and C3H/HeJ mice (Ghezzi et al, 1986b; Shedlofski et al, 1987; Bertini et al, 1988). Purified interleukin-1 (IL-1) produced the same effect when incubated directly with mouse or rat hepatocytes (Peterson and Renton, 1986b; Ghezzi et al, 1986b). These studies suggested that the alterations in hepatic cytochrome

P450 seen with endotoxin are consistent with the idea that they may be mediated, at least in part, by IL-1.

Two additional cytokines that have received a great deal of attention are tumor necrosis factor (TNF) and interleukin-6 (IL-6), both of which are released by macrophages after endotoxin administration (Ertel et al, 1991). The order of release of various cytokines during sepsis is somewhat controversial but it appears that TNF is released first and in turn results in the release of IL-1 and IL-6 (Ertel et al, 1991). TNF- α (cachectin) is predominantly derived from macrophages and monocytes, whereas TNF- β (lymphotoxin) is derived from T cells and B cells (Whicher and Evans, 1990). Small amounts of TNF play an important protective role in host defense against bacteria but high circulating levels of TNF during overwhelming gram-negative bacterial infection can cause septic shock (Havell and Sehgal, 1991). Recombinant human TNF caused a significant depression in hepatic microsomal cytochrome P450, AHH and ethoxycoumarin deethylase activities after *in vivo* administration to mice (Ghezzi et al, 1986a). Bertini et al (1989) demonstrated that the supernatants from cultures of human monocytes, incubated with recombinant human TNF- α , were capable of suppressing ethoxycoumarin deethylase activity after their addition to isolated rat hepatocytes. The *in vitro* incubation of human recombinant TNF directly with isolated rat hepatocytes did not effect drug metabolism capacity in these cells (Ghezzi et al, 1986a ; Bertini et al, 1988). This suggested that a factor released from the monocytes as a result of stimulation by TNF was mediating the effect on the hepatocytes. The supernatants displayed high IL-1 activity in a thymocyte proliferation assay (this had also been previously shown by Dinarello et al (1986)). When the supernatants from the monocyte cultures, stimulated by TNF, were treated with rabbit anti-human IL-1 antiserum, their ability to depress cytochrome P-450-mediated metabolism in the hepatocytes was

totally blocked. This suggests that IL-1 is the mediator of the effect of TNF on liver cytochrome P450.

Interleukin-6 (IL-6) (previously called IFN β 2, B cell stimulatory factor-2 and hepatocyte stimulating factor) was originally described in 1980. Its main sites of production are monocytes, macrophages, fibroblasts, and endothelial cells. Bacterial endotoxins, TNF, and IL-1 stimulate its production, and it is probably the major physiological inducer of acute phase protein synthesis in the liver (Whicher and Evans, 1990). It is known that LPS can produce an acute phase response and that cytokines such as IL-1, TNF and IL-6 are important mediators of this response. This response phenomena results in a myriad of physiological effects including major changes in the synthesis and secretion of many hepatic proteins (Heinrich et al, 1990). As discussed above, the cytochrome P450 enzyme system is suppressed after LPS administration and hence there has been interest in determining what role, if any, that IL-6 plays in this effect. LPS can directly stimulate the release of IL-6 from monocytes and macrophages. LPS stimulates the release of IL-1 and TNF- α from monocytes and macrophages that can in turn stimulate the release of IL-6 from endothelial cells and fibroblasts (Heinrich et al, 1990). The *in vivo* administration of recombinant human IL-6 to rats for 24 hours had no effect on hepatic microsomal cytochrome P450 content (Wright and Morgan, 1991). The incubation of IL-6 with isolated mouse hepatocytes for 20 hours resulted in a significant decrease in total cytochrome P450 content (Bryden et al, 1991), and Williams and co-workers (1991) recently demonstrated that recombinant human IL-6 was able to completely block the induction of cytochrome P450_{IIB} in isolated rat hepatocytes. To date, these are the only three reports on the effect of IL-6 on cytochrome P450-mediated metabolism.

There have been recent proposals and some clinical trials of various combinations of cytokines used in an attempt to kill tumor cells. They have also been proposed to be used

for 'rescue' after high dose chemotherapy or in conjunction with chemotherapy schedules to accelerate hemopoietic recovery (Lanz et al, 1991). Considering the evidence for cytokine-mediated depression of cytochrome P450, caution is warranted for the concurrent administration of cytokines and drugs with narrow therapeutic indices, or drugs that require activation by cytochrome P450.

MECHANISM FOR THE SUPPRESSION OF CYTOCHROME P450 BY INTERFERON

The mechanism by which interferon causes a suppression of cytochrome P450 is now reasonably well understood. Interferon has no effect on cytochrome P450 when incubated directly with hepatic microsomes (Parkinson et al, 1982) but Renton (1987) showed that recombinant human interferon- α (IFN- α -CON₁) significantly decreased total cytochrome P450 content after a 19 hour incubation with intact mouse hepatocytes. This suggested that the effect of interferon was exerted directly on the hepatocyte and did not involve other cell types. However, an earlier study (Renton et al, 1978b) showed that murine interferon caused a significant induction of total cytochrome P450 levels when incubated with isolated hepatocytes for 24 hours. In this study the interferon was added to the hepatocytes after they had been cultured for 24 hours and had lost approximately 80-90% of their cytochrome P450 content. Mannering and Deloria (1986) reported that interferon had no effect on cytochrome P450 levels when incubated with mouse hepatocytes. Therefore, the issue of whether or not other cell types are involved in the mechanism by which interferon depresses cytochrome P450 levels in hepatocytes is still not completely resolved.

It is now generally accepted that many of the antiviral effects of interferons are the result of an inhibition of viral protein synthesis (Mannering and Deloria, 1986). Many

studies have also investigated the effects of interferon-inducing agents and interferon on cytochrome P450 apoprotein synthesis. The administration of poly rI•rC for 24 hours along with ¹⁴C-labelled amino acids indicated that the incorporation of amino acids into a cytochrome P450 rich fraction of hepatic microsomes was depressed in poly rI•rC treated mice (Singh and Renton, 1984). This agreed with the previous report of Zerkle et al (1980); however, the results of this group suggested the possibility of some degree of isozyme selectivity of the effect. These two studies were the first to suggest that the loss of cytochrome P450 was due to a loss of apoprotein and that the effect was not part of a generalized decrease in the synthesis of all microsomal proteins. In fact, Singh and Renton (1984) showed that poly rI•rC treatment resulted in a significant increase in the total incorporation of labelled amino acids into microsomal protein. A more recent study (Gooderham and Mannering, 1990) demonstrated that poly rI•rC treatment also resulted in a significant increase in total hepatic mRNA levels. Thus, the rate of synthesis of some proteins in the liver was increased by poly rI•rC while the synthesis of cytochrome P450 appeared to be depressed.

An issue that had not been resolved was whether the decrease in apoprotein levels resulted solely from an inhibition of synthesis or also involved apoprotein destruction. Gooderham and Mannering (1986) showed that poly rI•rC significantly depressed the incorporation of radiolabelled leucine into rough endoplasmic reticulum proteins and also appeared to accelerate the loss of ¹⁴C-labelled protein from rough endoplasmic reticulum in mice. These authors suggested that poly rI•rC inhibits the synthesis and accelerates the degradation of proteins of the hepatic endoplasmic reticulum but this study did not differentiate between cytochrome P450 and other hepatic proteins. In a similar study, Moochala et al (1989) showed that the incorporation of labelled methionine into microsomal proteins with molecular weights consistent with those of cytochrome P450 was depressed

following interferon- α -CON₁ administration. This interferon also caused a significant reduction of total hepatic cytochrome P450 content as well as aminopyrine N-demethylase and benzo(a)pyrene hydroxylase activities after 24 hours in hamsters (Moochala and Renton, 1991a). Although this interferon decreased the synthesis of certain hepatic microsomal proteins there was no effect on the degradation rate of these same proteins. The total *in vitro* translational capacity of hepatic RNA remained unchanged in these experiments but the translation of proteins with molecular weights in the region of cytochrome P450 was depressed, suggesting that interferon decreases the levels of mRNA that code for the apoprotein portion of cytochrome P450. Renton et al (1987) also investigated the effect of poly rI•rC on the induction of cytochrome P450IVA by clofibrate. After treatment for 3 days with clofibrate, rats were treated with poly rI•rC for 24 hours at which point total hepatic RNA was isolated and translated in an *in vitro* cell free translation system containing ³⁵S-methionine. Poly rI•rC had no effect on the total translational capacity of the liver but the binding of an antibody to cytochrome P450IVA apoprotein was depressed by 50%. This data is consistent with the hypothesis that interferon depresses cytochrome P450 levels by decreasing apoprotein synthesis but has little effect on other proteins.

Renton and Knickle (1990) provided the first direct evidence indicating that interferon could decrease cytochrome P450 mRNA. Total hepatic RNA was isolated from clofibrate-treated rats and a cDNA probe for cytochrome P450IVA1 mRNA was utilized to identify this specific mRNA. After 6 hours of treatment with poly rI•rC, cytochrome P450IVA1 mRNA levels were significantly reduced but there was no effect on total hepatic microsomal P450 content or lauric acid hydroxylase activity (lauric acid hydroxylation is catalyzed by cytochrome P450IVA1; Parker and Orton, 1980). After 24 hours of treatment, cytochrome P450IVA1 mRNA levels, total cytochrome P450 content and lauric acid

hydroxylase activities were all significantly reduced. The decrease in mRNA levels preceded the loss in apoprotein indicating that the decrease in mRNA was most likely responsible for the loss in apoprotein.

Several other groups have since confirmed that interferon can effect cytochrome P450 mRNA levels. The administration of recombinant rat interferon- γ to male rats for 24 hours resulted in a significant decrease in cytochrome P450III_{A2} mRNA, apoprotein and P450III_{A2}-dependent androstenedione 6 β -hydroxylase activity (Craig et al, 1990). The administration of poly rI \cdot rC to male rats resulted in a significant decrease in total hepatic microsomal cytochrome P450 content and P450II_{C11} mRNA levels (Morgan and Norman, 1990). A time course study revealed that the decrease in mRNA preceded the decrease in apoprotein consistent with the findings of Renton and Knickle (1990). A subsequent study by Morgan (1991) demonstrated that poly rI \cdot rC caused a significant reduction in the female specific cytochrome P450II_{C12} apoprotein and mRNA levels in female rats; however, in this study the decrease in mRNA levels did not precede the loss of apoprotein. This suggested that there may be differences in the mechanisms by which interferon suppresses different forms of cytochrome P450, or that the mechanisms involved are different in male and female rats. In each study, the magnitude of the suppression of cytochrome P450 mRNA levels observed after the *in vivo* administration of interferon and interferon inducers could entirely account for the loss of apoprotein that was observed.

The mechanism by which interferon causes a decrease in cytochrome P450 mRNA remains to be established. To date, there have been no reports on the effect of interferon on the transcriptional activity of cytochrome P450 genes, nor has there been any reports on the effect of interferon on cytochrome P450 mRNA stability. It has however been established that the effect of interferon in hamsters requires the de novo synthesis of an intermediate protein (Moochala et al, 1989; Renton and Knickle, 1990). Both puromycin and

actinomycin D completely blocked the ability of interferon- α -CON₁ to cause a suppression of hepatic cytochrome P450 levels. This finding was consistent with the previous finding of Parkinson et al (1982) that administered interferons were no longer detectable in serum after 4 hours while cytochrome P450 levels were still significantly suppressed after 24 hours. The intermediate protein that mediates the effect of interferon has not been identified. Interferons that have no antiviral activity in a given species also do not suppress cytochrome P450 in that species (Singh et al, 1982; Parkinson et al, 1982; Singh and Renton, 1984; Mochala and Renton, 1989b). Therefore, it is possible that the loss in cytochrome P450 is an extension of the establishment of an antiviral state.

Interferon activates 2-5A oligoadenylate synthetase that then activates an endonuclease that in turn cleaves viral mRNA. Interferon also activates a kinase pathway that results in the phosphorylation and inactivation of a protein synthesis initiation complex (eIF2). It is generally accepted that the 2-5A-dependent endonuclease cleaves both host and viral RNAs although this issue remains controversial (Baglioni, 1978; Mannering and Deloria, 1986). It is therefore possible that 2-5A synthetase is the intermediate protein that is produced by interferon that activates an endonuclease which in turn cleaves cytochrome P450 mRNA. However, there is no direct evidence to support this hypothesis at this time.

Xanthine oxidase generates reactive oxygen species that are known to be involved in phagocyte-mediated microbicidal activity (Baglioni, 1978). Poly rI•rC and purified mouse interferon increased liver and serum xanthine oxidase levels approximately 3-4 fold after their administration to mice (Ghezzi et al, 1984). A subsequent study (Ghezzi et al, 1985) demonstrated that the free radical scavenger N-acetylcysteine and the xanthine oxidase inhibitor allopurinol protected against the interferon-mediated depression of hepatic cytochrome P450 levels, and suggested that reactive oxygen species may play a role in the interferon-mediated loss of cytochrome P450. Interferon- α -CON₁ produced a significant

decrease in hepatic microsomal cytochrome P450 content after 24 hours in hamsters, and a significant increase in hepatic xanthine oxidase levels (Moochala and Renton, 1991b). Both of these effects were blocked by actinomycin D. The administration of poly rI•rC had no effect on hepatic microsomal P450 levels in chicks that are not capable of forming xanthine oxidase (Moochala and Renton, 1991b). The incubation of milk xanthine oxidase and hypoxanthine directly with hepatic microsomes resulted in a significant loss of cytochrome P450 (Ghezzi et al, 1985; Moochala and Renton, 1991b). These results are consistent with the idea that the intermediate protein that is induced by interferon may be xanthine oxidase and that free radicals are responsible for the loss of cytochrome P450. As suggested by Renton and Knickle (1990), the free radicals may destroy cytochrome P450 mRNAs and/or affect the production of mature mRNA species, rather than directly destroy cytochrome P450.

The hypothesized role of xanthine oxidase however remains controversial. Hepatic xanthine oxidase can be inactivated in animals by including tungsten in the diet (Johnson et al, 1974). Mannering et al (1988) demonstrated that the addition of tungstate to drinking water for 10 days depressed hepatic xanthine oxidase activities to approximately 10% of control levels, but had no effect on cytochrome P450 levels. The administration of poly rI•rC to these mice produced a significant loss of cytochrome P450 but the levels of xanthine oxidase that were achieved were only 10% of those achieved in mice receiving poly rI•rC without tungstate. In this study, N-acetylcysteine did not protect against the effect of poly rI•rC on cytochrome P450; furthermore, the incubation of milk xanthine oxidase and hypoxanthine, directly with hepatic microsomes did not have any effect on cytochrome P450 levels. These authors concluded that the induction of xanthine oxidase and the loss of cytochrome P450 during interferon treatment are not causally related phenomena.

MECHANISM OF THE EFFECT OF ENDOTOXIN ON CYTOCHROME P450

Severe infections with LPS-producing gram-negative bacteria can produce fever, hyper- and hypoglycemia, hypotension, widespread organ dysfunction, and in some cases, death (Gilbert, 1960; Westphal, 1975). The administration of endotoxin to experimental animals results in a myriad of effects on the liver (Utili et al, 1977) including an inhibition of cytochrome P450-mediated drug metabolism which can occur at doses that do not produce significant histological changes. TNF, IL-1, and IL-6 are all released in response to endotoxin (Ertel et al, 1991). One or more of these cytokines are likely to be involved in the mechanism by which endotoxin causes a suppression of cytochrome P450-mediated metabolism, although conclusive evidence for this has yet to be demonstrated. As a result, attempts to elucidate the mechanism of the effect on cytochrome P450 have included studies with endotoxin and with the cytokines it releases.

Indirect evidence for inhibition of apoprotein synthesis was offered by Williams and Szentivanyi (1983). They showed that *E. coli* endotoxin blocked the induction of cytochrome P450 by phenobarbital. Although the exact mechanism by which phenobarbital induces cytochrome P450 is not completely understood, it is known that the induction requires an increase in apoprotein synthesis. The effect of endotoxin was therefore consistent with an ability to interfere with synthesis of the apoprotein. The administration of 1mg/kg endotoxin to mice for 24 hours resulted in a decrease in the level of PB1 apoprotein (PB1 is now named cytochrome P450IIC6) as determined by Western blotting and a semi-purified anti-PB1 antibody. An apparent decrease in PB1 mRNA was observed on a Northern blot (Stanley et al, 1988). In this study the cDNA and antibody probes that were used were developed for use in rats but were used in mice and thus their isozyme

selectivity is unknown. Nevertheless, it appeared that endotoxin was capable of suppressing the synthesis of some cytochrome P450 apoproteins.

A dose of 1mg/kg of E. coli endotoxin administered to male rats for 24 hours significantly suppressed cytochrome P450IIC11 apoprotein levels (cytochrome P450IIC11 is a male-specific isozyme) by approximately 40% as determined on Western blots using a polyclonal antibody (Morgan, 1989). In the same study, the level of cytochrome P450IIC11 mRNA levels were suppressed to approximately 15% of control levels. A time course indicated that the decrease in mRNA preceded the decrease in apoprotein and suggested that the change in apoprotein level is most likely the result of the change in mRNA. In this same study, endotoxin caused a significant depression of cytochrome P450IIC12 mRNA and apoprotein levels (P450IIC12 is a female-specific isozyme) determined with the use of a monoclonal antibody. After 24 hours, apoprotein levels were suppressed to approximately 40% of control levels and mRNA levels were decreased to approximately 10% of control levels. In contrast to the experiment with male rats, the decrease in cytochrome P450IIC12 mRNA did not precede the loss of apoprotein and suggested that other mechanisms, such as an effect on translation and/or protein degradation, may also be involved.

Microsomal levels of cytochrome P450IIC11 and P450IIC12 can account for up to half of the cytochrome P450 in male and female rats respectively (Morgan, 1989). Therefore, a suppression of these isozymes likely plays a significant role in the overall decrease in total cytochrome P450 content that is observed after endotoxin administration. The administration of E. coli endotoxin to male rats for 24 hours resulted in a 95% inhibition of cytochrome P450IIC11 gene transcription rates determined in hepatic nuclei using a nuclear runoff transcription assay (Wright and Morgan, 1990). Therefore, the suppression of cytochrome P450IIC11 in male rats is most likely the result of a change in

the transcriptional rate of the IIC11 gene but there have been no studies to date of the effect of endotoxin on cytochrome P450 mRNA stability. The effect of endotoxin on hepatic cytochrome P450 may be mediated, at least in part, by IL-1 (Ghezzi et al, 1986a; Ghezzi et al, 1986b; Shedlofski et al, 1987; Bertini et al, 1988; Bertini et al, 1989). Recombinant human IL-1- α caused a significant reduction in cytochrome P450IIC12 mRNA and apoprotein levels 24 hours after *in vivo* administration to female rats, but the transcriptional rate of the cytochrome P450IIC12 gene was not changed at this time (Wright and Morgan, 1991).

The growth hormone secretion pattern is the key regulator of the expression of cytochrome P450IIC11 and P450IIC12 which are two of the constitutive sex-specific isoforms found in male and female rats respectively. Neonatal androgens 'program' the secretion pattern of somatostatin and growth-hormone-releasing factor that in turn determines the type of growth hormone secretion pattern in adult rats (Kobliakov et al, 1991). Endotoxin has been shown to affect growth hormone levels in male rats (Kasting and Martin, 1982). 24 hours after administration of E. coli endotoxin, plasma growth hormone levels were significantly attenuated. After 48 hours, peak growth hormone levels during normal pulses of release were greater than in controls and they occurred with greater frequency. Therefore, it is possible that endotoxin-mediated alterations in growth hormone secretion pattern may be responsible for the effects on cytochrome P450IIC11 and IIC12.

The acute phase response (APR) can be triggered by infection or endotoxin in an attempt by the host to minimize tissue damage and to clear the infecting pathogen as rapidly as possible. It is characterized by a local reaction that involves activation of macrophages, fibroblasts, and endothelial cells and a systemic reaction resulting in fever, leukocytosis, activation of complement and clotting cascades, and significant changes in plasma concentrations of several proteins referred to as acute phase proteins (Heinrich et al, 1990;

Stadnyk and Gauldie, 1991). The liver is the major organ for the synthesis of the acute phase proteins. Alpha-2-macroglobulin and alpha-1-acid glycoprotein are examples of acute phase proteins where plasma concentrations increase 10-100 fold during an acute phase response; fibrinogen and haptoglobin have been shown to increase 2-10 fold. Plasma albumin concentrations decrease during an APR and hence it is referred to as a negative acute phase protein (Heinrich et al, 1990). It is now accepted that IL-6 is the primary regulator of acute phase proteins in the liver; however, some acute phase genes are directly induced by IL-1 and TNF (Heinrich et al, 1990; Stadnyk and Gauldie, 1991). Endotoxin can directly stimulate the release of IL-6 from monocytes and macrophages. LPS also stimulates the release of IL-1 and TNF- α from monocytes and macrophages that can in turn stimulate the release of IL-6 from endothelial cells and fibroblasts (Heinrich et al, 1990). The injection of ^{125}I -labelled IL-6 to rats showed that 30% was found in the liver after 20 minutes, and the liver is considered a major target organ for IL-6 (Heinrich et al, 1990). A similar study showed that 80% of the hepatic IL-6 was found on the surface of hepatocytes and a minor component on the surface of nonparenchymal cells (Sonne et al, 1990). The IL-6 receptor has been sequenced for a human natural killer cell line (YT) but little is known about the hepatic IL-6 receptor. The signal transduction pathway used by IL-6 to activate acute phase protein synthesis is not completely understood but it is believed that there are interleukin-6 responsive elements within acute phase protein genes (Heinrich et al, 1990). Poli and Cortese (1989) showed that human recombinant IL-6 stimulates the activation of an IL-6-dependent DNA binding protein (IL-6 DBP) which in turn binds to the promoter region of the hemopexin gene. Similar studies with the haptoglobin gene and the human c-reactive protein promoter led to the same conclusions, and led the authors to suggest this may be a general mechanism involved for the induction of transcription of all acute phase protein genes (Poli and Cortese, 1989).

It is possible that the decrease in hepatic cytochrome P450 levels, that results from endotoxin administration, is simply a consequence of the hepatic synthesis of acute phase proteins. As suggested by Morgan (1989), the loss in cytochrome P450 may reflect the diversion of the hepatic transcriptional 'machinery' to strongly induced secreted gene products. While I agree that this possibility exists, there is evidence to suggest that this is not the case. The administration of recombinant human IL-6 to rats did not produce a significant change in hepatic microsomal cytochrome P450 content, P450IIC12 apoprotein, or P450IIC12 mRNA; however, it did produce a significant decrease (approximately 50%) in albumin mRNA (Wright and Morgan, 1991) suggesting that an acute phase response had been activated without effecting cytochrome P450 levels. IL-1 may be the primary mediator of the effect of LPS on cytochrome P450. Human recombinant IL-1- α significantly decreased ethoxycoumarin deethylase activity not only in the liver but also in the lung, heart and spleen (Bertini et al, 1988). This suggests a more general effect on cytochrome P450 and is not consistent with the effect being an extension of the activation of acute phase genes that reside predominantly in the liver. A dose of 0.1 mg/mouse of LPS resulted in a significant decrease in hepatic microsomal ethoxycoumarin deethylase activity without producing a change in serum iron concentrations (Bertini et al, 1989). As hypoferrinemia is a marker of the acute phase response, this suggested that the acute phase response and the loss of cytochrome P450 can be 'separated' and that although the cytokines that produce the acute phase response may also depress cytochrome P450, the mechanisms involved may be entirely different.

LISTERIA MONOCYTOGENES - AN OVERVIEW

Listeria monocytogenes is a gram-positive, nonsporeforming bacterium with many different virulent strains (Gray and Killinger, 1966). By 1955, Listeria monocytogenes had

been isolated and identified from at least 27 animal species (Murray, 1955). A significant proportion of the human population (approximately 5%) periodically harbour listeria in the intestinal tract (Bojson - Moller, 1972) but the World Health Organization estimated disease rates to be only 0.003% (Editor, 1988). The groups most at risk from listeriosis are pregnant women, neonates, the immunocompromised and the elderly.

In the last 10-15 years there have been numerous outbreaks of listeriosis around the world, most of which have been attributed to contaminated foods such as raw vegetables, milk, and cheese (Kvenberg, 1988). The only major outbreak in Canada occurred in Nova Scotia in 1981 and identified contaminated coleslaw as the probable vehicle of transmission (Schlech et al, 1983). Several cases were also reported in 1988 in New Brunswick associated with contaminated lobster. The remainder of cases occur sporadically and although the prevalence of listeriosis in the general population is low, associated mortality may be as high as 10 - 20% (Cossart and Mengaud, 1989; Stecha et al, 1989). Listeria monocytogenes grows over a wide range of temperatures (3 - 45°C), it tolerates high salt concentrations and the food preservative sodium nitrite, it grows at pH 6-9 and survives in both wet and dry environments, occasionally even escaping destruction during Pasteurization (Gill, 1988). For these reasons, it creates a dilemma for food processors and regulatory agencies serving the food and dairy industries (Wehr, 1987). The most common presentation of adult listeriosis is meningitis, primary bacteremia, endocarditis, and non-meningeal central nervous system infection (Lamont et al, 1988). A combination of ampicillin and an aminoglycoside is the most commonly administered form of treatment (Lamont et al, 1988).

The administration of high doses of Listeria monocytogenes to mice can cause death within 3 or 4 days as a result of multiple necrotic lesions in the liver and spleen (Wilder and Sword, 1967). Lower doses of the bacterium produce a reversible infection lasting

approximately 7-10 days (MacKanness, 1962; Mitsuyama et al, 1978). Within 10 minutes after intravenous administration, 75 - 90% of the inoculum is recovered in the liver. The vast majority of the remainder is found in the spleen and less than 1% can be demonstrated in the blood (MacKanness, 1962; Mitsuyama et al, 1978). After 6 hours, the liver load of the bacteria decreases approximately 10-fold then gradually increases to reach a peak between days 2 and 4, declining thereafter and no longer detectable by day 9 (Mitsuyama et al, 1978). A similar time course is observed after intraperitoneal administration (Czuprynski et al, 1988).

Listeria monocytogenes can survive and grow within macrophages and hence it has been used as a classical model of intracellular parasitism (Lamont et al, 1988; Cossart and Mengaud, 1989). The immune response to Listeria monocytogenes is purely cell-mediated as antibodies are not formed during primary infection and are not protective in passive transfer (North and Deissler, 1975; Miki and MacKanness, 1964). Infection results in phagocytosis of the listeria which will kill some of the bacteria while other colonies survive and cause the dissolution of the membrane surrounding the phagosome. Surviving listeria colonies then become encapsulated by actin filaments that form a tail and allow the bacterium to reach the cell surface and become part of an extension of the membrane that is then phagocytosed by a neighboring macrophage. This cycle continues and allows listeria to move from cell to cell remaining intracellular (Tilney and Portnoy, 1989).

The immunological response during murine listeriosis has received a great deal of attention in the last 10 years. In the early stages of infection there is significant infiltration of neutrophils into the liver (MacKanness, 1962; North, 1970). Many believe that mobilization of neutrophils into sites of infection may be of prime importance in resistance to listeriosis (Czuprynski et al, 1984; Campbell, 1988; Kauffman, 1988; Kratz and Kurlander, 1988), as neutrophils express excellent bactericidal activity against listeria

in vitro (Campbell, 1988; Czuprynski et al, 1984). Others argue that neutrophils simply provide a residence for listeria bacteria but play no part in antimicrobial immunity (North, 1970; Kongshavn and Skamene, 1984). After the administration of a sublethal dose of Listeria monocytogenes, the liver load of the bacteria decreases approximately 10-fold during the first 6 hours (Mitsuyama et al, 1978). This early decrease in liver burden may involve neutrophils but has also been attributed to Kupffer cell-mediated phagocytosis (Mitsuyama et al, 1978; Kongshavn and Skamene, 1984), as the presence of listeria in the liver stimulates the proliferation of resident Kupffer cells (North, 1970). Monocytes have also been shown to populate infective foci in the liver during a primary listeria infection North (1970). Subsequent studies suggested that immigrant monocytes are the major effectors of defense and that the role of resident Kupffer cells is simply to facilitate containment of the bacteria (Kauffman, 1988; North, 1970, Kauffman, 1987).

Independent of the relative contributions of Kupffer cells, neutrophils, and immigrant monocytes, many bacteria are killed early in the infection but some bacteria evade the "attack" and replicate to reach a peak liver load between day 2 and 4 (Mitsuyama et al, 1978). After day 4, the number of viable listeria colonies in the liver steadily decreases until all bacteria have been eliminated by day 10. This second state of resistance is due to the activation of microbicidal macrophages via cytokines released from T lymphocytes (Kongshavn and Skamene, 1984). The establishment of acquired cellular resistance involves both CD4⁺ (L3T4⁺) T cells and CD8⁺ (Ly-2⁺) T cells (Kauffman et al, 1985; Kauffman, 1987; Sasaki et al, 1990). It was shown that T cells able to transfer protection and delayed-type hypersensitivity were sensitive to *in vitro* treatment with an antiserum, either against L3T4 or Ly-2 plus complement. Therefore, as noted by Kauffman (1987), at least two major types of host-parasite interaction likely occur during listeriosis. Listeria is phagocytosed by macrophages and a portion of the bacteria is presented on the surface of

the macrophages in association with class II MHC (major histocompatibility complex) molecules where it is then recognized by CD4⁺ helper T cells. This interaction, along with the release of IL-1 from the antigen-presenting macrophages, results in proliferation of the helper T cells and the release of IFN- γ , and TNF- β which activate other macrophages rendering them more efficient at killing listeria bacteria. Macrophages can also present listeria antigens on their surface in association with class I MHC molecules that are in turn recognized by CD8⁺ cytotoxic T cells. This interaction, in the presence of IL-2 produced from the interaction of macrophages and helper T cells, results in the activation of cytotoxic cells and the lysis of infected cells. In this regard, both CD4⁺ and CD8⁺ T lymphocytes appear to cooperate with macrophages in terminating listeria infection. The release of cytokines from T lymphocytes not only serves to activate macrophages but may also serve to recruit and focus macrophages at infective foci (Campbell, 1988; Mielke et al, 1988; Rosen et al, 1989).

Neutrophils, Kupffer cells, monocytes and T cells all appear to be involved in the immune response that is initiated during listeria infection. Listeria monocytogenes has also been shown to be capable of infection and proliferating in murine embryo fibroblasts (Havell, 1986a) and the human enterocyte-like cell line Caco-2 (Gaillard et al, 1987). The study of the interaction of Listeria monocytogenes bacteria and these various cell types *in vitro*, along with *in vivo* models of murine listeriosis, has revealed that many different cytokines appear to play a significant role in the immune response that is mounted against the bacterium.

Listeria monocytogenes stimulates the expression of IL-1 on the surface of mononuclear phagocytes (Kurt-Jones et al, 1985), and the capacity of peritoneal macrophages to produce IL-1 has been shown to increase through the course of listeria infection (Petit et al, 1988). One of the key roles of IL-1 is to act as a mitogen causing the

proliferation of antigen-specific T lymphocytes which will in turn activate macrophages and increase their listericidal capacity. It may also serve to increase neutrophil accumulation in the infected tissue by increasing their adherence to capillary endothelial cells (Kurlander et al, 1989). The administration of murine recombinant IL-1- α to mice, 48 hours prior to the administration of Listeria monocytogenes, significantly reduced both the peak bacterial burden and the time-lag before the bacteria began to be cleared from the spleens and livers of the mice (Czuprynski et al, 1988). The IL-1-stimulated accumulation of neutrophils in the spleen paralleled an increase in the *in vitro* antilisterial activity of splenic leukocytes and a decrease in the number of bacteria recovered from the spleen *in vivo* (Kurlander et al, 1989). Based on these findings, the authors suggested the possibility of administering IL-1 to immunocompromised patients during opportunistic infections like listeriosis (Czuprynski et al, 1988).

Tumor necrosis factor- α (TNF- α) is produced by macrophages in response to many stimuli including endotoxin (Beulter et al, 1986), whereas TNF- β is derived from T cells and B cells (Whicher and Evans, 1990). TNF production was demonstrated *in vivo* after intravenous injection of a lethal dose of listeria monocytogenes by Havell (1987). This study also demonstrated that the administration of anti-murine recombinant TNF- α immunoglobulin G to sublethally infected mice resulted in death. Nakane et al (1988) confirmed this finding and demonstrated that the lethal effect of the anti-TNF antibody was likely due to an inhibition of the production of activated listericidal macrophages. The administration of human TNF- α to mice for 2 days prior to Listeria monocytogenes infection significantly reduced the number of viable listeria measured in the spleen 24 hours later. This effect was attributed to a TNF-mediated increase in phagocyte antibacterial activity (Kurlander et al, 1989). Anti-TNF antibody was shown to produce death in sublethally infected nude mice (nude mice do not possess mature T cells) and thereby

extended and confirmed the previous studies that suggested a role for TNF in augmenting macrophage activation (Hauser et al, 1990). TNF- α serum concentrations were significantly elevated in a case of neonatal listeriosis and may have played a major role in the lethal septic shock that developed (Girardin et al, 1989). This accumulation of evidence supporting a significant beneficial role yet potentially toxic role for TNF during listeriosis may warrant its monitoring and modulation in the clinical arena during listeriosis.

The most extensively studied cytokine during Listeria monocytogenes infection is interferon. Spleen and peritoneal cells from listeria-infected mice were shown to produce increased amounts of interferon- γ upon *in vitro* challenge with heat-killed Listeria monocytogenes (Buchmeier and Schreiber, 1985). Interferon- γ was not detectable in the serum of mice infected with listeria but monoclonal anti-interferon- γ prolonged the duration of the infection as a result of a lack of generation of activated macrophages. Havell (1986b) was also unable to detect interferon- γ in the sera of mice infected with Listeria monocytogenes but interferon α/β was detectable from day 1 to 5 reaching a peak on day 2. Listeria infected fibroblasts have also been shown to produce interferon α/β (Havell, 1986a). The human neonate is known to be extremely susceptible to infection with Listeria monocytogenes and this may be the result of a decreased ability to produce interferon in response to the infection (Bortolussi et al, 1989).

The listericidal role of interferon remains controversial. An *in vitro* study with bacteria infected peritoneal macrophages demonstrated that interferon- γ prevented the bacteria from escaping the phagolysosome and accessing the cytoplasm, thereby inhibiting cell-to-cell spread of the bacteria (Portnoy et al, 1989). The source of the interferon- γ during listeriosis is assumed by most to be T lymphocytes (Buchmeier and Schreiber, 1985; Nakane et al, 1990). Early studies failed to detect interferon- γ in serum from listeria-infected mice (Buchmeier and Schreiber, 1985; Havell, 1986b). A more recent study did

demonstrate listeria-induced interferon- γ in serum that was shown to reach a peak on day 2 (Nakane et al, 1990). T cell mediated immunity is not generated until after day 2 and therefore it was possible that interferon may also be produced by cells other than T cells. This idea was introduced by Dunn and North (1991a) who demonstrated that the source of interferon- γ released early in the infection are a group of $CD4^- CD8^- Thy-1^+ NK1.1^+$ asialo- GM_1^+ natural killer (NK) cells. Depletion of NK cells resulted in a loss of interferon- γ secreting cells and in exacerbation of infection.

There are many different pathogenic strains of Listeria monocytogenes and there has been extensive investigation into the determination of virulence factors associated with this bacterium. The most frequently studied of these factors is the hemolysin that is secreted during the course of a listeria infection. This protein has a molecular weight of 60,000 daltons and is also referred to as listeriolysin O (Geoffroy et al, 1987). It appears that secretion of this hemolysin is essential for intracellular survival and spread in host tissues and it is now accepted that hemolysin disrupts the phagocytic membrane and allows the escape of the bacterium into a cytoplasm of host cells, as originally suggested by Kingdon and Sword (1970). All clinical isolates are hemolytic and produce a zone of hemolysis on blood agar medium (Geoffroy et al, 1987).

Kathariou et al (1987) constructed a non-hemolytic variant strain of Listeria monocytogenes (M3D) by inserting the tetracycline resistance transposon Tn 916 into the DNA of a virulent hemolytic strain. When tested in a mouse model, this M3D strain appeared to have lost the virulence of the parental strain, as intravenous administration was not followed by multiplication of the bacteria within host cells. The numbers of bacteria isolated from the spleens declined sharply after their introduction and were virtually eliminated within one day. A similar study using a different non-hemolytic strain of Listeria monocytogenes, demonstrated that hemolysin production was necessary for the *in vivo*

induction of T cell mediated immunity (Berche et al, 1987). Studies with avirulent non-hemolytic mutants were consistent with a role for listeriolysin in conferring virulence. However, it was not known if transposon insertion also effected other genes adjacent to the hemolysin gene that also may code for virulence factors. Although all virulent strains exhibit a hemolytic phenotype, no strict correlation between the levels of hemolysin produced *in vitro* and virulence has been found (Kathariou et al, 1988).

FORMULATION OF THE PROBLEM

Different types of infection and modes of immune stimulation have been shown to depress cytochrome P-450-mediated metabolism some of which include viral infections, vaccines, and endotoxins from gram (-) bacteria. The respective mechanisms by which these agents cause this depression are reasonably well understood. The interferon-mediated decrease in cytochrome P450 requires the de novo synthesis of an intermediate protein that, in turn, leads to a loss of cytochrome P450 mRNA; the degradation of cytochrome P450 apoprotein appears not to be involved. It is not known if the effect of interferon is direct on the hepatocyte or involves other cell types. The mechanism by which endotoxin depresses cytochrome P450 also involves a loss of mRNA and there is evidence to suggest that the effect involves the release of interleukin-1 from macrophages. The phagocytosis of latex beads or dextran sulfate by Kupffer cells has been shown to result in a decrease of cytochrome P450 in adjacent hepatocytes. This effect could be demonstrated within one hour and was consistent with a mechanism involving the destruction of cytochrome P450.

To date, we are aware of only five reports regarding the effect of active bacterial infections on cytochrome P450. In none of these reports was the mechanism involved determined. One of the bacteria that was shown to depress cytochrome P450 was Listeria monocytogenes. Listeria is phagocytosed by Kupffer cells and the capacity of macrophages to produce many cytokines including IL-1 is increased through the course of listeria infection. The intracellular killing of listeria within macrophages involves free radical production and the administration of high doses of this bacteria can cause liver damage. Azri and Renton (1987) demonstrated a loss of hepatic microsomal cytochrome P450 content after the administration of a hemolytic form of Listeria monocytogenes to mice. This effect did not correlate with a change in xanthine oxidase or heme oxygenase activity, it

occurred in the absence of a detectable change in serum interferon levels, and was not attenuated by pre-treatment with the free-radical scavenger α -tocopherol. The focus of the present study was to determine the mechanism by which this effect occurred.

OBJECTIVES

The primary objectives of this study were:

- A. To determine if the depressant effect of Listeria monocytogenes on hepatic cytochrome P450 is isozyme specific.
- B. To establish the time course of the effect.
- C. To determine if the mechanism involves an inhibition of synthesis or a degradation of cytochrome P450 apoprotein and to establish which steps are involved.
- D. To determine if the effect is direct on the hepatocyte or involves other hepatic cell types.
- E. To determine the role of hemolysin in the effect.

MATERIALS AND METHODS

Chemicals and Reagents

The following companies supplied the substances used in the experimental portion of this thesis; common laboratory reagents are not included:

Sigma Chemical Company

- albumin (A3782)
- bromophenol blue
- calf thymus DNA
- collagenase
- diethylpyrocarbonate (DEPC)
- dimethylsulfoxide
- erythrosine B
- ethidium bromide
- ethoxyresorufin
- insulin
- lubrol PX
- molecular weight markers (Western blots)
- N,N'-methyl-bis-acrylamide
- N,N,N',N'-tetramethyl ethylenediamine (TEMED)
- NADPH
- β -naphthoflavone
- phenol
- resorufin
- salmon sperm DNA

- Tween 20
- xylene cyanole

Merck Chemical Company

- coomasie blue

Molecular Probes Inc.

- benzyloxyresorufin
- pentoxyresorufin

Aldrich Chemical Company

- aminopyrine

Precision Biologicals

- 5% TSA blood agar plates \pm moxalactam

Eli Lilly Canada Inc.

- moxalactam

Betcon Dickson and Company

- Falcon[®] culture flasks
- Flacon[®] polypropylene tubes

B. and S. H. Thompson and Co. Ltd.

- nylon mesh (for filtering liver cells)

Flow Laboratories Inc.

- DMEM media
- fungizone
- L-15 media
- trypan blue

Gibco Laboratories

- fetal calf serum (Cat. No. 200-6140 AJ)
- gentamicin
- glutamine
- penicillin/streptomycin
- trypsin/EDTA (0.25% trypsin/1mM EDTA)

Calbiochem

- bisbenzamide H33258 (Hoechst - DNA binding fluorochrome)

Bio-Rad Laboratories

- BIO-DOT - slot blot apparatus
- mixed bed resin (AG 501-X8)
- Vacuum Blotter (gel transfer apparatus)

Stovall Life Sciences Inc.

- hybridization water bath

DuPont

- Adenosine 5'-triphosphate (γ -³²P)
- GeneScreen*Plus*

Difco

- brain heart infusion broth (BHI)

Pharmacia

- polynucleotide kinase (T4 kinase)

Bethesda Research Laboratories

- RNA ladder (0.24 - 9.5 kb)

Nalge Company

- Nalgene filters (0.2 and 0.45 μ m)

UVP Inc.

- transilluminator

Animals and Treatment

Male Swiss Balb/c mice (25-35 g) were obtained from Charles River Laboratories, Montreal, Quebec. The mice were maintained (5 per cage) on clay chip bedding and allowed to acclimatize for at least one week. Animals were fed standard Purina Rat Chow and water ad libitum. All *Listeria monocytogenes* suspensions were injected

intraperitoneally (i.p.) in a volume of 0.2 ml PBS at pH = 7.4; control mice received an equal volume of sterile PBS. During the infectious period, mice were maintained in cages covered with protective filter bonnets in a laminar flow hood. In experiments that investigated the effect of listeria infection on cytochrome P450IA1, mice were induced with β -naphthoflavone at a dose of 40 mg/kg as outlined in Table 4. β -naphthoflavone was prepared at a concentration of 12 mg/ml in olive oil and all injection volumes were 0.1 ml (i.p.).

Bacteria

Listeria monocytogenes strain 15U (serotype 4b) is a clinical isolate obtained from Dr. Robert Bortolussi (Bortolussi et al, 1984), Izaak Walton Killam Hospital, Halifax, Nova Scotia. The M3D strain was provided by Dr. Sophia Kathariou (Kathariou et al, 1987), Public Health Service, Centers for Disease Control, Atlanta, Georgia. Aliquots of both strains were stored in brain-heart infusion broth (BHI) containing 20% glycerol at -70°C . For each experiment, an aliquot of listeria was thawed and 50 μL was added to 10 ml of BHI and grown for 18 hours at 37°C . The bacterial suspension was then centrifuged at 3,000 rpm for 15 minutes to pellet the bacteria. The supernatant was discarded and the bacteria were resuspended in sterile phosphate buffered saline (PBS) at pH = 7.4. The bacteria were then washed three additional times after 5 minute centrifugation periods. Bacterial concentrations were determined spectrophotometrically at a wavelength of 620 nm; an absorbance of 0.6 is equivalent to 5×10^8 colony forming units (CFU)/ml. The bacterial suspensions were then adjusted to the desired concentration for *in vitro* experiments or for intraperitoneal administration to mice (injection volumes were 0.2 ml). BHI was prepared by dissolving 13.7 g of Difco BHI in distilled sterile filtered H_2O and

INFECTIOUS PERIOD	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
12	BNF	BNF	BNF	BNF	BNF + LISTERIA AT 11:00 PM	SACRIFICE	-	-
24	BNF	BNF	BNF	BNF	BNF + LISTERIA AT 11:00 AM	SACRIFICE	-	-
48	BNF	BNF	BNF	BNF + LISTERIA AT 11:00 AM	BNF	SACRIFICE	-	-
96	BNF	BNF	BNF	BNF + LISTERIA AT 11:00 AM	BNF	BNF	BNF	SACRIFICE

TABLE 4 - BNF INDUCTION PROTOCOL

diluting to 1 L. This solution was then autoclaved and aliquoted into sterile screw cap polypropylene tubes which were stored at 4°C.

Preparation of Hepatic Microsomes

Mice were killed by cervical dislocation, the peritoneum was soaked with 70% ethanol, livers were removed immediately and rinsed in sterile filtered KCl (1.15%), and the tissue was homogenized in a glass homogenizer. All homogenates were kept on ice until centrifugation at 10,000 x g for 10 minutes at 4°C in a Beckman J2-21 centrifuge. The supernatants were then transferred with a pasteur pipette to ultracentrifuge tubes and centrifuged at 100,000 x g for 40 minutes at 4°C in an IEC/B-60 ultracentrifuge. The microsomal pellets were resuspended (50% suspension by liver weight) and homogenized in glycerol buffer (25% 200 mM KH_2PO_4 , pH = 7.4, 20% glycerol, 0.23% KCl). Microsomes were stored at -70°C.

Protein Determination

Protein concentrations in microsomes, liver homogenates, or hepatocytes were determined by the method of Lowry et al (1951) using albumin as a standard. A standard curve was constructed for each analysis and all determinations were taken from the linear portion of the curve. Solution 1 contained: 49 ml of 2% sodium carbonate in 0.1% NaOH, 0.5 ml of 2% sodium potassium tartrate, and 0.5 ml of 1% copper sulfate. Solution 1 (5 ml) was added to 1 ml of diluted microsomes (microsomes were diluted 1:100 with distilled H_2O), or 1 ml of liver homogenate (diluted 1:500 in 1.15% KCl), or 1ml of diluted hepatocytes (diluted 1:100 in H_2O), and incubated for 10 minutes at room temperature. Phenol (0.5 ml of 1N) was added and the tubes were mixed and incubated at room temperature for 30 minutes; 1N phenol contained 50% of 2N phenol Folin-Ciocalteu

reagent and 50% H₂O. Absorbance determination was carried out using a Beckman DU-70 spectrophotometer at a wavelength of 700 nm.

Cytochrome P450 Determination

Hepatic microsomes were diluted to approximately 1.25 mg/ml by adding 0.5 ml of microsomes to 0.25 ml of 1 M KH₂PO₄ (pH = 7.4) and 1.25 ml of 1.15% KCl. The samples were mixed, transferred to a cuvette, and reduced with a few crystals of sodium dithionite. A baseline spectrum was then established in a Beckman DU-70 spectrophotometer from 400-500 nm. Carbon monoxide was then bubbled through the samples for 30 seconds and a second spectrum obtained. The concentration of cytochrome P450 was determined using an extinction coefficient of 91 mM⁻¹ cm⁻¹ for the 450/490 nm difference and was expressed as nmoles P450/mg protein.

EROD, BROD, PROD Determinations

Substrate activities were determined by a method similar to that described by Burke et al (1985). The conditions used are summarized below. All analyses were done using a Perkin Elmer spectrofluorimeter with an excitation wavelength of 510 nm for assessment of EROD (ethoxyresorufin-O-dealkylase) activity or 530 nm for BROD (benzyloxyresorufin-O-dealkylase) and PROD (pentoxyresorufin-O-dealkylase) activity; an emission wavelength of 586 nm was used for all substrates. Protein concentrations were varied in order to ensure linear reaction rates for at least 5 minutes. A volume of microsomal suspension that represented the desired protein content was diluted to 2 ml with 0.1 M KH₂PO₄(pH = 7.4). Substrate was then added and the mixture was incubated in a water bath at 37°C for two minutes. Stock substrates were prepared in DMSO, working solutions contained 10% DMSO, and the final DMSO concentration in the cuvette was approximately 0.2%. After

establishing a stable baseline, the reactions were initiated by the addition of 125 μM NADPH (10 μl of 25 mM NADPH in 0.1M KH_2PO_4 ; pH = 7.4). The reaction rate was measured directly by the increasing fluorescence of the reaction mixture due to the production of the product resorufin. A resorufin standard (10 μl of 10 μM resorufin) was used each day to calibrate the instrument. Activities were expressed as nmoles resorufin formed / min. / mg protein.

Animal Treatment	Activity	Microsomal Protein (mg)	Substrate Concentration (μM)
Uninduced	EROD	0.25	0.60
Uninduced	BROD	0.25	0.12
Uninduced	PROD	0.25	0.20
BNF-induced	EROD	0.083	0.60
BNF-induced	BROD	0.083	0.12
BNF-induced	PROD	0.045	0.20

For the single experiment that involved the direct incubation of hepatic microsomes with 15U listeria at 37°C for 24 hours, 1 mg of microsomal protein was used for the assessment of EROD, BROD, and PROD activities.

The determination of EROD activity in the cell suspension experiments was carried out as follows: 0.1×10^6 viable hepatocytes were diluted to 3.2 ml with PBS at pH = 7.4. Ethoxyresorufin (40 μl of 31 μM in 10% DMSO) was added to give a final substrate

concentration of 0.4 μM and a final DMSO concentration of 0.1%. This solution was incubated in a circulating water bath at 37°C for 2 minutes and a baseline fluorescence recording was established in a Perkin Elmer fluorimeter at an excitation wavelength of 510 nm and an emission wavelength of 586 nm. The cell solutions were gently mixed by a stirring device that fit onto the top of the glass cuvettes. Once a stable baseline was established, 50 μl of 25 mM NADPH (in PBS at pH = 7.4) was added to initiate the reaction. The reaction was measured directly by the increasing fluorescence of the reaction mixture due to the production of the product resorufin. Activities were expressed as nmoles resorufin formed/per minute/ μg DNA or 10^6 cells or 10^6 viable cells. The same procedure was used for the determination of EROD activity in the adherent cell culture experiments except that 0.2×10^6 viable hepatocytes were used.

BROD activity in the cell suspension experiments was determined as follows:

0.1×10^6 viable hepatocytes were diluted to 3.2 ml with PBS at pH = 7.4. Benzyloxyresorufin (50 μl of 10 μM in 10% DMSO) was added to give a final substrate concentrations of 0.15 μM and a final DMSO concentration of 0.1%. An identical procedure to that used for assessing EROD activity (as noted above) was then followed except that the excitation wavelength was 530 nm. Activities were expressed as nmoles resorufin formed/per minute/ μg DNA or 10^6 cells or 10^6 viable cells. The same procedure was used for the determination of BROD activity in the adherent cell culture experiments.

After induction with β -naphthoflavone (a '3-methylchloanthrene-type'(3-MC) inducer), hepatic microsomal EROD activity increased approximately 10-30 fold. In 3-MC induced rats, 82% of EROD activity was shown to be catalyzed by cytochrome P450IA1 (Kelley et al, 1987). Similarly, pentoxyresorufin is preferentially metabolized by cytochrome P450IIB1/2 after phenobarbital induction (Lubet et al, 1985). BROD activity is induced by both βNF and phenobarbital in rats and it is considered a substrate for both

phenobarbital and β NF-induced isozymes (Burke et al, 1985). In uninduced rats, EROD activity was inhibited by 78% in the presence of an antibody directed against cytochrome P450IA2 and was inhibited by only 6% by an antibody against cytochrome P450IA1 (Kelley et al, 1987). An antibody directed against cytochrome P450IIC11 with cross reactivity to cytochrome P450IIC6, inhibited EROD activity in hepatic microsomes from uninduced rats by 74% (Nakajima et al, 1990). These two studies suggested EROD activity in control rats is predominantly supported by cytochrome P450IA2, IIC11, and possibly IIC6. PROD activity, in control rats, is not inhibited by an antibody directed against cytochrome P450IIB1/2 (Waxman et al, 1987), whereas Chang (1991) showed a 90% inhibition of PROD activity with an antibody against cytochrome P450IIC11, in agreement with the findings of Nakajima et al (1990). All of these reports are from studies carried out in rats and it is not known if the same patterns of isozyme activity holds exactly true in mice. However, by assessing EROD, BROD, and PROD activities in uninduced mice, the status of many different isozymes is likely being assessed.

Microsomal Aminopyrine N-demethylase Activity

Each sample containing microsomes (1.2 mg), aminopyrine (5 mM), and semicarbazide solution (7.5 mM) was incubated in a water bath at 37°C for 2 minutes, after which 50 μ L of NADPH (final concentration of 1mM) was added and this mixture was incubated in a shaking water bath at 37°C for 10 minutes. The reaction was terminated by the addition of 1 ml of 5% ZnSO₄ and 1 ml of 5% Ba(OH)₂ and the samples were placed on ice for 15 minutes. The samples were centrifuged at 2,000 r.p.m. for 10 minutes and 2 ml of the supernatant was transferred to a clean tube and incubated with 1 ml of Nash's reagent (15 g ammonium acetate, 0.2 ml acetylacetone, 0.3 ml glacial acetic acid, diluted to 100 ml) for 20 minutes at 60°C. The samples were then allowed to cool to room

temperature. A blank sample that contained distilled H₂O instead of aminopyrine was carried through the identical procedure and absorbance was determined at a wavelength of 412 nm. The concentration of HCHO produced during the reaction was calculated from a formaldehyde standard curve and microsomal activities were expressed as nmoles HCHO formed /mg/ hour.

Aminopyrine is a substrate which is known to be demethylated by many non-inducible and inducible cytochrome P450 isozymes (Guengerich et al, 1982). In control rats, purified cytochrome P450IIC11 has high activity towards the N-demethylation of aminopyrine (Morgan et al, 1985), and Chang (1991) observed a 35% inhibition of activity in the presence of an antibody directed against cytochrome P450IIC11.

In Vitro Incubation of Microsomes with 15U Listeria

Pooled hepatic microsomes from control mice were divided into eight petri dishes (2.5 ml of microsome per dish). PBS (170µL) was added to the four controls and 1 x 10⁴ CFU of 15U listeria (in 170 µL) was added to the four treated dishes. Samples were then placed in an incubator at 37°C for 12 hours at which point a solution of penicillin and streptomycin was added. After a total of 24 hours, the microsomes were analyzed for protein, P450, EROD, BROD and PROD activities as discussed above.

Determination of Bacterial Liver Burden

After the specified infectious period, livers were homogenized, serial dilutions of the homogenates were prepared in PBS, and 100 µL of each dilution was added to a 5% TSA blood agar plate; bacterial colonies were spread out on the surface of the agar plate with a glass rod. The plates were incubated at 37°C for approximately 24 hours and the number of CFU of listeria were enumerated. In some experiments, TSA plates contained 30 mg/L

moxalactam. To determine bacterial burden in the cell culture experiments, 100 μ L of cell culture media was taken from each flask, immediately before the addition of penicillin and streptomycin, and diluted 1:10,000 with sterile PBS. A 100 μ L aliquot of this solution was added to a 5% TSA blood agar plate.

Histological Examination of Liver Sections

Centrilobular sections of liver were removed from both control and infected mice. The sections were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH = 7.2) for approximately 4 hours. The sections were then post fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate for 1 hour. Sections were dehydrated using graded alcohols and embedded in TAAB embedding resin. Sections (60 nm) were stained with uranyl acetate and lead citrate and were examined using a Phillips 300 electron microscope.

Isolation of Mouse Hepatocytes

Hepatocytes were prepared by the method of Renton (1987). Mice were anesthetized with sodium pentobarbital (approximately 100 mg/kg i.p.) and the peritoneum was soaked with 70% ethanol. A midline abdominal incision was made and the chest was opened, the intestines were displaced to the left and the portal vein was exposed. The inferior vena cava was cannulated via an incision made in the right atrium and the portal vein was cut. The perfusion was started at a flow rate of 10 ml/minute with calcium-free HEPES buffer (see below) and continued for approximately one minute to flush blood from the liver. Following perfusion with the HEPES buffer, the flow was changed to a collagenase solution (see below) at 10 ml/minute for 5 minutes; residual collagenase was flushed from the liver with HEPES buffer for one minute. The liver was excised and placed in a sterile petri dish containing cell culture media in which it was gently raked to release the cells. The

cells were then filtered through sterile filter nets (243 μm) and suspended in a volume of 40 ml of media in a sterile screw cap tube. An aliquot of this cell solution was then combined with an equal volume of 0.08% erythrosine B (in PBS at pH = 7.4) and added to a hemocytometer for determination of cell yield and viability. The average yield was approximately 50×10^6 hepatocytes (along with nonparenchymal cells) in agreement with the yields reported by Klaunig et al (1981) and Maslansky and Williams (1982).

All procedures were carried out under aseptic conditions. Before each experiment, perfusion lines were flushed with 70% ethanol and sterile H_2O . Sterility of the perfusion apparatus was confirmed by plating perfusion effluent on agar plates. All perfusion solutions were either sterile filtered or autoclaved and surgical equipment was soaked in 70% ethanol and briefly flamed.

The following solutions were used for liver perfusions:

Calcium-free HEPES Buffer:

- NaCl 41.5 g
- KCl 2.5 g
- HEPES 12.0 g

Adjust to pH = 7.4 and dilute to 500 ml. On the day of the experiment this was diluted 1:10, fungizone (0.25 $\mu\text{g}/\text{ml}$) and moxalactam (30 mg/L) were added and the solution was then re-adjusted to pH 7.4 and sterile filtered. Moxalactam was prepared by dissolving 130 mg in 10 ml of 0.04 N HCl for 1.5 hours then diluting to 100 ml with 0.1 M KH_2PO_4 at pH = 7.4 This solution was then sterile filtered and stored in aliquots at -70°C for a maximum of 2 months.

Collagenase Solution:

- 25 mg of Type IV collagenase in 50 ml of HEPES buffer, sterile filtered

L-15 Media (used for suspension cultures at 25°C)

- L-15 medium 13.91 g
- Insulin..... 8 mg
- Albumin..... 0.5 g
- Glucose..... 1.5 g
- Fetal Calf Serum..... 100 ml

Adjust pH to 7.4, dilute to 1L and sterile filter. On the day of the experiment fungizone (0.25 µg/ml) and moxalactam (30 mg/L) were added.

Cell Suspension Experiments

Liver cells (hepatocytes and nonparenchymal cells) were suspended in L-15 media and counted in a hemocytometer. For the experiments summarized in Figure 12, cells were divided into two flasks with each flask containing 18×10^6 viable hepatocytes (along with nonparenchymal cells) in a volume of 50 ml of L-15 media (0.5×10^6 viable hepatocytes/ml). 15U listeria (5×10^6 CFU) was added to the treated flask at zero time and an equal volume of PBS was added to the control flask. The cells were maintained in suspension on a rotating mixer at 25°C for 12 hours at which time a combination of penicillin (100 U/ml) and streptomycin (100 µg/ml) was added to eliminate extracellular bacteria, thereby preventing a significant change in media pH which results if the bacteria are allowed to multiply uninhibited for greater than 12 hours. After a total of 24 hours, the hepatocytes from each flask were separated from nonparenchymal cells by centrifuging the cell mixture at 50 x g for 2 minutes to pellet the hepatocytes and the supernatant fraction was discarded. The hepatocytes were resuspended in PBS and centrifugation was repeated two additional times. For the determination of cytochrome P450 content, 125 µl of 4% Lubrol PX (Sigma) was added to 0.9 ml of the hepatocyte solution and this mixture was

mixed for 10 seconds and centrifuged at $50 \times g \times 1$ minute. The supernatant was transferred to a cuvette and cytochrome P450 content was determined as described for the determination of microsomal cytochrome P450. Lubrol (4%) was prepared by diluting 4 g of lubrol PX to 100 ml with 0.1M KH_2PO_4 (pH = 7.4). The data was expressed as nmoles P450/ 10^6 cells with each treated value expressed as a percent of the corresponding control of that particular experiment, with each experiment giving an n of 1.

For the experiments summarized in Figures 13, liver cells (hepatocytes and nonparenchymal cells) were resuspended in L-15 media and counted in a hemocytometer. The cells were then split into two flasks with each flask containing 8×10^6 viable hepatocytes along with nonparenchymal cells in a volume of 40 ml (0.2×10^6 viable hepatocytes/ml). 15U listeria (5×10^6 CFU) was added to the treated flask at zero time and an equal volume of PBS was added to the control flask. The cells were maintained in suspension on a rotating mixer at 25°C for 12 hours at which point a combination of penicillin (100 U/ml) and streptomycin (100 $\mu\text{g}/\text{ml}$) were added. After a total of 24 hours, the hepatocytes from each flask were separated from nonparenchymal cells by centrifuging the cell mixture three times at $50 \times g \times 1$ minute. The final hepatocyte pellet was resuspended in 4.5 ml PBS and analyzed for cell count, viability, total cellular protein, DNA content, P450 content, EROD and BROD activities. Four separate experiments were carried out.

Cellular DNA content was determined by the method of Lubarca and Paigen (1980). A solution of 1 x TNE (10 x TNE contains 100mM Tris base, 10 mM EDTA, and 1.0 M NaCl at a pH of 7.4) was sterile filtered through a $0.45 \mu\text{m}$ filter and to 50 ml of this solution, 5 μL of 1 mg/ml H33258 (in sterile water) fluorochrome was added to give a final fluorochrome concentration of 0.1 $\mu\text{g}/\text{ml}$. A calf thymus DNA standard was prepared at a concentration of 10 $\mu\text{g}/\text{ml}$ in 1 x TNE. A 1 ml aliquot of the hepatocyte solution was

homogenized for 30 seconds in a Polytron homogenizer. Under minimum light conditions, 3.2 ml of DNA assay solution was added to a glass cuvette and a baseline was obtained in a Perkin Elmer fluorimeter at an excitation wavelength of 365 nm and an emission wavelength of 456 nm. The solution in the cuvette was gently mixed with a stirring apparatus that fit onto the top of the cuvette. After a stable baseline was obtained, 16 μ l of homogenized cell sample was added and the relative increase in fluorescence was measured; all samples were run in duplicate. This procedure was repeated for the calf thymus DNA standard. Different volumes of sample were measured ranging from 6 μ l to 64 μ l in order to ensure linearity of the assay. The H33258 dye is specific for DNA (it does not measure RNA) and works equally well with whole-cell homogenates or purified preparations of DNA (Labarca and Paigen, 1980). The same procedure was used for the determination of DNA content in the adherent cell culture experiments except that 0.5 ml aliquots were homogenized.

Adherent Cell Culture Experiments

The experimental protocol is outlined in Figure 1. Freshly isolated liver cells (hepatocytes and nonparenchymal cells) were suspended in DMEM media and counted in a hemocytometer; 5×10^6 viable hepatocytes along with nonparenchymal cells were then placed in a two flasks in a total volume of 20 ml. The remaining solution of liver cells was centrifuged four times at $50 \times g \times 1$ minute to obtain hepatocytes at a purity greater than 90%. 5×10^6 viable 'pure' hepatocytes (purity > 90%) were then placed in two flasks in a volume of 20 ml. This procedure yielded a total of four flasks; two of which contained hepatocytes and nonparenchymal cells and two contained 'pure' hepatocytes. All cells were placed in a humidified Forma Scientific incubator at 37°C and 5% CO₂. After approximately 3 hours, the flasks were removed, non-adherent cells were discarded, and 20 ml of fresh DMEM media was added. Treated flasks were infected with 1×10^4 CFU of

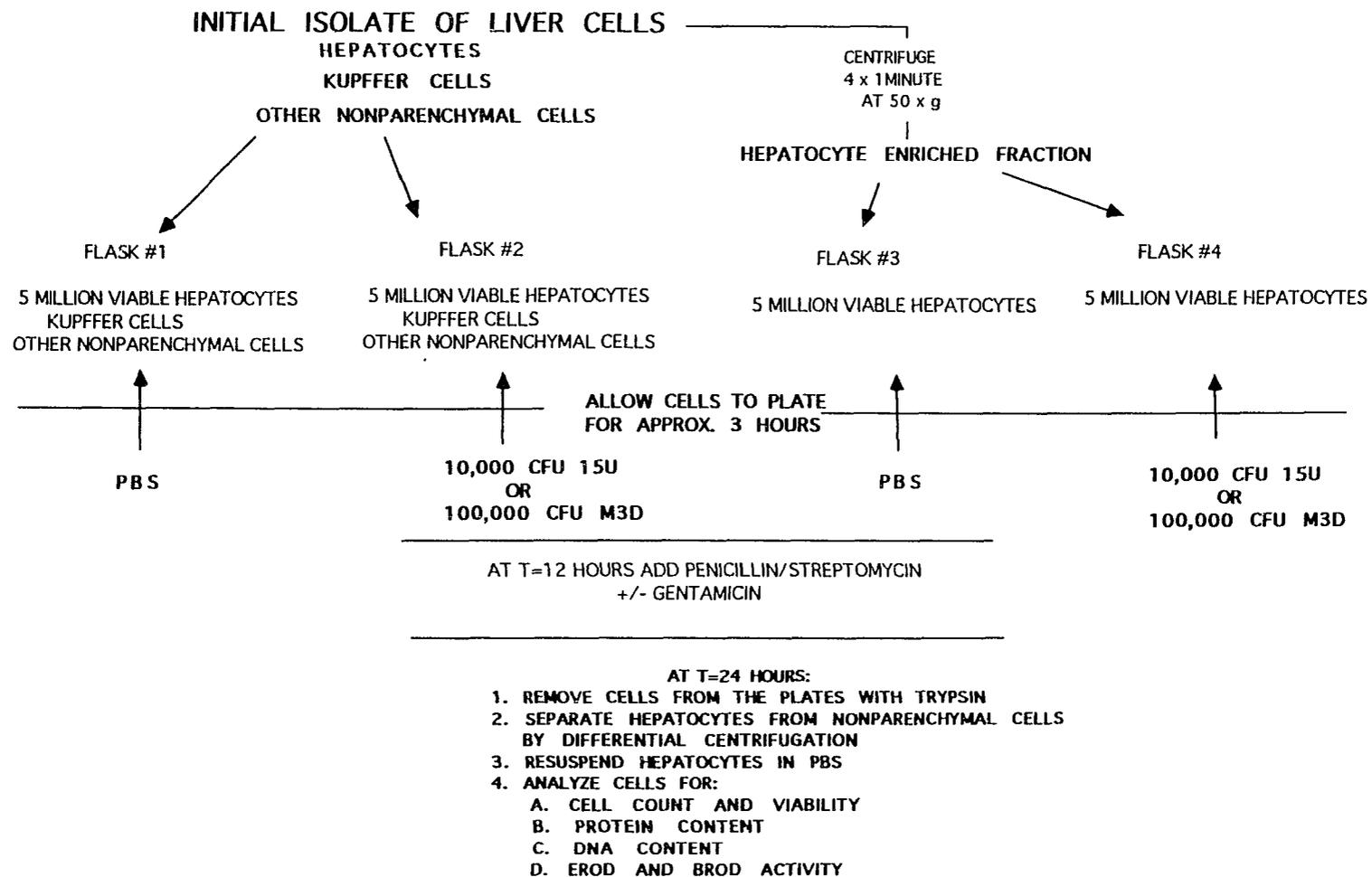


FIGURE 1 METHOD FOR PREPARATION OF ADHERENT LIVER CELL CULTURES

15U listeria or 1×10^5 CFU of M3D listeria and control flasks received an equal volume of sterile PBS. The reason for using the higher initial dose of the M3D strain was that it grows at a slower rate than the 15U strain in DMEM media at 37°C. After a 12 hour incubation period, a combination of penicillin (200 U/ml) and streptomycin (200 µg/ml) were added; gentamicin (200 µg/ml) was also added to M3D containing flasks (and the corresponding control flasks) since this non-hemolytic mutant is streptomycin resistant. The incubations were terminated 24 hours after the addition of the listeria. Cells were washed with 20 ml of PBS and then removed from the surface of the flasks with a solution of 0.125% trypsin and 0.5 mM EDTA. The trypsinized cells were then added to 20 ml of DMEM media containing 10% fetal calf serum and spun at 50 x g x 2 minutes. The supernatant was discarded and the hepatocyte pellet was resuspended in 4 ml of PBS. The cells were centrifuged twice more at 50 x g x 1 minute and the final hepatocyte pellet was resuspended in 2 ml of PBS.

DMEM Media

DMEM.....	13.44 g
NaHCO ₃	3.7 g
Glutamine	2 mM
Fetal Calf Serum.....	100 ml
Albumin.....	0.5 g
Insulin.....	8 mg

- adjusted to pH = 7.4; dilute to 1 L; sterile filter
- on the day of the experiment, fungizone (0.25 µg/ml) was added in addition to moxalactam at 30 mg/L. Moxalactam was omitted from the media for the M3D experiments as it proved to be inhibitory towards this strain.

Hepatic RNA Isolation

Immediately after each mouse was killed, approximately 2/3 of one liver lobe was rinsed in KCl and then frozen in liquid nitrogen; the remainder of the liver was used to prepare microsomes. Once frozen, the liver section was broken into small pieces using a mortar and pestle, placed in cryovials, and stored at -70°C . Total hepatic RNA was isolated by the method of Chomczynski and Sacchi (1987) with the following modifications.

- i) Approximately 0.1 g of liver was extracted
- ii) Samples were centrifuged at room temperature
- iii) RNA pellets were washed three times with 75% ethanol
- iv) RNA pellets were air dried not vacuum dried
- v) The final dried RNA pellet was resuspended in 0.5 ml DEPC-treated water

The final RNA pellets were dissolved in 0.5 ml DEPC H_2O and stored at -20°C .

For RNA quantitation, 20 μL of RNA was added to 0.98 ml of H_2O , mixed, and transferred to a quartz cuvette. The RNA concentration was determined by the absorbance at 260 nm assuming that an optical density of 1, in a 1 cm pathlength cell, corresponds to 40 μg RNA/ml (Maniatis et al, 1982). Gloves were worn at all times to prevent RNA destruction from accidental contamination with skin ribonucleases.

The following is a list of solutions used for the RNA extraction procedure:

Diethylpyrocarbonate treated water (DEPC H_2O)

- | | |
|-------|--------------------------------|
| 1 ml | diethylpyrocarbonate |
| 1.5 L | Millipore H_2O |
- allow DEPC to dissolve, autoclave

10% sarcosyl

10 g N-Lauroyl sarcosine
 100 ml H₂O

Stock extraction buffer

50 g guanidine thiocyanate
 58.6 ml DEPC H₂O
 3.5 ml 0.75 M sodium citrate (pH = 7)
 5.3 ml 10% sarcosyl

Heat at 60°C until dissolved, store in a brown bottle covered in foil

Solution D

20 ml stock extraction buffer
 144 µL 2-mercapto-ethanol

Chloroform: isoamyl alcohol

4.9 ml chloroform
 0.1 ml isoamyl alcohol

RNA Gel Electrophoresis

The hepatic RNA was separated in a 1.33% agarose gel containing 2.2 M formaldehyde. The gel was prepared by melting 2 g of agarose in 108 ml of DEPC H₂O and boiling for approximately 3 minutes. This solution was allowed to cool to 65°C and 15 ml of 10 x buffer A (see below), 27 ml formaldehyde, 7.5 µL of ethidium bromide (10 mg/ml) were added. The mixture was allowed to cool to 55°C and then poured onto a gel tray (25 cm x 15 cm) and allowed to solidify for 1 hour, and submerged in 1 x buffer A in the electrophoresis chamber.

The RNA samples (15 or 20 μg) were prepared for gel electrophoresis by lyophilization and reconstitution in 4.5 μL DEPC H_2O . Solution B (15.5 μL) was added, the samples were mixed, incubated at 60°C for 10 minutes, and placed on ice. Loading buffer (5 μL) was added, the samples were mixed and briefly centrifuged. Samples were added to the wells of the gel and the RNA was separated at 38 V for approximately 21 hours (800 volt hours). Gels were photographed on an ultraviolet transilluminator.

The following is a list of solutions for the RNA gel electrophoresis procedure:

10 x Buffer A (prepared in distilled water at pH = 7)

- 0.4 M 3(N-morpholino) propane - sulfonic acid (MOPS)
- 199 mM sodium acetate
- 20 mM EDTA

This solution was sterile filtered through a 0.45 μm filter

Solution B:

- 40 μL of 10 x buffer A
- 50 μL of formaldehyde
- 200 μL of formamide (this was deionized with mixed bed resin AG 501-X8)

Loading Buffer

- 0.5 ml of glycerol
- 0.2 ml of 0.4% bromophenol blue, 0.4% xylene cyanol
- 4 μL of 0.25 M EDTA (pH = 7)
- 0.296 ml DEPC H_2O

Transfer of RNA from the Agarose Gel to GenescreenPlus Membranes

The agarose gels were washed five times with distilled deionized H₂O, 50 mM NaOH for 30 minutes, 100 mM Tris-HCl for 30 minutes, and finally washed five additional times with distilled deionized H₂O. The gels were transferred in a BIO-RAD Vacuum Blotter in a solution of 10 x SSC (1.5 M NaCl, 0.15 M Na citrate) for 3 hours at a vacuum pressure of 5 mm Hg. After transfer, the GenescreenPlus membrane was soaked in 2 x SSC for 15 minutes, air dried for approximately 1 hour and then baked at 80°C for 2 hours. Northern blots were stored desiccated at 4°C. Each gel was restained with ethidium bromide (0.5 µg/ml in water) for 30 minutes and visualized on an ultraviolet transilluminator to ensure that the transfer was complete.

Slot Blot Analysis of RNA

For βNF-induced mice, 15 µg of RNA was diluted to 340 µL with DEPC H₂O, followed by the addition of 160 µL formaldehyde, and 500 µL of deionized formamide. Samples were heated at 60°C for 15 minutes then cooled on ice. A range of concentrations of each RNA sample were prepared by two-fold serial dilution with DEPC H₂O. Samples were added to the GenescreenPlus membrane via a BIO-RAD BIO-DOT™ slot blot apparatus under gentle vacuum. The final slot blot loads (0.2 ml/slot) were: 3.0, 1.5, 0.75, 0.188, 0.093, 0.047, 0.023 and 0.012 µg. Each set of dilution tubes were used to prepare duplicate blots. One blot of each pair was hybridized with the cytochrome P450IA probe and the second was hybridized with the oligo(dT)₁₈ probe.

For uninduced mice used for the assessment of cytochrome P450IIC11 mRNA levels, 100 µg of RNA was diluted to 340 µL with DEPC H₂O, followed by the addition of 160 µL formaldehyde, and 500 µL of deionized formamide; the same procedure used for βNF-induced mice was then followed. The final slot blot loads (0.2 ml/slot) were: 20, 10, 5,

2.5, 1.25, 0.625, 0.31 and 0.16 µg of RNA. Each set of dilution tubes were used to prepare duplicate blots. One blot of each pair was hybridized with the cytochrome P450IIC11 probe and the second was hybridized with the oligo(dT)₁₈ probe. All slot blots were air dried for approximately one hour and then baked at 80°C for two hours. Slot blots were stored desiccated at 4°C.

Hybridization of Northern Blots

Northern blots were hydrated in 2 x SSPE for 5 minutes and prehybridized in 12.5 ml of prehybridization solution (in a heat-sealing bag) for 4 hours at 42°C in a hybridization water bath. After the prehybridization period, this solution was replaced with 12.5 ml of hybridization solution which contained: 6 x SSPE, 1% SDS, 0.1% Ficoll, 0.1% bovine serum albumin, 0.1% polyvinyl pyrrolidone, and 5 µL of ³²P-labelled cDNA probe. Northern blots carried out with the cytochrome P450IA cDNA probe were hybridized at 48°C for approximately 18 hours and were washed in 2 x SSC at room temperature for 10 minutes followed by four 10 minute washes at 64°C in 2 x SSC (10 x SSC contains 1.5 M NaCl and 0.15 M Na citrate). Northern blots carried out with the cytochrome P450IIC11 cDNA probe were hybridized at 60°C for 3 hours and were washed at room temperature for 10 minutes followed by four 10 minute washes at 60°C in 2 x SSC.

The prehybridization solution contained the following:

- 6 x SSPE (20 x SSPE contains 3 M NaCl, 0.2 M Na H₂PO₄ and 0.02 M EDTA at pH = 7.4)
- 1% SDS
- 0.1% Ficoll

- 0.1% Bovine serum albumin
- 0.1 mg/ml Sheared salmon sperm DNA
- 0.1% Polyvinyl pyrrolidone

Hybridization of Slot Blots

Slot blots were prehybridized for 4 hours at 42°C by the same procedure outlined for the Northern blots except that the volume of the prehybridization solution was 4.5 ml. After the prehybridization period, the solution was replaced with 4.5 ml of hybridization solution along with 5 µl of ³²P-labelled cDNA probe. Slot blots were hybridized and washed by the same procedure outlined for the Northern blots.

The effect of listeria infection on total mRNA levels was determined using the second blot of each pair and an oligo(dT)₁₈ probe by a modification of the method of Harley (1987). Slot blots were prehybridized at 42°C for 1 hour in 4.5 ml of a solution containing: 6 x SSPE, 1% SDS, 0.1% Ficoll, 0.1% bovine serum albumin, and 0.1 mg/ml sheared salmon sperm DNA. After the prehybridization period, this solution was replaced with 6 ml of a solution containing: 5 x SSC, 0.1% Ficoll, 0.1% bovine serum albumin, 0.1% polyvinyl pyrrolidone, 8.6 mg Na₂HPO₄, and 30 µL of ³²P-labelled oligo(dT)₁₈ probe. The slot blots were hybridized at room temperature for 1 hour and were then washed 4 x 5 minutes with 2 x SSC at room temperature.

Nucleotide Sequences of the Hybridization Probes

The cytochrome P450IA cDNA probe is a complement to nucleotides 1259 - 1281 of the murine cytochrome P450IA1 mRNA sequence (Kimura et al, 1984). This sequence was compared to other gene sequences in GenBank (included with Beckman's Microgenie™) using the MicroGenie Sequence Analysis Program (Queen and Korn,

1984). It was determined that this probe sequence is also complimentary to the mouse cytochrome P₃450 sequence and is complimentary to all but one nucleotide of the mouse P₂450 sequence (cytochrome P450IA2). No other matches with greater than 65% homology were detected. The cytochrome P450IA cDNA sequence was synthesized at the Regional DNA Synthesis Laboratory at The University of Calgary. Its sequence was:

5' - d(GTGCTGTGGGGGATGGTGAAGGG) - 3'.

The cytochrome P450IIC11 cDNA probe is a compliment to nucleotides 1429 - 1461 of the murine cytochrome P450IIC11 mRNA sequence (Yoshioka et al, 1990). The cytochrome P450IIC11 cDNA sequence was synthesized at The Marine Gene Probe Laboratory of Dalhousie University. Its sequence was:

5' - d (CGGAAAAGGAAAGACACCAAGGTTTCTAGGCCT) - 3'

No matches to other known sequences with greater than 65% homology were detected; one region of the mouse cytochrome P450IIC12 sequence (female-specific) was 60% homologous.

5'-End-Labeling of cDNA Probes

The 5' - OH ends of the cytochrome P450IA and P450IIC11 oligomers were labelled with ³²P by a modification of the method of Maxam and Gilbert (1980) as follows: 5 μL of 0.05 M dithiothreitol in 0.01 M Tris HCl/1 mM EDTA, 5 μL of 0.01 M MgCl₂/0.5 M Tris HCL, 2 μL of 25 mM spermidine in 0.01 M Tris/HCL/1 mM EDTA, 20 units of T4 polynucleotide kinase, 60 pmoles of [γ-³²P] ATP, and 48 pmoles of oligomer were combined in a volume of 50 μL. The labelling reaction was carried out at 37°C for approximately 18 hours and terminated by the addition of 4.5 μL of 0.25 M EDTA, mixing and boiling for 5 minutes; probes were stored at -70°C. The cytochrome

P450IIC11 cDNA probe was labelled by the same procedure as the cytochrome P450IA1 probe except that 60 pmoles of oligomer was added to the reaction mixture.

The oligo(dT)₁₈ probe was labelled by the method of Harley (1987) as follows: 12 µL of 10 x T4 buffer (0.5 M Tris HCl (pH = 8), 0.1 M MgCl₂, 50 mM dithiothreitol, final pH = 8), 20 units of T4 polynucleotide kinase, 18 pmoles of [γ -³²P] ATP, 60 pmoles of oligo(dT)₁₈ probe, and 99 µL sterile H₂O were combined and the labelling reaction was carried out at 37°C for 1 hour, terminated by the addition of 8 µL of 0.25 M EDTA and 72 µL of sterile H₂O, and mixing for 20 seconds.

Autoradiography

After completion of the washing procedure, all blots were placed on filter paper and covered in plastic wrap. The blots were exposed to X-ray film (Kodak XAR-5) in X-ray cassettes containing intensifying screens (Cronex Lightning Plus). Blots hybridized with the cytochrome P450IA and P450IIC11 probes were exposed for an appropriate length of time at -70°C (approximately 1-4 days). Autoradiography of oligo(dT)₁₈ blots was carried out at room temperature.

Densitometry

The intensities of the bands on the slot blot autoradiograms were determined using a video densitometer (Bio-Rad Model 620) and the 1-D Analyst™ software program which measures peak height in optical density units. Duplicate slot blots were prepared for each experiment. One blot of each pair was hybridized with the cytochrome P450IA or IIC11 cDNA probe and the second of each pair was used to ensure equal total mRNA loading by hybridizing it with an oligo(dT)₁₈ probe which binds to all eukaryotic mRNA. Each slot blot row contained eight dilutions of RNA and autoradiographic exposure was adjusted to

ensure that at least three of the dilutions were on the linear portion of the densitometric scan. The optical density of a single band within this linear region was expressed as a ratio of the optical density of the RNA standard of that blot (the standard was hepatic RNA from a β NF-induced control mouse that was loaded onto all blots). The same procedure was repeated for the second blot of the pair hybridized with the oligo(dT)₁₈ probe. The cytochrome P450 mRNA content was then corrected for total mRNA loading and this final ratio was used for statistical analysis.

Western Blot Analysis of Microsomal Protein

Hepatic microsomes were prepared from β NF-induced mice after 12, 24, 48 and 96 hours of infection with *Listeria monocytogenes*. The microsomal proteins were separated by electrophoresis and the resulting Western blot was probed with an antibody directed against cytochrome P450IA1 apoprotein. Preliminary studies demonstrated that this rabbit anti-rat cytochrome P450IA1 antibody will also bind to murine cytochrome P450IA1. Virtually no binding was evident in lanes containing microsomal protein from uninduced mice but intense bands were present in lanes containing microsomes from β NF-induced mice. The apparent molecular weight of the target protein was between 45 and 58.5 kd, consistent with the molecular weight of murine cytochrome P450IA1 apoprotein (55kd). Murine cytochromes P₁450 and P₃450 (P450IA2) also have the same molecular weight and are also induced by 3-MC type inducers (Gonzalez et al, 1984; Negishi and Nebert, 1979; Kimura et al, 1986; Ikeda et al, 1983), and it is therefore possible that the antibody will cross-react to some extent with these apoproteins.

Microsomal samples were prepared by adding an equal volume of sample and Laemmli buffer in an eppendorf tube and boiling for 3 minutes. Microsomal protein (20-25 μ g) was added to each well in a volume of 10 μ L. Proteins were separated using

stacking minigels (1.25 mm). The 7.5% running gels were prepared by combining: 3.75 ml of stock acrylamide, 3.75 ml of 4 x running gel buffer, 75 μ L of ammonium persulfate, 7.5 ml distilled H₂O, and 15 μ L TEMED (N,N,N',N'-tetramethyl ethylenediamine). The running gel was layered into the gel holders with a Pasteur pipette and allowed to set for approximately 30 minutes. A 3% stacking gel was prepared by combining 0.5 ml of acrylamide stock, 2 ml of distilled H₂O, 2.5 ml of 2 x stacking buffer, 50 μ L ammonium persulfate, and 10 μ L TEMED. The stacking gel was then added to the gel holder and allowed to set for 30 minutes.

After the addition of samples and molecular weight markers to the wells, protein stacking was carried out at 75 volts in 1 x electrode buffer for approximately 20 minutes; proteins were then separated in the running gel at 125 volts. Proteins were transferred from the gel to nitrocellulose membranes using a semi-dry electrophoretic transfer system (Tyler Research Instruments), at 25 volts for 15 minutes in 1 x transfer buffer (25 mM Tris base, 150 mM glycine, 20% methanol, 0.05% SDS). Transfer efficiency was determined by staining the gel with coomassie blue. The resulting Western blot analysis was then carried out using a rabbit anti-rat cytochrome P450IA1 primary antibody (Oxygene) and an anti-rabbit IgG secondary antibody (Sigma) linked to alkaline phosphatase for detection.

The Western blots were blocked with 50 ml of blotto buffer at room temperature for one hour then washed 3 x 10 minutes with 0.05% Tween 20 in PBS. The membrane was then incubated with the primary antibody overnight at 4°C, and again washed 3 x 10 minutes with 0.05% Tween 20 in PBS. The secondary antibody was then added and incubated with the membrane for one hour at room temperature, followed by three ten minute washes. The membrane was equilibrated with alkaline phosphatase assay buffer for 2 - 3 minutes which was then replaced by alkaline phosphatase mix until bands were adequately visible. The reaction was terminated with alkaline phosphatase stop buffer.

The following is a list of solutions that were used for the Western Blots:

Stock Acrylamide

- acrylamide 29 g
- N,N'-methyl-bis-acrylamide 1 g
- distilled H₂O 70 ml

Ammonium Persulfate

- 100 mg/ml in water, prepared fresh on the day of the experiment

4 x Running Gel Buffer

- Tris base 16.95 g
- distilled H₂O 90 ml
- adjust pH to 8.8
- add 4 ml of 10% SDS
- dilute to 100 ml (stored at 4°C)

2 x Stacking Gel Buffer

- Tris base 3 g
- distilled H₂O 75 ml
- adjust pH to 6.8
- add 2 ml of 10% SDS
- dilute to 100 ml (store at 4°C)

10 x Electrode Buffer

- Tris base 7.56 g
- glycine 36 g
- distilled H₂O 150 ml
- adjust pH to 8.3
- add 25 ml of 10% SDS
- dilute to 250 ml (stored at 4°C)

1 x Transfer Buffer

- Tris base 3.03 g
- glycine 11.25 g
- distilled H₂O 700 ml
- add 200 ml methanol
- dilute to 1L
- just before use, add 10% SDS to give a final concentration of 0.05% SDS

Coomassie Blue Stain

- coomassie blue (0.2%) 1 g
- methanol (25%) 125 ml
- acetic acid (10%) 50 ml
- water (65%) 325 ml

Destaining Solution

- methanol 10%
- acetic acid 10%
- water 80%

Laemmli Buffer

- Tris HCl 489 mg
- distilled H₂O 10 ml
- adjust pH to 6.8
- glycerol 5 ml
- bromophenol blue 0.5 mg
- 10% SDS 5 ml
- dilute to 25 ml
- aliquots were stored at -20°C

Blotto Buffer

- 5% (w/v) carnation milk (low fat) and 2% BSA in PBS

Alkaline Phosphatase Assay Buffer

- 100 mM Tris HCl
- 100 mM NaCl
- 50 mM MgCl₂
- adjust to pH = 9

Alkaline Phosphatase Stop Buffer

- 20 mM Tris HCl
- 5 mM EDTA
- adjust to pH = 8

Nitroblue Tetrazolium (NBT)

- 50 mg of NBT in 1 ml of 70% dimethylformamide (stored at 4°C)

5-bromo-4-chloro-3-indolyl phosphate (BCIP)

- 25 mg of BCIP in 500 μ L of 100% dimethylformamide

Final Alkaline Phosphatase Mix

- 10 ml alkaline phosphatase assay buffer
- 66 μ L NBT
- 33 μ L BCIP

STATISTICAL METHODS

The unpaired Student's t test was used for statistical comparison between the treated group and corresponding control group for all *in vivo* experiments except for the statistical comparison of the data of Figure 6 for which Dunnett's test was used, and Figure 7 in which a paired-sample t test was used. The statistical comparison of differences in hepatocyte viability was carried out using a paired-sample t test of the arcsine transformed data. A one-sample t test was used for statistical comparison in all *in vitro* cell culture experiments. A p value less than 0.05 was considered significant; * designates $p < 0.05$, ** designates $p < 0.01$. The error bars on all graphs are the standard error of the mean.

RESULTS

TIME COURSE OF CYTOCHROME P450 LOSS DURING 15U LISTERIA INFECTION AND COMPARISON WITH INFECTION WITH THE M3D STRAIN

The time course for the loss of hepatic microsomal cytochrome P450 during a bacterial infection was examined in male Swiss Balb/c mice infected with 1×10^6 colony forming units (CFU) of a hemolytic and virulent strain of *Listeria monocytogenes* (15U) (Figure 2). A significant reduction in cytochrome P450 was observed after 48 hours of infection and this suppression was maintained for at least 3 days. An avirulent non-hemolytic strain of *Listeria* (M3D) had no effect on microsomal cytochrome P450 content after 48 hours of infection as shown in Figure 3. Further analysis of liver microsomes isolated from mice after 48 hours of infection with 15U *Listeria* indicated that not only was total cytochrome P450 content reduced but the capacity of this enzyme system to metabolize 4 test substrates, ethoxyresorufin, benzyloxyresorufin, pentoxyresorufin, and aminopyrine was also significantly reduced to approximately 40-50% of control values (Figure 4). The M3D strain had no effect on EROD, BROD, or PROD activities in hepatic microsomes (Figure 5).

The same dose of the two strains of *Listeria* was administered in each of these experiments; however, the liver loads of the two strains of the bacteria were significantly different at the time of sacrifice (Figure 3). After 48 hours of infection with the 15U strain, the average number of bacteria per liver was 1.0×10^5 CFU whereas no detectable colonies of the M3D strain were observed after the same infectious period.

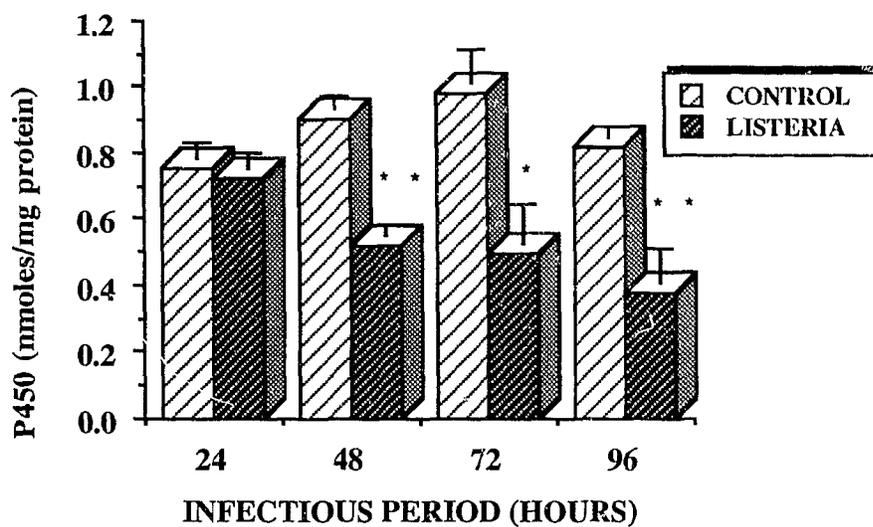
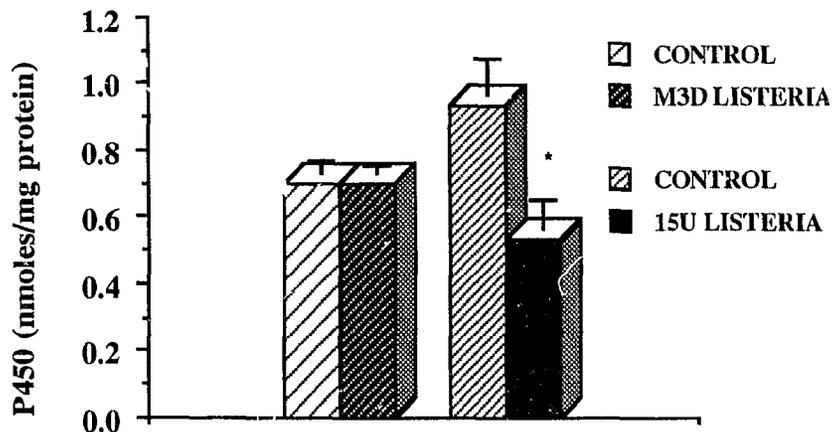


FIGURE 2

TIME COURSE OF THE EFFECT OF *IN VIVO* INFECTION WITH 1×10^6 CFU OF 15U LISTERIA

Mice were infected with 1×10^6 CFU of 15U listeria and sacrificed after 24-96 hours; control mice received an equal volume of sterile PBS. Cytochrome P450 levels were determined in hepatic microsomes and expressed per mg of microsomal protein. Statistical analysis was carried out using an unpaired Student's *t* test (n=4).



LIVER LOAD 15-U = 1.8×10^5 CFU

LIVER LOAD M3D = 0 CFU

FIGURE 3

EFFECT OF 48 HOURS OF *IN VIVO* INFECTION WITH 1×10^6 CFU OF M3D AND 15U LISTERIA

Mice were infected with 1×10^6 CFU of either 15U (n=4) or M3D (n=5) listeria and sacrificed after 48 hours of infection; control mice received an equal volume of sterile PBS. Cytochrome P450 levels were determined in hepatic microsomes and the liver loads of the bacteria from blood agar plates containing aliquots of the liver homogenates. Statistical analysis was carried out using an unpaired Student's *t* test.

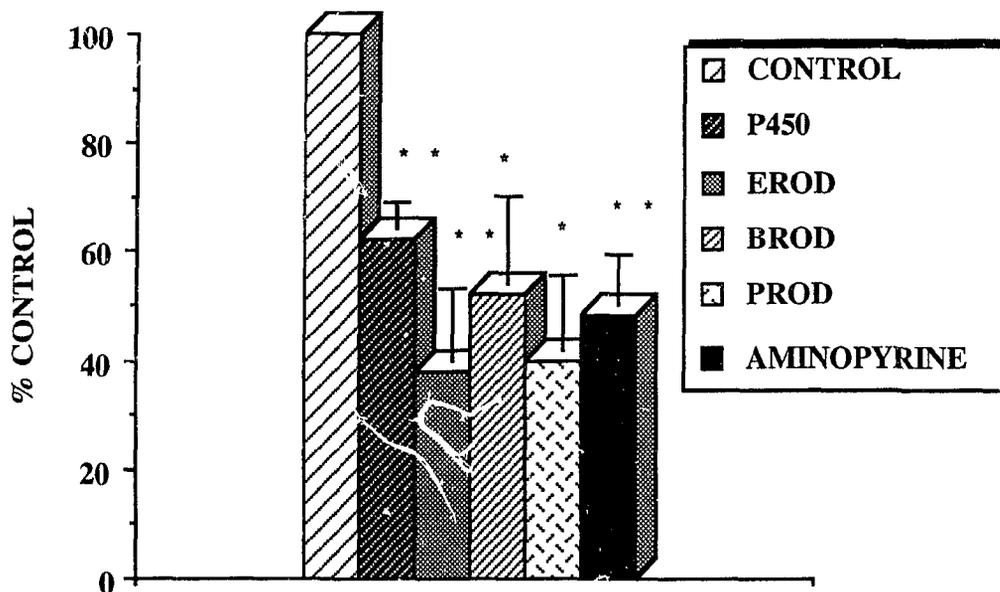


FIGURE 4

EFFECT OF 48 HOURS OF *IN VIVO* INFECTION WITH 1×10^6 CFU OF 15U LISTERIA ON SUBSTRATE METABOLISM CAPACITY

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours. Hepatic microsomal cytochrome P450 content, EROD, BROD, PROD, and aminopyrine N-demethylase activities were determined and expressed as a % of the mean control levels determined in mice treated for the same period with an equal volume of sterile PBS. The mean control levels were: cytochrome P450 = 0.70 ± 0.02 nmoles /mg protein; EROD = 27.8 ± 0.3 pmoles resorufin/mg /min; BROD = 4.6 ± 0.4 pmoles resorufin/mg/min; PROD = 1.7 ± 0.2 pmoles resorufin/mg /min and aminopyrine N-demethylase = 253 ± 18.7 μ moles HCHO/mg/hour. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test (n=5).

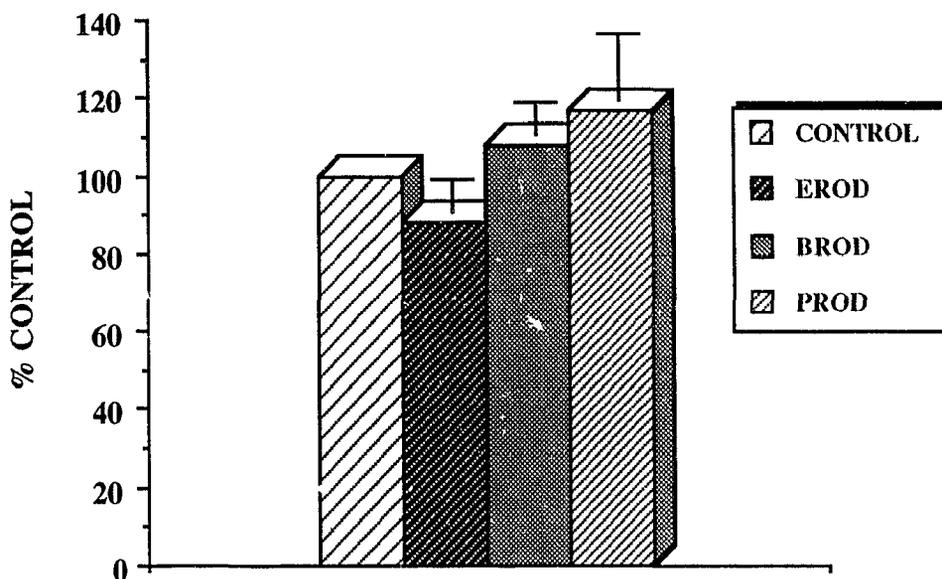


FIGURE 5

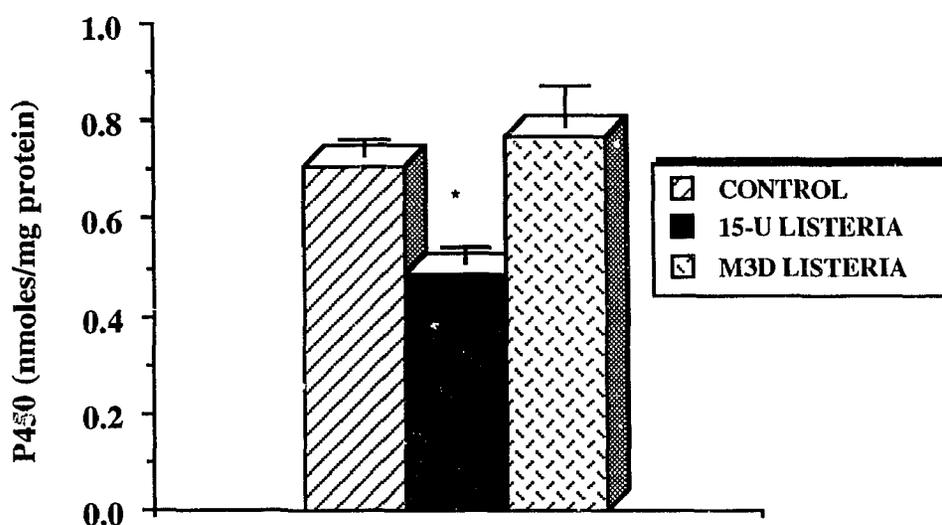
EFFECT OF 48 HOURS OF *IN VIVO* INFECTION WITH 1×10^6 CFU OF M3D LISTERIA ON SUBSTRATE METABOLISM CAPACITY

Mice were infected with 1×10^6 CFU of M3D listeria for 48 hours. Hepatic microsomal cytochrome P450 content, EROD, BROD, and PROD, were determined and expressed as a % of the mean control levels determined in mice treated for the same period with an equal volume of sterile PBS. The mean control levels were: cytochrome P450 = 0.76 ± 0.06 nmoles /mg protein; EROD = 60 ± 11 pmoles resorufin/mg /min; BROD = 4.4 ± 0.6 pmoles resorufin/mg/min; PROD = 1.0 ± 0.05 pmoles resorufin/mg /min. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test ($n=5$).

The next experiments were designed to achieve more comparable liver loads of the two strains of the bacteria prior to measuring cytochrome P450 content and substrate metabolism capacity in hepatic microsomes. Mice were injected intraperitoneally (i.p.) with phosphate-buffered saline (as control), 1×10^5 CFU of 15U listeria, or 1×10^8 CFU of the M3D strain and sacrificed 48 hours later. Cytochrome P450 content was depressed to 68% of control by the 15U strain whereas the M3D strain had no effect (Figure 6). Even though there was a 1,000-fold difference in administered dose, the liver load for the M3D strain was only one third of that for the 15U strain. Achieving exactly equal liver loads of the two strains in an *in vivo* experiment proved to be very difficult and therefore for future studies we utilized a liver cell culture system in which the bacterial load administered to the cells could be controlled.

THE EFFECT OF *IN VIVO* 15U LISTERIA INFECTION ON BODY WEIGHT, LIVER WEIGHT, AND LIVER HISTOLOGY

After 48 hours of *in vivo* infection with 1×10^6 CFU of 15U listeria, microsomal cytochrome P450 content, EROD, BROD, PROD, and aminopyrine N-demethylase activities were significantly decreased to approximately 40-60% of control levels (Figure 4). During this 48 hour infectious period, the mice did not demonstrate any obvious behavioral changes and previous studies have shown that the mice were not febrile (Azri and Renton, 1987). No change in body weight or liver weight occurred (Figures 7 and 8) and histological examination of liver slices taken from mice after 65 hours of infection did not indicate any significant changes (Figures 9A and 9B).



LIVER LOAD 15-U = 2.7×10^4 CFU

LIVER LOAD M3D = 9.0×10^3 CFU

FIGURE 6

EFFECT OF 48 HOURS OF *IN VIVO* INFECTION WITH 1×10^5 CFU OF 15U LISTERIA AND 1×10^8 CFU OF M3D LISTERIA

Mice were infected with 1×10^5 CFU of 15U or 1×10^8 CFU of M3D listeria and sacrificed after 48 hours of infection; control mice received an equal volume of sterile PBS. Cytochrome P450 levels were determined in hepatic microsomes and the liver loads of the bacteria from blood agar plates containing aliquots of the liver homogenates. Statistical analysis was carried out using Dunnett's test (n=5).

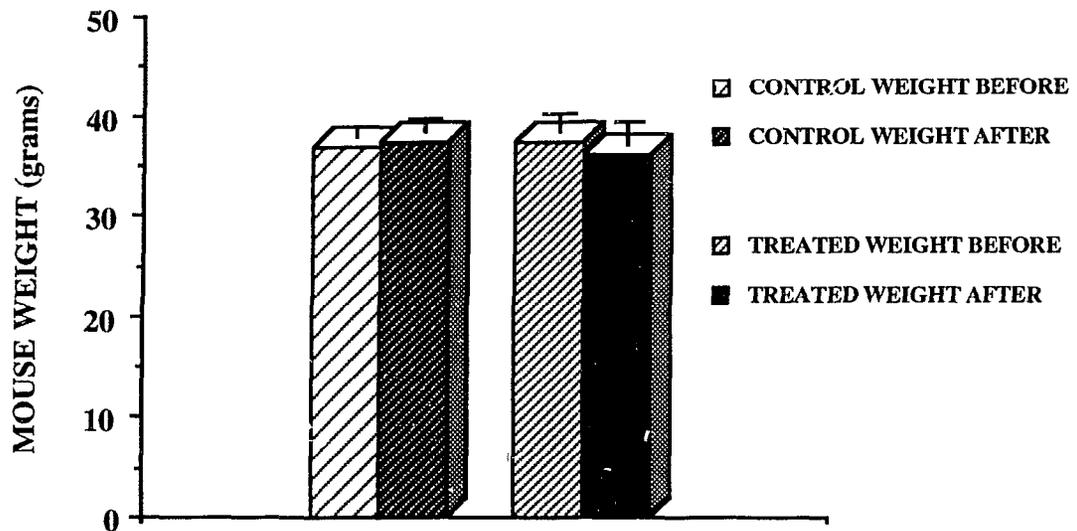


FIGURE 7

EFFECT OF 48 HOURS *IN VIVO* INFECTION WITH 1×10^6 CFU OF 15U LISTERIA ON BODY WEIGHT

Mice were weighed and infected with 1×10^6 CFU of 15U listeria for 48 hours and weighed again after sacrifice; control mice received an equal volume of sterile PBS.

Statistical analysis was carried out using a paired-sample Student's *t* test ($n=4$).

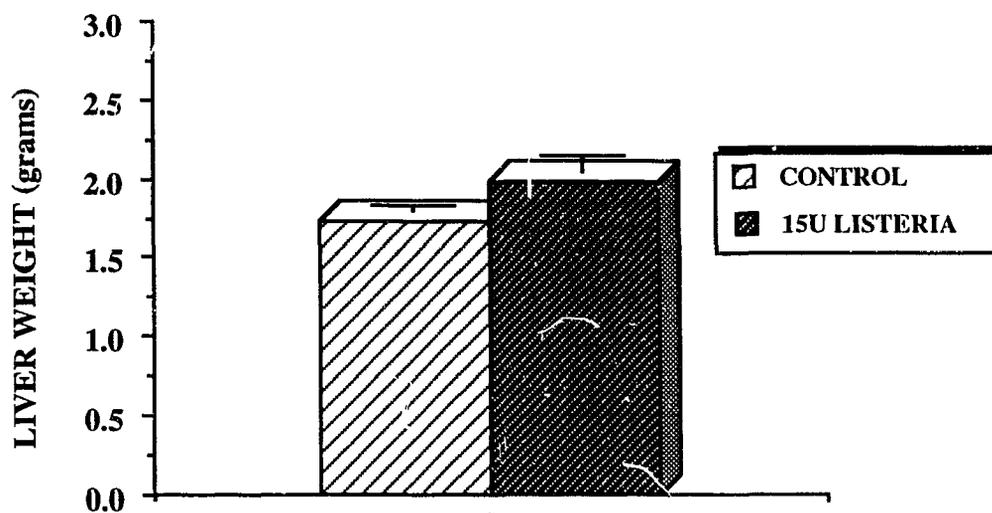


FIGURE 8

EFFECT OF 48 HOURS *IN VIVO* INFECTION WITH 1×10^6 CFU OF 15U LISTERIA ON LIVER WEIGHT

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours and livers were weighed after sacrifice; control mice received an equal volume of sterile PBS. Statistical analysis was carried out using an unpaired Student's *t* test ($n=4$).

FIGURE 9**EFFECT OF 65 HOURS OF *IN VIVO* INFECTION WITH 1×10^6 CFU OF 15U
LISTERIA ON LIVER ULTRASTRUCTURE**

Mice were sacrificed after 65 hours of infection with 1×10^6 CFU of 15U listeria. Livers were removed, fixed in formaldehyde, and sectioned for EM analysis; control mice were treated for the same period of time with sterile PBS. Magnification = 4730x

A. EM section from a control liver

B. EM section from a treated liver

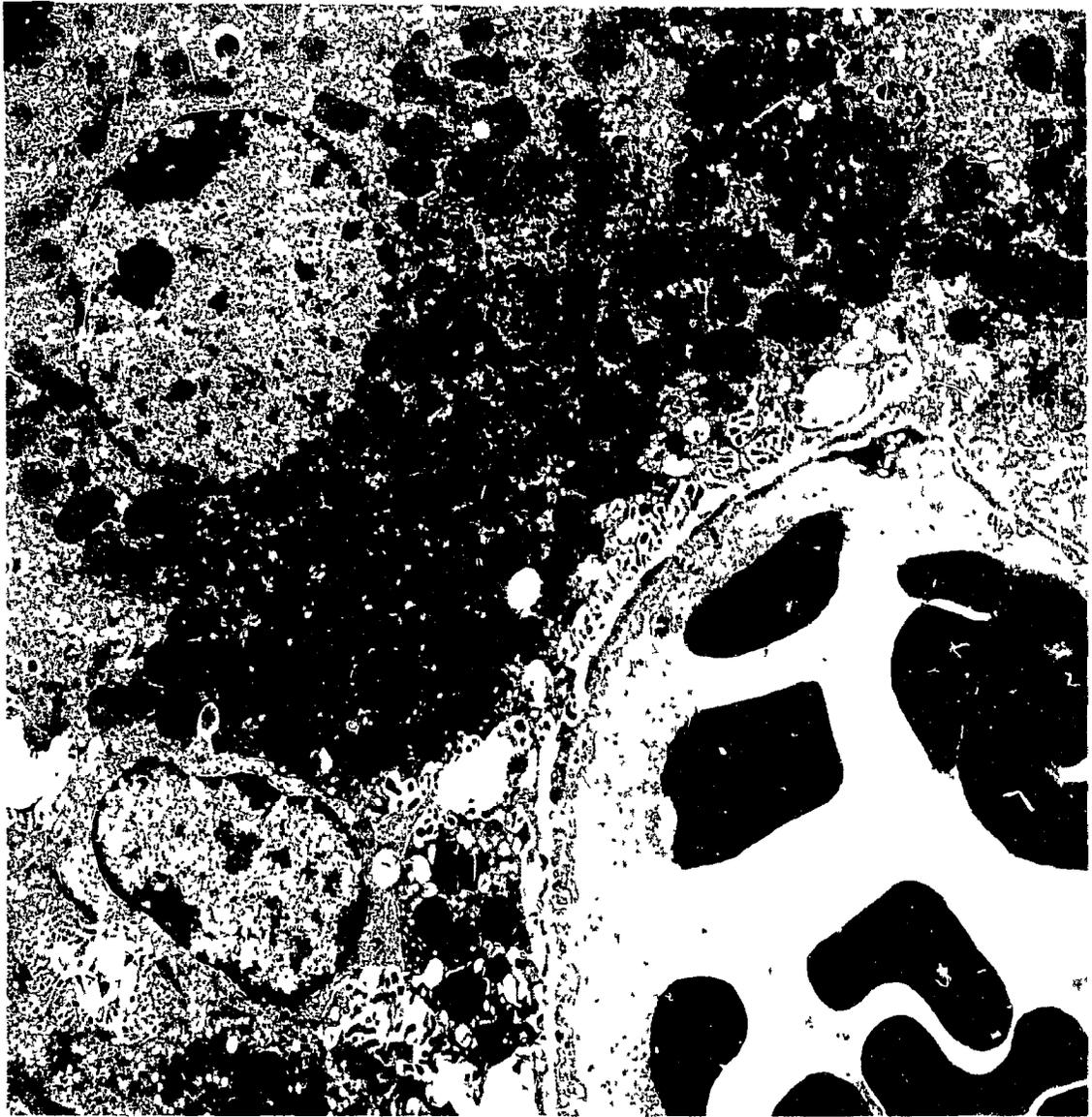


FIGURE 9A

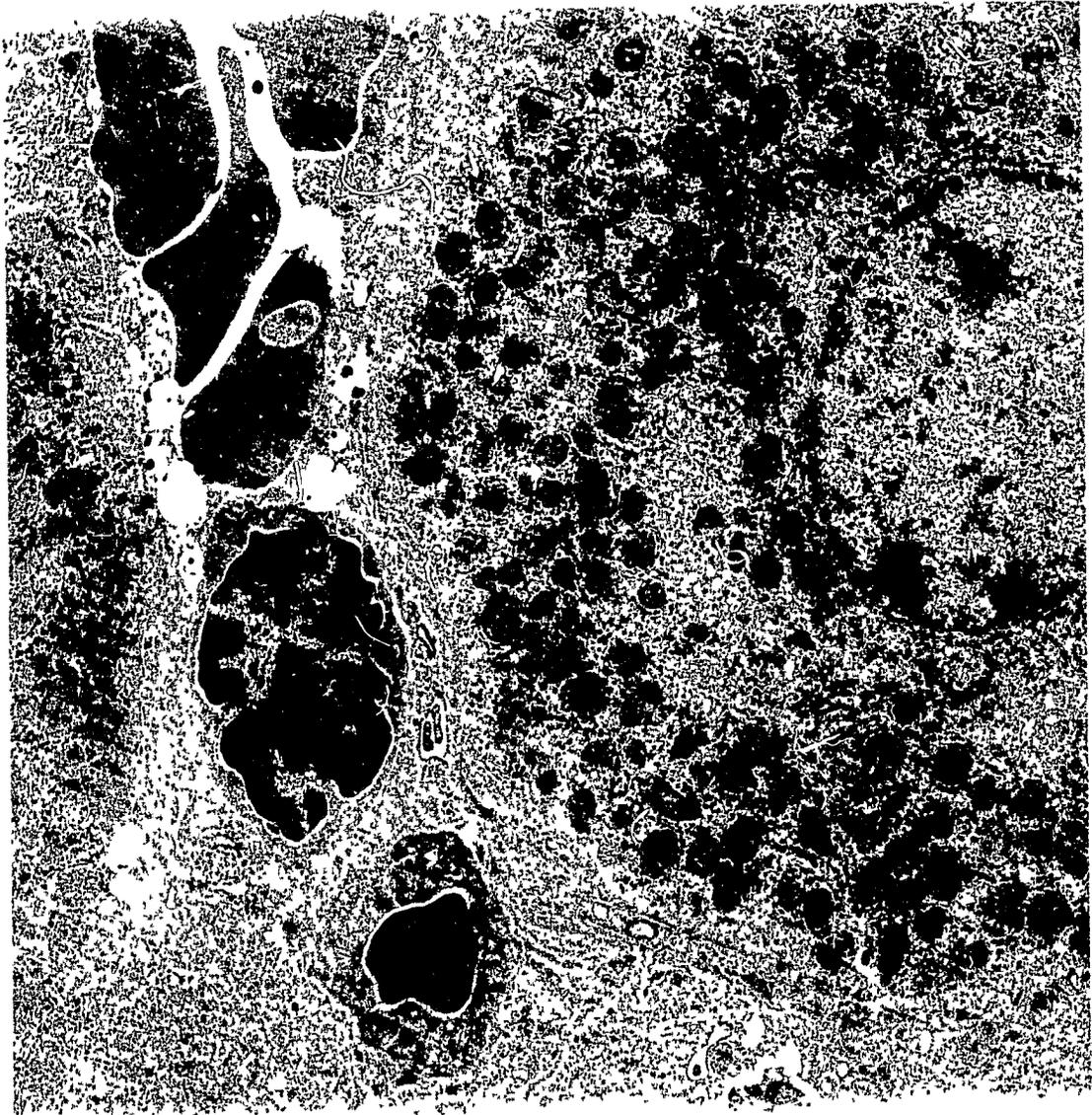


FIGURE 9B

THE EFFECT OF 15U LISTERIA INFECTION ON CYTOCHROME P450-MEDIATED METABOLISM WHEN INCUBATED DIRECTLY WITH HEPATIC MICROSOMES

When 15U listeria was incubated directly with hepatic microsomes the bacteria had no effect on cytochrome P450, EROD, BROD, or PROD activities as illustrated in Figure 10.

THE EFFECT OF 15U LISTERIA INFECTION ON HEPATOCYTES MAINTAINED IN SUSPENSION

A suspended culture system in which the liver cells (hepatocytes + nonparenchymal cells) were constantly mixed on a rotating device was used for these experiments. To minimize cell clumping and maintain detectable cytochrome P450 levels, the liver cell suspensions were incubated at 25°C. A comparison of the relative rate of loss of cytochrome P450 at 37°C versus 25°C is shown in Figure 11. At the lower temperature adequate hepatocyte viability and cytochrome P450 content were maintained.

The percent loss of cytochrome P450 in hepatocytes incubated with 15U listeria for 24 or 48 hours is shown in Figure 12. Cytochrome P450 was decreased 40% and 20% after 24 and 48 hour incubations respectively. In addition to a significant decrease in cytochrome P450 content, 15U listeria caused a 20-30% decrease in ethoxyresorufin-O-dealkylase (EROD) and benzyloxyresorufin-O-dealkylase (BROD) activities after a 24 hour incubation. The mean percent loss for cytochrome P450, EROD, and BROD is shown in Figures 13A, 13B, and 13C with the results expressed by DNA content, total cell number, and viable cell number respectively. This loss of drug biotransformation occurred in the absence of a significant change in viability or total hepatocyte protein content. For the remainder of the cell culture experiments, DNA content was chosen to normalize the data.

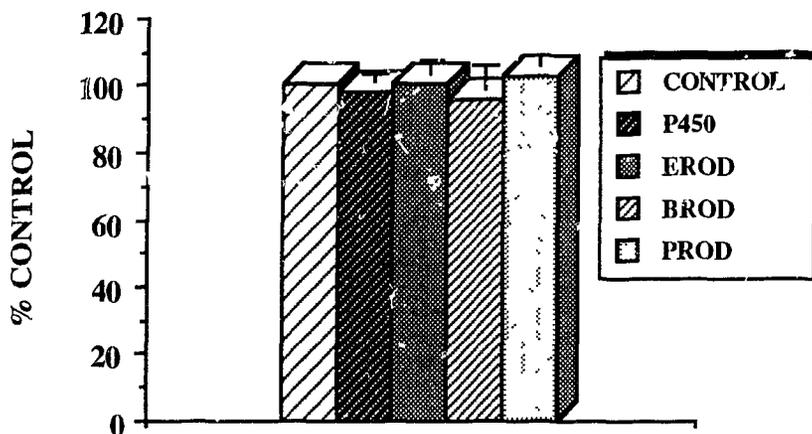


FIGURE 10

EFFECT OF A 24 HOUR *IN VITRO* INCUBATION OF 15U LISTERIA WITH HEPATIC MICROSOMES

PBS or 1×10^4 CFU of 15U listeria was added to aliquots of pooled control microsomes. The microsomes were incubated at 37°C for 12 hours at which point penicillin and streptomycin were added. After 24 hours the microsomes were analyzed and the results of the treated microsomes were expressed as a % of control. The mean control levels were: cytochrome P450 = 0.25 ± 0.01 nmoles /mg protein; EROD = 2.9 ± 0.12 pmoles resorufin/mg /min; BROD = 0.56 ± 0.07 pmoles resorufin/mg/min and PROD = 0.59 ± 0.07 pmoles resorufin/mg /min. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test ($n=4$).

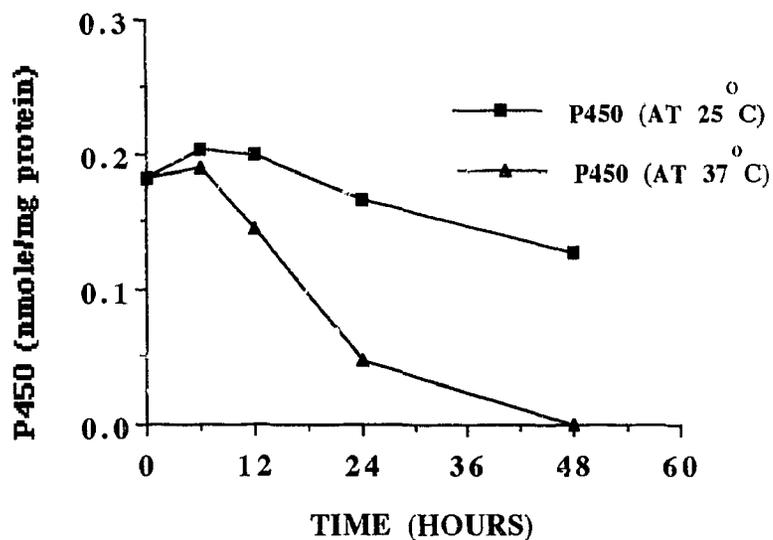


FIGURE 11

EFFECT OF INCUBATION TEMPERATURE ON CYTOCHROME P450 LOSS IN CONTROL HEPATOCYTES MAINTAINED IN SUSPENSION

Hepatocytes at a density of 0.5×10^6 cells/mL (along with nonparenchymal cells) were maintained in suspension at 25°C or 37°C. After the specified periods, the hepatocytes in each flask were analyzed for cytochrome P450 and protein content.

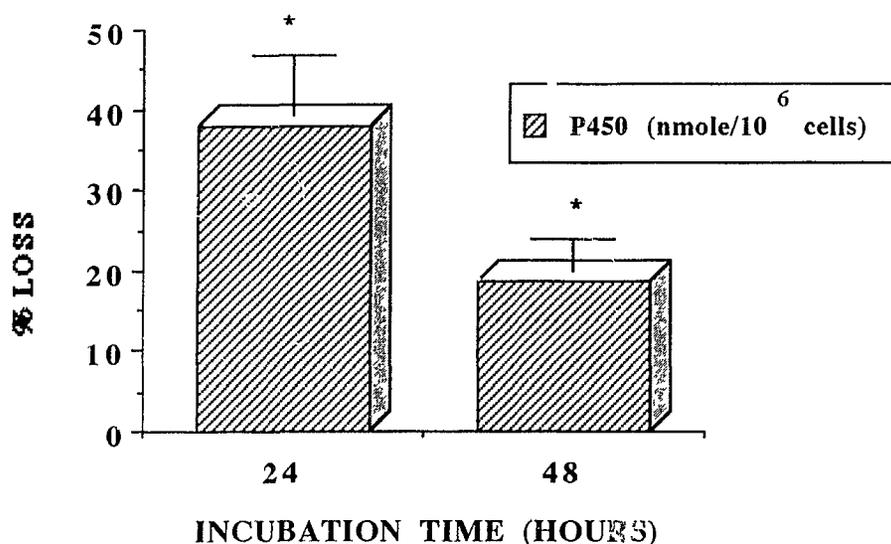


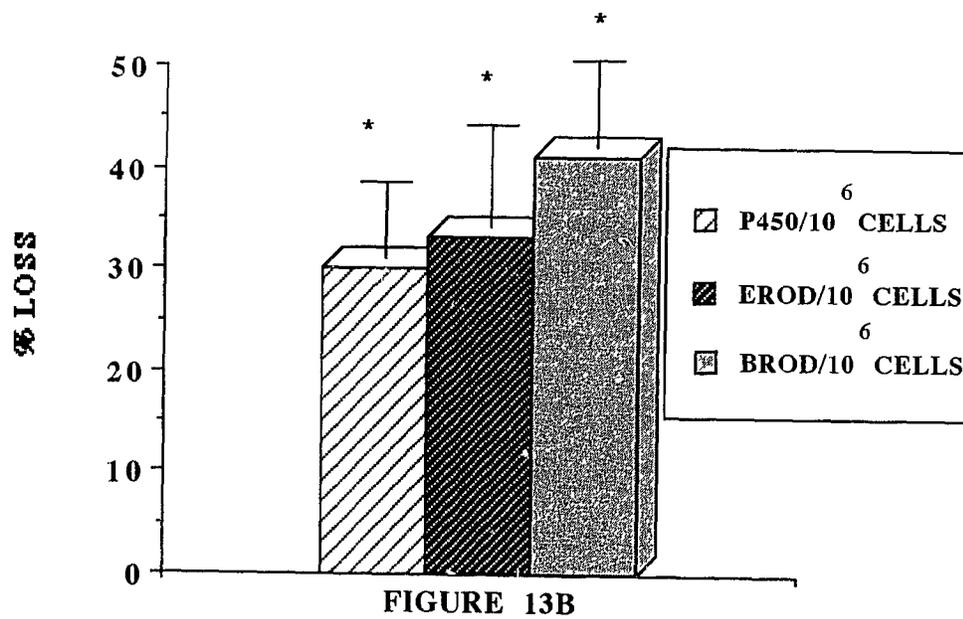
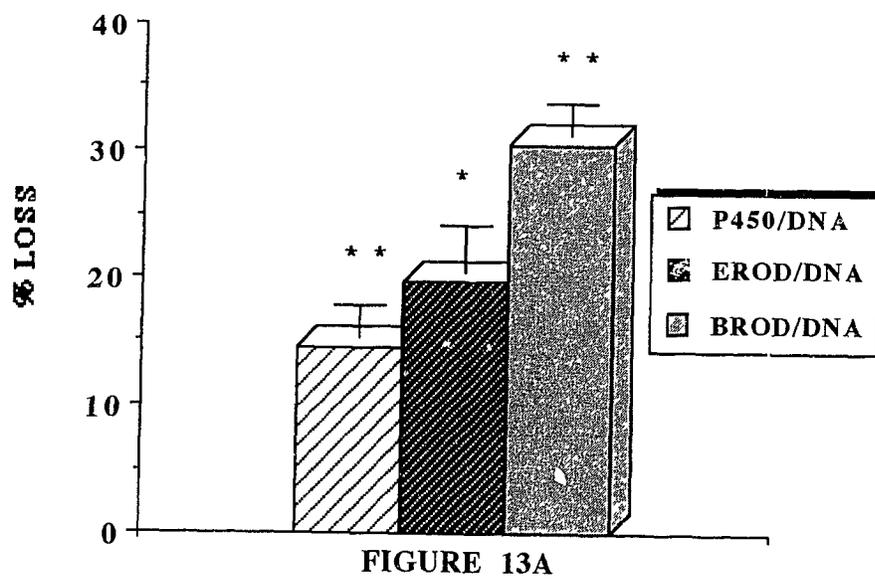
FIGURE 12

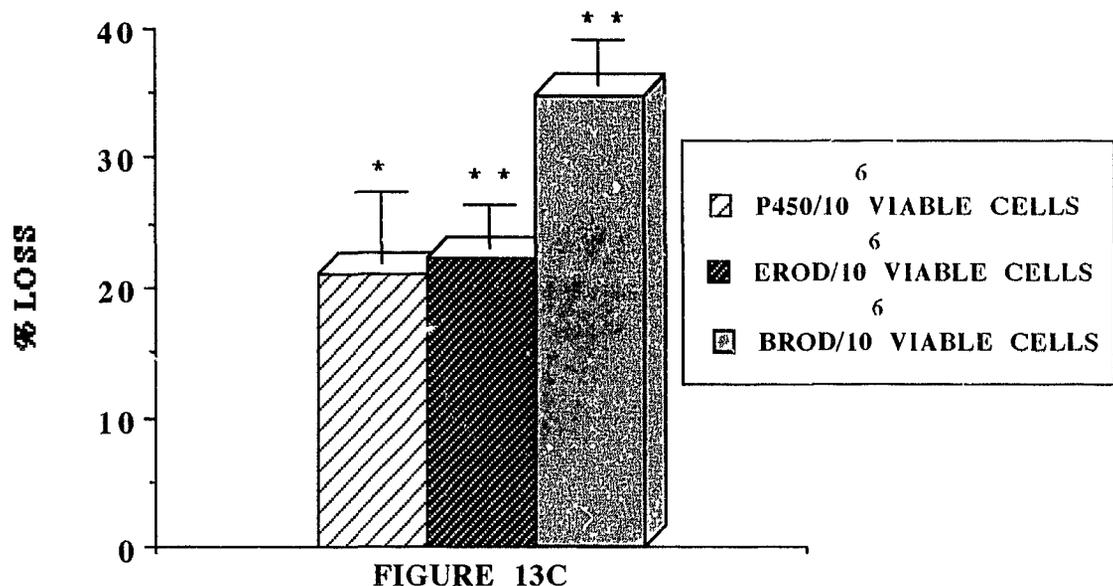
EFFECT OF *IN VITRO* INCUBATION TIME WITH 15U LISTERIA ON CYTOCHROME P450 LEVELS IN HEPATOCYTES MAINTAINED IN SUSPENSION

Hepatocytes at a density of 0.5×10^6 viable cells/mL were maintained in suspension along with Kupffer cells and other nonparenchymal cells at 25°C. Sterile PBS was added to the control flasks and 5×10^6 CFU of 15U listeria was added to the treated flasks at t=0. The cells were maintained in suspension at 25°C for 12 hours at which point a combination of penicillin and streptomycin were added. After 24 or 48 hours the cytochrome P450 content of the hepatocytes in each flask were determined. The treated levels were expressed as a % of the corresponding control and then subtracted from 100 to yield % loss. The mean control cytochrome P450 content at t=24 hours was 0.60 ± 0.25 and at t=48 hours was 0.38 ± 0.06 nmoles/ 10^6 cells. Statistical analysis was carried out using a one-sample *t* test (n=3).

FIGURE 13**EFFECT OF 24 HOURS OF *IN VITRO* INCUBATION WITH 15U LISTERIA ON CYTOCHROME P450, EROD, AND BROD IN HEPATOCYTES MAINTAINED IN SUSPENSION**

Hepatocytes at a density of 0.2×10^6 viable cells/mL were maintained in suspension along with Kupffer cells and other nonparenchymal cells at 25°C. Sterile PBS was added to the control flasks and 5×10^6 CFU of 15U listeria was added to the treated flasks at $t=0$. The cells were then maintained in suspension at 25°C for 12 hours at which point a combination of penicillin and streptomycin were added. After 24 hours the cytochrome P450 content, EROD, and BROD activities of the hepatocytes in each flask were determined. The treated levels were expressed as a % of the corresponding control and then subtracted from 100 to yield % loss. The enzyme levels were expressed by DNA content, total cell number, and viable cell number in Figures 12A, 12B, and 12C respectively. The mean control levels were cytochrome P450 = 0.04 ± 0.01 nmoles/ μ g DNA, EROD = 2.4 ± 0.6 and BROD = 0.38 ± 0.05 pmoles resorufin/ μ g DNA/min. Statistical analysis was carried out using a one-sample *t* test ($n=4$).





THE EFFECT OF 15U AND M3D LISTERIA INFECTION ON HEPATOCYTES CULTURED IN THE PRESENCE AND ABSENCE OF NONPARENCHYMAL CELLS

To determine if the effect of listeria is direct on the hepatocyte or if it requires the presence of nonparenchymal cells, a series of experiments were designed that involved the incubation of 15U and M3D listeria with isolated hepatocyte preparations and with cultures containing hepatocytes in addition to nonparenchymal cells (as described in the methods section; see Figure 1). The results of these experiments are shown in Figures 14-17. The 15U strain of listeria had no effect on EROD activity in isolated hepatocyte cultures but did produce a significant loss of EROD activity when incubated with cultures containing hepatocytes and nonparenchymal cells (Figure 14). The M3D strain had no effect on EROD activity in isolated hepatocyte cultures or in cultures containing hepatocytes and nonparenchymal cells (Figure 15). At the peak of infection ($t=12$ hours, immediately before the addition of penicillin and streptomycin), the average number of CFU of listeria was 8.8×10^4 for the 15U strain and 5.1×10^4 for the M3D strain.

The effect of 15U listeria on BROD activity is shown in Figure 16. This strain of listeria caused a significant reduction in BROD activity in cultures of isolated hepatocytes and in cultures containing isolated hepatocytes and nonparenchymal cells, but the M3D strain has no effect on BROD activity in either type of culture (Figure 17). The effect of the 15U listeria on EROD and BROD activity in cultures containing hepatocytes and nonparenchymal cells occurred in the absence of a change in viability or total cell protein as shown in Figure 18.

FIGURE 14**EFFECT OF 24 HOURS OF *IN VITRO* INCUBATION WITH 15U LISTERIA ON EROD ACTIVITIES IN ADHERENT HEPATOCYTE CULTURES AT 37⁰C**

Cultures of isolated hepatocytes or hepatocytes along with Kupffer cells and other nonparenchymal cells were prepared as described in the methods section (Figure 1). After a 24 hour incubation with 15U listeria the EROD activity was determined in the treated cells and expressed relative to the corresponding control. The mean control hepatocyte EROD activities were 1.56 ± 0.3 and 1.70 ± 0.4 pmoles/ μ g DNA/min for isolated hepatocyte and hepatocyte + nonparenchymal cell cultures respectively. Statistical analysis was carried out using a one-sample *t* test (n=4).

FIGURE 15**EFFECT OF 24 HOURS OF *IN VITRO* INCUBATION WITH M3D LISTERIA ON EROD ACTIVITIES IN ADHERENT HEPATOCYTE CULTURES AT 37⁰C**

Cultures of isolated hepatocytes or hepatocytes along with other nonparenchymal cells were prepared as described in the methods section (Figure 1). After a 24 hour incubation with M3D listeria the EROD activity was determined in the treated cells and expressed relative to the corresponding control. The mean control hepatocyte EROD activities were 4.60 ± 2.8 and 4.68 ± 2.5 pmoles/ μ g DNA/min for isolated hepatocyte and hepatocyte + nonparenchymal cell cultures respectively. Statistical analysis was carried out using a one-sample *t* test (n=3).

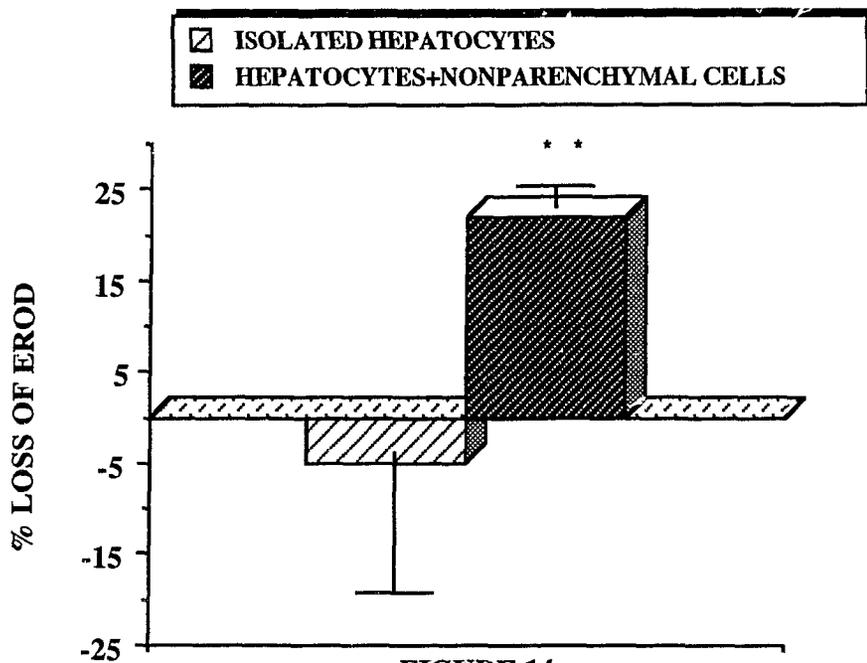


FIGURE 14

BACTERIAL BURDEN = 8.8×10^4 CFU

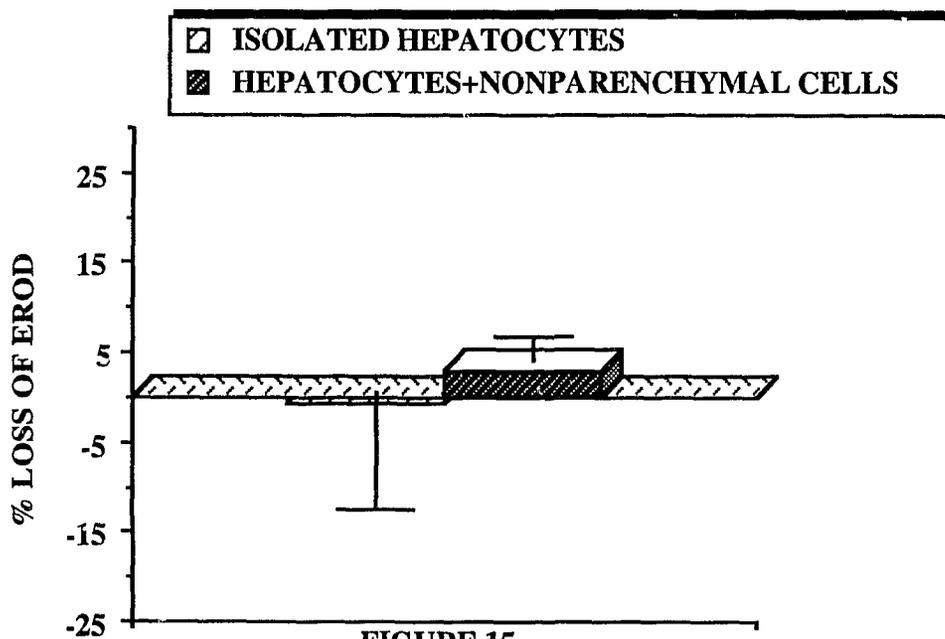


FIGURE 15

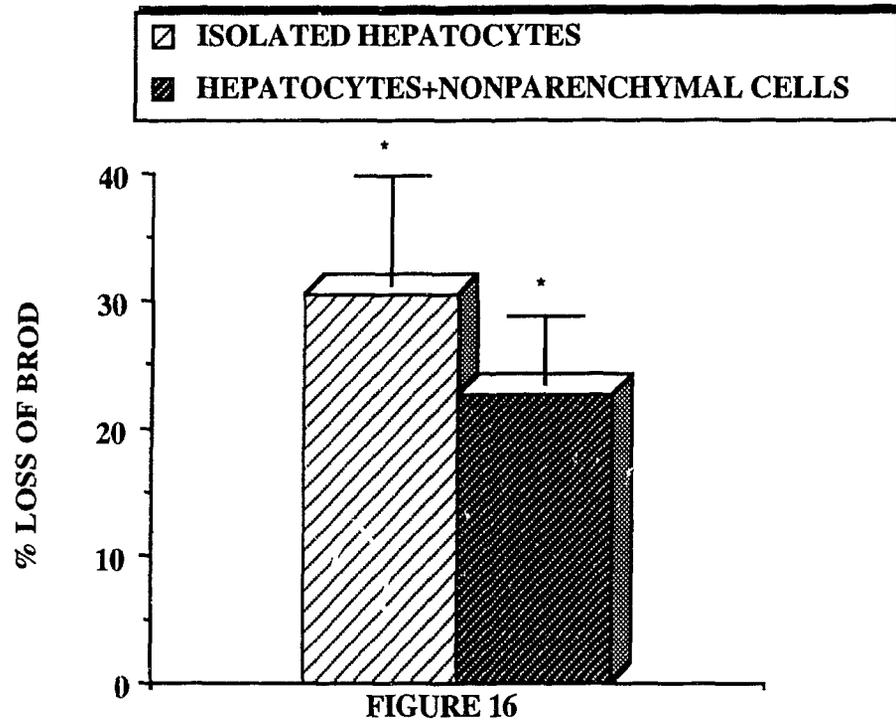
BACTERIAL BURDEN = 5.1×10^4 CFU

FIGURE 16**EFFECT OF 24 HOURS OF *IN VITRO* INCUBATION WITH 15U LISTERIA ON BROD ACTIVITIES IN ADHERENT HEPATOCYTE CULTURES AT 37⁰C**

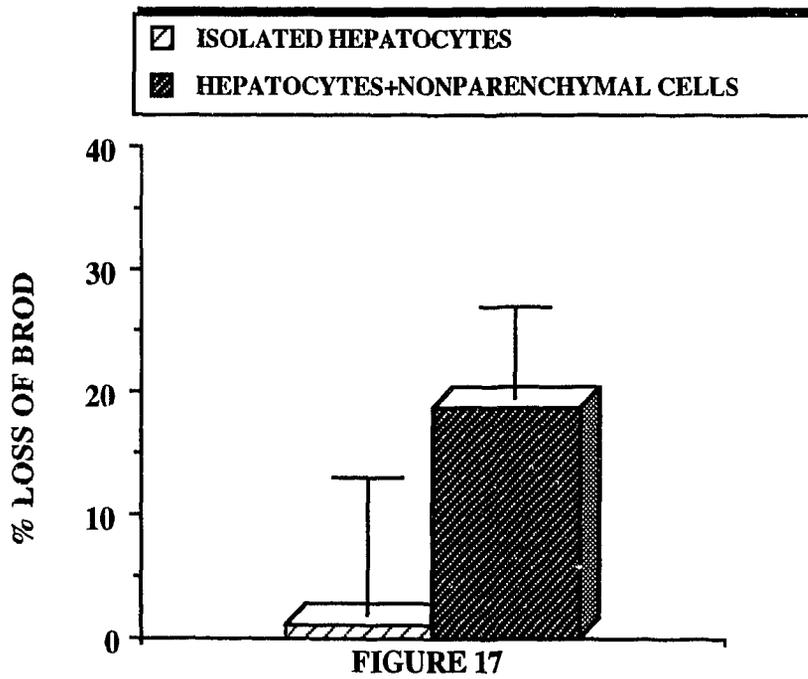
Cultures of isolated hepatocytes or hepatocytes along with Kupffer cells and other nonparenchymal cells were prepared as described in the methods section (Figure 1). After a 24 hour incubation with 15U listeria the BROD activity was determined in the treated cells and expressed relative to the corresponding control. The mean control hepatocyte BROD activities were 0.31 ± 0.06 and 0.25 ± 0.08 pmoles/ μ g DNA/min for isolated hepatocyte and hepatocyte + nonparenchymal cell cultures respectively. Statistical analysis was carried out using a one-sample *t* test (n=4).

FIGURE 17**EFFECT OF 24 HOURS OF *IN VITRO* INCUBATION WITH M3D LISTERIA ON BROD ACTIVITIES IN ADHERENT HEPATOCYTE CULTURES AT 37⁰C**

Cultures of isolated hepatocytes or hepatocytes along with other nonparenchymal cells were prepared as described in the methods section (Figure 1). After a 24 hour incubation with M3D listeria the BROD activity was determined in the treated cells and expressed relative to the corresponding control. The mean control hepatocyte BROD activities were 0.38 ± 0.12 and 0.45 ± 0.28 pmoles/ μ g DNA/min for isolated hepatocyte and hepatocyte + nonparenchymal cell cultures respectively. Statistical analysis was carried out using a one-sample *t* test (n=3).



BACTERIAL BURDEN = 8.8×10^4 CFU



BACTERIAL BURDEN = 5.1×10^4 CFU

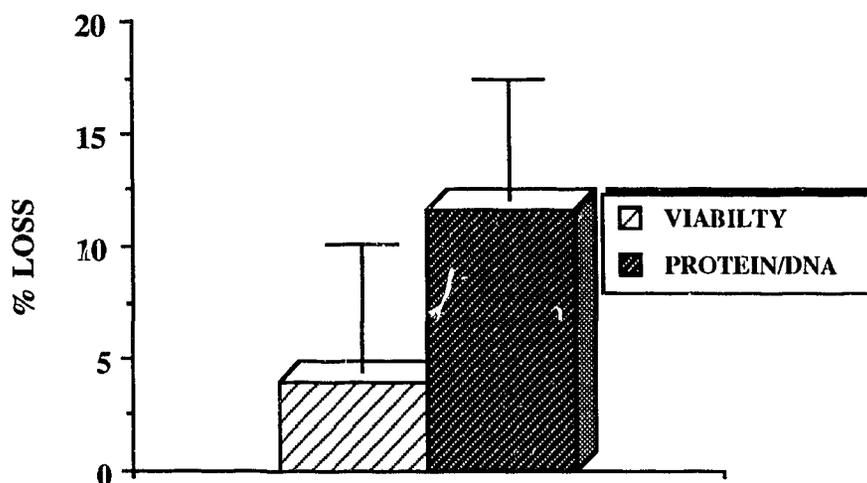


FIGURE 18

EFFECT OF 24 HOURS OF *IN VITRO* INCUBATION WITH 15U LISTERIA ON THE VIABILITY AND TOTAL CELLULAR PROTEIN CONTENT OF ADHERENT HEPATOCYTE CULTURES AT 37⁰C

Cultures of hepatocytes along with Kupffer cells and other nonparenchymal cells were prepared as described in the methods section (Figure 1). After a 24 hour incubation with 15U listeria the viability and total cellular protein of the hepatocytes was determined.

Statistical analysis of the changes in protein content was carried out using a one-sample *t* test (n=4). The analysis of the changes in viability was carried out using a paired-sample *t* test on the arcsine transformed viability data (n=4).

THE *IN VIVO* EFFECT OF 15U LISTERIA INFECTION ON CYTOCHROME P450IA mRNA LEVELS

After induction with β NF, mice were infected for 48 hours with 1×10^6 CFU of 15U listeria (see Table 4 of the methods section). This resulted in a 40% reduction in total cytochrome P450 levels and a 65% reduction in EROD activity (Figure 19). No significant change in total liver protein or microsomal protein concentrations was observed in these animals (Figures 20 and 21).

The RNA from these animals was separated in an agarose-formaldehyde gel with each sample lane containing 15 μ g of total RNA along with 20 μ g of RNA ladder, as shown in Figure 22. From left to right, lanes 2 and 3 are uninduced control mice, lanes 6-8 are from mice induced with β NF and treated for 48 hours with sterile PBS (induced-control), lanes 10-12 contain RNA from mice induced with β NF and treated for 48 hours with 15U listeria (induced-treated). The gel was then transferred to a nylon membrane by vacuum transfer with a transfer efficiency of virtually 100%, and the resulting Northern blot was probed with a cDNA oligomer directed against cytochrome P450IA mRNA as shown in Figure 23. The apparent molecular size of the mRNA hybridized to the probe was approximately 2.4 kb (actual size of the IA1 mRNA is 2.6 kb). The density of the bands in lanes 10-12, from the β NF-induced listeria-treated mice, are approximately 10-fold lower than those of the β NF-induced control mice (lanes 6-8). The listeria treatment reduced cytochrome P450IA mRNA to a level comparable to that found in uninduced control mice (lanes 2 and 3).

To accurately quantitate the magnitude of this difference, duplicate slot blots were prepared and hybridized with the cytochrome P450IA cDNA probe and to an oligo(dT)₁₈ probe which binds to all mRNA. Autoradiographs of representative slot blots probed with

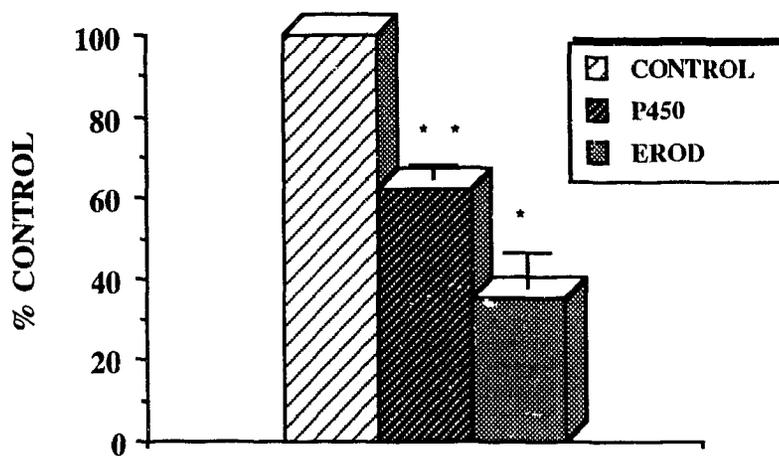


FIGURE 19

**EFFECT OF 48 HOURS OF *IN VIVO* INFECTION WITH 15U LISTERIA ON
CYTOCHROME P450 AND EROD ACTIVITY AFTER BNF INDUCTION**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours after being induced for 3 days with BNF (see Table 4 in the methods section); control mice were also induced with BNF and received an equal volume of sterile PBS. Cytochrome P450 levels and EROD activities were determined in hepatic microsomes and are expressed as a % of control levels. The mean control cytochrome P450 was 0.74 ± 0.03 nmoles/mg protein and the mean control EROD was 532 ± 113 pmoles resorufin/mg/min. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test ($n=5$).

FIGURE 20**EFFECT OF 48 HOURS OF *IN VIVO* INFECTION WITH 15U LISTERIA ON TOTAL LIVER PROTEIN AFTER β NF INDUCTION**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours after being induced for 3 days with β NF (see Table 4 in the methods section); control mice were also induced with β NF and received an equal volume of sterile PBS. Total liver protein concentrations were determined from whole liver homogenates. Statistical analysis was carried out using an unpaired Student's *t* test (n=5).

FIGURE 21**EFFECT OF 48 HOURS OF *IN VIVO* INFECTION WITH 15U LISTERIA ON MICROSOMAL PROTEIN AFTER β NF INDUCTION**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours after being induced for 3 days with β NF (see Table 4 in the methods section); control mice were also induced with β NF and received an equal volume of sterile PBS. Microsomal protein concentrations were determined from the hepatic microsomes prepared at the time of sacrifice. Statistical analysis was carried out using an unpaired Student's *t* test (n=5).

LIVER PROTEIN
(mg/g liver)

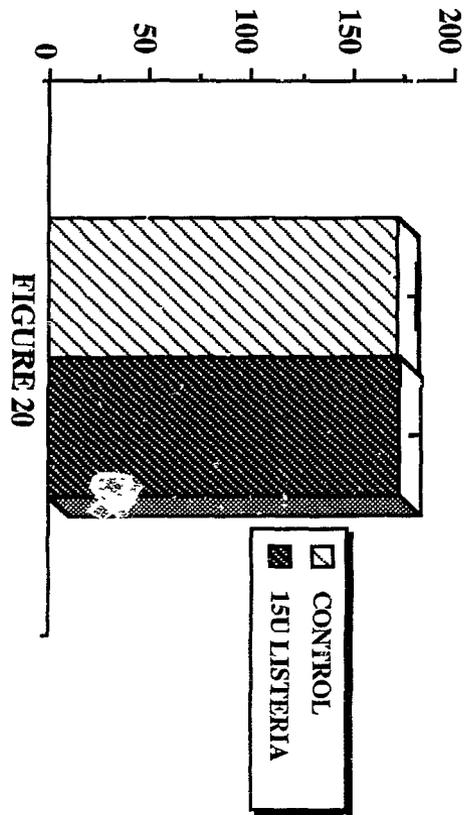


FIGURE 20

MICROSOMAL PROTEIN
(mg/g liver)

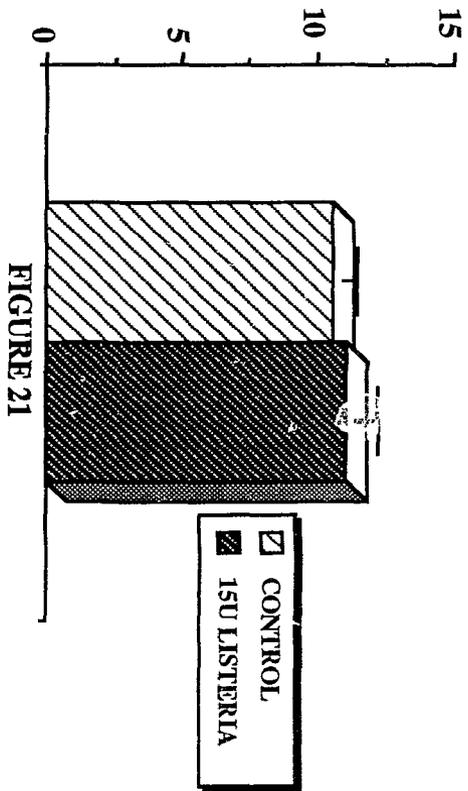


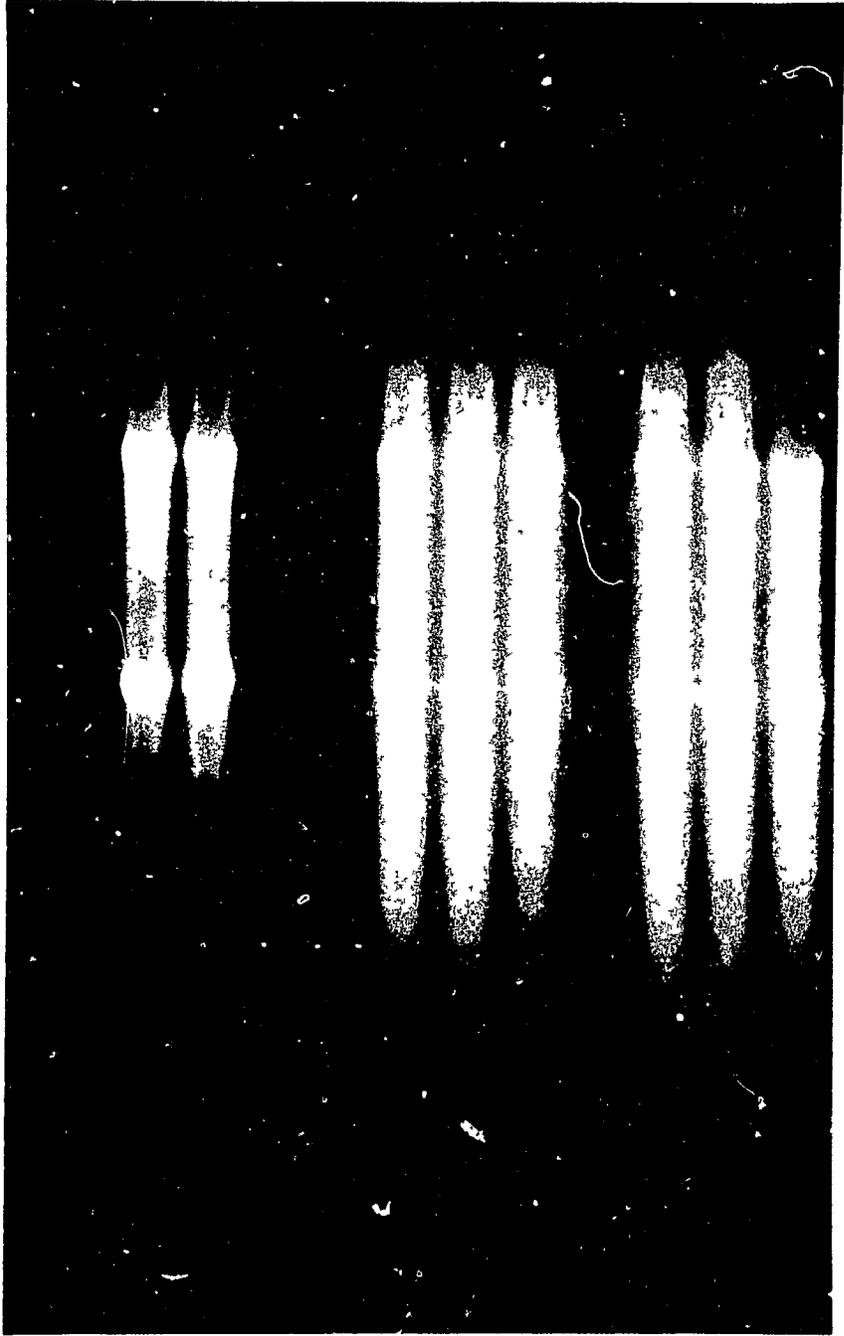
FIGURE 21

FIGURE 22

AGAROSE FORMALDEHYDE GEL - EFFECT OF 48 HOURS *IN VIVO* LISTERIA INFECTION ON CYTOCHROME P450IA mRNA LEVELS AFTER β NF INDUCTION

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours after being induced for 3 days with β NF (see Table 4 in the methods section); control mice were also induced with β NF and received an equal volume of sterile PBS. Lanes contain 15 μ g of total RNA as follows: from left to right, lanes 2 and 3 are uninduced control mice, lanes 6-8 are from mice induced with β NF and treated for 48 hours with sterile PBS (induced-control), lanes 10-12 contain RNA from mice induced with β NF and treated for 48 hours with 15U listeria (induced-treated).

1 2 3 4 5 6 7 8 9 10 11 12



23S

18S

FIGURE 22

FIGURE 23**NORTHERN BLOT - EFFECT OF 48 HOURS *IN VIVO* LISTERIA INFECTION ON CYTOCHROME P450IA mRNA LEVELS AFTER β NF INDUCTION**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours after being induced for 3 days with β NF (see Table 4 in the methods section); control mice were also induced with β NF and received an equal volume of sterile PBS. Lanes contain 15 μ g of total RNA as follows: from left to right, lanes 2 and 3 are uninduced control mice, lanes 6-8 are from mice induced with β NF and treated for 48 hours with sterile PBS (induced-control), and lanes 10-12 contain RNA from mice induced with β NF and treated for 48 hours with 15U listeria (induced-treated). The blot was hybridized with a cDNA directed against cytochrome P450IA mRNA.

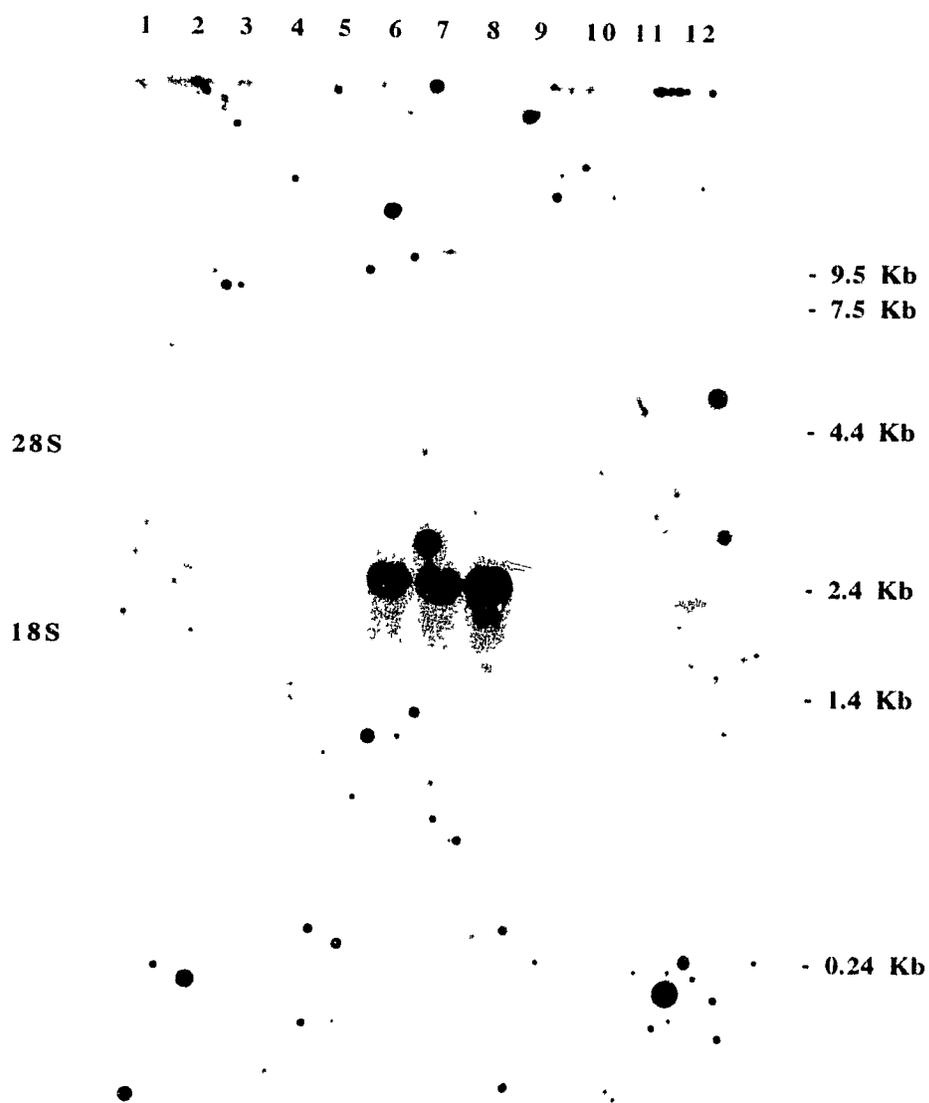


FIGURE 23

the P450IA cDNA and the oligo(dT)₁₈ probes are shown in Figures 24 and 25 respectively. The density of the bands in the two treated rows are significantly less than those of the three control rows (Figure 24). Each lane contains serial 1:2 dilutions of RNA beginning with a first slot blot load of 3 µg and thus from top to bottom the slots contain 3.0, 1.5, 0.75, 0.188, 0.093, 0.047, 0.023 and 0.012 µg. The intensity of the top lane of the two treated rows corresponds approximately with the intensity of the fourth (1:8 dilution) or fifth (1:16 dilution) band of the control samples. This is in agreement with the Northern blot (Figure 23) which showed cytochrome P450IA mRNA levels in treated lanes were approximately 10% of those in control lanes. Approximately equal blot intensities were seen in all of the slots hybridized with the oligo(dT)₁₈ probe (Figure 25), indicating that listeria treatment did not effect total mRNA levels.

A summary of the effect of 48 hours *in vivo* infection with 15U listeria is illustrated in Figure 26. Cytochrome P450 was significantly depressed to 62% of control, EROD activity was significantly reduced to 35% of control, and cytochrome P450IA mRNA levels depressed to 21% of control.

THE EFFECT OF 48 HOURS *IN VIVO* LISTERIA INFECTION ON CYTOCHROME P450IA1 APOPROTEIN LEVELS AFTER BNF INDUCTION

Microsomes from control and infected mice (48 hours with 1×10^6 CFU of 15U listeria) were separated by electrophoresis and the resulting Western blot was probed with an antibody directed against cytochrome P450IA1 apoprotein as shown in Figure 27. The apparent molecular weight of the protein to which the antibody bound is slightly less than 58.5 kd (actual molecular weight of murine cytochrome P450IA1 apoprotein is 55 kd). Although equal quantities of total microsomal protein were loaded in each lane, it is

FIGURE 24**SLOT BLOT - EFFECT OF 48 HOURS *IN VIVO* LISTERIA INFECTION ON
CYTOCHROME P450IA mRNA LEVELS AFTER β NF INDUCTION**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours after being induced for 3 days with β NF (see Table 4 in the methods section); control mice were also induced with β NF and received an equal volume of sterile PBS. Each lane contains serial 1:2 dilutions of sample beginning with 3 μ g of total RNA. C=control samples, T=treated samples, and S=standard. The slot blot was hybridized with a cDNA directed against cytochrome P450IA mRNA.

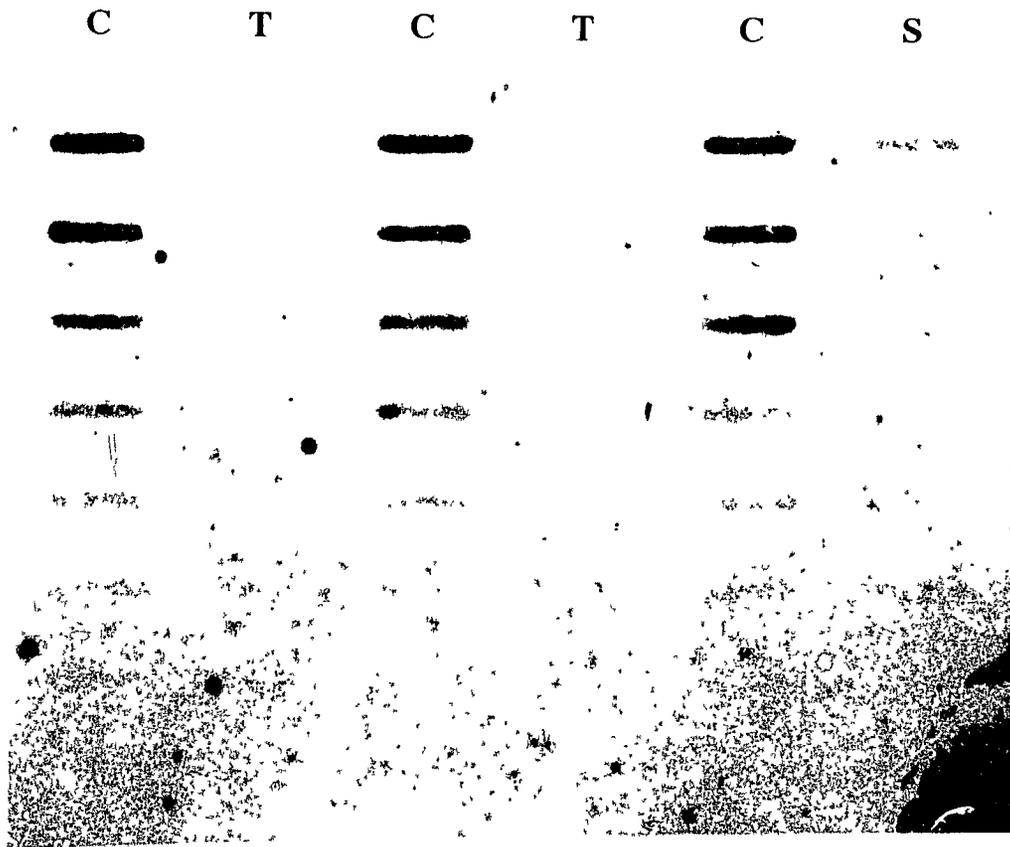


FIGURE 24

FIGURE 25**SLOT BLOT - EFFECT OF 48 HOURS *IN VIVO* LISTERIA INFECTION ON TOTAL mRNA LEVELS AFTER β NF INDUCTION**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours after being induced for 3 days with β NF (see Table 4 in the methods section); control mice were also induced with β NF and received an equal volume of sterile PBS. Each lane contains serial 1:2 dilutions of sample beginning with 3 μ g of total RNA. C=control samples, T=treated samples, and S=standard. The slot blot was hybridized with an oligo(dT)₁₈ probe.

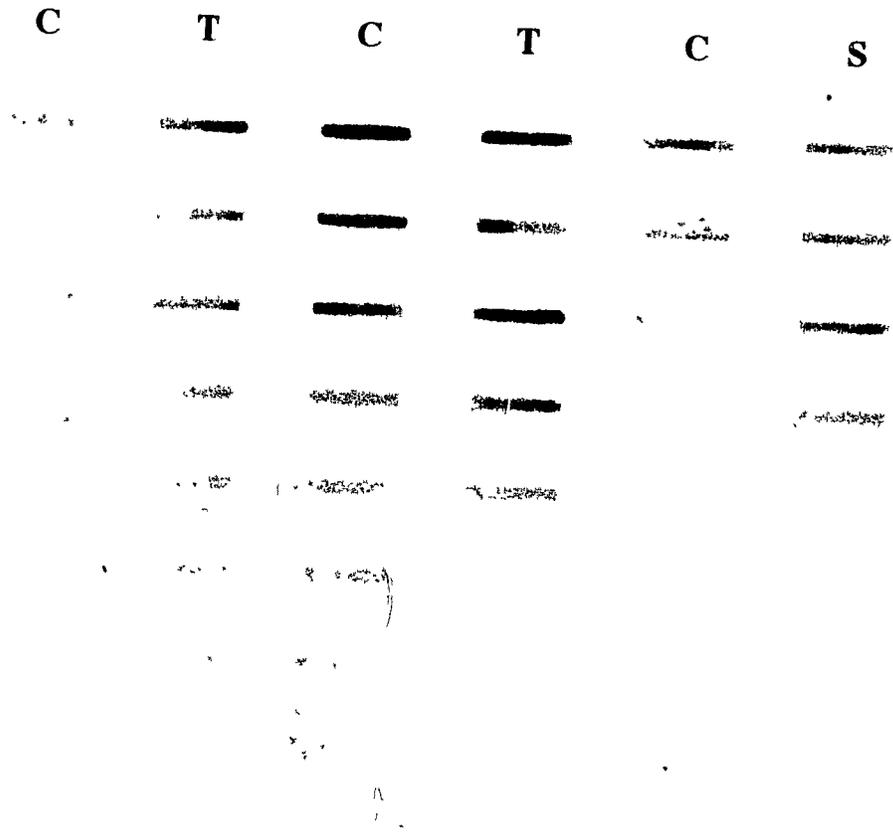


FIGURE 25

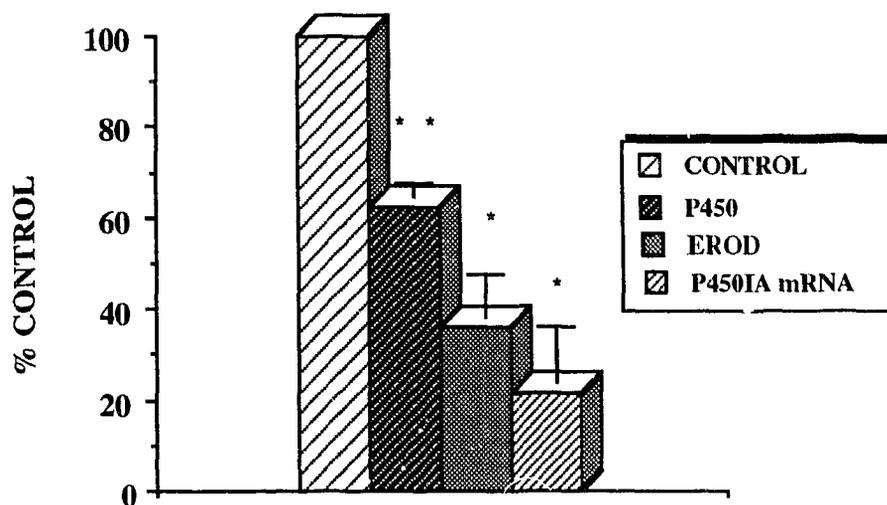


FIGURE 26

EFFECT OF 48 HOURS OF *IN VIVO* INFECTION WITH 15U LISTERIA ON CYTOCHROME P450, EROD, AND CYTOCHROME P450IA mRNA LEVELS AFTER β NF INDUCTION

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours after being induced for 3 days with β NF (see Table 4 in the methods section); control mice were also induced with β NF and received an equal volume of sterile PBS. Cytochrome P450 levels and EROD activities were determined in hepatic microsomes, and cytochrome P450IA mRNA levels were determined from duplicate slot blots that were hybridized with a cytochrome P450IA cDNA and standardized with an oligo(dT)₁₈ probe. All results are expressed as a % of control levels. The mean control cytochrome P450 was 0.74 ± 0.03 nmoles/mg protein, the mean control EROD was 532 ± 113 pmoles resorufin/mg/min and the mean control cytochrome P450IA mRNA level was 5.8 ± 1.3 (arbitrary units) Statistical analysis was carried out on the raw data using an unpaired Student's *t* test ($n=5$).

FIGURE 27**WESTERN BLOT - EFFECT OF 48 HOURS OF *IN VIVO* INFECTION WITH 15U LISTERIA ON CYTOCHROME P450IA1 APOPROTEIN LEVELS AFTER β NF INDUCTION**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours after being induced for 3 days with β NF (see Table 4 in the methods section); control mice were also induced with β NF and received an equal volume of sterile PBS. After electrophoretic separation of hepatic microsomes on a gel, a western blot was prepared and probed with an antibody directed against cytochrome P450IA1 apoprotein. C=control samples and T=treated samples.

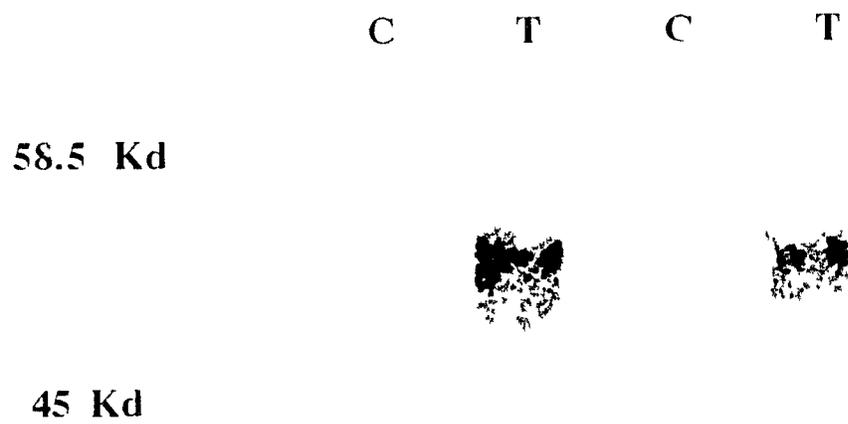


FIGURE 27

obvious that the quantity of IA1 apoprotein in the lanes containing samples from infected mice are significantly less than that in the control lanes. The magnitude of the difference was approximately equal to that which was observed for the cytochrome P450IA mRNA levels (Figures 23 and 24).

TIME COURSE OF THE EFFECT OF *IN VIVO* LISTERIA INFECTION ON CYTOCHROME P450IA1 mRNA LEVELS, APOPROTEIN CONTENT, AND EROD ACTIVITY AFTER BNF INDUCTION

To establish the time course for the effect of listeria infection on the loss of cytochrome P450IA mRNA and EROD activity, a series of mice were infected for 12, 24, 48, and 96 hours after BNF induction as described in Table 4 of the methods section. Duplicate slot blots were prepared for each time point and hybridized with the cytochrome P450IA cDNA and oligo(dT)₁₈ probes. EROD activity was determined in hepatic microsomes from the same mice. The results obtained in this time course experiment are summarized in Figure 28. Listeria produced an increase in the levels of cytochrome P450IA mRNA at t=12 hours, which was followed by a decrease at t=24 and t=48 hours. Cytochrome P450IA mRNA levels were not significantly different from control levels at t=96 hours. Listeria produced an increase in EROD activity at t=24 hours which was followed by a significant decrease at t=48 and t=96 hours. The same pattern was observed on a Western blot that summarizes the time course of the effect of listeria infection on cytochrome P450IA apoprotein levels (Figure 29).

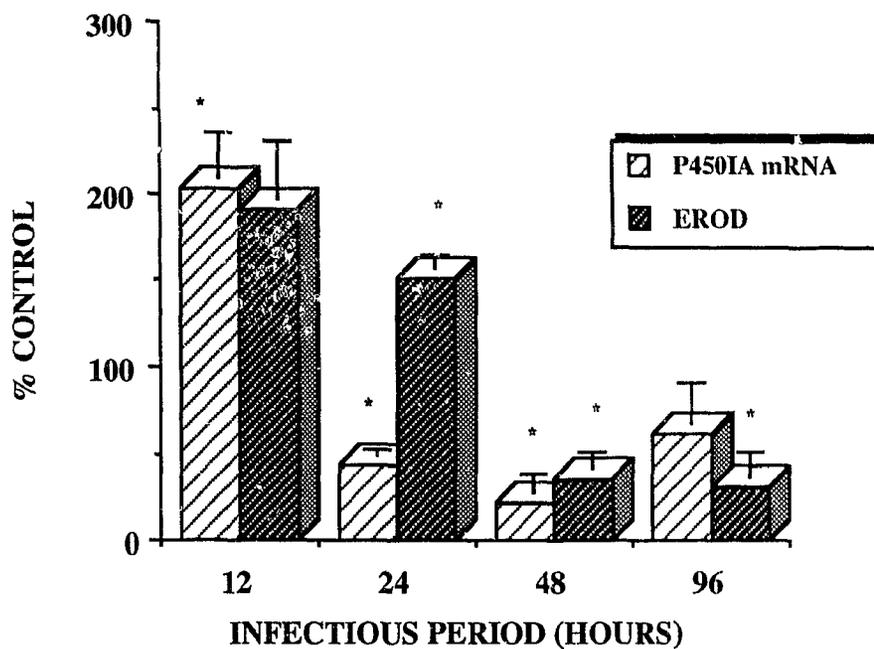


FIGURE 28

TIME COURSE OF THE EFFECT OF *IN VIVO* INFECTION WITH 15U LISTERIA ON CYTOCHROME P450IA mRNA LEVELS AND EROD ACTIVITY AFTER BNF INDUCTION

Mice were infected with 1×10^6 CFU of 15U listeria after induction with BNF as described in Table 4 of the methods section; control mice were also induced with BNF and received an equal volume of sterile PBS. EROD activity was determined in hepatic microsomes and cytochrome P450IA mRNA levels were determined from slot blots as described in Figure 26. All results are expressed as a % of control. The mean control EROD values for 12, 24, 48, and 96 hours were: 300 ± 94 , 430 ± 80 , 532 ± 113 , and 810 ± 158 pmoles resorufin/mg/min respectively. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test.

FIGURE 29**WESTERN BLOT - TIME COURSE OF THE EFFECT OF *IN VIVO* INFECTION WITH 15U LISTERIA ON CYTOCHROME P450IA1 APOPROTEIN LEVELS AFTER β NF INDUCTION**

Mice were infected with 1×10^6 CFU of 15U listeria after induction with β NF as described in Table 4 of the methods section; control mice were also induced with β NF and received an equal volume of sterile PBS. After electrophoretic separation of hepatic microsomes on a gel, a western blot was prepared and probed with an antibody directed against cytochrome P450IA1 apoprotein. A pair of samples for each time point are shown; C=control samples and T=treated samples.

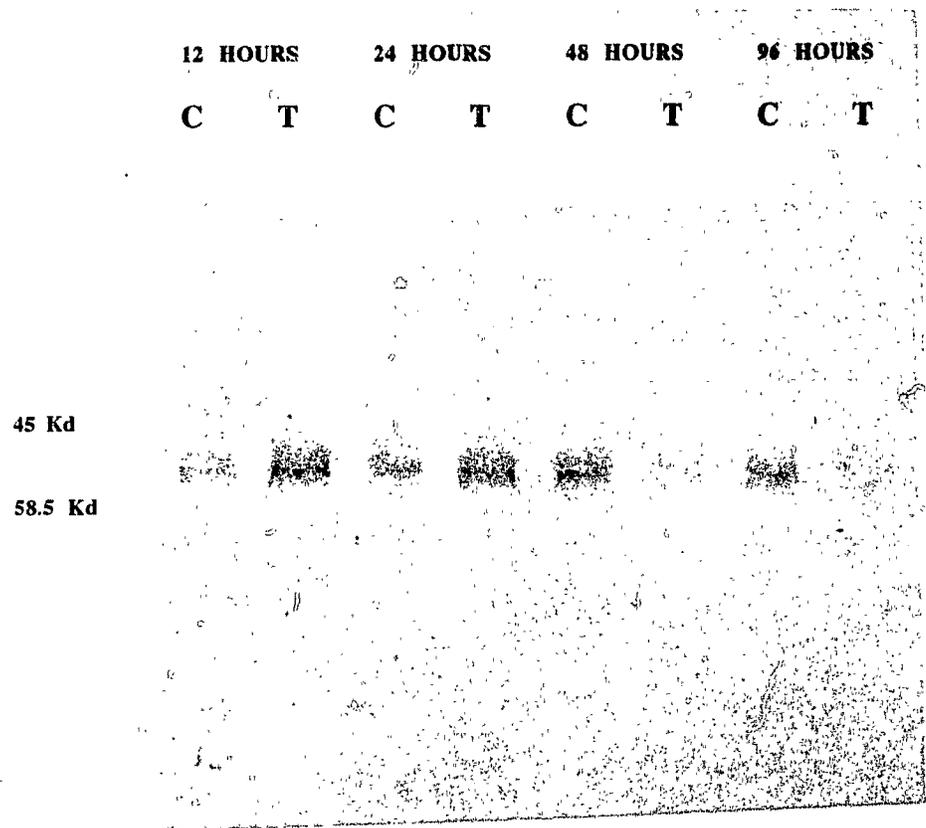


FIGURE 29

Twelve hours of listeria infection significantly increased P450IA mRNA levels but did not have any effect on total cytochrome P450, BROD, or PROD activities (Figure 30). Forty eight hours of listeria infection not only decreased cytochrome P450IA mRNA and EROD activity but also significantly decreased cytochrome P450 content along with BROD and PROD activities (Figure 31). The maximum suppression of EROD activity occurred when the liver load of the bacteria was at a maximum as shown in Figure 32.

To ensure equal loading of mRNA, the slot blots were hybridized with an oligo(dT)₁₈ probe which binds to the poly(A) tail of eukaryotic mRNA. The time course of the effect of listeria infection on cytochrome P450IA mRNA levels is presented with and without correction for oligo(dT)₁₈ binding (Figure 33), clearly demonstrating that exactly the same pattern is observed in both cases.

THE EFFECT OF 48 HOURS *IN VIVO* LISTERIA INFECTION ON CYTOCHROME P450IIC11 mRNA AND AMINOPYRINE N-DEMETHYLASE ACTIVITY

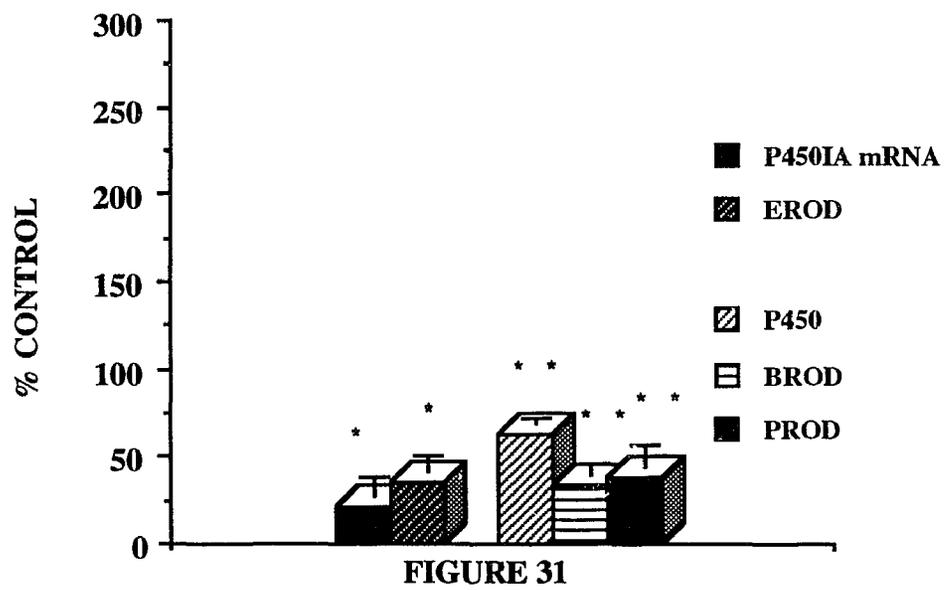
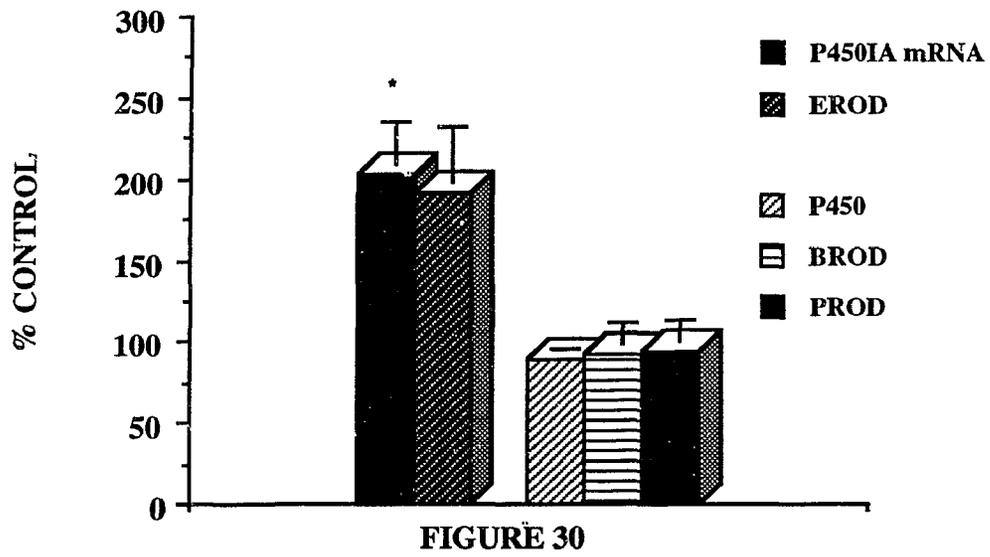
The effect of listeria infection on the levels of cytochrome P450IIC11, a male-specific isozyme that is constitutively expressed and that does not require induction for detection, was the focus of the next set of experiments. The effect of listeria infection on cytochrome P450IIC11 mRNA is illustrated in Figure 34 as a Northern blot containing from left to right: hepatic RNA from female mice in lanes 2 and 3, lanes 6-8 are from male mice treated for 48 hours with sterile PBS, and lanes 10-12 contain RNA from male mice treated for 48 hours with 15U listeria. The apparent molecular size of the target RNA was slightly greater than 1.4 kb (actual size of murine IIC11 mRNA is 1.6 kb). The mean intensity of lanes 10-12 (infected-male) is less than the mean intensity of lanes 6-8

FIGURE 30**EFFECT OF 12 HOURS *IN VIVO* LISTERIA INFECTION AFTER β NF INDUCTION ON CYTOCHROME P450IA mRNA, EROD, P450, BROD, AND PROD ACTIVITIES**

Mice were infected with 1×10^6 CFU of 15U listeria for 12 hours after being induced for 4 days with β NF (see Table 4 in the methods section); control mice were also induced with β NF and received an equal volume of sterile PBS. P450IA mRNA levels were determined from duplicate slot blots that were hybridized with a P450IA cDNA and standardized with an oligo(dT)18 probe. Hepatic microsomal cytochrome P450 content, EROD, BROD, and PROD were determined and expressed as a % of the mean control levels determined in mice treated for the same period with an equal volume of sterile PBS (n=6). The mean control levels were: EROD = 300 ± 94 pmoles resorufin/mg /min; cytochrome P450 = 0.83 ± 0.08 nmoles /mg protein; BROD = 19.3 ± 4.9 and PROD = 9.8 ± 2.2 pmoles resorufin/mg /min. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test (n=6).

FIGURE 31**EFFECT OF 48 HOURS *IN VIVO* LISTERIA INFECTION AFTER β NF INDUCTION ON CYTOCHROME P450IA mRNA, EROD, P450, BROD, AND PROD ACTIVITIES**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours after being induced for 4 days with β NF (see Table 4 in the methods section); control mice were also induced with β NF and received an equal volume of sterile PBS. P450IA mRNA levels were determined from duplicate slot blots that were hybridized with a P450IA cDNA and standardized with an oligo(dT)18 probe. Hepatic microsomal cytochrome P450 content, EROD, BROD, and PROD were determined and expressed as a % of the mean control levels determined in mice treated for the same period with an equal volume of sterile PBS (n=5). The mean control levels were: EROD = 532 ± 113 pmoles resorufin/mg /min; P450 = 0.74 ± 0.03 nmoles /mg protein; BROD = 15.0 ± 2.0 and PROD = 9.2 ± 1.0 pmoles resorufin/mg /min. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test (n=5).



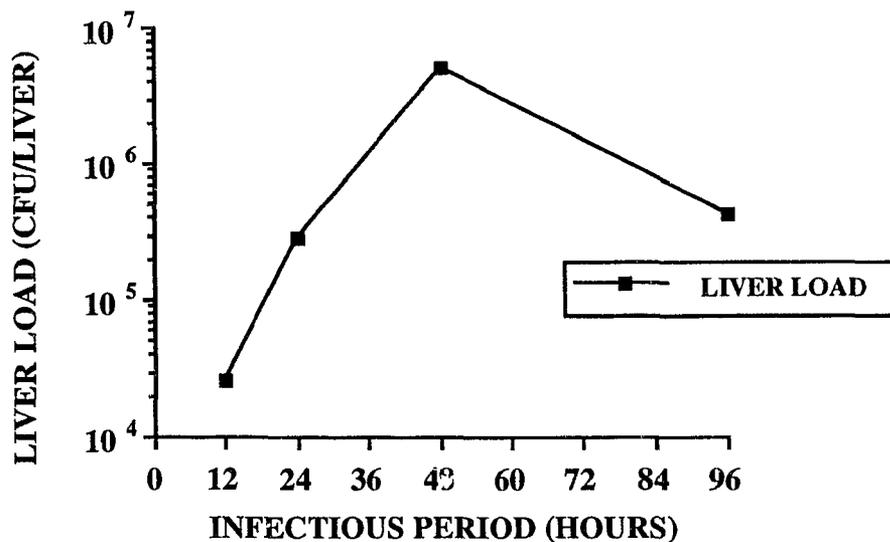


FIGURE 32

TIME COURSE OF BACTERIAL LIVER LOAD DURING INFECTION WITH 15U LISTERIA AFTER BNF INDUCTION

Mice were infected with 1×10^6 CFU of 15U listeria after induction with BNF as described in Table 4 of the methods section; control mice were also induced with BNF and received an equal volume of sterile PBS. Liver loads of the bacteria were determined from blood agar plates containing aliquots of the liver homogenates. Each point represents the mean liver load of 5 mice for that infection period.

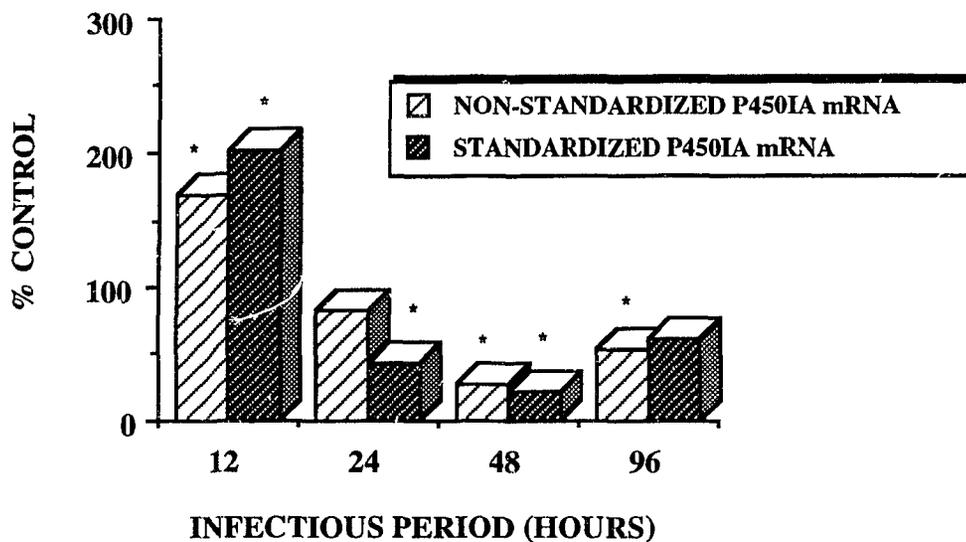


FIGURE 33

TIME COURSE OF THE EFFECT OF *IN VIVO* INFECTION WITH 15U LISTERIA AFTER β NF INDUCTION ON CYTOCHROME P450IA mRNA LEVELS

EXPRESSED WITH AND WITHOUT CORRECTION FOR OLIGO(dT)₁₈ BINDING

Mice were infected with 1×10^6 CFU of 15U listeria after induction with β NF as described in Table 4 of the methods section; control mice were also induced with β NF and received an equal volume of sterile PBS. Standardized cytochrome P450IA mRNA levels were determined from duplicate slot blots that were hybridized with a cytochrome P450IA cDNA and standardized with an oligo(dT)₁₈ probe. Non-standardized cytochrome P450IA mRNA levels were determined from slot blots that were hybridized only with a cytochrome P450IA cDNA. All results are expressed as a % of control. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test.

FIGURE 34**NORTHERN BLOT - EFFECT OF 48 HOURS *IN VIVO* LISTERIA INFECTION ON
CYTOCHROME P450IIC11 mRNA LEVELS**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours, control mice received an equal volume of sterile PBS. Lanes contain 20 μ g of total RNA as follows: from left to right, lanes 2 and 3 are female mice, lanes 6-8 are from male mice treated for 48 hours with sterile PBS, and lanes 10-12 contain RNA from male mice treated for 48 hours with 15U listeria. The blot was hybridized with a cDNA directed against cytochrome P450IIC11 mRNA.

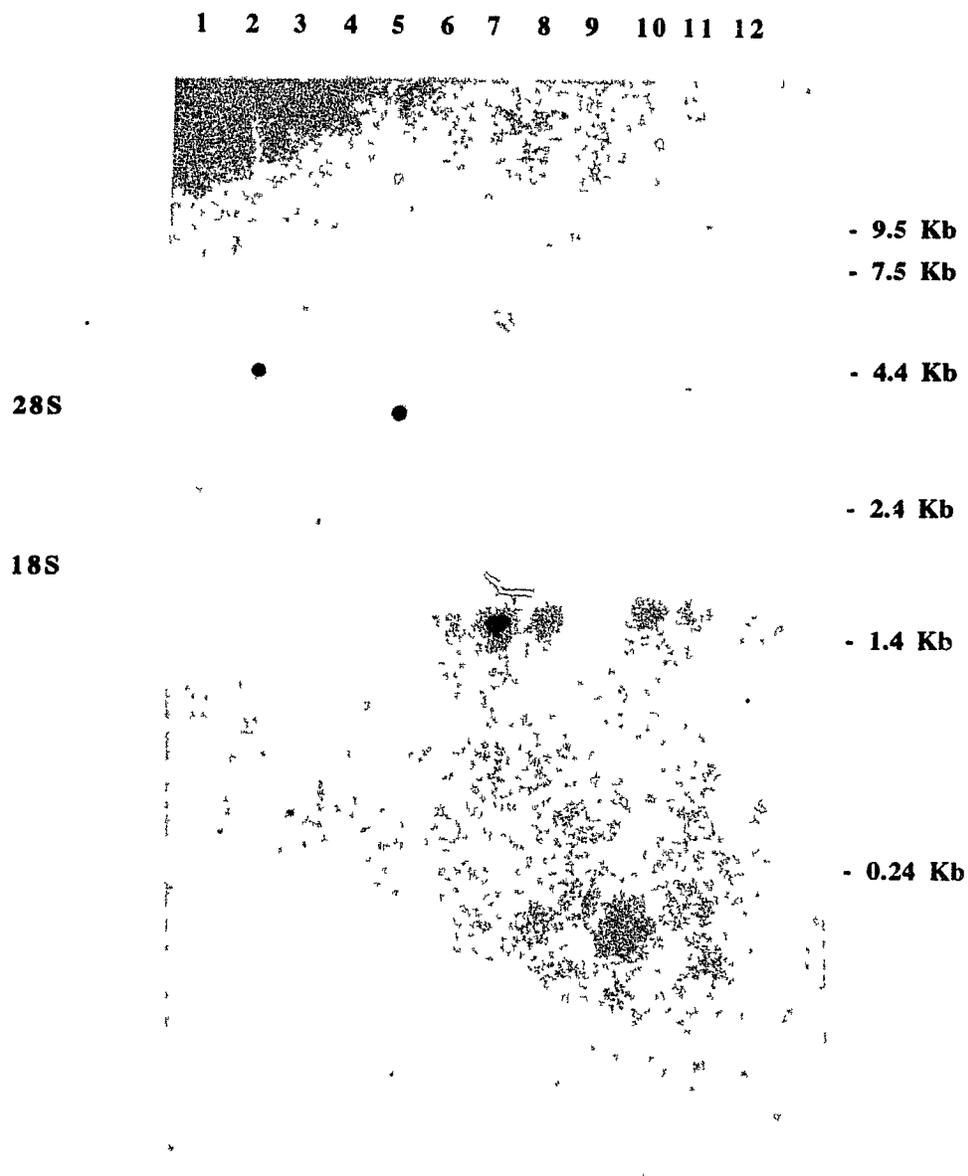


FIGURE 34

(control -male); virtually no binding occurred in lanes 2 and 3 which contain RNA from female mice.

To quantitate the magnitude of the difference, slot blots were prepared and hybridized with the cytochrome P450IIC11 cDNA and standardized with the oligo(dT)₁₈ probe (Figures 35 and 36). The intensities of the two treated lanes were less than the three control lanes for the slot blot probed with the cytochrome P450IIC11 cDNA (Figure 35). The first slot load for these blots was 20 µg along with serial 1:2 dilutions thereof. The intensity of the 20 µg slot of the two treated samples corresponded approximately with the 10 µg slot of the three control samples (50% reduction). However, the intensities of all rows were approximately equal when the slot was hybridized with the oligo(dT)₁₈ probe (Figure 36). *Listeria* caused a significant depression in cytochrome P450IIC11 mRNA, aminopyrine N-demethylase, and total cytochrome P450 content as shown in Figure 37. Cytochrome P450IIC11 shows high activity for aminopyrine N-demethylation in the rat (Morgan et al, 1985; Chang, 1991), and was therefore used to assess the functional status of this isozyme during listeriosis.

TIME COURSE OF THE EFFECT OF *IN VIVO* LISTERIA INFECTION ON CYTOCHROME P450IIC11 mRNA LEVELS

To establish the time course for the loss of cytochrome P450IIC11 mRNA and aminopyrine N-demethylase activity, mice were infected for 12, 48, or 96 hours. The results obtained in this time course are summarized in Figure 38. *Listeria* infection for 12 hours did not effect IIC11 mRNA levels nor did it have any effect on aminopyrine N-demethylase activity. Infection for 48 hours resulted in a significant decrease in IIC11 mRNA and aminopyrine N-demethylase activity; the IIC11 mRNA levels recovered to

FIGURE 35**SLOT BLOT - EFFECT OF 48 HOURS *IN VIVO* LISTERIA INFECTION ON
CYTOCHROME P450IIC11 mRNA LEVELS**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours, control mice received an equal volume of sterile PBS. Each lane contains serial 1:2 dilutions of sample beginning with 20 μ g of total RNA. C=control samples, T=treated samples, and S=standard. The slot blot was hybridized with a cDNA directed against cytochrome P450IIC11 mRNA.

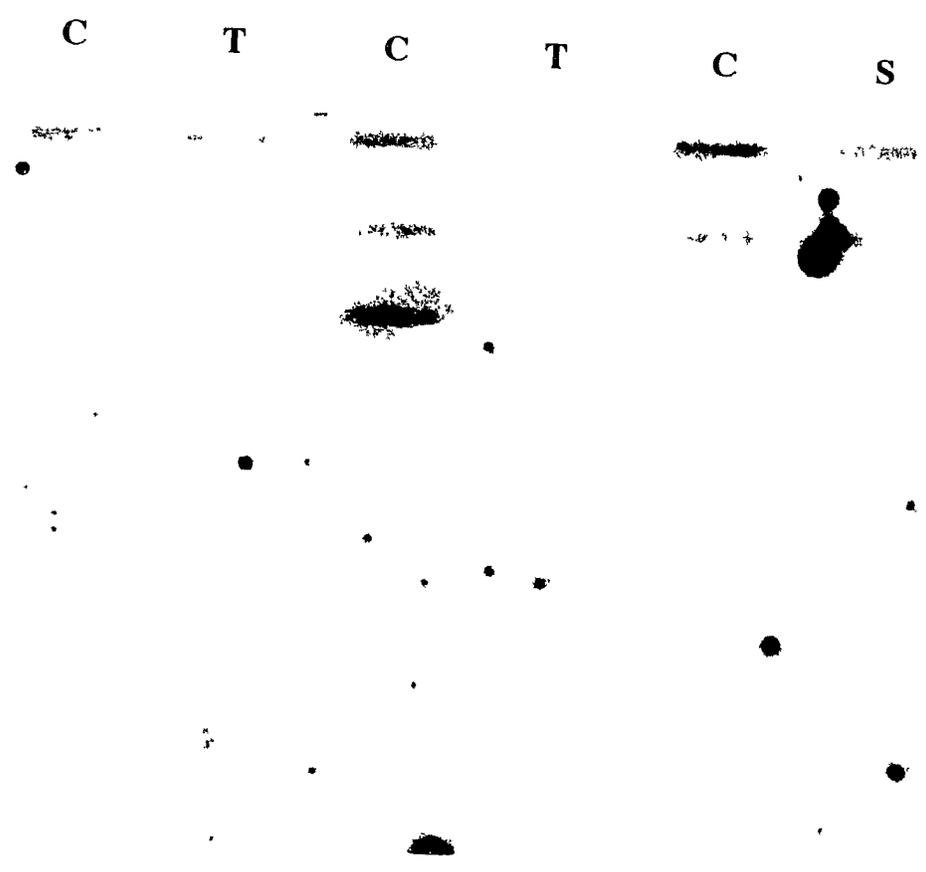


FIGURE 35

FIGURE 36**SLOT BLOT - EFFECT OF 48 HOURS *IN VIVO* LISTERIA INFECTION ON TOTAL mRNA LEVELS**

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours, control mice received an equal volume of sterile PBS. Each lane contains serial 1:2 dilutions of sample beginning with 20 μ g of total RNA. C=control samples, T=treated samples, and S=standard. The slot blot was hybridized with an oligo(dT)₁₈ probe.

C T C T C S

FIGURE 36

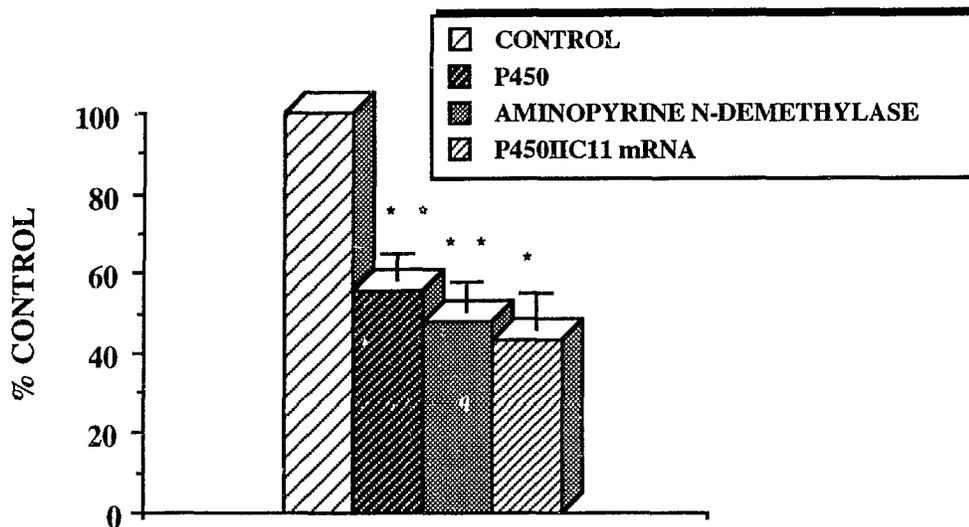


FIGURE 37

EFFECT OF 48 HOURS *IN VIVO* INFECTION WITH 15U LISTERIA ON
CYTOCHROME P450, AMINOPYRINE N-DEMETHYLASE AND CYTOCHROME
P450IIC11 mRNA LEVELS

Mice were infected with 1×10^6 CFU of 15U listeria for 48 hours, control mice received an equal volume of sterile PBS. Cytochrome P450 levels and aminopyrine N-demethylase activities were determined in hepatic microsomes, and cytochrome P450IIC11 mRNA levels were determined from duplicate slot blots that were hybridized with a cytochrome P450IIC11 cDNA and standardized with an oligo(dT)₁₈ probe. All results are expressed as a % of control levels. The mean control cytochrome P450 was 0.48 ± 0.02 nmoles/mg protein, the mean control aminopyrine N-demethylase activity was 260 ± 10 nmoles HCHO/mg/hour, and the mean control cytochrome P450IIC11 mRNA level was 2.1 ± 0.4 (arbitrary units). Statistical analysis was carried out on the raw data using an unpaired Student's *t* test ($n=5$).

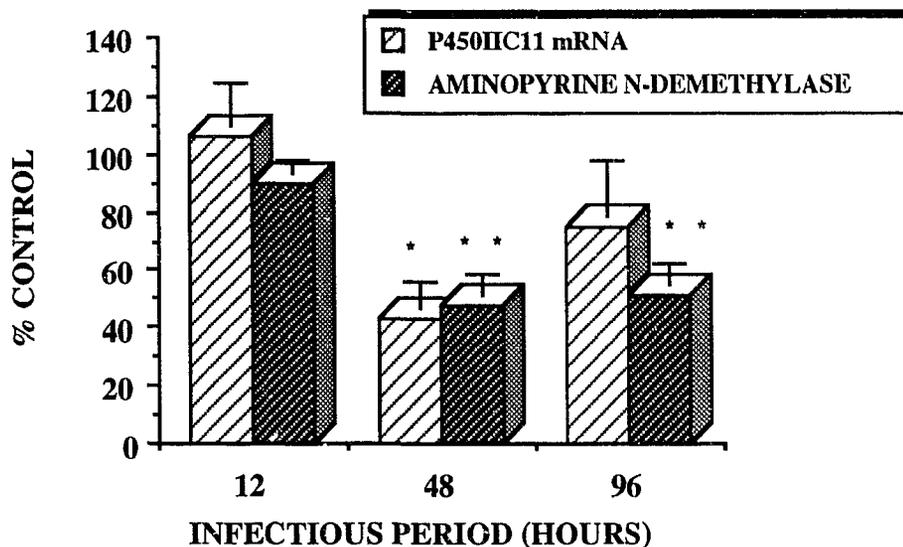


FIGURE 38

TIME COURSE OF THE EFFECT OF *IN VIVO* LISTERIA INFECTION ON CYTOCHROME P450IIC11 mRNA LEVELS AND AMINOPYRINE N-DEMETHYLASE ACTIVITIES

Mice were infected with 1×10^6 CFU of 15U listeria for 12, 48, and 96 hours; control mice received an equal volume of sterile PBS. Aminopyrine N-demethylase activities were determined in hepatic microsomes, and cytochrome P450IIC11 mRNA levels were determined from duplicate slot blots that were hybridized with a cytochrome P450IIC11 cDNA and standardized with an oligo(dT)₁₈ probe. All results are expressed as a % of control levels. The mean control aminopyrine N-demethylase activities at t=12, 48, and 96 hours were: 255 ± 8 , 260 ± 10 , and 267 ± 14 nmoles HCHO/mg/hour respectively. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test (n=5).

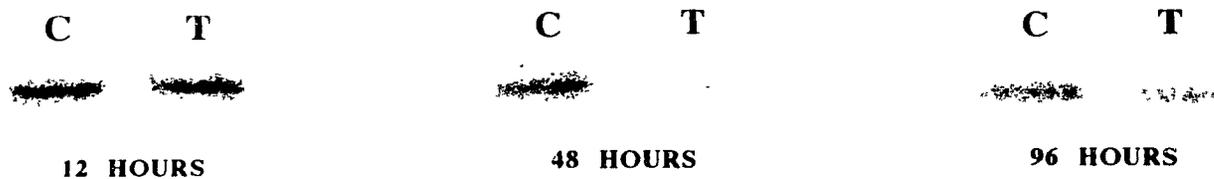
normal levels by $t=96$ hours but aminopyrine N-demethylase activity was still significantly reduced at this time point. A representative pair of samples for each slot blot is shown in Figure 39. The upper and lower panels represent RNA samples hybridized with the cytochrome P450IIC11 cDNA and oligo(dT)₁₈ probes respectively. The liver load of the bacteria for the same time course is shown in Figure 40.

To ensure equal loading of mRNA, the slot blots were hybridized with an oligo(dT)₁₈ probe which binds to the poly(A) tail of eukaryotic mRNA. The time course of the effect of listeria infection on cytochrome P450IIC11 mRNA levels is presented with and without correction for oligo(dT)₁₈ binding (Figure 41), clearly demonstrating that the same pattern is observed in both cases.

FIGURE 39**TIME COURSE OF THE EFFECT OF 15U LISTERIA ON CYTOCHROME P450IIC11 AND TOTAL mRNA**

Mice were infected with 1×10^6 CFU of 15U listeria for 12, 48, or 96 hours. Duplicate slot blots were prepared for each time point and hybridized with the cytochrome P450IIC11 cDNA and oligo(dT)₁₈ probes respectively. The upper and lower panels contain a representative pair of samples for each time point hybridized to the cytochrome P450IIC11 cDNA and oligo(dT)₁₈ probes respectively; C=control samples and T=treated samples.

CYTOCHROME P450IIC11 PROBE



OLIGO(dT)₁₈ PROBE

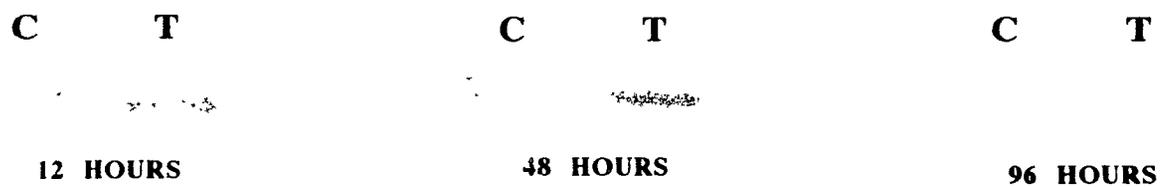


FIGURE 39

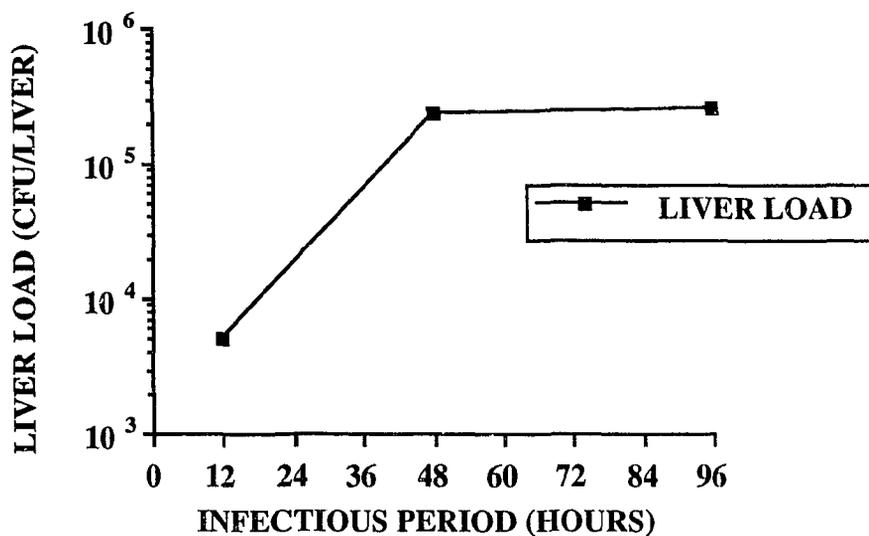


FIGURE 40

**TIME COURSE OF BACTERIAL LIVER LOAD DURING INFECTION WITH 15U
LISTERIA**

Mice were infected with 1×10^6 CFU of 15U listeria for 12, 48, and 96 hours; control mice received an equal volume of sterile PBS. Liver loads of the bacteria were determined from blood agar plates containing aliquots of the liver homogenates. Each point represents the mean liver load of 5 mice for that infection period.

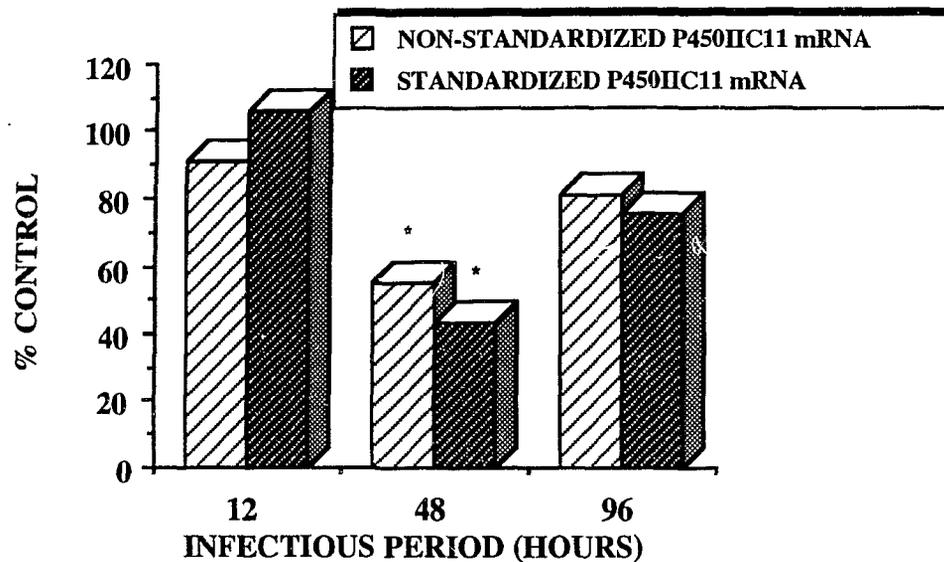


FIGURE 41

TIME COURSE OF THE EFFECT OF *IN VIVO* INFECTION WITH 15U LISTERIA ON CYTOCHROME P450IIC11 mRNA LEVELS EXPRESSED WITH AND WITHOUT CORRECTION FOR OLIGO(dT)₁₈ BINDING

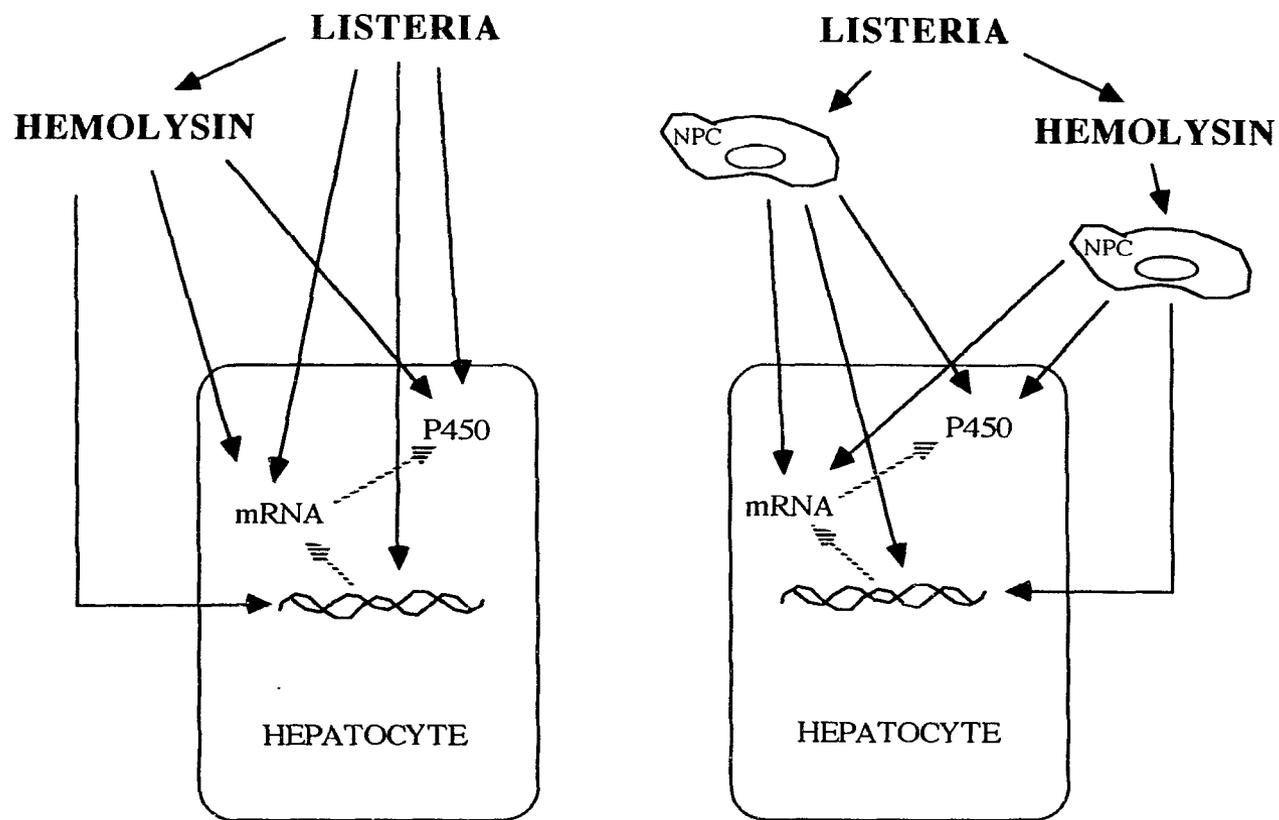
Mice were infected with 1×10^6 CFU of 15U listeria for 12, 48, and 96 hours; control mice received an equal volume of sterile PBS. Standardized cytochrome P450IIC11 mRNA levels were determined from duplicate slot blots that were hybridized with a cytochrome P450IIC11 cDNA and standardized with an oligo(dT)₁₈ probe. Non-standardized cytochrome P450IIC11 mRNA levels were determined from slot blots that were hybridized only with a cytochrome P450IIC11 cDNA. All results are expressed as a % of control. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test ($n=5$).

DISCUSSION

In a large number of studies, a variety of infections and modes of immune stimulation have been shown to effect cytochrome P450-mediated metabolism (Table 3). We are aware of only three published studies with rodents and two with humans regarding the effect of active bacterial infections on cytochrome P450. As indicated in the introduction of this thesis, the mechanism by which viral infections, cloned interferons, and LPS (from gram (-) bacteria) decrease hepatic cytochrome P450 levels is reasonably well established. The mechanism by which an active infection with a gram (+) bacterium can decrease cytochrome P450 levels was not known prior to the commencement of this thesis.

The working hypothesis contained a number of possible pathways by which cytochrome P450 levels could be diminished during listeriosis (Figure 42). The mechanism may have involved a direct effect of listeria and/or the hemolysin it secretes on the endoplasmic reticulum of parenchymal cells resulting in the degradation of cytochrome P450. Alternatively, the effect could result from an interaction of listeria and/or the hemolysin with nonparenchymal cells and the subsequent release of a factor that acts on the hepatocyte. This loss could also result from an inhibition of one or more of the steps involved in the synthesis of cytochrome P450 apoprotein. The experiments described in the thesis were designed to determine which possibilities played key roles in the depression of hepatic microsomal cytochrome P450 that was observed during Listeria monocytogenes infection in mice.

The initial studies were designed to establish the time course of the effect of 15U listeria on cytochrome P450-mediated metabolism and to establish whether or not the effect was isozyme specific. The administration of a sublethal dose of the hemolytic 15U strain



ABBREVIATIONS : NPC : NONPARENCHYMAL CELL

FIGURE 42 PATHWAYS FOR THE MECHANISM OF LISTERIA-MEDIATED DEPRESSION OF HEPATIC CYTOCHROME P450

of Listeria monocytogenes produced a significant reduction in total hepatic microsomal cytochrome P450 content after 48 hours of infection and this suppression was maintained for at least 3 days. After 8 days, cytochrome P450 levels in treated mice had returned to control levels and bacterial colonies of listeria could no longer be detected in the liver. The virulent strain of listeria not only decreased total hepatic microsomal P450 content but it also significantly reduced the capacity of this enzyme system to catalyze various reactions such as EROD, BROD, PROD, and aminopyrine N-demethylase. These reactions are catalyzed by a series of different isozymes in uninduced mice (see Methods section), and the results indicated that listeria infection produces a general effect on numerous if not all cytochrome P450 isozymes. A generalized effect on all isozymes has also been observed in virtually all of the studies carried out with interferon, interferon-inducers, and endotoxin in uninduced animals that have been reported to date (Table 3), but there have been a few notable exceptions. Recently, Stanley et al (1991) reported that interferon- α did not have a significant effect on benzphetamine N-demethylase or BROD activity in mice, and Zerkle et al (1980) had previously reported that poly rI \cdot rC selectively depressed only 3 of 6 electrophoretically separated species of cytochrome P450 that unfortunately were not identified. Although the administration of recombinant rat interferon- γ or naturally derived rat interferon α/β resulted in a significant decrease in the activity of cytochrome P450IIB1/2 and IIIA, in the same study it had no effect on cytochrome P450IIC11 or IIA after its administration to rats (Craig et al, 1990).

In the present study, listeria infection did not cause overt liver damage and was not capable of degrading cytochrome P450 apoproteins *in vitro*. The direct incubation of 15U Listeria monocytogenes with hepatic microsomes for 24 hours had no effect on cytochrome P450 content, EROD, BROD, or PROD activities, a result which is consistent with the previous report that interferon and endotoxin also have no effect when incubated directly

with hepatic microsomes (Parkinson et al, 1982; Egawa and Kasai, 1979). Histological examination of liver sections during listeria infection did not indicate any change in the appearance of the endoplasmic reticulum. In some sections, bacteria were present in sinusoidal cells but bacteria were not observed intrahepatocellularly. These observations indicated that the mechanism by which 15U listeria decreases P450-mediated metabolism cannot be due to a direct destruction of P450 in the endoplasmic reticulum, nor is it merely an extension of overt liver damage. The mechanism is also not likely to result from generalized illness in the mouse, as the infected mice did not display any obvious behavioral changes and no differences were observed in animal or liver weights.

To determine if 15U listeria was capable of suppressing cytochrome P450 levels in hepatocytes maintained in culture, bacteria were incubated with liver cell cultures in the presence and absence of hepatic nonparenchymal cells. *In vivo* studies established that 15U listeria decreased cytochrome P450-mediated metabolism within 48 hours and cytochrome P450IA mRNA levels within 24 hours. The immunological response to listeria during the first 48 hours involves neutrophils, Kupffer cells, and monocytes but does not involve T cells which are not generated until after day 2 (MacKanness, 1962; North, 1970; Campbell, 1988; Czuprynski et al, 1984; Mitsuyama et al, 1978; Kongshavn and Skamene, 1984; Kauffman, 1988; Dunn and North, 1991a). Therefore, the proliferation of antigen-specific T cells is not required for the depressant effect of listeria on cytochrome P450 that is observed within the first 48 hours. If the *in vivo* effect of listeria is predominantly due to a direct effect on the hepatocyte and/or if it is the result of an interaction of listeria with hepatic nonparenchymal cells, the incubation of listeria with mixed liver cell preparations *in vitro* should also produce a depression of cytochrome P450-mediated metabolism.

One of the principal limiting factors of *in vitro* studies with hepatocytes is the reported rapid loss of cytochrome P450 content, as the half-life of cytochrome P450 in

adherent cultures (the cells remain adherent to the substratum) of mouse hepatocytes at 37°C has been reported to range from 12 - 20 hours (Renton et al, 1978b; Maslansky and Williams, 1982). The loss of cytochrome P450 in the present studies proved to be even more rapid when hepatocytes were maintained in suspension at 37°C (Figure 11). In addition, the cells were subject to rapid decreases in viability that resulted from cell clumping. At 25°C, cell clumping was minimized and adequate maintenance of cytochrome P450 was achieved for the duration of the experiments. A recent report by Blankson et al (1991) also reported an attenuation of the loss of cytochrome P450 in adherent cultures of rat hepatocytes maintained at 30°C.

In mixed liver cell suspensions (hepatocytes + nonparenchymal cells) maintained at 25°C, 15U listeria caused a 40% reduction in the cytochrome P450 content and a 20-30% decrease in EROD and BROD activities of hepatocytes after a 24 hour *in vitro* incubation. Listeria had no significant effect on total cell protein or viability. This was consistent with the effect of *in vivo* listeria infection except that the magnitude of the response was slightly smaller and a significant effect occurred within 24 hours. This more rapid onset of the effect is not surprising as the *in vitro* model involves the direct addition of a high dose of the bacteria to liver cells which contrasts with the *in vivo* situation in which liver burden actually decreases during the first 6 hours and does not reach a peak until day 2-4 (Mitsuyama et al, 1978). Optimum growth of Listeria monocytogenes occurs at 30-37°C but it will grow at temperatures ranging from 3 to 45°C (Gray and Killinger, 1966). At 22°C, listeria grows at approximately 50% of the rate observed at 37°C (Stecha et al, 1989). Furthermore, human monocytes have been shown to exhibit greater listericidal activity when they were maintained in suspension rather than adherent to glass coverslips (Czuprynski et al, 1984). Therefore, the decreased magnitude of the response observed *in vitro* at 25°C is not due to an inability of the bacteria to grow at 25°C, nor is it due to an inability of liver

macrophages or neutrophils to phagocytose listeria when in suspension. These cell suspensions contained hepatocytes, Kupffer cells, and other nonparenchymal cells and therefore this system did not allow for us to distinguish whether that the effect was direct on the hepatocyte or involved an indirect interaction with nonparenchymal cells. Ideally, the next logical step would have been to infect a pure fraction of isolated hepatocytes maintained in suspension at 25°C with 15U listeria. Unfortunately, cell clumping in such preparations did not allow for this set of experiments to be completed. This required that subsequent cell culture experiments be carried out in adherent liver cell cultures in which the liver cells remain fixed to the substratum. Nevertheless, the studies at 25°C allowed us to conclude that the effect of listeria infection on cytochrome P450 is predominantly due to an interaction within the liver and does not require the influence of extrahepatic cells or factors.

In order to determine which cell types were involved in the listeria-mediated decrease in hepatic cytochrome P450, a series of experiments were designed that involved the incubation of 15U listeria with pure fractions of isolated hepatocytes compared to cultures containing nonparenchymal cells in addition to hepatocytes. These cultures were prepared as outlined in Figure 1 of the methods section and contained only cell types capable of adhering to the surface of the culture flasks. This cell culture system circumvents problems of cell clumping but leads to more rapid loss of cytochrome P450, as the incubation temperature was 37°C. The 15U strain of listeria produced a 25% loss of EROD activity when incubated with cultures containing hepatocytes and nonparenchymal cells but had no effect on EROD activity in pure hepatocyte cultures. The loss of EROD activity is therefore most likely due to an indirect effect of listeria, and/or the hemolysin it secretes, that involves the nonparenchymal cells. The adherent nonparenchymal cells are most likely predominantly Kupffer cells and thus this data is consistent with a mechanism that involves the release of a factor from Kupffer cells as previously suggested by Peterson and Renton

(1984). In contrast, BROD activity was decreased by 25-30% in both cultures containing hepatocytes and nonparenchymal cells as well as in cultures of pure hepatocytes. BROD activity can therefore be depressed as a result of a direct action of the bacteria and/or the hemolysin it secretes on the hepatocyte itself. These studies established that listeria is capable of suppressing cytochrome P450-mediated metabolism in the hepatocyte by both direct and indirect mechanisms. The differential effect suggests varying susceptibilities of different isozymes of cytochrome P450. The effect of 15U listeria on EROD and BROD activity occurred in the absence of a significant change in viability or total protein content consistent with the observation *in vivo* that the effect is not due to a generalized 'toxic' effect on the hepatocyte.

Previous studies from our laboratory have demonstrated that factors released from phagocytic cells are capable of suppressing cytochrome P450 in hepatocytes. The incubation of Kupffer cells with dextran sulfate or latex particles on one side of a Marbrook vessel for 16 hours resulted in a significant decrease in the cytochrome P450 content of hepatocytes maintained on the other side of the chamber (Peterson and Renton, 1984; Peterson and Renton, 1986a); no effect was observed after direct incubation with hepatocytes. When the supernatants of cultures of human monocytes and LPS were incubated with rat hepatocytes, a significant reduction in ethoxycoumarin deethylase activity resulted (Ghezzi et al, 1986b). LPS was shown to have no effect when incubated directly with isolated rat hepatocytes. Monocytes and Kupffer cells have been shown to synthesize and release IL-1 in response to endotoxin and IL-1 receptors have been demonstrated on rat hepatocytes (Dinarello, 1984; Sujita et al, 1990). Purified human IL-1 can depress ethoxycoumarin deethylase activity when incubated directly with rat hepatocytes for 18 hours (Ghezzi et al, 1986b). This suggested that monocyte or macrophage-derived cytokines such as interleukin-1 may mediate the depression of cytochrome P450 that is

observed after immune stimulation with agents such as endotoxin. Endotoxin has also been shown to stimulate the *in vitro* release of TNF from murine peritoneal macrophages (Beulter et al, 1986). Bertini et al (1989) demonstrated that the supernatants from cultures of human monocytes, incubated with recombinant human TNF- α , were capable of suppressing ethoxycoumarin deethylase activity after their addition to isolated hepatocytes. Another cytokine, also known to be released from monocytes and macrophages in response to endotoxin, is interleukin-6 (Heinrich et al, 1990) and it also has been shown to decrease cytochrome P450 content after incubation with isolated mouse hepatocytes (Bryden et al, 1991). This evidence suggests that many factors released from phagocytic cells are capable of suppressing drug metabolism in hepatocytes and support the idea that infective agents can lower cytochrome P450 in the hepatocyte by an indirect action on other cell types.

The capacity of peritoneal macrophages to produce IL-1 and interferon has been shown to increase through the course of listeria infection (Petit et al, 1988; Buchmeier and Schreiber, 1985); TNF and IL-6 are also produced during listeria infection (Havell, 1987; Havell and Sehgal, 1991) but their respective sources were not identified. The cytokines released by listeria-stimulated Kupffer cells have not been identified but it is possible that a cytokine is involved in the Kupffer cell-mediated suppression of EROD activity.

It is known that certain isozymes have different stabilities in hepatocyte cultures (Guguen-Guillouzo and Guillouzo, 1983; Steward et al, 1985; Lindsay et al, 1989). In rat hepatocytes, the levels of cytochrome P450IIC11 and IIC6 apoprotein do not decrease during the first 24 hours whereas cytochrome P450IIA1 apoprotein decreases by greater than 80% during this period. Nakajima et al (1990) provided evidence for a role of cytochrome P450IIC11/IIC6 in supporting EROD activity in control rat microsomes. If the same isozyme pattern holds true in mice then it is possible that EROD activity in hepatocytes is supported by relatively stable isozymes. The isozymes that support BROD

activity in control mouse hepatocytes have not been clearly identified. It is possible that there is a relationship between isozyme stability in culture and susceptibility to a direct effect of 15U listeria but it is not clear how such a relationship could be conferred.

Independent of a direct or indirect effect and independent of the involvement of hemolysin, the mechanism involved in the decrease in cytochrome P450 was the focus of the final section of the thesis. Ethoxyresorufin is preferentially metabolized by cytochrome P450IA1 in mice induced with a '3-Methylcholanthrene-type' (3-MC) inducer such as β -naphthoflavone. After induction with β -naphthoflavone, listeria caused a 65% reduction in EROD activity within 48 hours of infection. The amount of P450IA1 apoprotein was also significantly decreased as measured by Western blot analysis using an antibody directed against cytochrome P450IA1. The apparent molecular weight of the protein bound to the antibody was consistent with the molecular weight of murine cytochrome P450IA1 (55 kd) and appeared as a single band (Negishi and Nebert, 1979); our laboratory has previously shown a lack of any significant binding on Western blots containing microsomes from uninduced mice (unpublished results). This indicates that the apoprotein identified by the antibody was indeed cytochrome P450IA1. The decreases in EROD activity and cytochrome P450IA1 apoprotein were not accompanied by a change in microsomal or total liver protein concentrations, consistent with a relatively selective effect of the bacteria.

The loss of EROD activity and cytochrome P450IA1 apoprotein could have resulted from a decrease in its rate of synthesis and/or an increase in its rate of degradation. Previous reports from our laboratory have suggested that the depression of hepatic cytochrome P450 during immune stimulation is due to an inhibition of synthesis and is not the result of destruction of cytochrome P450 apoprotein (Moochala et al, 1989; Moochala and Renton, 1991a). To determine definitively if listeria causes a suppression of apoprotein synthesis that results from a loss of mRNA, hepatic RNA was measured after various

infectious periods. A significant depression of cytochrome P450IA1 mRNA was evident after 48 hours of infection with 15U listeria in β NF-induced mice. A Northern blot revealed that listeria infection decreased cytochrome P450IA1 mRNA levels to approximately 10% of control levels. The target mRNA to which the cDNA oligomer hybridized had an apparent molecular size of 2.4 kb consistent with the actual size of murine cytochrome P450IA1 mRNA which is 2.6 kb (Kimura et al, 1984). The binding was specific and no bands were seen in regions that correspond to murine cytochrome P₂450 and P₃450 mRNA (P450IA2) which have molecular sizes of 1.8 and 1.9 kb respectively (Kimura et al, 1984; Genbank - Beckman's MicroGenie™, 1991). Very little hybridization was evident in control mice that were not induced with β -naphthoflavone consistent with the findings of Kimura et al (1986). The magnitude of the suppression of cytochrome P450IA1 mRNA after 48 hours of infection was approximately 80% as determined by slot blot analysis and could entirely account for the loss of apoprotein and EROD activity that was observed at the same time. This study therefore demonstrates that listeria decreases cytochrome P450IA1 levels predominantly by a pretranslational mechanism. A previous report by Morgan (1989) documented a similar loss of cytochrome P450IIC11 mRNA after the administration of endotoxin to control rats, and poly rI•rC has been shown to cause a significant depression of cytochrome P450IVA in clofibrate-induced rats (Renton and Knickle, 1990). This is the first report that we are aware of that conclusively demonstrates that an active infection of any kind results in a significant loss of cytochrome P450 mRNA.

Listeria infection did not affect total hepatic mRNA levels as determined by oligo(dT)₁₈ hybridization. This demonstrates that listeria has a selective effect on hepatic protein synthesis. The rate of synthesis of many hepatic proteins appears to be unaffected while the synthesis of some proteins such as cytochrome P450 are significantly depressed.

This type of selectivity has also been previously documented following the administration of poly rI•rC and recombinant interferon (Singh and Renton, 1984; Moochala and Renton, 1991a). These studies demonstrated that the total incorporation of radiolabelled amino acids into the microsomal fraction was unaffected while those microsomal proteins that had molecular weights consistent with those of cytochrome P450 were significantly depressed.

If the effect of listeria results predominantly from a decrease in cytochrome P450 mRNA levels, then the decrease in mRNA levels must be observed prior to the loss of apoprotein and/or enzyme activity. The time lag between mRNA and apoprotein loss would depend on the half-life of the isozyme of interest. In β NF-treated rats, most isozymes have half-lives ranging from 15-37 hours (Shiraki and Guengerich, 1984). To assess the time course of the effect of listeria, a series of mice were induced with β -naphthoflavone and were then infected for 12, 24, 48 or 96 hours. A significant increase in cytochrome P450IA1 mRNA was observed after 12 hours of infection, accompanied by a 2-fold increase in EROD activity. This effect was a selective effect on cytochrome P450IA1 as listeria had no effect on total cytochrome P450 content, BROD or PROD activities at this time. This apparent potentiation of induction by immune stimulation has been observed by others. Ragland et al (1971) observed a potentiation of the inducing effect of DDT on ethylmorphine N-demethylation during hepatitis virus infection in mallard ducks, Stanley and co-workers (1988) reported a potentiation of 3MC-mediated induction of EROD activity, cytochrome P450IA mRNA and apoprotein, in rats challenged with endotoxin, and Kato et al (1963) reported enhanced phenobarbital induction in mice infected with murine hepatitis virus. Hepatic microsomal EROD activity was significantly increased after 24 hours of infection as a result of the increase in cytochrome P450IA1 mRNA at t=12 hours, but EROD activity was significantly reduced after 48 and 96 hours of infection. The time course of listeria infection on cytochrome P450IA1 apoprotein content paralleled the effect

on EROD activity. Hepatic cytochrome P450IA1 mRNA was significantly reduced after 24 and 48 hours of infection and had returned to control levels by 96 hours. This entire time course clearly demonstrates that any changes in mRNA preceded the changes in EROD activity and also shows that mRNA levels return to normal levels prior to the reversal of the effect on EROD activity. Therefore, the results clearly indicate that the decrease in EROD activity that was observed during Listeria monocytogenes infection occurs by a pretranslational mechanism in β NF-induced mice.

Further studies indicated that Listeria monocytogenes infection also decreased another isozyme cytochrome P450IIC11 by a pretranslational mechanism. Cytochrome P450IIC11 is a male-specific isozyme that is constitutively expressed. Aminopyrine N-demethylase activity and the levels of cytochrome P450IIC11 mRNA were decreased by approximately 50% after a 48 hour listeria infection. A single band was observed on a Northern blot indicating an apparent molecular size of 1.6 kb consistent with the reported murine cytochrome P450IIC11 mRNA sequence length (Yoshioka et al, 1990). Virtually no hybridization was observed in lanes containing RNA from female mice, in agreement with the lack of this isozyme in female rats (Strom et al, 1988). Aminopyrine N-demethylation is not a specific substrate assay for cytochrome P450IIC11 but this isozyme does demonstrate a high activity toward aminopyrine N-demethylation (Morgan et al, 1985; Chang, 1991), and therefore this substrate was used to assess the functional status of this isozyme during listeriosis. In a time course study, listeria had no effect on cytochrome P450IIC11 mRNA or aminopyrine N-demethylation after 12 hours of infection, but both were significantly suppressed by 48 hours. Cytochrome P450IIC11 mRNA levels in infected mice were not significantly different from control after 96 hours while aminopyrine N-demethylase activity remained significantly suppressed at this time. The magnitude of the suppression of mRNA at various times of infection could entirely

account for the loss of isozyme activity indicating that a pretranslational effect on the synthesis of cytochrome P450IIC11 apoprotein is responsible for the loss of this isozyme. These observations paralleled our findings that an induced form of cytochrome P450IA1 is also decreased as a result of a loss of mRNA, and suggests that listeria may depress many or even all cytochrome P450 isozymes by a similar mechanism.

The decrease in cytochrome P450 mRNA during listeria infection could result from a decrease in the transcriptional rate of the genes of those isozymes known to be affected, or result from mRNA destruction. To date, there has only been one report that has assessed the effect of immune stimulation on the transcriptional rate of cytochrome P450 genes, and there have been no reports on the effect of immune stimulation on cytochrome P450 mRNA stability. The administration of *E. coli* endotoxin to male rats for 24 hours resulted in a 95% inhibition of the transcription rate of the cytochrome P450IIC11 gene while the transcription rate of the β -actin gene was unchanged in hepatic nuclei using a nuclear runoff transcription assay (Wright and Morgan, 1990). This is the first and only report of the effect of an immune stimulant on cytochrome P450 transcription rates.

The hemolysin secreted by the 15U strain is thought to be a principal virulence factor as a result of its ability to disrupt the phagocytic membrane allowing escape of the bacterium into the cytoplasm of host cells and subsequent spread to neighboring cells. It was possible that the hemolysin also plays a role in the loss of cytochrome P450 that is observed during infection with 15U listeria. A dose of 1×10^6 CFU of 15U listeria resulted in a significant depression of total cytochrome P450 content, EROD, BROD, and PROD activities after 48 hours of infection; the average liver load at the time of sacrifice was 1.0×10^5 CFU/liver. The administration of the same dose of an avirulent form of listeria (M3D), that does not secrete hemolysin, for 48 hours had no effect on total hepatic microsomal P450 content, EROD, BROD, or PROD activities; however, no colonies of the

M3D strain were detectable in liver homogenates at the time of sacrifice. The lack of effect on cytochrome P450 during infection with the M3D strain and the absence of bacterial colonies in liver homogenates at the time of sacrifice could be interpreted in two ways. Firstly, it is possible that the maintenance of a critical liver burden of bacteria is a prerequisite for an effect on cytochrome P450, and secondly that hemolysin production is a necessary component of the effect of listeria on hepatic drug metabolism capacity. In an attempt to achieve equal liver loads of the two strains of the bacteria, mice were infected with 1×10^5 CFU of the hemolytic 15U strain and 1×10^8 CFU of the non-hemolytic M3D strain for 48 hours. Even though there was a 1,000-fold difference in administered dose, the liver load of the M3D strain was only one third of that for the 15U strain at the time of sacrifice. Cytochrome P450 content was depressed to 68% of control by the 15U strain whereas the M3D strain had no effect. This rapid clearance of strains that do not produce hemolysin has been previously documented (Kathariou et al, 1987; Gaillard et al, 1986; Berche et al, 1987). Non-hemolytic strains are phagocytosed and antigens thereof are presented on the surface of macrophages (Cluff and Ziegler, 1987), but these strains cannot escape from the phagosome and therefore cannot grow intracellularly (Cossart and Mengaud, 1989; Portnoy et al, 1988; Mounier et al, 1990; Gaillard et al, 1987). The M3D strain was constructed by inserting a tetracycline resistance transposon into the DNA of a hemolytic strain (Kathariou et al, 1987). The average reversion rate (spontaneous loss of the transposon and return to the hemolytic phenotype) of this mutant was determined to be 3×10^{-4} and therefore the administration of doses greater than 1×10^8 CFU carries the risk of the production of enough hemolytic revertants to initiate an infection and immune response characteristic of the hemolytic strain. For this reason, *in vivo* experiments with the M3D strain using higher doses were not continued and subsequent experiments that

addressed the role of liver burden were carried out in a liver cell culture system, in which the number of bacteria added to the cells could be controlled.

The hemolytic 15U strain of listeria resulted in a significant loss of EROD and BROD activities in mixed liver cell cultures but a comparable liver load of the M3D strain had no effect. This suggested that the presence of the hemolysin is an essential component of the mechanism, but it is not clear if the effect of 15U listeria is due solely to the presence of the hemolysin or whether it is the result of the hemolysin along with the intact bacteria. To distinguish between these two possibilities would require the addition of purified hemolysin to liver cell cultures as proposed in the future work section.

The time course suggested that bacterial burden does not correlate with cytochrome P450 suppression. In β NF-induced mice, bacterial liver load was the same after 24 and 96 hours of infection but cytochrome P450IA mRNA levels were only suppressed at $t=24$ hours. Similarly, liver load in uninduced mice was the same after 48 and 96 hours of infection yet cytochrome P450IIC11 mRNA was only suppressed at $t=48$ hours. One of the difficulties in interpreting this data is that liver burden was determined from whole liver homogenates which, during an infection with listeria, would include homogenates of hepatocytes, Kupffer cells, monocytes, neutrophils, and other cell populations; moreover, the homogenates would include intracellular and extracellular bacteria. Therefore, it is possible the intracellular liver load within a specific cell type (i.e. Kupffer cells) does directly correlate with the loss of cytochrome P450.

CLINICAL RELEVANCE

The experimental portion of this thesis demonstrates that an active bacterial infection with Listeria monocytogenes results in a significant depression of cytochrome P450-mediated metabolism in mice. Only two reports have been published to date that have indicated a change in drug clearance during a bacterial infection in humans. The lack of reports suggesting this association is likely the result of the current focus on the potential deleterious effects of viral infections and recombinant interferons. In the last 15 years, there have been numerous reports of alterations of drug metabolism during viral infections in humans, and in the last six years there have been four reports of altered drug clearance during the clinical use of interferon- α , all of which could be predicted to occur based on the results of animal studies with similar agents. The incidence of Listeria monocytogenes infection in the general population is low but, if the capacity of listeria to depress cytochrome P450 proves to be an effect that is common to many different types of bacterial infections, then more clinical reports in this regard are likely in the future. These studies serve to emphasize the warning that drugs with narrow therapeutic indices should be administered with caution during infectious disease caused by bacteria as well as viruses.

CONCLUSIONS

The following conclusions were drawn from the data presented in this thesis:

1. Listeria monocytogenes (strain 15U) produced a reversible depression of numerous cytochrome P450 isozymes after intraperitoneal administration to male Swiss (Balb/c) mice.
2. *Listeria* significantly depressed EROD and BROD activity in hepatocytes maintained in culture. The mechanism involved a direct effect on the hepatocyte and the release of a factor from hepatic nonparenchymal cells. The results indicated a role for the hemolysin as the non-hemolytic M3D strain had no effect in liver cell cultures.
3. *Listeria* decreased the levels of cytochrome P450IA1 and P450IIC11 by a pretranslational mechanism involving a loss of P450 mRNA.
4. *Listeria* had no effect on total hepatic mRNA or protein content, and did not produce remarkable histological change in liver sections.

PROPOSALS FOR FUTURE WORK

1. Establish the effect of listeria infection on the transcriptional rate of murine cytochrome P450IA1 and P450IIC11 genes.
2. Establish the effect of listeria infection on the stability of cytochrome P450 mRNA in hepatocytes maintained in culture.
3. Compare the effect of purified hemolysin with intact hemolytic listeria on the drug metabolism capacity of hepatocytes maintained in culture.
4. Establish whether or not protection against the depressant effect of in vivo infection with 15U listeria on cytochrome P450 is conferred by the pre-treatment of mice with an anti-listeriolysin antibody.
5. Determine if other types of gram (+) bacteria are capable of causing a significant depression of cytochrome P450-mediated metabolism.

REFERENCES

- Anolik, R., Kolski, G.B., Schaible, D.H. and Ratner, J. Transient alteration of theophylline half-life: possible association with herpes simplex infection. *Ann. Allergy* 49: 109-111, 1982.
- Apte, R.W., Ascher, O. and Pluznik, D.H. Genetic analysis of generation of serum interferon by bacterial lipopolysaccharide. *J. Immunol.* 119: 1898-1902, 1977.
- Azri, S. and Renton, K.W. Depression in mixed function oxidase during infection with *Listeria monocytogenes*. *J. Pharmacol. Exp. Ther.* 243: 1089-1094, 1987.
- Azri, S. and Renton, K. W. Factors involved in the depression of hepatic mixed function oxidase during infections with *Listeria monocytogenes*. *Int. J. Immunopharmac.* 13: 197-204, 1991.
- Babior, B.M. Oxygen-dependent microbial killing by phagocytes. *N. Engl. J. Med.* 298: 659-668, 1978.
- Baglioni, C. Interferon-induced enzymatic activities and their role in the antiviral state. *Cell* 17: 255-264, 1979.
- Baron, S., Tying, S.K., Fleischmann, W.R., Coppenhaver, D.H., Niesel, D.W., Klimpel, G.R., Stanton, G.J. and Hughes, T.K. The interferons. Mechanisms of action and clinical applications. *J. Am. Med. Assoc.* 266: 1375-1383, 1991.
- Berche, P., Gaillard, J-L and Sansonetti, P.J. Intracellular growth of *Listeria monocytogenes* as a prerequisite for in vivo induction of T cell-mediated immunity. *J. Immunol.* 138: 2266-2271, 1987.
- Bertini, R. and Ghezzi, P. Depression of liver drug metabolism in sarcoma-bearing mice. Evidence for a circulating factor and dissociation from lipolytic activity. *Eur. J. Cancer Clin. Oncol.* 24: 1845-1849, 1988.
- Bertini, R., Bianchi, M., Villa, P. and Ghezzi, P. Depression of liver drug metabolism and increase in plasma fibrinogen by interleukin-1 and tumor necrosis factor: a comparison with lymphotoxin and interferon. *Int. J. Immunopharmac.* 10: 525-530, 1988.
- Bertini, R., Bianchi, M., Erroi, A., Villa, P. and Ghezzi, P. Dexamethazone modulation of in vivo effects of endotoxin, tumor necrosis factor, and interleukin-1 on liver cytochrome P-450, plasma fibrinogen, and serum iron. *J. Leukoc. Biol.* 46: 254-262, 1989.
- Beutler, B., Krochin, N., Milsark, I.W., Lundke, C. and Cerami, A. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science* 232: 977-980, 1986.

- Blankson, E.A., Chenery, R.J. and Paine, A.J. Cytochrome P450 maintenance and diazepam metabolism in cultured rat hepatocytes. *Biochem. Pharmacol.*42: 1241-1245, 1991.
- Bojsen-Moller, J. Human listeriosis. Diagnostic, epidemiological and clinical studies. *Acta Pathologica Et Microbiologica Scandinavica. Section B, Supplement Number 229:* 1-157, 1972.
- Bortolussi, R., Campbell, N. and Krause, V. Dynamics of *Listeria monocytogenes* type 4b infection in pregnant and infant rats. *Clin. Inv. Med.* 7: 273-279, 1984.
- Bortolussi, R., Issekutz, T., Burbridge, S. and Schellekens, H. Neonatal host defense mechanisms against *Listeria monocytogenes* infection: the role of lipopolysaccharides and interferons *Ped. Res.* 25: 311-315, 1989.
- Breimer, D.D. and Schellens, J.H.M. A 'cocktail' approach to assess in vivo oxidative drug metabolism in humans. *Trends Pharmacol. Sci.* II: 223-225, 1990.
- Bryden, K.E., Renton, K.W. and Singh, G. Cytokine mediated loss of cytochrome P-450. *Proc. of Can. Fed. of Biol. Sci. (Kingston) 1991 (Abstract).*
- Buchmeier, N.A. and Schreiber, R.D. Requirement on endogenous interferon- γ production for resolution of *Listeria monocytogenes* infection. *Proc. Natl. Acad. Sci. USA* 82: 7404-7408, 1985.
- Burke, D.M., Thompson, S., Elcombe, C.R., Halpert, J., Haaparanta, T. and Mayer, R.T. Ethoxy-, pentoxy- and benzyloxy- phenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P450. *Biochem. Pharmacol.*34: 3337-3345, 1985.
- Calandra, T., Baumgartner, J.D. and Glauser, M.P. Anti-lipopolysaccharide and anti-tumor necrosis factor/cachetin antibodies for the treatment of gram negative bacteremia and septic shock. In: *Bacterial Endotoxins: Cytokine Mediators and New Therapies for Sepsis*, eds. Struk, A., van Deventer, S.J.H., ten Cate, J.W., Buller, H.R., Thijs, L.G. and Levin, J., New York, Wiley-Liss, Inc., pp. 141-159, 1991.
- Campbell, P.A. What T-cells tell macrophages to do during resistance to listeriosis. *Adv. Exp. Med. Biol.* 239: 13-22, 1988.
- Cantrell, E. and Bresnick, E. Benzpyrene hydroxylase activity in isolated parenchymal and nonparenchymal cells of rat liver. *J. Cell Biol.* 52: 316-321, 1972.
- Chang, K. C., Lauer, B.A., Bell, T.D. and Chai, H. Altered theopylline pharmacokinetics during acute respiratory viral illness. *The Lancet*, May 27: 1132-1133, 1978.
- Chang, T.W.H. Ph.D. Thesis. University of British Columbia, Department of Pharmaceutical Sciences, 1991.
- Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162: 156-159, 1987.

- Clark, C.J. and Boyd, G. Theophylline pharmacokinetics during respiratory viral infection. *Lancet* 1: 492, 1979.
- Cluff, C.W. and Ziegler, H.K. Inhibition of macrophage-mediated antigen presentation by hemolysin-producing *Listeria monocytogenes*. *J. Immunol.* 139: 3808-3812, 1987.
- Cooper, D.H., Estabrook, R.W. and Rosenthal, O. Spectral characteristics of the oxygen activating enzyme for adrenal cortical C-21 hydroxylation of steroids. *Endocrine Soc., Atlantic City, N.J. Abstract 7, p.4, 1963.*
- Cooper, D.Y., Levin, S.S., Narasimhula, S., Rosenthal, O. and Estabrook, R.W. Photochemical action spectrum of the terminal oxidase of mixed function oxidase systems. *Science* 147: 400-402, 1965.
- Cossart, P. and Mengaud, J. *Listeria monocytogenes*. A model system for the molecular study of intracellular parasitism. *Mol. Biol. Med.* 6: 463-474, 1989.
- Coto, J.A. and Williams, J.F. Effect of endotoxin to differentially affect cytochrome P450 monooxygenase activities of untreated rats and animals induced with phenobarbital or 3-Methylcholanthrene. *Int. J. Immuno. Pharmac.* 11: 623-628, 1989.
- Craig, P.I., Mehta, I., Murray, M., McDonald, D., Astrom, A., van der Meide, P.H. and Farrell, G.C. Interferon down regulates the male-specific cytochrome P450III_{A2} in rat liver. *Mol. Pharmacol.* 38: 313-318, 1990.
- Czuprynski, C.J., Henson, P.M. and Campbell, P.A. Killing of *Listeria monocytogenes* by inflammatory neutrophils and mononuclear phagocytes from immune and nonimmune mice. *J. Leuk. Biol.* 35: 193-208, 1984.
- Czuprynski, L., Brown, J.F., Young, K.M. Cooley, A.J. and Kurtz, R.S. Effects of murine recombinant interleukin 1 α on the host response to bacterial infection. *J. Immunol.* 140: 962-968, 1988.
- DeMaeyer, E. and DeMaeyer-Guignard, J. Consideration on mouse genes influencing interferon production and action in Interferon 1, 1979 (L. Gresser, ed.). Academic Press, New York: 75-100, 1979.
- Dinarello, C.A. Interleukin-1. *Rev. Infect. Dis.* 6: 51-95, 1984.
- Dinarello, C.A., Cannon, J.G., Wolff, S.M., Bernheim, H.A., Beutler, B., Cerami, A., Figari, I.S., Palladino, M.A. Jr. and O'Connor, J.V. Tumor necrosis factor (cachetin) is an endogenous pyrogen and induces production of interleukin-1. *J. Exp. Med.* 163: 1433-1450, 1986.
- Dunn, P.L. and North, R.J. Early gamma interferon production by natural killer cells is important in defense against murine listeriosis. *Infect. Immun.* 59: 2892-2900, 1991a.

- Dunn, P.L. and North, R.J. Resolution of primary murine listeriosis and acquired resistance to lethal secondary infection can be mediated predominantly by Thy-1⁺ CD4⁻ CD8⁻ cells. *J. Inf. Dis.* 164: 869-877, 1991b.
- Editor, *The Lancet* 8598: 1351, 1988.
- Egawa, K. and Kasai, W. Endotoxic glycolipid as a potent depressor of the hepatic drug-metabolizing enzyme systems in mice. *Microbiol. Immunol.* 23: 87-94, 1979.
- Egawa, K., Yoshida, M. and Kasai, N. An endotoxin-induced serum factor that depresses hepatic δ -aminolevalinic acid synthetase activity and cytochrome P-450 levels in mice. *Microbiol. Immunol.* 25: 1091-1096, 1981.
- Eichelbaum, M. Genetic polymorphism of sparteine/debrisoquine oxidation. *ISI Atlas of Science: Pharmacology* : 243-251, 1988.
- El Azhary, R. and Mannering, G.J. Effects of interferon inducing agents (polyriboinosinic acid - polyribocytidylic acid, tilorone) on hepatic hemoproteins (cytochrome P450, catalase, tryptophan 2, 3-dioxygenase, mitochondrial cytochromes), heme metabolism, and cytochrome P450-linked monooxygenase systems. *Mol. Pharmacol.* 15: 698-707, 1979.
- El Azhary, R. Renton, K.W. and Mannering, G.J. Effect of interferon inducing agents (Polyriboinosinic acid•polyribocytidylic acid and tilorone) on the heme turnover of hepatic cytochrome P450. *Mol. Pharmacol.* 17: 395-399, 1980.
- Ertel, W., Morrison, M.H., Wang, P., Zheng, F., Ayala, A. and Chandry, I.H. The complex pattern of cytokines in sepsis. Association between prostaglandins, cachectin, and interleukins. *Ann. Surg.* 214: 141-148, 1991.
- Farquhar, D., Loo, T.L., Gufferman, J.U., Hershi, E.M. and Luna, M.A. Inhibition of drug metabolism enzymes in the rat after Bacillus Calmette-Guerin treatment. *Biochem. Pharmacol.* 25: 1529-1535, 1976.
- Fleetham, J.A., Nakatsu, K. and Munt, P.W. Theophylline pharmacokinetics and respiratory infections. *Lancet* 2: 898, 1978.
- Flohe, S., Heinrich, P.C., Schneider, J., Wendel, A. and Flohe, L. Time course of IL-6 and TNF- α release during endotoxin-induced endotoxin tolerance in rats. *Biochem. Pharmacol.* 41: 1607-1614, 1991.
- Forsyth, J.S., Moreland, T.A. and Ryelance, G.W. The effect of fever on antipyrine metabolism in children. *Br. J. Clin. Pharmacol.* 13: 811, 1982.
- Franklin, M.R. and Finkle, B.S. Effect of murine gamma-interferon on the mouse liver and its drug metabolizing enzymes. *J. Interferon Res.* 5: 265-272, 1985.
- Gaillard, J.L., Berche, P. and Sansonetti, P. Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of *Listeria Monocytogenes*. *Infect. Immun.* 52: 50-55, 1986.

- Gaillard, J.L., Berche, P., Mounier, J., Richard, S. and Sansonetti, P. In vitro model of penetration and intracellular growth of *Listeria monocytogenes* in the human enterocyte-like cell line Caco-2. *Infect. Immun.* 55: 2822-2829, 1987.
- Geoffroy, C., Gaillard, J-L., Alouf, J.E. and Berche, P. Purification, characterization, and toxicity of the sulfhydryl-activated hemolysin listeriolysin O from *Listeria monocytogenes*. *Infect. Immun.* 55: 1641-1646, 1987.
- Ghezzi, P., Bianchi, M., Montovani, A., Spreafico, F. and Salmons, M. Enhanced xanthine oxidase activity in mice treated with interferon and interferon inducers. *Biochem. Biophys. Res. Commun.* 119: 144-149, 1984.
- Ghezzi, P., Bianchi, M., Gianera, L., Landolfo, S. and Salmons, M. Role of reactive oxygen intermediates in the interferon-mediated depression of hepatic drug metabolism and protective effect of N-acetylcysteine in mice. *Cancer Res.* 45: 3444-3447, 1985.
- Ghezzi, P., Saccardo, B. and Bianchi, M. Recombinant tumor necrosis factor depresses cytochrome P450-dependent microsomal drug metabolism in mice. *Biochem. Biophys. Res. Commun.* 136: 316-321, 1986a.
- Ghezzi, P., Saccardo, B., Villa, P., Rossi, V., Bianchi, M. and Dinarello, C.A. Role of interleukin-1 in the depression of liver drug metabolism by endotoxin. *Infect. Immun.* 54: 837-840, 1986b.
- Gibson, G.G. and Skett, P. Introduction to drug metabolism. Chapman and Hall, New York, 1986.
- Gilbert, R.P. Mechanisms of the hemodynamic effects of endotoxin. *Physiol. Rev.* 40: 245, 1960.
- Gill, P. Is listeriosis a foodborne illness? *J. Infect.* 17: 1-5, 1988.
- Girardin, E., Berner, M., Grau, G.E., Dayer, J.M., Roux-Lombard, P. and Suter, S. Tumor necrosis factor in neonatal listeriosis: a case report. *Eur. J. Pediatr.* 148: 644-645, 1989.
- Gonzalez, F.J., Tukey, R.H. and Nebert, D.W. Structural gene products of the Ah locus. Transcriptional regulation of cytochrome P₁450 and P₂450 mRNA levels by 3-methylcholanthrene. *Mol. Pharm.* 26: 117-121, 1984.
- Gooderham, N.J. and Mannering, G.J. Depression of cytochrome P-450 and Alterations of drug metabolism in mice treated with the interferon inducer polyribinosinic acid•polyribocytidylic acid. *Arch. Biochem. Biophys.* 250: 418-425, 1986.
- Gooderham, N.J. and Mannering, G.J. In vitro translational activity of messenger-RNA from mice treated with the interferon inducer, polyribinosinic acid•polyribocytidylic acid. *Biochem. Pharmacol.* 39: 865-871, 1990.

- Gorodischer, R., Krasner, J., McDevitt, J.J., Nolan, J.P. and Yaffe, S. Hepatic microsomal drug metabolism after administration of endotoxin in rats. *Biochem. Pharmacol.* 25: 351-353, 1976.
- Gray, J.D., Renton, K.W. and Hung, O.R. Depression of theophylline elimination following BCG vaccination. *Br. J. Clin. Pharmacol.* 16: 735-737, 1983.
- Gray, M.L. and Killinger, A.H. *Listeria monocytogenes* and listeria infections. *Bact. Rev.* 30: 309-382, 1966.
- Greenwald, M. and Koren, G. Viral induced changes in theophylline handling in children. *Am. J. Asthma Allergy Peds.* 3: 162, 1990.
- Guengerich, F.P. and Turvy, C.G. Comparison of levels of several human microsomal cytochrome P450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical liver samples. *J. Pharmacol. Ther.* 256: 1189-1194, 1991.
- Guengerich, F.P., Dannan, G.A., Wright, S.T., Martin, M.V. and Kaminski, L.S. Purification and characterization of liver microsomal cytochrome P450: electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or β -naphthoflavone. *Biochemistry* 21: 6019-6030, 1982.
- Guguen-Guillouzo, C. and Guillouzo, A. Modulation of functional activities in cultured rat hepatocytes. *Mol. Cell Biochem.* 53/54: 35-36, 1983.
- Haak-Frendscho, M., Young, K.M. and Czuprynski, C.J. Treatment of mice with human recombinant interleukin-2 augments resistance to the facultative intracellular pathogen *Listeria monocytogenes*. *Infect. Immun.* 57: 3014-3021, 1989.
- Harley, C.B. Hybridization of oligo(dT) to RNA on nitrocellulose. *Gene. Anal. Techn.* 4: 17-22, 1987.
- Hauser, T., Frei, K., Zinkernagel, R.M. and Leist, T.P. Role of tumor necrosis factor in *Listeria* resistance of nude mice. *Med. Microbiol. Immunol.* 179: 95-104, 1990.
- Havell, E.A. Synthesis and secretion of interferon by murine fibroblasts in response to intracellular *Listeria monocytogenes*. *Infect. Immun.* 54: 787-792, 1986a.
- Havell, E.A. Augmented induction of interferons during *Listeria monocytogenes* infection. *J. Infect. Dis.* 153: 960-969, 1986b.
- Havell, E.A. Production of tumor necrosis factor during murine listeriosis. *J. Immunol.* 139: 4225-4231, 1987.
- Havell, E.A. and Sehgal, P.B. Tumor necrosis factor-independent IL-6 production during murine listeriosis. *J. Immunol.* 146: 756-761, 1991.

- Heinrich, P.C., Castell, J.V. and Andus, T. Interleukin-6 and the acute phase response. *Biochem. J.* 265: 621-636, 1990.
- Ikeda, T., Altieri, M., Chen, Y., Nakamura, M., Tukey, R.H. and Nebert, D.W. Characterization of cytochrome P₂450 (20-S) mRNA. Association with the P₁-450 genomic gene and differential response to the inducers 3-Methylcholanthrene and isosafarole. *Eur. J. Biochem.* 134: 13-18, 1983.
- Ishizaki, S., Furuhashi, K., Kaneta, S. and Fujihira, E. Reduced drug metabolism in isolated hepatocytes from adjuvant arthritic rats. *Res. Commun. Chem. Pathol. Pharmacol.* 39: 261-276, 1983.
- Issekutz, T.B., Evans, J. and Bortolussi, R. The immune response of human neonates to *Listeria monocytogenes* infection. *Clin. Invest. Med.* 7: 281-286, 1984.
- Johnson, J.L., Rajagopalan, K.V. and Cohen, H.J. Molecular basis of the biological function of molybdenum: effect of tungsten on xanthine oxidase and sulfite oxidase in the rat. *J. Biol. Chem.* 249: 859-866, 1974.
- Jonkman, J.H.G., Nicholson, K.G., Furrow, P.R., Eckert, M., Grasmeyer, G., Oosterhuis, B., de Noord, O.E. and Guentert, T.W. Effects of α -interferon on theophylline pharmacokinetics and metabolism. *Br. J. Clin. Pharmacol.* 27: 795, 1989.
- Juchau, M.R. Substrate specificities and functions of the P450 cytochromes. *Life Sciences* 47: 2385-2394, 1990.
- Kasting, N.W. and Martin, J.B. Altered release of growth hormone and thyrotropin induced by endotoxin in the rat. *Am. J. Physiol.* 243: E332-E337, 1982.
- Kathariou, S., Metz, P., Hof, H. and Goebel, W. Tn 916-induced mutations in the hemolysin determinant affecting virulence of *Listeria monocytogenes*. *J. Bacteriol.* 159: 1291-1297, 1987.
- Kathariou, S., Rocourt, J., Hof, H. and Goebel, W. Levels of *Listeria monocytogenes* hemolysin are not directly proportional to virulence in experimental infections of mice. *Infect. Immun.* 56: 534-536, 1988.
- Kato, R., Nakamura, Y. and Chiesara, E. Enhanced phenobarbital induction of liver microsomal drug-metabolizing enzymes in mice infected with murine hepatitis virus. *Bioc. Pharmacol.* 12: 365-370, 1963.
- Kato, K., Nakane, A., Minigawa, T., Kasai, W., Yamamoto, K., Sato, W. and Tsuroka, W. Human tumor necrosis factor increases the resistance against *Listeria* infection in mice. *Med. Microbiol. Immunol.* 178: 337-346, 1989.
- Kauffman, S.H.E. Acquired resistance to facultative intracellular bacteria: relationship between persistence, cross-reactivity at the T-cell level, and capacity to stimulate cellular immunity of different *Listeria* strains. *Infect. Immun.* 45: 234-241, 1984.

- Kauffmann, S.H.E. Possible role of helper and cytolytic T lymphocytes in antibacterial defense: conclusions based on a murine model of listeriosis. *Reviews of Infectious Diseases* 9: Supplement 5, S650-S659, 1987.
- Kauffmann, S.H.E. Which T-cells are relevant to resistance against *Listeria monocytogenes* infection? *Adv. Exp. Med. Biol.* 239: 135-150, 1988.
- Kauffman, S.H.E., Hug, E., Vath, U. and Muller, I. Effective protection against *Listeria monocytogenes* and delayed-type hypersensitivity to listerial antigens depend on cooperation between specific L3T4⁺ and Lyt2⁺ T-cells. *Infect. Immun.* 48: 263-266, 1985.
- Keller, G.A., West, M.A., Cerra, F.B. and Simmons, R.L. Multiple systems organ failure. Modulation of hepatocyte protein synthesis by endotoxin activated kupffer cells. *Ann. Surg.* 201: 87-95, 1985.
- Kelley, M., Hantelle, P., Safe, S., Levin, W. and Thomas, P.E. Co-induction of cytochrome P-450 isozymes in rat liver by 2,4,5,2',4',5' - hexachlorobiphenyl or 3-methoxy-4-aminoazobenzene. *Mol. Pharm.* 32: 206-211, 1987.
- Kimura, S., Gonzalez, F.J. and Webert, D.W. The murine Ah locus. Comparison of the complete cytochrome P₁450 and P₃450 cDNA nucleotide and amino acid sequences. *J. Biol. Chem.* 259: 10705-10713, 1984.
- Kimura, S., Gonzalez, F.J. and Nebert, D.W. Tissue-specific expression of the mouse dioxin-inducible P₁450 and P₃450 genes: Differential transcriptional activation and mRNA stability in liver and extrahepatic tissues. *Mol. and Cell. Biol.* 6: 1471-1477, 1986.
- Kingdon, G.C. and Sword, C.P. Effects of *Listeria monocytogenes* hemolysin on phagocytic cells and lysosomes. *Infect. Immun.* 1: 356-362, 1970.
- Klaunig, J.E., Goldblatt, P.J., Hinton, D.E., Lipsky, M.M., Chako, J. and Trump, B.F. Mouse liver culture. I. Hepatic isolation. *In Vitro* 17: 913-925, 1981.
- Kobliakov, V., Popova, N. and Rossi, L. Regulation of the expression of the sex-specific isoforms of cytochrome P-450. *Eur. J. Biochem.* 195: 585-591, 1991.
- Kongshavn, P.A.L. and Skamene, E. The role of natural resistance in protection of the murine host from listeriosis. *Clin. Invest. Med.* 7: 253-257, 1984.
- Koop, D.R., Chernosky, A. and Bruss, E.P. Identification and induction of cytochrome P450 2E1 in rat kupffer cells. *The Journal of Pharmacology and Experimental Therapeutics* 258: 1072-1076, 1991.
- Koren, G. and Greenwald, M. Decrease in theophylline clearance causing toxicity during viral epidemics. *J. Asthma* 22: 75, 1985.

- Kraemer, M. J., Furukawa, C.T., Koup, J.R., Shapiro, G.G., Pierson, W.E. and Bierman, C.W. Altered theopylline clearance during an influenza outbreak. *Pediatrics* 69: 476-480, 1982.
- Kratz, S.S. and Kurlander, R.J. Characterization of the pattern of inflammatory cell influx and cytokine production during the murine host response to *Listeria monocytogenes*. *J. Immunol.* 141: 598-606, 1988.
- Kurlander, R.J., Hoffman, M. Kratz, S.S. and Gates, J. Comparison of the effects of $IL-1\alpha$ and $TNF-\alpha$ on phagocyte accumulation and murine antibacterial immunity. *Cell Immunol.* 123: 9-22, 1989.
- Kurt-Jones, E.A., Virgin, H.W. and Unanue, E.R. Relationship of macrophage Ia and membrane IL-1 expression to antigen presentation. *J. Immunol.* 135: 3652-3654, 1985.
- Kvenberg, J.E. Outbreaks of listeriosis/*Listeria*-contaminated foods. *Microbiol. Sciences* 5: 355-358, 1988.
- Labarca, C. and Paigen, K. A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem.* 102: 344-352, 1980.
- Lamont, R.J., Postlewaite, R. and MacGowan, A.P. *Listeria monocytogenes* and its role in human infection. *J. Infect.* 17: 7-28, 1988.
- Lanz, L., Brugger, W., Bross, K. and Mertelsmann, R. Combination of cytokines: current status and future prospects. *British Journal of Haematology* 79: Supp. 1: 96-104, 1991.
- Leeson, G.A., Biedenbach, S.A., Chan, K.Y., Gibson, J.P. and Wright, G.J. Decrease in the activity of the drug-metabolizing enzymes of rat liver following the administration of tilorone hydrochloride. *Drug Metab. Dispos.* 4: 232-238, 1976.
- Leimeister-Wachter, M., Haffner, C., Domann, E., Goebel, W. and Chakraborty, T. Identification of a gene that positively regulates expression of listeriolysin, the major virulence factor of *Listeria monocytogenes*. *Proc. Natl. Acad. Sci. USA* 87: 8336-8340, 1990.
- Leimeister-Wachter, M., Domann, E. and Chakraborty, T. Detection of a gene encoding a phosphatidylinositol-specific phospholipase C that is co-ordinately expressed with listeriolysin in *Listeria monocytogenes*. *Molecular Microbiology* 5: 361-366, 1991.
- Lindsay, C.K., Rotondo, D., Grant, M.H. and Hawksworth, G.M. Maintenance of mixed-function oxidase activities in primary cultures of rat hepatocytes. *Biochem. Soc Trans.* 17: 188-189, 1989.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. Protein determination with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275, 1951.

- Lubet, R.A., Mayer, R.T., Cameron, J.W., Nims, R.W., Burke, M.D., Wolff, T. and Guengerich, F.P. Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P450 by phenobarbital and other xenobiotics in the rat. *Arch. Biochem Biophys.* 238: 43-48, 1985.
- MacKanness, M.B. Cellular resistance to infection. *The Journal of Experimental Medicine* 116: 381-406, 1962.
- Maniatis, T., Fritsch, E.F. and Sambrook, J. *Molecular cloning: A Laboratory Manual*, USA: Cold Spring Harbour Laboratory, 1982.
- Mannering, G.J. and Deloria, L.B. The pharmacology and toxicology of the interferons: an overview. *Ann. Rev. Pharmacol. Toxicol.* 26: 455-515, 1986.
- Mannering, G.J., Deloria, L. B. and Abbott, V. Role of xanthine oxidase in the interferon-mediated depression of the hepatic cytochrome P-450 system in mice. *Cancer Res.* 48: 2107-2112, 1988.
- Maslanski, C.J. and Williams, G.M. Primary cultures and the levels of cytochrome P450 in hepatocytes from mouse, rat, hamster, and rabbit liver. *In vitro* 18: 683-693, 1982.
- Maxam, A.M. and Gilbert, W. Sequencing end-labelled DNA with base-specific chemical cleavages. *Methods Enzymol.* 65: 499-560, 1980.
- McCarthy, J.S., Furner, R.L., VanDyke, K. and Snitzel, R. Effects of malarial infection on host microsomal drug-metabolizing enzymes. *Biochem. Pharmacol.* 19: 1341-1349, 1970.
- Mielke, M., Ehlers, S. and Hahn, H. The role of T-cell subpopulations in cell mediated immunity to facultative intracellular bacteria. *Infection* 16: Suppl. 2, S123-S127, 1988.
- Miki, K. and MacKanness, G.B. The passive transfer of acquired resistance to *Listeria monocytogenes*. *J. Exp. Med.* 120: 93-103, 1964.
- Mitsuyama, M., Takeya, K., Nomoto, K. and Shimotori, S. *J. Gen. Microbiol.* 106: 165-171, 1978.
- Moochala, S.M. and Renton, K.W. Effects of the interferon inducing agent, Poly rI•rC on the synthesis of hepatic cytochrome P-450. *Asia Pac. J. Pharmacol.* 4: 83-88, 1989a.
- Moochala, S.M. and Renton, K.W. and Stebbing, N. Induction and depression of cytochrome P450-dependent mixed-function oxidase by a cloned consensus α -interferon (IFN- α CON₁) in the hamster. *Biochem. Pharmacol.* 38: 439-447, 1989b.
- Moochala, S.M. and Renton, K.W. The effect of IFN- α -CON₁ on hepatic cytochrome P-450 protein synthesis and degradation in hepatic microsomes. *Int. J. Immunopharmac.* 13: 903-912, 1991a.

- Moochala, S.M. and Renton, K.W. A role of xanthine oxidase in the loss of cytochrome P450 evoked by interferon. *Can. J. Physiol. Pharmacol.* 69: 944-950, 1991b.
- Morgan, E.T. Suppression of constitutive cytochrome P-450 gene expression in livers of rats undergoing an acute phase response to endotoxin. *Mol. Pharmacol.* 36: 699-707, 1989.
- Morgan, E.T. Suppression of P450IIC12 gene expression and elevation of active messenger ribonucleic acid levels in the livers of female rats after injection of the interferon inducer poly rI•rC. *Biochem. Pharmacol.* 42: 51-57, 1991.
- Morgan, E.T., MacGeoch, C. and Gustafsson, J-A. Sexual differentiation of cytochrome P-450 in rat liver. Evidence for a constitutive isozyme as the male-specific 16 α -hydroxylase. *Mol. Pharm.* 27: 471-479, 1985.
- Morgan, E.T. and Norman, C.A. Pretranslational suppression of cytochrome P450h (IIC11) gene expression in rat liver after administration of interferon inducers. *Drug Metabolism and Disposition* 18: 649-653, 1990.
- Mounier, J., Ryter, A., Coquis-Rondon, M. and Sansonetti, P.J. Intracellular and cell to cell spread of *Listeria monocytogenes* involves interaction with F-actin in the enterocyte-like cell line Caco-2. *Infect. Immun.* 58: 1048-1058, 1990.
- Munson, A.E., Regelson, E., Lawrence, W. and Wooles, W.R. Biphasic response of the reticuloendothelial system induced by pyran copolymer. *J. Reticuloendothel. Soc.* 5: 590, 1968.
- Murray, E.G.D. A characterization of listeriosis in man and other animals. *Can Med. Assoc. J.* 72: 99-103, 1955.
- Murray, M. and Reidy, G.F. Selectivity in the inhibition of mammalian cytochromes P-450 by chemical agents. *Pharmacol. Rev.* 42: 85-101, 1990.
- Nakajima, T., Elovaara, E., Park, S.S., Gelboin, H.V., Heitanen, E. and Vainio, H. Monoclonal antibody - directed characterization of benzene, ethoxyresorufin, and pentoxyresorufin metabolism in rat liver microsomes. *Biochem. Pharmacol.* 40: 1255-1261, 1990.
- Nakane, A., Minigawa, T. and Kato, K. Endogenous tumor necrosis factor (cachectin) is essential to host resistance against *Listeria monocytogenes* infection. *Infect. Immun.* 56: 2563-2569, 1988.
- Nakane, A., Numata, A., Asano, M., Kohanawa, M., Chen, Y. and Minigawa, T. Evidence that endogenous gamma interferon is produced early in *Listeria monocytogenes* infection. *Infect. Immun.* 58: 2386-2388, 1990.
- Nebert, D.W., Welson, D.R., Coon, M.J., Estabrook, R.W., Fehereisen, R., Fujii-Kuriyama, Y., Gonzalez, F.J., Guengerich, F.P., Gunsalus, I.C., Johnson, E.F., Loper, J.C., Sato, R., Waterman, M.R., and Waxman, D.J. The P450 superfamily: update on

- new sequences, gene mapping, and recommended nomenclature. *DNA Cell. Biol.* 10: 1-14, 1991.
- Negishi, M. and Nebert, D.W. Structural gene products of the Ah locus. Genetic and immunochemical evidence for two forms of mouse liver cytochrome P450 induced by 3-methylcholanthrene. *J. Biol. Chem.* 254: 11015-11023, 1979.
- North, R.J. The relative importance of blood monocytes and fixed macrophages to the expression of cell mediated immunity to infection. *J. Exp. Med.* 132: 521-534, 1970.
- North, R.J. and Deissler, J.F. Nature of 'memory' in T-cell mediated antibacterial immunity: cellular parameters that distinguish between the active immune response and a state of memory. *Infect. Immun.* 12: 761-767, 1975.
- Notermans, S.H.W., Dufrenne, J., Leimeister-Wachter, M., Domann, E. and Chakraborty, T. Phosphatidyl inositol-specific phospholipase C activity as a marker to distinguish between pathogenic and nonpathogenic listeria species. *Applied and Environmental Microbiology* 57: 2666-2670, 1991.
- Okuno, H., Kitao, Y., Takasu, M., Kano, H., Kunieda, K., Seki, T., Shiozaki, Y. and Sameshima, Y. Depression of drug metabolizing activity in the human liver by interferon- α . *Eur. J. Clin. Pharmacol.* 39: 365-367, 1990.
- Omura, T. and Sato, R. The carbon monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* 239: 2370-2378, 1964.
- Park, B.K. and Bröckenridge, A.M. Clinical implications of enzyme induction and enzyme inhibition. *Clin Pharmacokinet.* 6: 1-24, 1981.
- Parker, G.L. and Orton, G.C. Induction of oxyisobutyrate of hepatic and kidney microsomal cytochrome P450 with specificity towards hydroxylation of fatty acids. In *Biochemistry, Biophysics and Regulation of Cytochrome P450*, ed. Gustafsson, J.A. et al., Elsevier: North-Holland Biomedical Press, pp. 373-377, 1980.
- Parkinson, A. Lasker, J., Kramer, M.J., Huang, M.T., Thomas, P.E., Ryan, D.E., Reik, L.M., Norman, R.L., Levin, W. and Conney, A.H. Effects of three recombinant human leukocyte interferons on drug metabolism in mice. *Drug Metabolism and Disposition* 10: 579-585, 1982.
- Peterson, T.C. and Renton, K.W. Depression of cytochrome P-450-dependent drug biotransformation in hepatocytes after the activation of the reticuloendothelial system by dextran sulfate. *Journal of Pharmacology and Experimental Therapeutics* 229: 299-304, 1984.
- Peterson, T.C. and Renton, K.W. Kupffer cell factor mediated depression of hepatic parenchymal cell cytochrome P-450. *Biochem. Pharmacol.* 35: 1491-1497, 1986a.

- Peterson, T.C. and Renton, K.W. The role of lymphocytes, macrophages and interferon in the depression of drug metabolism by dextran sulfate. *Immunopharmacology* 11: 21-28, 1986b.
- Petit, J.C., Burghoffer, B., Richard, G. and Daguët, G.L. Kinetics of interleukin-1 production by macrophages during infection with *Listeria monocytogenes*. *Int. J. Immunopharmac.* 10: 875-878, 1988.
- Poli, V. and Cortese, R. Interleukin-6 induces a liver-specific nuclear protein that binds to the promoter of acute-phase genes. *Proc. Natl. Acad. Sci.* 86: 8202-8206, 1989.
- Porter, T.D. and Coon, M.J. Cytochrome P450. Multiplicity of isoforms, substrates and catalytic and regulatory mechanisms. *J. Biol. Chem.* 266: 13469-13472, 1991.
- Portnoy, D.A., Lacks, P.S. and Hinrichs, D.J. Role of hemolysin for the intracellular growth of *Listeria monocytogenes*. *J. Exp. Med.* 167: 1459-1471, 1988.
- Portnoy, D.A., Schreiber, R.D., Connelly, P. and Tilney, L.G. γ interferon limits access of *Listeria monocytogenes* to the macrophage cytoplasm. *J. Exp. Med.* 170: 2141-2146, 1989.
- Queen, C. and Korn, L.J. A comprehensive sequence analysis program for the IBM personal computer. *Nucl. Acids Res.* 12: 581-599, 1984.
- Quinn, J.P., Axelrod, G. and Brodie, B.B. Species, strain, and sex differences in metabolism of hexobarbitone, aminopyrine, antipyrine and aniline. *Biochem. Pharmacol.* 1: 152-159, 1958.
- Raetz, C.R.H., Ulevitch, R.J., Wright, S.D., Sibley, C.H., Ding A. and Nathan, C.F. Gram-negative endotoxin: an extraordinary lipid with profound effects on eukaryotic signal transduction. *FASEB J.* 5: 2652-2660, 1991.
- Ragland, W.L., Friend, M., Trainder, D.O. and Sladek, W.E. Interaction between duck hepatitis virus and DDT in ducks. *Res. Comm. Chem. Path. Pharm.* 2: 236-244, 1971.
- Renton, K.W. Letter. *The Lancet* 2: 160-161, 1978a.
- Renton, K.W. The deleterious effect of *Bordetella pertussis* vaccine and Poly (rI • rC) on the metabolism and disposition of phenytoin. *The Journal of Pharmacology and Experimental Therapeutics* 208: 267-270, 1979.
- Renton, K.W. Effects of interferon inducers on viral infections on the metabolism of drugs. In *Advances in Immunopharmacology*, ed. Haddon, J. et al., Oxford: Pergamon Press, 17-24, 1981.
- Renton, K.W. Relationships between enzyme of detoxication and host defence mechanisms. In: *Biological Basis of Detoxication*. Ed. Caldwell, J. and Jacoby, W.B.: 307-324, Academic Press, New York, 1983.

- Renton, K.W. Cytochrome P-450-dependent monooxygenase systems in mouse hepatocytes In: *The Isolated Hepatocyte. Use in toxicology and xenobiotic biotransformations.* Rauckman, E.J. and Padilla, G.M. eds. Academic Press, Inc., pp. 69-92, 1987.
- Renton, K. W. and Mannering, G.J. Depression of the hepatic cytochrome P-450-dependent mono-oxygenase system by administered tilorone. *Drug Metab. and Disp.* 4: 223-231, 1976a.
- Renton, K. W. and Mannering, G.J. Depression of the hepatic cytochrome P-450-dependent mono-oxygenase system with administered interferon inducing agents. *Biochem. Biophys. Res. Commun.* 73: 343-348, 1976b.
- Renton, K.W., Deloria, L.B. and Mannering, G.J. Effects of polyribinosinic acid and polyribocytidilic acid and a mouse interferon preparation on cytochrome P450-dependent monooxygenase systems in cultures of primary mouse hepatocytes. *Mol. Pharmacol.* 14: 672-681, 1978b.
- Renton, K.W., Keyler, D.E. and Mannering, G.J. Depression of the inductive effects of phenobarbital and 3-Methylcholanthrene on ascorbic acid synthesis and hepatic cytochrome P450-linked monooxygenase systems by interferon inducers, poly rI•rC and tilorone. *Biochem. Biophys. Res. Comm.* 88: 1017-1023, 1979.
- Renton, K.W., Gray, J.D. and Hall, R.I. Decreased elimination of theophylline after influenza vaccination. *Can. Med. Assoc. J.* 123: 288-290, 1980.
- Renton, K.W., Moochala, S., Gibson, G.G. and Makowski, R. The interferon mediated loss in cytochrome P-452. In: *Drug Metabolism - from Molecules and Man*, ed. Benford, D.J. et al., London: Taylor and Francis, pp. 448-452, 1987.
- Renton, K.W. and Knickle, L.C. Regulation of cytochrome P-450 during infectious disease. *Can. J. Physiol. Pharmacol.* 68: 777-781, 1990.
- Rosen, H., Gordon, S. and Worth, R.J. Exacerbation of murine listeriosis by a monoclonal antibody specific for the type 3 complement receptor of myelomonocytic cells. *J. Exp. Med.* 170: 27-37, 1989.
- Rosenstreich, D.L., Vogel, S.N., Jacques, A.R., Wahl, L. M. and Oppenheim, J.J. Macrophage sensitivity to endotoxin: genetic control by a single codominant gene. *J. Immunol.* 121: 1664-1670, 1978.
- Ryan, D.E. and Levin, W. Purification and characterization of hepatic microsomal cytochrome P450. *Pharmacol. Ther.* 45: 153-239, 1990.
- Sasaki, T., Mieno, M., Uono, H., Yamaguchi, K., Usui, T., Hara, K., Shiku, H. and Nakayama, E. Roles of CD4⁺ and CD8⁺ cells, and the effect of administration of recombinant interferon γ in Listerial infection. *J. Exp. Med.* 171: 1141-1154, 1990.

- Schlech, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S. and Broome, C.V. Epidemic listeriosis - evidence for transmission by food. *N. Engl. J. Med.* 308: 203-206, 1983.
- Schmucker, D.L., Woodhouse, K.W., Wang, R.K., Wynne, H., James, O.F., McManus, M. and Kremers, P. Effect of age and gender on in vitro properties of human microsomal monooxygenases. *Clin. Pharmacol. Ther.* 48: 365-374, 1990.
- Shedlofski, S.I., Swim, A.T., Robinson, J.M., Gallicchio, V.S., Cohen, D.A. and McClain, C.J. Interleukin-1 (IL-1) depresses cytochrome P450 levels and activities in mice. *Life Sciences* 40: 2331-2336, 1987.
- Shiraki, H. and Guengerich, F.P. Turnover of membrane proteins: kinetics of induction and degradation of seven forms of rat liver microsomal cytochrome P450, NADPH-cytochrome P450 reductase, and epoxide hydrolase. *Arch. Biochem Biophys.* 235: 86-96, 1984.
- Singh, G. and Renton, K.W. Interferon-mediated depression of cytochrome P-450-dependent drug biotransformation. *Mol. Pharmacol.* 20: 681-684, 1981.
- Singh, G., Renton, K.W. and Stebbing, N. Homogeneous interferon from *E. coli* depresses hepatic cytochrome P-450 and drug biotransformation. *Biochemical and Biophysical Research Communications* 106: 1256-1261, 1982.
- Singh, G. and Renton, K.W. Inhibition of the synthesis of hepatic cytochrome P450 by the interferon-inducing agent poly rI•rC. *Can. J. Physiol. Pharmacol.* 62: 379-383, 1984.
- Sonne, J., Dossing, M., Loft, S. and Andreasen, P.B. Antipyrine clearance in pneumonia. *Clin. Pharmacol. Ther.* 37: 701-704, 1985.
- Sonne, O., Davidsen, O., Moller, B.K. and Petersen, C.M. Cellular targets and receptors for IL-6. I. In vivo and in vitro uptake of IL-6 in liver and hepatocytes. *Eur. J. Clin. Invest.* 20: 366-370, 1990.
- Sonnenfeld, G., Harned, C.L., Thaniyavarn, S., Huff, T., Mandel, A.D. and Nerland, D.E. Type II interferon induction and passive transfer depress the murine cytochrome P-450 drug metabolism system. *Antimicrob. Agents Chemother.* 17: 969-972, 1980.
- Soyka, L.F., Stephens, C.C., MacPherson, B.R. and Foster, R.S. Jr. Role of mononuclear phagocytes in decreased hepatic drug metabolism following administration of *corynebacterium parvum*. *Int. J. Immuno. Pharmac.* 1: 101-112, 1979.
- Stadnyk, A.W. and Gauldie, J. The acute phase protein response during parasitic infection. *Immunol. Today* April 1991: A7-A12.
- Stanley, L.A., Adams, D.J., Lindsay, R., Meehan, R.R., Liao, W. and Wolf, C.R. Potentiation and suppression of mouse liver cytochrome P-450 isozymes during the acute-phase response induced by bacterial endotoxin. *Eur. J. Biochem.* 174: 31-36, 1988.

- Stanley, L.A., Adams, D.J., Balkwill, F.R., Griffin, D. and Wolf, C.R. Differential effects of recombinant interferon α on constitutive and inducible cytochrome P450 isozymes in mouse liver. *Biochem. Pharmacol.* 42: 311-320, 1991.
- Starnes, H.F. Jr., Pearce, M.K., Tewari, A., Yim, J.H., Zou, J.C. and Abrams, J.S. Anti-IL-6 monoclonal antibodies protect against lethal *Escherichia coli* infection and lethal tumor necrosis factor- α challenge in mice. *J. Immunol.* 145: 4185-4191, 1990.
- Stecha, P.F., Heynen, C.A., Roll, J.T., Brown, J.F. and Czuprynski, C.J. Effects of growth temperature on the ingestion and killing of clinical isolates of *Listeria monocytogenes* by human neutrophils. *Journal of Clinical Microbiology* 27: 1572-1576, 1989.
- Steinberg, P., Lafranconi, W.M., Wolf, R., Waxman, D.J., Oesch, F., and Friedberg, T. Xenobiotic metabolizing enzymes are not restricted to parenchymal cells in the liver. *Mol. Pharm.* 32: 463-470, 1987.
- Steward, A.R., Dannan, G.A., Guzelian, P.S. and Guengerich, F.P. Changes in the concentration of seven forms of cytochrome P450 in primary cultures of adult rat hepatocytes. *Mol. Pharm.* 27: 125-132, 1985.
- Strom, A., Mode, A., Zaphiropoulos, P., Nilsson, A., Morgan, E. and Gustafsson, J. Cloning and pretranslational hormonal regulation of testosterone 16 α -hydroxylase (P450 16 α) in male rat liver. *Acta Endocrinologica (Copenh)* 118: 314-320, 1988.
- Sujita, K., Okuno, F., Tanaka, Y., Hirano, Y., Inamoto, Y., Eto, S. and Arai, M. Effect of interleukin-1 (IL-1) on the levels of cytochrome P-450 involving IL-1 receptor on the isolated hepatocytes of rat. *Biochemical and Biophysical Research Communications* 168: 1217-1222, 1990.
- Tatsukawa, K., Misuyama, M., Takeya, K. and Nomoto, K. Differing contribution of polymorphonuclear cells and macrophages to protection of mice against *Listeria monocytogenes* and *Pseudomonas aeruginosa*. *J. Gen. Microbiol.* 115: 161-166, 1979.
- Tilney, L.G. and Portnoy, D.A. Active filaments and the growth, movement and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. *J. Cell Biol.* 109: 1597-1608, 1989.
- Utili, R., Abernathy, C.O. and Zimmerman, H.J. Minireview. Endotoxin effects on the liver. *Life Sciences* 20: 553-568, 1977.
- Vozch, S., Powell, J. R., Riegelman, S., Costello, J.F., Sheiner, L.B. and Hopewell, P.C. Changes in theophylline clearance during acute illness. *J. Am. Med. Assoc.* 240: 1882-1884, 1978.
- Wakabayashi, G., Gefland, J.A., Burke, J.F., Thompson, R.C. and Dinarello, C. A specific receptor antagonist for interleukin-1 prevents *Escherichia coli*-induced shock in rabbits. *FASEB J.* 5: 338-343, 1991.

- Waxman, D.J., Lapenson, D.P., Park, S.S., Attisano, C. and Gelboin, H. Monoclonal antibodies inhibitory to rat hepatic cytochromes P-450: P-450 form specificities and use as probes for cytochrome P-450-dependent steroid hydroxylations. *Mol. Pharm.* 32: 615-624, 1987.
- Wehr, H.M. *Listeria monocytogenes* - a current dilemma. *J. Assoc. Off. Anal. Chem.* 70: 768-772, 1987.
- Weiner, M. and Olson, J.W. Inhibition of hepatic drug metabolism in VX₂ carcinoma-bearing rabbits. *Drug Metab. Disp.* 8: 139, 1980.
- Westphal, O. Bacterial Endotoxins. *Int. Archs. Allergy Appl. Immun.* 49: 1-43, 1975.
- Whicher, J.T. and Evans, S.W. Cytokines in disease. *Clin. Chem.* 36: 1269-1281, 1990.
- Wilder, M.S. and Sword, C.P. Mechanisms of pathogenesis in *Listeria monocytogenes* infection. *J. Bacteriol.* 93: 531-537, 1967.
- Williams, J. F. Induction of tolerance in mice and rats to the effect of endotoxin to decrease the hepatic microsomal mixed-function oxidase system. Evidence for a possible macrophage-derived factor in the endotoxin effect. *Int. J. Immunopharmac* 7: 501-509, 1985.
- Williams, J. F. and Szentivanyi, A. Depression of hepatic drug metabolizing enzyme activity by *B. pertussis* vaccination. *Eur J. Pharm.* 43: 281-284, 1977.
- Williams, J.F., Lowitt, S. and Szentivanyi, A. Endotoxin depression of hepatic mixed-function oxidase system in C3H/HeJ and C3H/HeN mice. *Immunopharmacology* 2: 285, 1980.
- Williams, J.F., Winters, A.L., Lowitt, S. and Szentivanyi, A. Depression of hepatic mixed-function oxidase activity by *B. pertussis* in splenectomized and athymic nude mice. *Immunopharmacology* 3: 101-106, 1981.
- Williams, J.F. and Szentivanyi, A. Investigation of adrenergic and prostaglandin influences in the endotoxin alteration of hepatic heme oxygenase, microsomal mixed-function oxidase, and glucocorticoid-induced tryptophan oxygenase activities. *Immunopharmacology* 6: 75-86, 1983.
- Williams, J. F. and Szentivanyi, A. Pharmacokinetic and pharmacodynamic parameters effected by RE cell activators. In: *The reticuloendothelial system, a comprehensive treatise*. Vol. 8; Pharmacology. Eds. Hadden, J.W. and Szentivanyi, A. Plenum Press, New York, pp. 1-25, 1985.
- Williams, S.J. and Farrell, G.C. Inhibition of antipyrine metabolism by interferon. *Br. J. Clin. Pharmac.* 22: 610-612, 1986.
- Williams, S.J., Baird-Lambert, J.A. and Farrell, G.C. Inhibition of theophylline metabolism by interferon. *The Lancet*, October 24 : 939-941, 1987.

- Williams, J.F., Bement, W.J., Sinclair, J.F. and Sinclair, P.R. Effect of interleukin-6 on phenobarbital induction of cytochrome P-450 IIB in cultured rat hepatocytes. *Biochemical and Biophysical Research Communications* 178: 1049-1055, 1991.
- Wooles, W.R. and Munson, A.E. The effect of stimulants and depressants of reticuloendothelial activity on drug metabolism. *J. Reticuloendothel. Soc.* 9: 108-119, 1971.
- Wright, K. and Morgan, E.T. Transcriptional and post-transcriptional suppression of P450IIC11 and P450IIC12 by inflammation. *FEBS Lett.* 27: 59-61, 1990.
- Wright, K. and Morgan, E.T. Regulation of cytochrome P-450 IIC12 expression by interleukin-1 α , interleukin-6, and dexamethasone. *Mol. Pharmacol.* 39: 468-474, 1991.
- Yoshioka, H., Lindberg, R., Wong, G., Ichikawa, T., Itakura, T., Burkhardt, B. and Negishi, M. Characterization and regulation of sex-specific mouse steroid hydroxylase genes. *Can. J. Physiol. Pharmacol.* 68: 754-761, 1990.
- Zerke, T.B., Wade, A.E. and Ragland, W.L. Selective depression of hepatic cytochrome P-450 hemoprotein by interferon inducers. *Biochem. Biophys. Res. Comm.* 96: 121-127, 1980.