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LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L'AVONS REÇUE

INTERFERON LINKED DEPRESSION OF HEPATIC CYTOCHROME P-450

by

© GURMIT SINGH

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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MARCH 1982

"My proof convinces the ignorant, and the wise man's proof convinces me. But he whose reasoning falls between wisdom and ignorance, I neither can convince him, nor can he convince me."

Khalil Gabran

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ABBREVIATIONS AND SYMBOLS USED IN THIS THESIS

ADP	Adenosine diphosphate
ALA	Aminolevulinic Acid
EDTA	Ethylene diaminetetra-acetic acid
g	Gram
Xg	Acceleration of gravity
M	Molar
3-Mc	3-Methylcholanthrene
NADH	Diphosphopyridine nucleotide, reduced form
NADPH	Triphosphopyridine nucleotide, reduced form
NADP	Triphosphopyridine nucleotide
NDV	New Castle Disease Virus
nm	Nanometers
OD	Change in absorbance
PB	Phenobarbital
Poly r (I.C)	Polyriboinosinic Acid Polyribocytidylic Acid
rpm	Revolutions per minute
λ	Wavelength

Prefixes for units of measurement:

n	nano (10^{-9})
μ	micro (10^{-6})
m	milli (10^{-3})

ABSTRACT

Cytochrome P-450 and related drug biotransformation in the liver is depressed during viral infections or following the administration of interferon inducing agents. Renton and Mannering proposed that depression of this enzyme system was mediated via interferon but provided no direct evidence for this hypothesis. In this thesis indirect evidence obtained using a genetic model and direct evidence using pure, homogenous cloned interferon provides the first proof that interferon causes a marked depression of hepatic drug biotransformation.

The depressant effect of interferon on hepatic cytochrome P-450 could be caused by changes in synthesis and/or degradation of the enzyme. Evidence obtained by using the turnover of labelled heme precursors and labelled amino acids, indicate that the decrease in steady-state levels of hepatic cytochrome P-450 resulted from changes in both the synthesis and degradation of the hemoprotein.

We conclude that an impairment of cytochrome P-450-mediated drug biotransformation occurs via an interferon-mediated interaction and that this effect may enhance the toxicity of drugs and exogenously administered chemicals.

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Finally I would like to dedicate this thesis to my parents and my fiancée Anju Joshi. Their moral support and encouragement made this work enjoyable.

PUBLICATIONS

Parts of this thesis have already been published as follows:

(a) Interferon mediated depression of cytochrome P-450 dependent drug biotransformation. G. Singh and K.W. Renton. Proc. Can. Fed. of Biol. Sci. 24: 399, 1981.

(b) Effect of Interferon on Hepatic Cytochrome P-450 drug metabolizing systems. Gurmit Singh, Kenneth W. Renton and Nowell Stebbing. Pharmacologist 23: 283, 1981.

(c) Interferon mediated depression of cytochrome P-450 dependent drug biotransformation. Gurmit Singh and Kenneth W. Renton. Molecular Pharmacology. 20: 681-684, 1981.

INTRODUCTION

SECTION A

(1) General Review of Hepatic Mixed Function Oxidase System

Drugs and other foreign compounds are predominantly metabolized in the microsomal fraction of the liver. These generally lipophilic compounds are converted to more hydrophilic and excretable products by a multistep metabolic process which usually involves initial oxidation and subsequent conjugation reactions. The enzyme systems primarily responsible for drug biotransformation are located in the hepatic endoplasmic reticulum which is a highly organized network of continous interconnected lipoprotein membranes. Homogenization of the liver results in the endoplasmic reticulum being pinched off, followed by membrane closure to form particulates called microsomes. The term "microsomes" therefore does not denote a morphological entity associated with the structure of a cell but is a pinched off fraction of the endoplasmic reticulum. Microsomes can be isolated by high-speed centrifugation of the post-mitochondrial supernatant.

Early studies (Brodie et al., 1955) demonstrated that drug metabolism was catalyzed in the microsomal fraction of the liver. This microsomal enzyme system was termed the hepatic mixed function oxidase system by Mason in 1957 in order to characterize the mixed function of the oxygen

molecule. Later Hayaishi (1962) termed it the monooxygenase system because the enzyme system incorporates only one atom of molecular oxygen into organic substrates with concomitant reduction of the second oxygen atom to water. Both of these terms are now used interchangeably in the literature. The enzyme system is capable of oxidizing a wide variety of different substrates by several diverse reactions including N-dealkylation, deamination, aromatic hydroxylation, sulphoxidation, O-dealkylation, S-oxidation and N-oxidation (Brodie, 1958; Gillette, 1966). Examples of these oxidation reactions include O-dealkylation of codeine to morphine followed by N-dealkylation of morphine to normorphine, sulfoxidation of chloropromazine to chlorpromazine sulfoxide, aromatic hydroxylation of phenobarbital to the parahydroxylphenyl derivative and deamination of amphetamine to phenyl acetone.

During mixed function oxidation of a xenobiotic, electrons are transfered from NADPH via an electron transport chain, which subsequently reacts with molecular oxygen to form an active oxygen intermediate. The active oxygen is then transferred to the substrate via the following simplified reaction scheme.

$$NADPH + P\text{-}450 + H^+ \longrightarrow P\text{-}450\text{-}H_2 + NADP^+$$

$$P\text{-}450\text{-}H_2 + O_2 \longrightarrow \text{"active oxygen"}$$

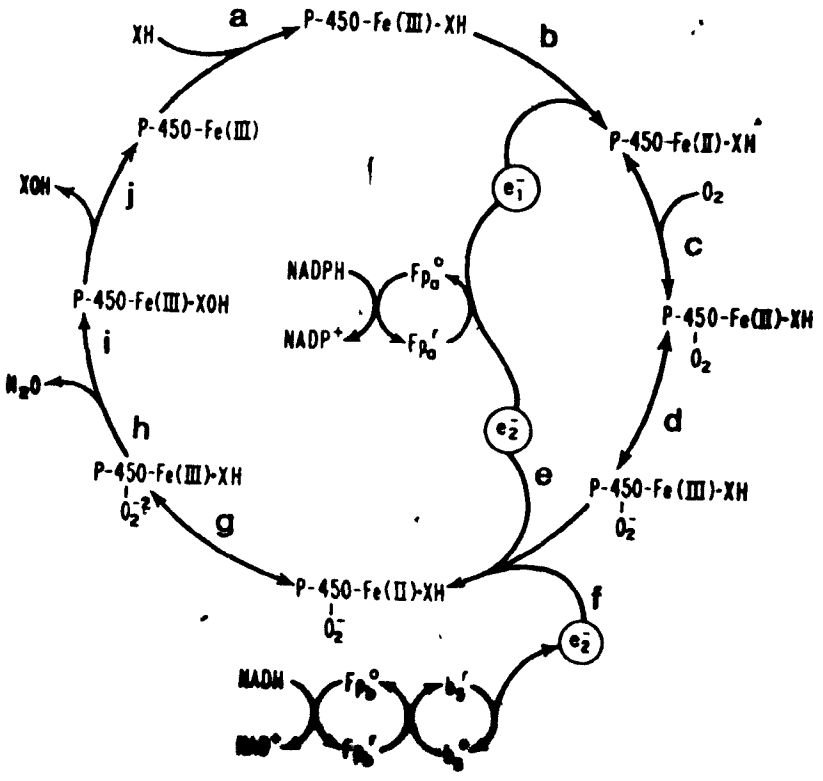
$$\text{active oxygen} + \text{substrate} \longrightarrow \text{oxidized drug} + P\text{-}450 + H_2O$$

$$NADPH + O_2 + \text{substrate} \longrightarrow NADP^+ + H_2O + \text{oxidized substrate}$$

The component P-450 of the mixed function oxidase system has been identified as a membrane bound hemoprotein sensitive to carbon monoxide (Omura, 1963). The other components of the electron transport system consist of cytochrome b_5 (Pappenheimer and Williams, 1954) and cytochrome C reductase (Phillips and Langdon, 1962). Cytochrome P-450 is the major component and central to the oxidation process by the enzyme system.

Several schemes have been proposed (Omura et al., (1965), Estabrook et al. (1971), Gander et al., (1980)) in attempts to include the involvement of cytochrome b_5 and NADH in the electron transport system and to achieve a stoichiometric reaction for the mixed function oxidase system. In a recent review, Gander et al. (1980) proposed a representative scheme for electron transport in the mixed function oxidase system. In this scheme, (illustrated on the following page) the substrate (XH) combines with oxidized P-450 (ferricytochrome P-450) to form a complex which is reduced by electrons from NADPH via the electron

transport chain which includes cytochrome c reductase. The reduced substrate P-450 complex (ferrocytochrome P-450) reacts with molecular oxygen to form a substrate cytochrome P-450-oxygen complex. Electrons from NADH via cytochrome b_5 may contribute to the second electron input on the cytochrome P-450 complex or the second electron may be derived from NADPH via cytochrome c reductase. This yields a complex with activated oxygen which then splits to yield water, oxidized drug (XOH) and oxidized form of cytochrome P-450 (ferricytochrome P-450).



(Gander and Mannering, 1980)

P-450 (III) = Ferricytochrome-P-450

P-450(II) = Ferrocytochrome P-450

b_5 = cytochrome b_5

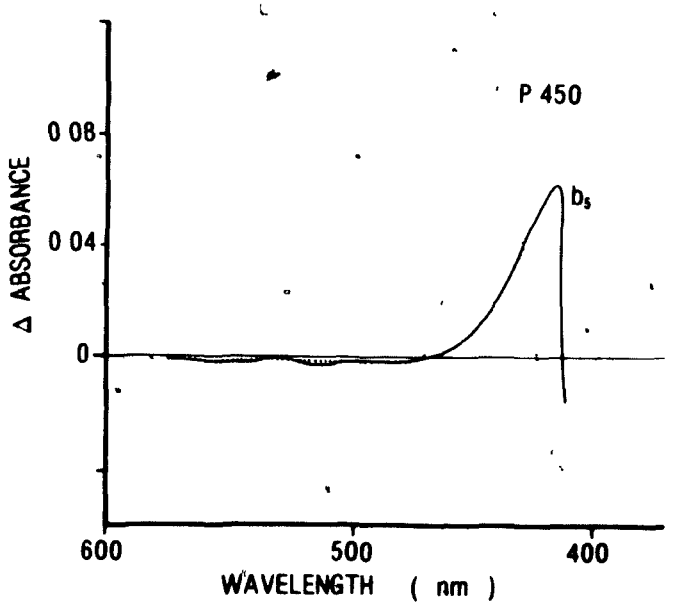
FP_a = NADPH-cytochrome P-450 reductase

FP_b = NADH-cytochrome b_5 reductase

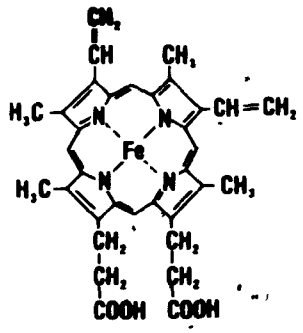
XH = substrate

(ii) Cytochrome P-450

Cytochrome P-450 is a hemoprotein with a peak absorbance of about 450 nm, when it is reduced and complexed with carbon monoxide (Omura, 1963). A typical carbon monoxide difference spectra, which exhibits a large peak at about 450 nm, and a deep trough at about 405 nm is illustrated below.



It is generally accepted that the prosthetic group of cytochrome P-450 is ferroprotoporphyrin IX (Protoheme IX) with the following structure.



PROTOHEME (IX)

The protein moeity of the purified cytochrome P-450 from liver yeilds a single polypeptide band on SDS-urea polyacrylamide gel electrophoresis (Imai and Sato, 1974; Ryan et al., 1975; Haugen and Coon, 1976). The molecular weight of the polypeptide chain of P-450 is estimated around 50,000 - 55,000 daltons by the method of gel electrophoresis (Huang et al., 1976). Cytochrome P-450 is found in various tissues including liver (Omura and Sato, 1964), the kidney (Ellin et al., 1972), small intestine (Takesue et al., 1968), lung (Matsubara et al., 1971) adrenal cortex (Estabrook et al., 1963), skin (Poland et al., 1974) testis (Betz et al., 1976) and placenta (Meigs, R.A. and Ryan, K.J., 1968).

Most cytochromes are involved in electron transport

chains as mere electron carriers, but cytochrome P-450, is involved in catalysing several types of redox reactions in addition to being a terminal electron carrier. Early attempts to solubilize and purify the enzyme system in its native form by conventional procedures failed because cytochrome P-450 was converted rapidly to an inactive form (cytochrome P-420). Ichikawa and Yamano (1967) solved this technical difficulty when they discovered that glycerol and other polyols stabilized detergent treated cytochrome P-450. Solubilization and resolution of catalytically active hydroxylating system from the microsomal membrane was achieved using a combination of ionic and nonionic detergents (Lu and Coon, 1968). Purification of cytochrome P-450 to homogeneity as defined by SDS electrophoresis and immunological techniques has now been achieved in several laboratories (Imai and Sato, 1974; Van der Hoeven et al., 1974; Haugen and Coon, 1976).

Warner et al. (1978) have been able to obtain high yields of cytochrome P-450 in solubilized form. These investigators incubated microsomes in a glycerol buffer with the nonionic detergent, Emulgen 911 and the ionic detergent cholate at room temperature and recovered approximately 90% of cytochrome P-450 in the solubilized form. The advantage in using room temperature was to avoid aggregation which occurs at 4°C. The solubilized microsomes were discriminated on the basis of molecular weight by passage

through a sephadex gel column. The fractions containing cytochrome P-450 were then separated on a DEAE-Anion exchange column. Finally the fractions were resolved by electrofocussing on SDS gels. The results obtained, indicated extensive heterogeneity in the cytochrome P-450.

Initial evidence for multiple forms of cytochrome P-450 was provided in 1966 by Sladek and Mannering. They showed that 3-methylcholanthrene-treated rats had a different species of cytochrome P-450 in liver microsomes. Physical separation of different forms of cytochrome P-450 from a single source of microsomes (Comai and Gaylor, 1973) has provided direct verification for the multiplicity of the molecular species of cytochrome P-450. Inducing agents such as phenobarbital and 3-methylcholanthrene cause the synthesis of new forms of cytochrome P-450 or a disproportionate increase in the synthesis of existing forms and has allowed the isolation of particular species of cytochrome P-450 (Ryan et al., 1975; Haugen et al., 1976). The Phenobarbital induced cytochrome is designated as P-450 LM_2 and has a reduced carbon monoxide spectrum with a maximum absorbance at 450 nm, a molecular weight of 48,700 and it hydroxylates ethylmorphine and benzaphetamine preferentially whereas the 3-methylcholanthrene induced cytochrome is designated as P-450 LM_4, and has a reduced CO spectrum with a maximum absorbance at 448 nm, a molecular weight of 53,300 and a high substrate selectivity for benzo

(a) pyrene. The sophistication of purification and isolation procedures has dramatically increased the number of species of cytochrome P-450 which can be isolated.

(iii) Factors which alter the steady-state levels of cytochrome P-450

The activity of microsomal mixed function oxidase is altered by various external factors and by abnormal physiological states such as changes in nutritional status, hormonal disturbances, disease or other pathological states. Since this enzyme system is responsible for the biotransformation of so many drugs, alterations in the steady-state levels of the enzyme become a major concern in the practise of therapeutics. Some of the factors which alter the steady-state levels of cytochrome P-450 are summarized below.

a) species. Williams (1967) has extensively reviewed interspecies differences in the ability of animals to metabolize xenobiotics by cytochrome P-450. Variations in metabolism can be quantitative or due to alternate pathways of metabolism. The alternate metabolite formation probably results from the presence in a particular species of a specific type of P-450 which is not found in others. Quantitive differences could be simply due to different steady-state levels of the enzyme in various species.

b) strain. Interstrain differences in the inducibility of various forms of cytochrome P-450 by 3-methylcholanthrene and phenobarbital have been reported in mice (Nebert, 1979; Vesell, 1968). Such interstrain differences have also been reported in rats (Mitoma et al., 1967).

c) age. Drug metabolism in the newborn is quite different from that in the adult. This was first reported by Fouts who determined that in the rabbit hepatic mixed function oxidase is deficent at birth and reaches adult levels at 4 weeks of age (Fouts et al., 1959). There have been several subsequent reports documenting the immaturity of various hepatic microsomal enzymes in various species of young animals (Gram et al., 1969; Basu et al., 1971; Macleod et al., 1972). In human newborns a deficiency in drug oxidations has been reported which is related to a deficiency in cytochrome P-450 (Aranda et al., 1974).

d. sex. Drug oxidation in male rats is higher than in female rats. Quinn et al. (1958) demonstrated that sleeping times for hexobarbital are longer in female rats compared to male rats and that the differences are due to an increased half life of the drug in female rats. El Defraway et al. (1974) found qualitative differences in the cytochrome P-450 of the two sexes and argued that the differences could be related to the hormonal status of the sexes. Other species, besides rats have negligible sex differences.

e) nutritional status. Several investigators have observed that the activity of drug-metabolizing enzymes is higher in rats fed on chow diets as compared with semi-synthetic diets (Wattenberg, 1972; Zeiger, 1975; Pantuck et al., 1975). It has also been shown that starvation of male mice for 36 hours depresses hepatic microsomal drug metabolism, measured both in vitro and in vivo (Dixon et al., 1960).

f) hormonal disturbances. Hormonal status has been shown to cause alterations in steady-state levels of cytochrome P-450 (Kato, 1977). For instance, castration of adult male rats decreases the activity of the sex-dependent hepatic microsomal enzymes and administration of androgens to these castrated animals increases the activity of the sex-dependent enzymes without affecting the sex-independent ones (Kato and Gillette, 1965). Wada et al., (1964) reported that the content of cytochrome P-450 was significantly decreased in liver microsomes from adrenalectomized male rats and that the decrease is restored by cortisone administration.

g) induction. Insecticides, herbicides, polycyclic hydrocarbons, various drugs and other chemicals are capable of inducing the microsomal mixed function oxidase system as shown by Axelrod (1956), Conney (1967) and others. Enzyme induction usually refers to a relative increase in mixed function oxidase activity which is determined by an increased rate of drug biotransformation. There are few

structural similarities between compounds in causing microsomal mixed function oxidase system induction except that most inducers are lipid soluble. Most inducers of the mixed function oxidase system can be segregated into two major classes (Conney, 1967). Phenobarbital (PB) is the prototype of one class, which stimulates the metabolism of a wide variety of substrates and is capable of inducing a number of cytochrome P-450 subspecies. Polycyclic aromatic hydrocarbons such as benzo (a) pyrene and 3-methylcholanthrene (3-MC) represent the other class of inducing agents. They induce a distinct species of cytochrome P-450 commonly known as cytochrome P-448 or P_1-450 and enhance the metabolism of a limited number of substrates.

h) inhibition. SKF-525A (β-diethylaminoethyl diphenyl propyl acetate) prolongs barbiturate sleeping time in rats and mice by inhibiting metabolism of the barbiturates by hepatic enzymes (Axelrod et al., 1954). Various other agents such as carbon monoxide (Kato, 1966), heavy metals (Tephyl and Hibbeln, 1971), immunosuppressive agents (Soyka, et al., 1976), antiviral agents (Renton and Mannering, 1976) have been reported to inhibit the microsomal mixed function oxidase system. Inhibition of the microsomal enzyme system has been demostrated to occur by various different mechanisms at different steps in the electron transport chain or by the destruction of a specific component of the mixed function oxidase system. Metyrapone (Ullrich and

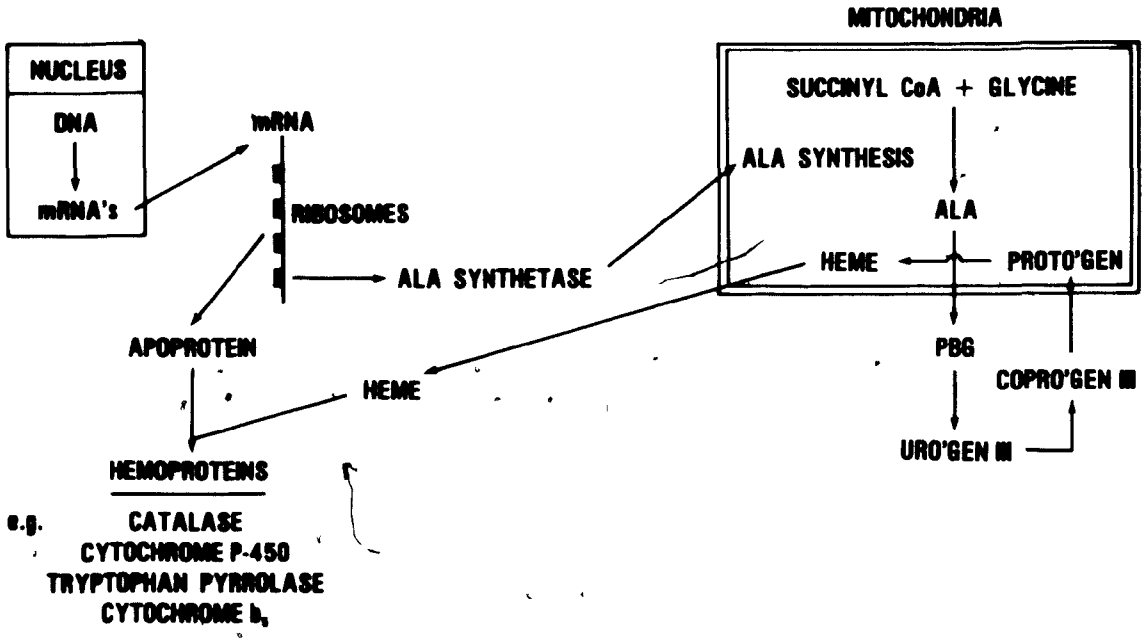
Schnabel, 1973) and carbon monoxide (Cooper et al., 1965) inhibit the mixed function oxidase system by binding directly to the cytochrome P-450. Cobalt choloride has been shown to inhibit drug biotransformation by inhibiting the biosynthesis of the heme component of cytochrome P-450 (Tephyl and Hibbeln, 1971) and by stimulation of the oxidative degradation of heme (Maines and Kappas, 1975).

SECTION B

(1) The turnover of Hepatic Hemoproteins.

Hemoproteins which have both a heme moiety and a protein moeity can be broadly divided into five major classes according to their functions (Granick and Gilder, 1947): (a) Oxygen transporting hemoproteins e.g Myoglobin and Hemoglobin; (b) Electron transport hemoproteins e.g. Mitochondrial proteins; (c) Hemoproteins which activate oxygen e.g. cytochrome oxidase, cytochrome P-450 and tryptophan pyrolase; (d) Hydrogen peroxide activating enzymes e.g. peroxidases and (e) those which decompose hydrogen peroxide e.g. catalases. Most of these hemoproteins have a rapid turnover rate relative to hemoglobin which remains in the erythrocyte, for the entire life of the cell, which is about 120 days in man (London, 1961) and about 60 days in the rat (Akeson et al., 1960). A summary of the biological half-lives of the hepatic hemoproteins is given in Table 1.

The biosynthesis of the hemoproteins is rather complex, since it requires a coordinated synthesis of the heme moeity and the apoprotein moeity followed by their assembly into the holohemoprotein. A diagramatic representation of biosynthesis of hemoproteins is given in the following scheme.



(Tait, 1978)

Table 1

Hemoprotein	Biological half live	Location	Reference
catalase	2 days	Peroxisomes	Poole et al., 1969
tryptophan pyrrolase	2 hours	cytoplasm	Schimke et al., 1965
cytochrome b	5.5 days	Mitochondria	Druyan et al., 1969
cytochrome c	6.1 days	Mitochondria	Druyan et al., 1969
cytochrome P-450	1-2 days	Endoplasmic reticulum	Levin and Kuntzman, 1969
cytochrome b_5	2 days	Endoplasmic reticulum	Greim et al., 1970

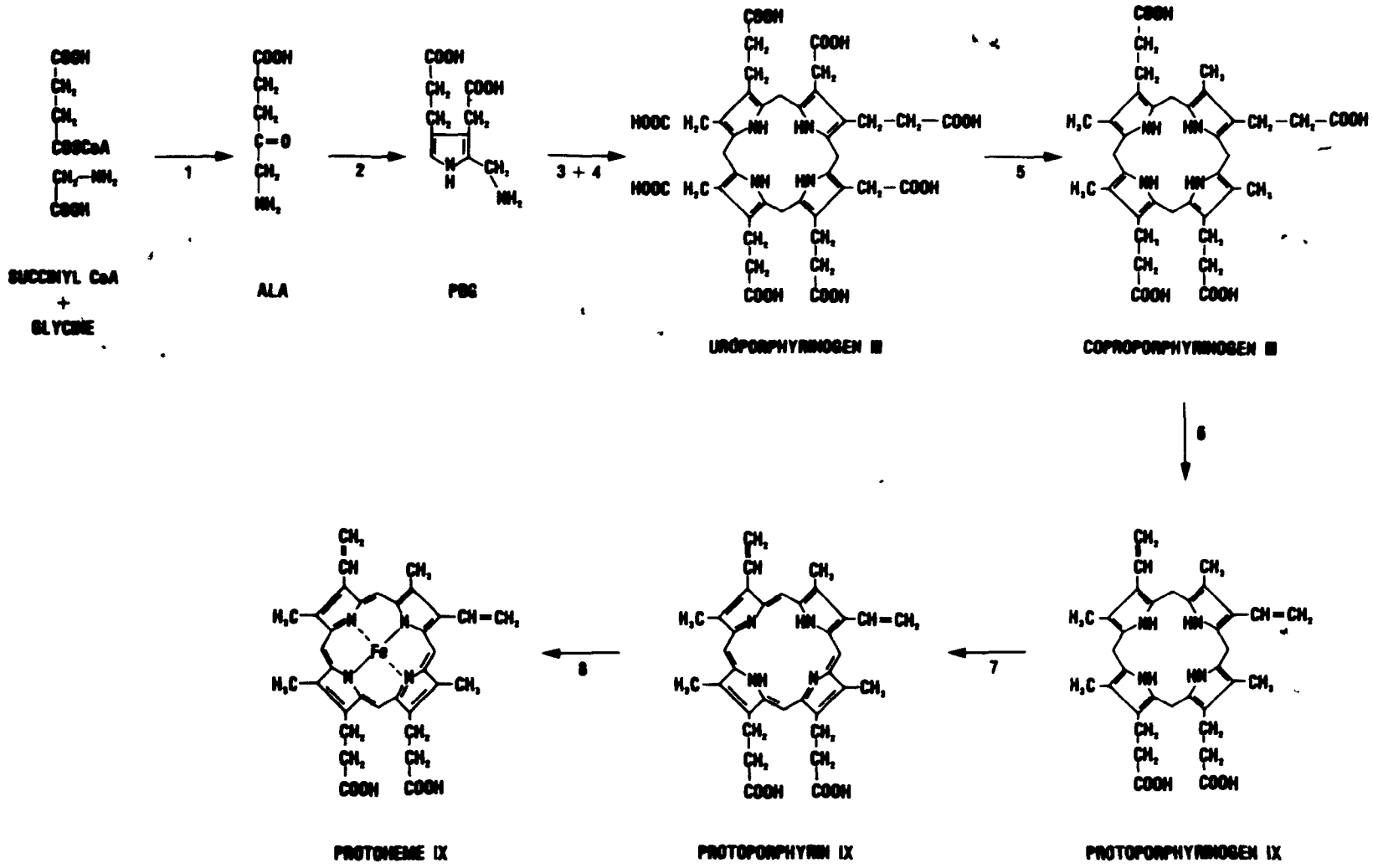
The half-lives of the hepatic hemoproteins can be measured accurately by using a dual label technique in which the heme and the apoprotein are simultaneously labelled. Heme is labelled using tritiated delta-aminolevulinic acid which is specifically incorporated into the heme moeity and on degradation of the hemoprotein, is not reutilized (Druyan et al., 1969). The apoprotein can be labelled in the same experiment utilizing [^{14}C]-amino acids (Schimke, 1973). Both the apoprotein and the heme moieties of the individual hemoproteins have been shown to turnover at the same rate (Tait, 1978).

(ii) Turnover of heme in cytochrome P-450.

The turnover of the heme moeity in cytochrome P-450 is an important process because heme serves as the prosthetic group for this hemoprotein enzyme. This is unlike most other prosthetic groups which are derived from dietary sources. The heme moeity is biosynthesized entirely in the hepatocyte via the metabolic pathways illustrated in Fig. 1. The heme moeity, being a prosthetic group for various hemoproteins including cytochrome P-450, has its synthesis finely regulated. Glycine and succinyl CoA are condensed to ALA by the rate-limiting enzyme ALA-synthetase (step 1 in Fig. 1) which is under negative feedback control (Tait, 1978). Excess heme which is not bound to protein causes a decrease in the activity, or in the amount of the enzyme and

FIGURE 1

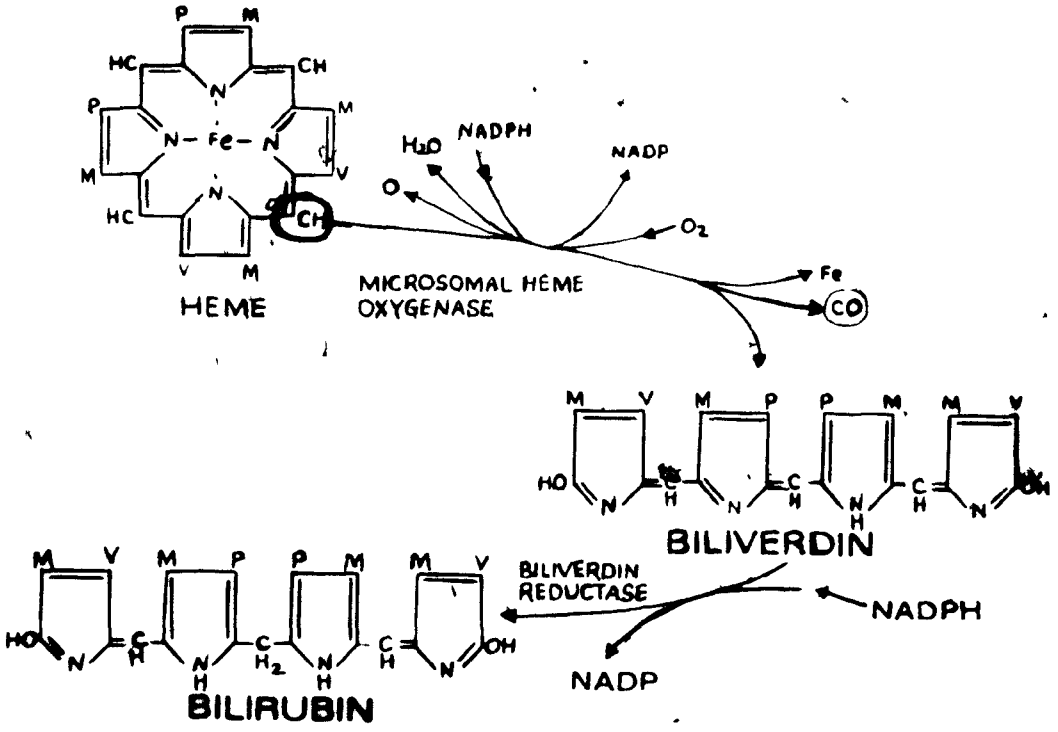
Biosynthesis of Heme Enzymes catalyzing the interconversions are: 1. ALA synthetase. 2. ALA dehydratase. 3. PBG deaminase. 4. Uroporphyrinogen III cosynthetase. 5. Uroporphyrinogen decarboxylase. 6. Coproporphyrinogen oxidase. 7. Protoporphrinogen oxidase. 8. Heme synthetase. (from Tait, G.H. (1978)).


SUCCINYL CoA
+
GLYCINE
1
ALA
2
PBG
3 + 4
UROPORPHYRINOGEN III
5
COPROPORPHYRINOGEN III
6
PROTOPORPHYRINOGEN IX
7
PROTOPORPHYRIN IX
8
PROTOHEME IX

when a deficiency of heme occurs, relative to the amount of apoprotein, the enzyme is derepressed and an increase in the activity, or the amount of ALA synthetase takes place. The molecular mechanism by which heme regulates the rate of formation of ALA is not completely understood, although mechanisms have been postulated for this effect. These have included the inhibition of the enzyme at the ribosomal level (Tyrrell and Marks, 1972), direct inhibition of the enzyme activity by heme in the mitochondria (Hayashi et al., 1972) or repression by heme of the formation of mRNA for ALA synthetase (Skea et al., 1971). The technical difficulty in measuring the amount of enzyme as opposed to the activity of the enzyme has hindered progress in resolving the exact molecular mechanism involved.

The degradation pathway of heme to bilirubin is shown in Fig. 2. The rate-limiting step for degradation of free heme is its conversion to biliverdin. Also formed at this stage is carbon monoxide which will be used in this thesis as a measure of heme breakdown. However it is controversial whether heme oxygenase is capable of degrading heme while it is attached to the apoprotein.

FIGURE 2



(iii) Turnover of Protein in Cytochrome P-450.

Omura et al. (1967) established heterogeneity in the turnover rates of several microsomal enzymes. This important observation implied that the proteins associated with the endoplasmic reticulum are synthesized and degraded at independent rates rather than an entire synthesis and destruction of the endoplasmic reticulum membrane. Thus drugs and hormones which can affect protein synthesis and degradation are capable of affecting specific proteins in the endoplasmic reticulum. This phenomenon is observed with a variety of drugs, most notably by phenobarbital, which

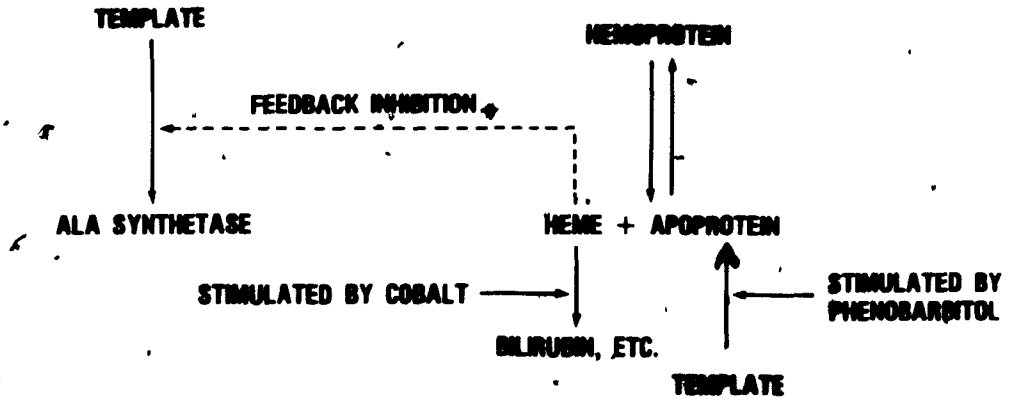
causes a proliferation of the endoplasmic reticulum and an increase in capacity to metabolize drugs and steroids (Conney and Burns, 1959; Remmer and Merker, 1965). Arias et al, (1969) and Kuriyama et al., (1969) showed that phenobarbital increased the rate of synthesis of cytochrome c reductase but little effect on cytochrome b_5. Dehlinger and Schimke (1972) later showed that phenobarbital also increased the content of cytochrome P-450 by increasing its rate of synthesis. Increased rate of de-novo synthesis of the apoprotein of cytochrome P-450 after phenobarbital administration has been subsequently shown by Correia and Meyer (1975) by adding heme in vitro to the isolated hepatic microsomal fraction from rats treated with phenobarbital plus cobalt. Recently Kumar et al. (1980) have shown a synchronized increase in cytochrome P-450 RNA messenger activity and the rate of apocytochrome P-450 synthesis. It is curious that phenobarbital increases biosynthesis of apocytochrome P-450 and several other proteins but it can also cause a decrease in synthesis of other proteins, which have not been identified (DuBois et al., 1980).

Turnover of the apoprotein has also been studied after the administration of aromatic hydrocarbons such as β-napthoflavone and 3-methylcholanthrene which induce a specific type of cytochrome P-450 named cytochrome P-448 or P_1-450 (Nebert and Gielen, 1971). Significant differences in the capacity to induce P_1-450 dependent-hydroxylase

systems occurs in different inbred strains. Utilizing the incorporation of ^{14}C- and ^{3}H-leucine into hepatic proteins in P_1-450 responsive and non responsive mice Haugen et al. (1976) have shown that enhanced formation of cytochrome P-448, is primarily the result of an increased rate of denovo protein synthesis rather than a decreased degradation rate or a conversion of pre-existing polypeptides. Recently Negishi and Kreibich (1978) have shown the coordinated apoprotein synthesis and the insertion of protoheme to form the holo cytochrome P-450 in the endoplasmic reticulum.

The emerging molecular mechanism for the turnover of cytochrome P-450 seems very complex and involves both the apoprotein synthesis and the heme moeity regulation in synchrony. However administration of drugs such as phenobarbital or cobalt can disrupt the steady-state turnover of apoprotein and of the heme moeity and thereby affect the level of cytochrome P-450. A possible scheme for the coordination of Heme and the Apoprotein is illustrated in Fig. 3.

FIGURE 3



SECTION C

(1) General review of Interferon.

Interferon was discovered by Isaacs and Lindenmann in 1957, (Lindenmann et al., 1957). On partial purification it has been demonstrated that interferon is a heterogenous class of proteins with a molecular weight range of 15,000 - 28,000 daltons, depending upon the cell, source and induction mode of its production (Chen et al., 1976). Due to the heterogeneity of interferon, and a widespread interest in interferon, an international committee has been set up to define and classify the interferons and the following definition has been adopted by the committee: "To qualify as an interferon a factor must be a protein which exerts virus non-specific, antiviral activity at least in homologous cells through cellular metabolic processes involving synthesis of both RNA and protein" (Stewart II, 1980). The following table (Table 2) shows the Interferon nomenclature for human and mouse interferons that the committee adopted.

TABLE 2

New Nomenclature	Old Nomenclature	
	Human	Mouse
IFN-α	Le (leukocyte), Type I, pH 2 stable foreign cell-induced	F (fast), C, type I, pH 2 stable
IFN-β	F (fibroblast), F1, Type I, pH 2 stable	S (slow), A, B, Type I, pH 2 stable
IFN-γ	II F (immune) Type II, T, pH 2 labile, antigen induced, mitogen-induced	immune (IIF), Type II, pH 2 labile, T, antigen-induced, mitogen-induced

(From Nature Vol. 286: 110, 1980).

Most nucleated cells have the capacity to produce interferon, when stimulated by an appropriate inducer. A variety of natural and synthetic agents are capable of inducing interferon production, such as viruses, double-stranded polynucleotides like poly r (I.C.), fungal extracts, bacteria and bacterial products, mitogens and some small synthetic molecules like tilorone (Stewart II, 1979). Interferon production is a process that requires denovo protein synthesis and is triggered by the inducer-cell interaction (Vilcek et al., 1969). Several structural genes for interferon production have been localized and

therefore the inducer may produce interferon by activating one or more gene loci (DeMaeyer and DeMaeyer-Guignard, 1979).

The resistance of cells to viruses is dependent on the concentration of the interferon present and the type of interferon (Stewart II, et al., 1969). Different species of animal, and different kinds of cell, show a particular pattern of relative resistance to various viruses when exposed to a type of interferon, and the same virus therefore may appear "sensitive" to interferon in one species and "resistant to it in another" (Krim, M., 1980). The mechanism by which interferon exerts its antiviral effect is still controversial and appears to be complex. Both viral transcriptional block and translational block in interferon-treated cells has been demonstrated (Joklik, W.K., 1977). Revel and Groner (1978) have described three different mechanisms by which viral mRNA translation can be inhibited, that is, an enhanced degradation of viral mRNA, an inhibition of chain initiation, and an inhibition of chain elongation. Therefore the interferon-induced antiviral state seems to be a multipronged-attack that can be directed to various stages of viral replication and maturation. This complexity can be used as an explaination as to why there is so far no instance of acquired resistance to interferon action by any virus.

In addition to antiviral activities, interferons seem to have other biological effects on cells. The non-antiviral effects of interferon such as antitumor activity (Stewart II, 1979), immunodepression (Ngan et al., 1976), cell-multiplication inhibition (Lee et al., 1972), surface alteration (Lindahl et al., 1976), enhanced phagocytosis (Huang et al., 1971), macrophage activation (Rabinovitch et al., 1977), enhanced protein synthesis (Rozee et al., 1969) and depressed protein synthesis (Beck et al., 1974) have been demonstrated. However conflicting reports have shown both enhancement and suppression of the same cellular functions and this is presumably due to the impurity of the interferon preparations used, source of interferon and variability in experimental procedures utilized. None the less, the idea that interferon can play a much broader role in cellular regulation than merely as a modulator of antiviral resistance has been generally accepted. The antitumor activity of interferons is probably mediated by the combination of its antiviral activity, its cell-multiplication-inhibiting activity and its immuno-modulatory actions. Interactions between the immune system and the interferon mediated defence system seems to be of great importance, because both systems are involved in the recognition of, and defence against virus-infected and neo-plastic cells (Krim, M., 1980). Due to the antiviral effect and potent antitumor effect on some types of tumors

interferon seems to have great potential as a drug for the future.

Various crude preparations of interferon have been demonstrated to have therapeutic effects in treatment of various human viral infections ,Jones et al. (1976); Merigan et al. (1973) in clinical trials. Horoszewicz et al. (1978) also showed that interferon caused marked tumor regression, both benign and malignant types in man. There is no strong evidence that tumors in humans are caused by virus infections, therefore at present there is no reason to think the antitumor action of interferons in humans is an extension of its antiviral activity. The inhibition of chemically or radiation-induced tumors, suggest that the antitumor action of interferon is based on something more than its antiviral activity. Some of the reasons why interferon may be an effective treatment for some forms of human cancer are (a) Inhibition of tumor cell growth (Gresser et al, 1972), (b) Activation of natural killer cells (Cantell, K., 1980), (c) Macrophage stimulation (Schultz et al., 1977), (d) Increase in histocompatibility antigen expression (Lindahl, et al., 1976). In addition the work of several laboratories (Glasgow et al., 1978; Gresser, I., and Bourali, C., 1970) have demonstrated an antitumor effect of interferon treatment in animals; this provides a rationale for attempting such clinical studies in humans.

A number of human cancers are currently being treated with human interferons, most frequently interferon from

human leukocytes. Results from adjuvant interferon treatment of human osteogenic sarcoma are suggestive of an inhibitory effect of interferon treatment on the spread of the tumor (Strander et al., 1978). Scarcity of human interferon has slowed the progress for more clinical trials.

SECTION D

Effect of viral infections on drug metabolism

Impaired drug metabolism has been reported during episodes of infection with influenza virus. Chang et al. (1978) initially described an increase in theophylline half life in patients who had upper respiratory tract viral infections. Similar changes in the kinetics of theophylline elimination were observed in subjects who were vaccinated with influenza virus vaccine (Renton et al., 1980). During an epidemic of influenza B in the Seattle area in 1980, Kraemer et al., (1981) reported the occurence of theophylline toxicity in eleven children. Each child had clinical evidence of a concurrent viral infection and six children demonstrated positive serologic evidence of influenza. These children had experienced no difficulties with their theophylline dosage. Encephalomycarditis virus infections in mice caused a decrease in drug elimination and depressed the steady-state levels of hepatic cytochrome P-450 (Renton, 1981b). Murine hepatitis virus impairs hexobarbital oxidation (Kato, et al., 1963) and depresses cytochrome P-450 in the liver (Budillon et al., 1972). To date only viruses of the influenza class have been identified as causing impairment of drug metabolism in man, however animal experiments suggest that other viral

infections will also cause a deleterious effect on drug metabolism.

Other agents which stimulate host defence mechanisms such as BCG (Farquar et al., 1976), E. coli endotoxin (Gorodischer et al., 1976), Plasmodium berghei (McCarthy et al., 1970), Corynebacterium parvum (Soyka et al., 1976) and M. butyricum (Carlson et al., 1975) have also caused decreased drug biotransformation and depressed steady-state levels of cytochrome P-450. Renton and Mannering (1976) and Leeson et al., (1976) reported a marked depression of cytochrome P-450 in rats following the administration of the interferon inducing agent tilorone. Other interferon inducers such as lipopolysaccharide (E. coli endotoxin), bacteria (pertussis vaccine), fungal products (statalon) and double-stranded nucleotides (poly r (I.C.)) also caused a decrease in the mixed function oxidase activity and depressed levels of hepatic cytochrome P-450 (Renton and Mannering, 1976). Renton (1981a) showed a temporal relationship between the loss of mixed function oxidase activity and the production of interferon following the administration of Poly r (I.C.). A common factor in most viral infections and agents which stimulate host defence mechanisms is interferon and therefore it could be implied as a mediator that causes the deleterious effect on hepatic drug biotransformation during infections.

The "Interferon hypothesis" put forward by Renton and Mannering seems to be attractive, however other reasons for

depression of cytochrome P-450 during viral infections, such as immune enhancement, reticuloendothelial cell stimulation, or a combination of more than one factor must be taken into account. Agents such as dextrans, latex beads and dextran sulfate which do not stimulate interferon production but are phagocytosed by the reticuloendothelial system in the liver also cause a decrease in cytochrome P-450 and related drug biotransformation (Renton, 1981). Using various molecular weights of maleic anhydride ether copolymers Barnes et al., (1979) demonstrated a correlation between the magnitude of the depression in drug biotransformation caused by these compounds and their anti-viral effect, anti-tumor effect and their ability to block phagocytosis. It is likely that alterations in other host defence mechanisms against an invading organism is also involved in depressing hepatic drug biotransformation.

SECTION E

Formulation of the Problem

Renton and Mannering (1976) proposed that the ability to depress the cytochrome P-450 dependent mixed function oxidase system was a common property of all interferon inducing agents and that this was likely related to the production of interferon itself. No direct evidence implicating a role for interferon could be provided at that time. The present study was undertaken to substantiate the "Interferon hypothesis". The major objectives of this study were:

(a) To determine that interferon itself causes a depression of hepatic cytochrome P-450.

(b) To establish the mechanism involved in the depression of hepatic mixed function oxidase system caused by interferon.

In establishing the mechanism by which the depression of cytochrome P-450 occurred various possibilities such as an overall effect on synthesis and degradation of hepatic cytochrome P-450, effect of interferon on the apoprotein and the effect on the protoheme of the cytochrome P-450 were considered.

MATERIALS AND METHODS

MATERIALS

A. CHEMICALS AND REAGENTS

Standard reagent grade laboratory chemicals were used in the entire study. Most of these reagents were manufactured or supplied by Fisher Scientific Co., Fairlawn, New Jersey, or, by J.T. Baker Chemical Co., Phillipsburg, New Jersey or by Sigma Chemical Co., St. Louis, Missouri.

Non standard reagents, and their suppliers are listed below.

Acetyl acetone: Fisher Scientific Co.

Acetone (distilled in glass) Caledon Labs. Ltd. Ont.

Aminopyrine (4-dimethylamino antipyrine): Aldrich Chem. Co.

Ascarite: A.H. Thomas Co. Philadelphia, PA., U.S.A.

Benzo[a] pyrene: Sigma Chemical Co.

Biofluor: New England Nuclear Canada Ltd., Que.

Bovine serum albumin: Sigma Chemical Co.

Calibration Kit for sephacryl S-200: Pharmacia, Montreal.

Carbon Monoxide: Union Carbide Canada Ltd., Halifax, N.S.

Cholic acid: Sigma Chemical Co.

^{14}C-Amino Acid mixture: NEN Canada Ltd., Que.

^{14}C-5-Aminolevulinic Acid: NEN Canada Ltd., Que.

Cytochrome C (horse heart): Sigma Chemical Co.

Disodium Monohydrogen phosphate: J.T. Baker Chemical Co.

D-glucose-6-phosphate: Sigma Chemical Co.

Emulgen 911: Generously supplied by Dr. M. Warner, Dept. of Pharmacology and Therapeutics, McGill University, Que.

Ethylene diamine-tetra-acetic acid: Fisher Scientific Co.

Ferrous sulphate: Fisher Scientific Co.

Glucose-6-Phosphate dehydrogenase: Sigma Chemical Co.

Glycine: Sigma Chemical Co.

Glycerol: Sigma Chemical Co.

^{3}H-Amino Acid mixture: NEN Canada Ltd., Que.

Hopcalite: MSA Canada Ltd., Ont.

NADH (Diphosphopyridine nucleotide, reduced form: Sigma Chemical Co.

NADP (triphosphopyridine nucleotide): Sigma Chemical Co.

NADPH (triphosphopyridine nucleotide, reduced form): Sigma Chemical Co.

Oxifluor-CO_2: NEN Canada Ltd., Que.

Phenol reagent: fisher Chemical Co.

Poly rI.rC: Sigma Chemical Co.

Potassium dihydrogen Phosphate: J.T. Baker Chemical Co.

Sephacryl S-200: Pharmacia, Montreal, Que.

Semicarbazide hydrochloride: Sigma Chemical Co.

Sodium dithionite: Fisher Scientific Co.

Succinic Acid: Sigma Chemical Co.

Trichloroacetic acid: Fisher Scientific Co.

UDPGA (uridine-5-diphosphoglucuronic acid): Sigma Chemical Co.

Whatman DE-52 Ion Exchange Cellulose. Mandel Scientific Co., Montreal.

B. ANIMALS

Male mice with an average weight between 20-25 gms obtained from Jackson Laboratories, Maine, U.S.A., were used in the entire study. The following strains of mice were utilized in the study: C57BL/6J; AKR/J; C_3H/HeJ; BalbC/J; and randomly bred Swiss strain. All mice were allowed to acclimatize for a period of at least four days after receipt from the breeder and were kept on clay chip bedding to avoid problems of induction from normal wood shaving bedding. Diet consisted of Purina rat chow and water ad libitum. No more than 8 mice were kept in a cage to avoid overcrowding.

C. INTERFERONS AND INTERFERON INDUCERS

Various types of interferons that were used in this study are listed in the table on the following page. The cloned interferons derived by recombinant DNA procedures

were supplied as part of a collaborative study between our laboratory and Dr. Nowell Stebbing of Genentech Inc. Calif. POLY rI.rC and Newcastle Disease Virus (NDV) were utilized as interferon inducers. NDV was generously supplied by Dr. S.H.S. Lee, Dept. of Microbiology, Dalhousie University, Halifax, N.S.

Supplier	Type	Animal Cell Source	Nomenclature
Calbiochem.	CAL-IFN	Mouse fibroblast	IFN-β
Genentech Inc.	CD-1	Mouse leukocyte	IFN-α
Genentech Inc.	Buffy Coat	Human leukocyte	IFN-α
Genentech Inc.	LeIF-A	Human cloned leukocyte	IFN-α
Genentech Inc.	LeIF-AD	Human hybridized cloned leukocyte	IFN-α

METHODS

A. Preparation of microsomes and other subcellular fractions.

All animals were killed by decapitation, and livers were excised immediately. The livers were rinsed in cold 1.15% KCL solution and weighed. The liver was then suspended in 10 mls of 1.15% KCl and homogenized in a glass homogenizer (10 strokes with a loose-fit pestle; and 1 stroke with a tight-fit pestle). The homogenate was centrifuged at 10,000Xg for 10 minutes in a Sorvall (RC-2) refrigerated centrifuge. The pellet obtained from the 10,000xg centrifugation contained unbroken cells, cell wall fragments, nuclei and mitochondria.

The supernatant was removed with a pasteur pipette and recentrifuged at 125,000xg for 40 minutes in an IEC/B-60 refrigerated ultra centrifuge, to obtain a microsomal pellet. The supernatant from the 125,000xg centrifugation was removed with a pasteur pipette. The microsomal pellet was resuspended in 1.15% KCl to yield a 50% suspension (i.e. 2X Vol. of KCl/gm of liver weight) and resuspended using a glass homogenizer with 5 strokes of a tight-fit pestle. All enzyme determinations were carried out using freshly prepared subcellular fractions.

B. Determination of Protein in Subcellular Fractions.

Protein was determined using a modified method of Lowry et al. (1951), using Bovine serum albumin as a standard.

Solution A: 49 mls of 2% $Na_2 CO_3$

0.5 mls of 2% NaK Tartrate

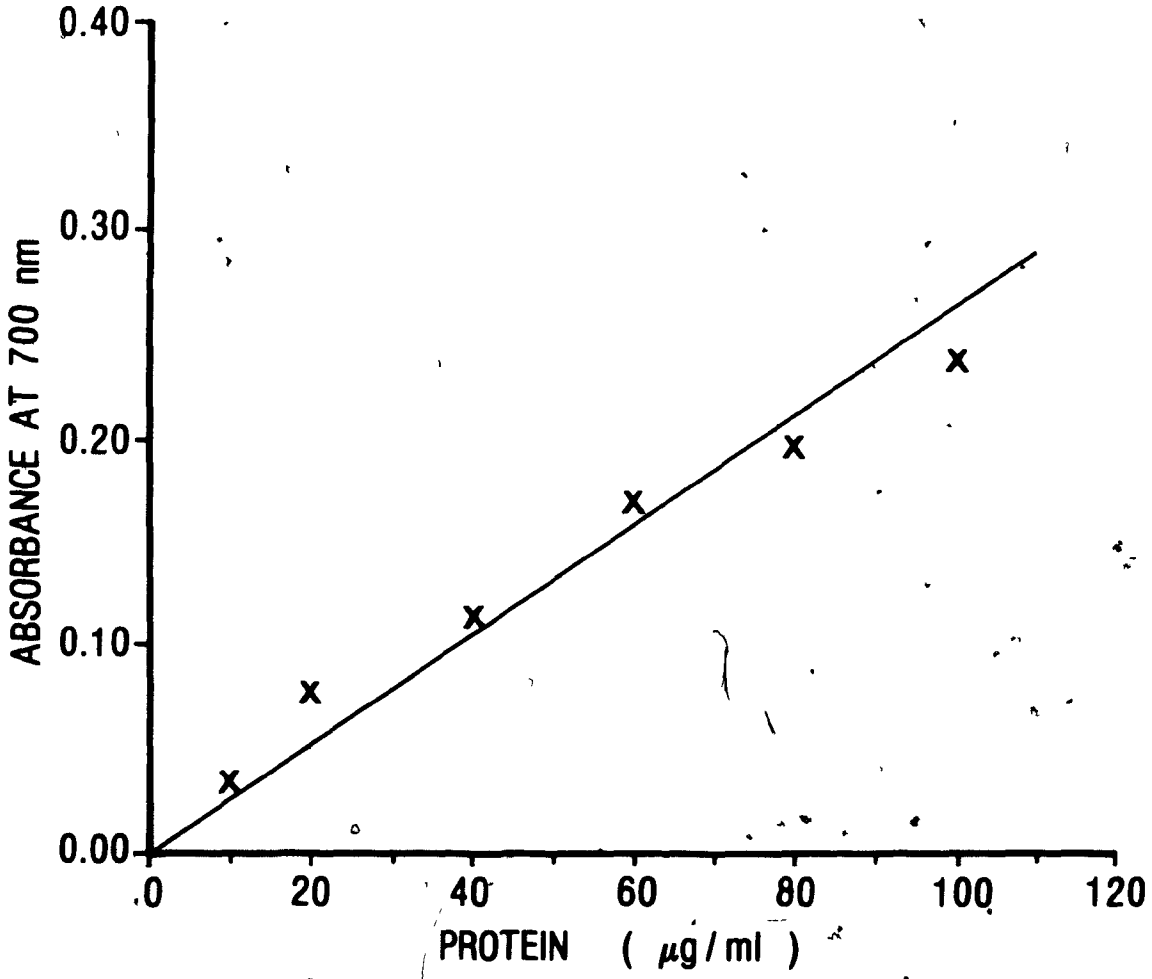
0.5 mls of 1% CU SO_4 $5H_2O$

Solution B: 1 volume Folin-Ciocalteau reagent diluted with 1 volume distilled water.

Microsomal suspensions were diluted 1:10 in distilled water. Distilled water was used as blank. One ml of diluted microsomes were mixed with five ml of solution A and allowed to stand for 10 minutes. Then 0.5 mls of solution B were added to the mixture and after mixing were incubated at room temperature for 30 minutes. Absorbances were determined at 700 nm using the blank as a zero absorbance reference on either a Unicam SP8-20 or a Turner 350 spectrophotometer. A linear standard curve of protein concentration versus absorbance is illustrated in Fig. 4.

FIGURE 4

Standard curve for protein determination. Protein standard solutions were prepared with lyophilized bovine serum albumin and protein was determined by the method of Lowry et al. (1951) values shown are duplicates done in one experiment. (Regression coefficient = 0.9931).


0.40
0.30
0.20
0.10
0.00
ABSORBANCE AT 700 nm
0
20
40
60
80
100
120
PROTEIN (µg / ml)

C. Determination of Microsomal Cytochrome P-450 and Cytochrome b_5.

Microsomes were diluted to a concentration of 1 mg microsomal protein per ml (with 0.5 ml phosphate buffer (1M, pH 7.4) and 1.15% KCl solution). The diluted microsomes were then divided equally between two spectrophotometer cuvettes, and a baseline spectrum from 550 nm to 400 nm was determined with a Unicam SP8-200 Spectrophotometer. A few crystals of sodium dithionite were then added to the sample cuvette and the spectrum redetermined. The molar concentration of cytochrome b_5 was calculated from the difference in absorption at 425 nm using an extinction coefficient of 171 nm^{-1} cm^{-1} (Omura and Sato, 1964).

The reference cuvette was then reduced with dithionite and carbon monoxide was bubbled into the sample cuvette and the spectrum redetermined. The molar concentration of cytochrome P-450 was calculated from the difference in absorption between 450 and 490 nm using an extinction coefficient of 91 $mM^{-1}cm^{-1}$ (Omura and Sato, 1964).

D. Determination of Microsomal Aminopyrine N-Demethylation.

The N-demethylation of aminopyrine in microsomes was determined by measuring the amount of formaldehyde formed (Sladek and Mannering, 1969). Formaldehyde was trapped as semicarbazone by semicarbazide and measured by the Nash reaction (Nash, 1953).

REAGENTS. a) $Ba(OH)_2 \cdot 8H_2O$ - Dissolve 50 gms in glass-distilled water to give 1 liter.

b) $ZnSO_4 \cdot 7H_2O$ - Dissolve 50 gms in glass-distilled water to give 1 liter.

c) Solution A: D-Glucose-6-Phosphate - 358 mg
NADP - 76 mg
1.15% KCl - 10 ml
Store in freezer in 1 ml aliquots.

d) Solution B: Semicarbazide HCl - 2.74 gms
Na_2HPO_4 $7H_2O$ - 15.11 gms
KH_2 PO_4 - 3.62 gms
1.15% KCl - 600 mls
Distilled H_2O - 400 mls
Adjust pH to 7.4 and store at 4°C.

e) NASH'S Reagent - Ammonium Acetate - 150 gms
Acetyl Acetone - 1 ml
Glacial acetic acid - 1.5 mls
Dissolve to give 500 ml solution with distilled water and store at 4°C.

f) Glucose-6-Phosphate dehydrogenase 100 unit dissolved in 0.2 mls 1.15% KCl to give 0.5 units/μL.

g) Aminopyrine - 57.8 mg/10 mls of 1.15% KCl to give 5 mM solution.

h) $MgCl_2$ - Dissolve 20.3 gms in 1 liter of distilled water.

i) MASTER MIX - 1.15% KCl 4.8 mls

Solution B 6.0 mls

Solution A 0.8 mls

$MgCl_2$ 0.4 mls

Glucose-6-phosphate dehydrogenase 10 ul

The reaction mixture contained 1.2 mls of master mix and 0.4 mls of aminopyrine which were incubated for 1 min at 37°C to generate triphosphopyridine nucleotide, reduced form (NADPH). Microsomes (0.4 mls of 3 mg/mg) were added to the reaction mixture and incubated for 10 minutes in a shaking bath at 37°C.

The rections were terminated and deproteinised by adding 1 ml of zinc sulphate followed by 1 ml of Barium hydroxide solutions. The mixtures were thoroughly agitated on a Vortex mixer after each addition, and then centrifuged at 2000xg for 5 minutes. Formaldehyde was assayed in 2 mls of the clear supernatant by adding 1 ml of Nash's reagent and incubating for 20 minutes at 60°C in a water bath. The absorbance of the cooled, clear samples was determined at 415 nm in a spectrophotometer. Reaction mixtures without

substrate (aminopyrine) were used as blanks and subtracted from the experimental incubation mixtures to correct for any formaldehyde or other material reacting with Nash reagent which may be formed from a non-substrate source. Results were expressed as specific activity (i.e. nmoles formaldehyde formed/mg microsomal protein/hour).

E. Determination of Microsomal Benzo (a) pyrene Hydroxylation.

The hydroxylation of benzo(a)pyrene in microsomes was determined by measuring the formation of non-polar fluorescent metabolites (Nebert and Gelboin, 1968).

The incubation of microsomes with benzo(a)pyrene results in oxidation to highly fluorescent products which can be readily detected by a flurometer. Incubation mixture contained 0.1 ml of benzo(a)pyrene, 0.5 ml of microsomes, 0.5 mls of 1.15% KCl and 1 ml of co-factor (NADPH, NADP dissolved in KH_2PO_4 buffer at pH 7.4). The mixture was incubated at 37°C for 10 minutes. The reaction was terminated with the addition of 3 mls of acetone and 7 mls of petroleum ether. The tubes were then capped and shaken for 90 minutes to extract the non-polar metabolites. Then 2 mls of the organic phase were removed and 1 ml of (1N) NaOH was added. The aqueous phase was read in the fluorometer using 400 nm excitation and 522 nm emission wavelengths. 3-OH benzo(a)pyrene was utilized to standardize the assay

procedure and results were expressed as nmoles of BP hydroxylated metabolites formed/mg protein/hr.).

F. Determination of Microsomal Total Heme.

Total microsomal heme content was measured by the pyridine hemochromogen method described by Falk (1964). Diluted microsomes (1 mg/ml) were mixed with 0.5 ml(N) NaOH and 0.5 mls of pyridine. The mixture was divided into two cuvettes and a pinch of sodium dithionite was added to one cuvette. The cuvettes were then scanned from 600 - 500 nm in a Unicam SP8-200 spectrophotometer. The results were calculated from the difference spectrum at 558 nm and expressed as nmoles of heme per mg of protein using an extinction coefficient of 31 $mM^{-1}cm^{-1}$.

G. Determination of Interferon Units in Plasma.

Determination of interferon in plasma was carried out by Dr. S. Lee, Dept. of Microbiology, Dalhousie University. Interferon was assayed in L.929 cell monolayers by the plaque reduction method using vesicular stomatitis virus (Indiana strain) as the challenge virus (O'Shaughnessy, et al., 1972). The amount of interferon required for 50 percent plaque reduction was defined as 1 plaque reduction dose ($1PRD_{50}$ unit).

H. Incorporation of L-[^{14}C] Amino Acid Mixture in the Microsomes.

A mixture of ^{14}C-labelled amino acids (15 highly purified amino acids, sp. activity 55.0 mCi/matom carbon) were injected intraperitoneally (i.p) into mice (2.5 μCi/mouse). Two hours later the mice were killed by decapitation and microsomes prepared as described earlier. Microsomal pellets obtained from 125,000 xg centrifugation were resuspended in buffered detergent using a glass-homogenizer. Microsomes from 10 mice were pooled for solubilization in the buffered detergent. The buffered detergent was composed of 10 mM sodium phosphate, 0.2% Emulgen 911 (v/v), 0.5% sodium cholate (w/v), 0.1 mM EDTA, and 20% glycerol (v/v), with the final pH adjusted to 7.4. The suspension was allowed to incubate at room temperature (23-24^{o}C) for 3 hours, at which time approximately 90% of the cytochrome P-450 was solubilized.

I. Separation of Microsomal Proteins on Sephacryl S-200 Column.

Initial fractionation of microsomes was carried out using a sephacryl S-200 column.

Sephacryl S-200 is a covalently cross-linked allyl dextran with N, N'-methylene bisacrylamide to give a rigid gel. Separation of microsomal proteins is based upon their molecular weights. The gel was packed in a column (100 x 2.5 cm) obtained from Pharmacia and equilibrated with the

buffered detergent described above. The column was loaded with 10 mls of heme solution (1 mg/ml). This procedure prior to microsomal fractionation minimized the damage to the cytochrome P-450 in the microsomes. A 10 ml aliquot of the ^{14}C-labelled solubilized microsomal preparation were then introduced onto the column and the column was eluted with the buffered detergent at room temperature. A flow rate of 0.5 ml/min was maintained and 4-ml fractions were collected. Each fraction was monitored for radioactivity, cytochrome P-450 and cytochrome b_5. The fractions which contained cytochrome P-450 were pooled and concentrated to 10 mls with the use of millipore immersible CX-30 ultrafiltration unit (Millipore Corp.).

J. Separation of Cytochrome P-450 on DEAE-cellulose Anion Exchange Column.

Diethylaminoethyl cellulose (DEAE-cellulose, Whatman DE-52) was equilibrated with the buffered detergent described above and packed in 50 x 2.5 cm columns obtained from Pharmacia. The 10 mls of the concentrated fractions obtained from the sephacryl column were loaded on the DEAE-column and developed first with 100 mls of the buffered detergent, and then with a 200 ml linear gradient of NaCl from 0 to 0.25 M incorporated in the buffered detergent. The entire procedure was carried out at room temperature. A flow rate of 0.5 ml/min was maintained, and 4 ml fractions

were collected. Cytochrome P-450 and cytochrome b_5 were determined in each fraction. Radioactivity in each fraction was determined using 0.5 mls of each fraction in 10 mls of Bioflour.

Similar to the sephacryl column, the DEAE-cellulose column was loaded with 5 mls of heme solution and eluted with buffered detergent prior to loading the concentrated fractions of the solubilized microsomes on the column.

K. Incorporation of [^{14}C] -ALA into Various Subcellular Fractions.

Mice were injected at various times with Poly r (I.C.) and 1 hour before sacrifice they received i.p. 2.5 µl of ^{14}C- Aminolevulinic Acid (ALA) (sp. act. 49.0 mCi/mmol) per mouse. Control animals were injected with saline. The liver was excised and homogenized with a glass homogenizer Radioactivity of whole homogenate was determined in 0.1 ml of the whole liver homogenate added to 10 mls of Biofluor. Microsomes were prepared as described earlier and radioactivity was determined in 0.1 ml of each subcellular fraction.

L. Determination of [^{14}C]-Amino Acids Incorporation in Various Subcellular Fractions, at Different Time Periods.

A similar protocol as above was carried out to study the incorporation of amino acids at various time intervals

after the treatment with Poly r (I.C.). The pellet obtained from the 10,000 xg was resuspended with a glass homogenizer homogenizer and proteins precipitated using trichloroacetic acid (TCA) and 0.1 ml of the mixture was added to 10 mls of Biofluor for counting. Liquid scintillation counting was carried out in MARK III Searle Analytic Inc. Model 6880 Liquid Scintillation System.

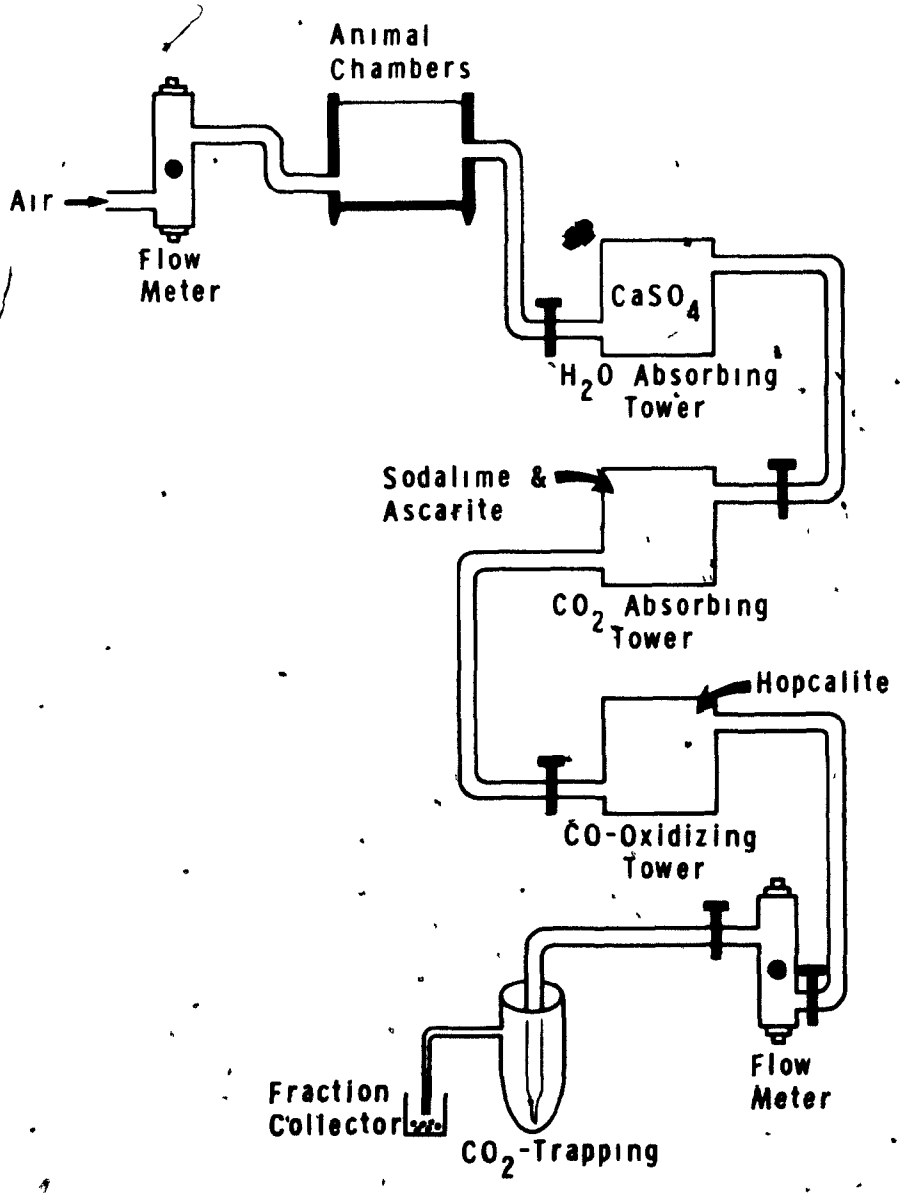
M. Catabolism of Heme In Vivo.

Cytochrome P-450 degradation was determined by measuring the amount of ^{14}CO in expired air following the administration of 5-^{14}C-ALA. In mammals carbon monoxide originates solely from the oxidized alpha-methene bridge carbon atom of heme (Ludwig et al., 1957). This implies that for each mole of heme degraded, 1 mole of CO and 1 mole of bilirubin are formed. 5-^{14}C-ALA specifically labels the alpha-methene bridge carbon in the porphyrin ring. On cleavage of the heme ring this carbon is converted to carbon monoxide. Landaw and Winchell (1966) demonstrated the validity of such an experiment to measure cytochrome P-450 breakdown.

At different time periods following the administration of Poly r (I.C.) mice received 5-^{14}C-ALA (2.0 μCi/mouse; sp. act. 49.0 mCi/mmol) i.p. The animals were then placed in a sealed chamber connected to the apparatus illustrated in Fig. 5. The air from the chamber was passed through a

FIGURE 5

In Vivo breath collection systems. Schematic representation of apparatus used to measure ^{14}CO following the catabolism of labelled Heme.


Animal
Chambers
Air
Flow
Meter
$CaSO_4$
H_2O Absorbing
Tower
Sodalime &
Ascarite
CO_2 Absorbing
Tower
Hopcalite
CO-Oxidizing
Tower
Flow
Meter
Fraction
Collector
CO_2-Trapping

tower containing calcium sulphate to trap water followed by a tower containing soda lime and ascarite to trap expired carbon dioxide. The carbon monoxide (^{14}CO) was then passed through a tower containing the catalyst hopcalite which converted ^{14}CO to $^{14}CO_2$. $^{14}CO_2$ was then bubbled into oxyfluor which trapped carbon dioxide (CO_2-absorbant/scintillation cocktail combination, New England Nuclear). Samples were collected every 10 minutes in a fraction collector and counted in a scintillation counter. Two animals were studied simultaneously using two apparatus in series.

N. Statistical Methods

1. The students t test for unpaired data was utilized in this thesis to determine statistical signficance of the difference between two means. Analysis of variance, F-test, was used to determine statistical difference when three or more groups were compared. significance throughout these studies is defined at the 5% level, i.e. $p<0.05$.

The data for catabolism of heme in vivo was fitted to a double exponential equation which described the two compartment model for cytochrome P-450 as described by Levin and Kuntzman (1969). The rate constants and half-lives were determined using the computer program PEEL-SMOOTH which fits data to two compartment model using the following equation.

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

RESULTS

A. Effect of Interferon inducers on Hepatic Cytochrome P-450.

The effects of the administration of Poly r (I.C) on hepatic microsomal protein, cytochrome P-450 and cytochrome b_5 levels, and aminopyrine N-demethylase activity are summarized in Table 3. Loss of cytochrome P-450 and aminopyrine N-demethylase activity were observed in five separate strains of mice treated with Poly r (I.C) for 24 hours. These results are similar to those reported by Renton and Mannering (1976) in rats.

Poly r (I.C) had no effect on Hepatic microsomal cytochrome P-450 when added to microsomes in vitro.

B. Indirect evidence for the involvement of interferon in the depression of Hepatic cytochrome P-450.

We utilized strains of mice carrying the high ($1F\text{-}1^h$) or low ($1F\text{-}1^l$) production allele (DeMaeyer and coworkers, 1979) at the 1F-1 locus to demonstrate that depression of hepatic cytochrome P-450 can be correlated with circulating interferon levels.

In C57BL/6J mice which carry the allele for the high production of interferon at the 1F-1 locus the mean circulating level of interferon was 2443 PRD_{50} units/ml, twenty four hours after the administration of NDV (Table 4). At the same time cytochrome P-450 levels and

TABLE 3

Effect of Poly r(I.C) on Hepatic Cytochrome P-450..

STRAIN	TREATMENT	PROTEIN mg/ml	CYTOCHROME P-450 nmoles/mg Protein	CYTOCHROME b_5 nmoles/mg Protein	AMINOPYRINE N-demethylase Actvity nmoles HCHO formed/ mg Protein/hr.
Swiss	Saline	5.84 ± 0.29	0.747 ± 0.054	0.306 ± 0.015	329 ± 11
"	Poly r(I.C)	4.45 ± 0.16*	0.445 ± 0.021*	0.258 ± 0.002	221 ± 8*
AKR/J	Saline	5.92 ± 0.28	0.717 ± 0.034	0.252 ± 0.011	445 ± 9
"	Poly r(I.C)	5.14 ± 0.25	0.420 ± 0.031*	0.187 ± 0.007*	212 ± 20*
Balbc/J	Saline	5.30 ± 0.14	0.871 ± 0.064	0.293 ± 0.006	460 ± 35
"	Poly r(I.C)	4.92 ± 0.26	0.587 ± 0.047*	0.245 ± 0.023	350 ± 20*
C57BL/6J	Saline	6.99 ± 0.30	0.616 ± 0.044	0.170 ± 0.064	174 ± 2
"	Poly r(I.C)	4.69 ± 0.22*	0.353 ± 0.017*	0.202 ± 0.021	87 ± 10*
C_3H/HeJ	Saline	4.67 ± 0.18	0.535 ± 0.035	0.311 ± 0.016	321 ± 30
"	Poly r(I.C)	4.11 ± 0.26	0.363 ± 0.036*	0.257 ± 0.021	204 ± 25*

Animals were treated with Poly r(I.C) 10 mg/kg (i.p) 24 hours before sacrifice.

Each value is the mean ± S.E.M. of 4 individual animals.

Significantly different from control $p < 0.05$

aminopyrine N-demethylase activities were depressed by 35% and 48%, respectively in hepatic microsomes (Figures 6 and 7). In C_3H/HeJ mice which carry the allele for the low production of interferon at the 1F-1 locus the circulating levels of interferon were below our lowest limit of detection (40 PRD_{50} units/ml) twenty-four hours following treatment with NDV (Table 4). In this strain of mice NDV had no effect on the levels of cytochrome P-450 or aminopyrine-N-demethylase activity in hepatic microsomes (Figures 6 and 7).

Twenty-four hours following the administration of Poly r (I.C) which induces the formation of interferon via loci other than 1F-1, high concentrations of interferon were observed in the serum of both strains of mice (Table 4). In C57BL/6J mice cytochrome P-450 levels and aminopyrine-N-demethylase activities were decreased by 44% and 50% respectively 24 hours following the administration of Poly r (I.C) (Figures 8 and 9). In C_3H/HeJ mice cytochrome P-450 levels and aminopyrine N-demethylase activities were also decreased by 33% and 38% following the administration of Poly r (I.C) (Figures 8 and 9).

C. <u>Direct evidence for the depression of Hepatic cytochrome P-450 and drug biotransformation using crude preparations of interferon.</u>

Various crude preparations of interferon were tested in this study to establish a causal relationship between

TABLE 4

Circulating levels of interferon in inbred strains of mice following the administration of NDV or poly r(I.C)

STRAIN	TREATMENT	ALLELE	INTERFERON (PRD_{50}/units per ml).
C57BL/6J	Saline	IF-1^h	<40, <40, <40
C57BL/6J	NDV	IF-1^h	1448, 1702, 4178
C57BL/6J	Poly r(I.C)	IF-1^h	1742, 2010
C_3H/HeJ	Saline	IF-1^l	<40, <40, <40
C_3H/HeJ	NDV	IF-1^l	<40, <40, <40
C_3H/HeJ	Poly r(I.C)	IF-1^l	2118, 4376

Each value is the serum interferon level in an individual mouse.

Levels were determined 24 hours after the administration of NDV (5×10^7 p.f.u) or poly r(I.C) (10 mg/kg).

The amount of interferon required for 50% plaque reduction was defined as 1 plaque reduction dose (1 PRD_{50} Unit).

FIGURE 6

The effect of NDV on cytochrome P-450 and cytochrome b_5 levels in hepatic microsomes prepared from inbred strains of mice. C57BL/6J carry the high production allele at 1F-1 and C_3H/HeJ carry the low production allele at 1F-1. The animals were killed 24 hours following the administration of NDV. The open bars represents cytochrome P-450 levels and the shaded bars represents cytochrome b_5 levels. Each value is the mean $\pm$ S.E. of 6 individual mice.

* Significantly different from control, $p < 0.05$.

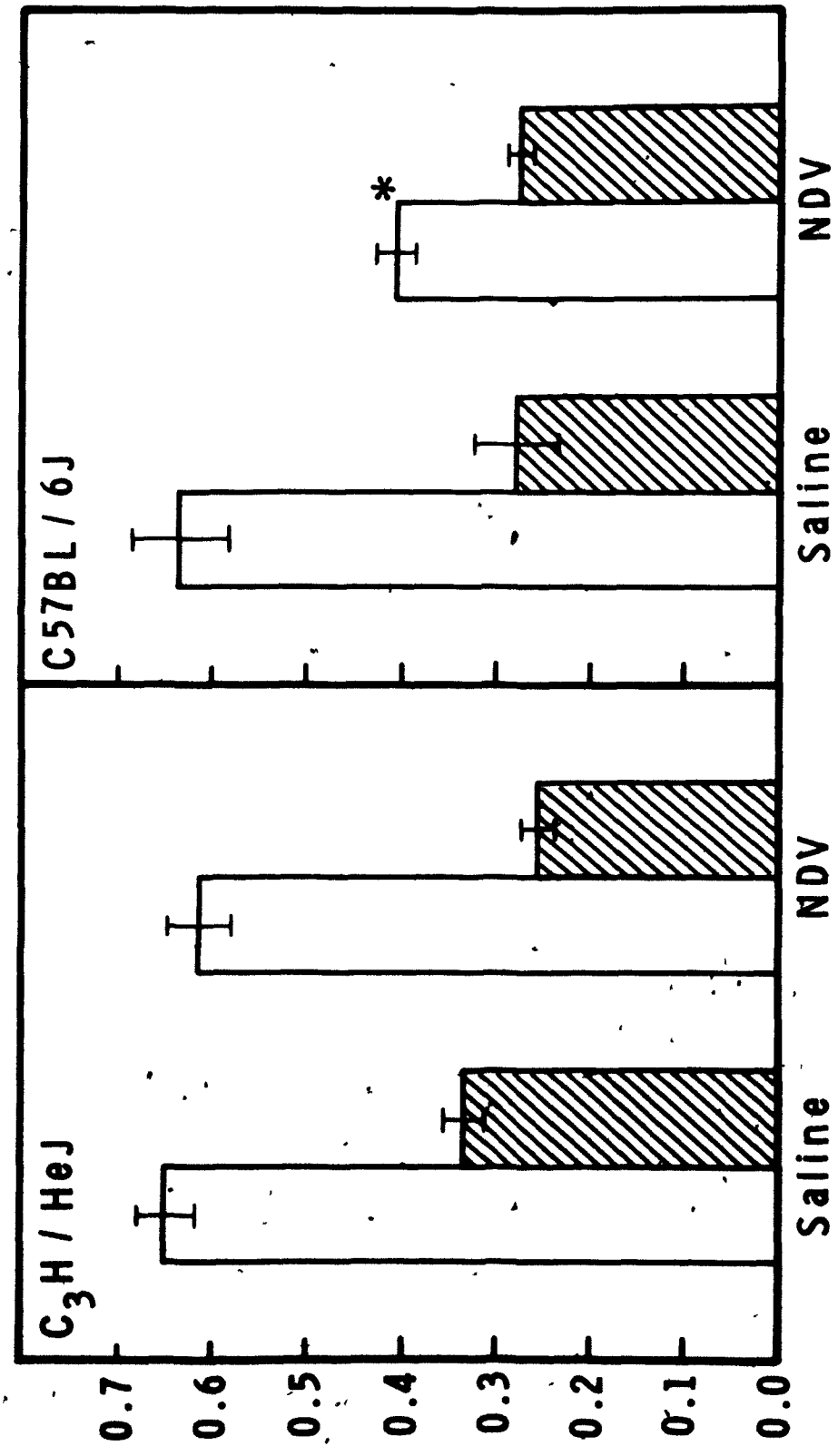

nmoles Hemoprotein / mg Protein
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
C3H / HeJ
C57BL / 6J
Saline
NDV
Saline
NDV
*

FIGURE 7

The effect of NDV on aminopyrine N-demethylase activity in hepatic microsomes prepared from inbred strains of mice. The animals were killed 24 hours after the administration of NDV. Activity is expressed as the amount of formaldehyde formed/mg protein/hour. Each bar represents the mean $\pm$ S.E. of 6 individual mice.

* Significantly different from control, $p < 0.05$.

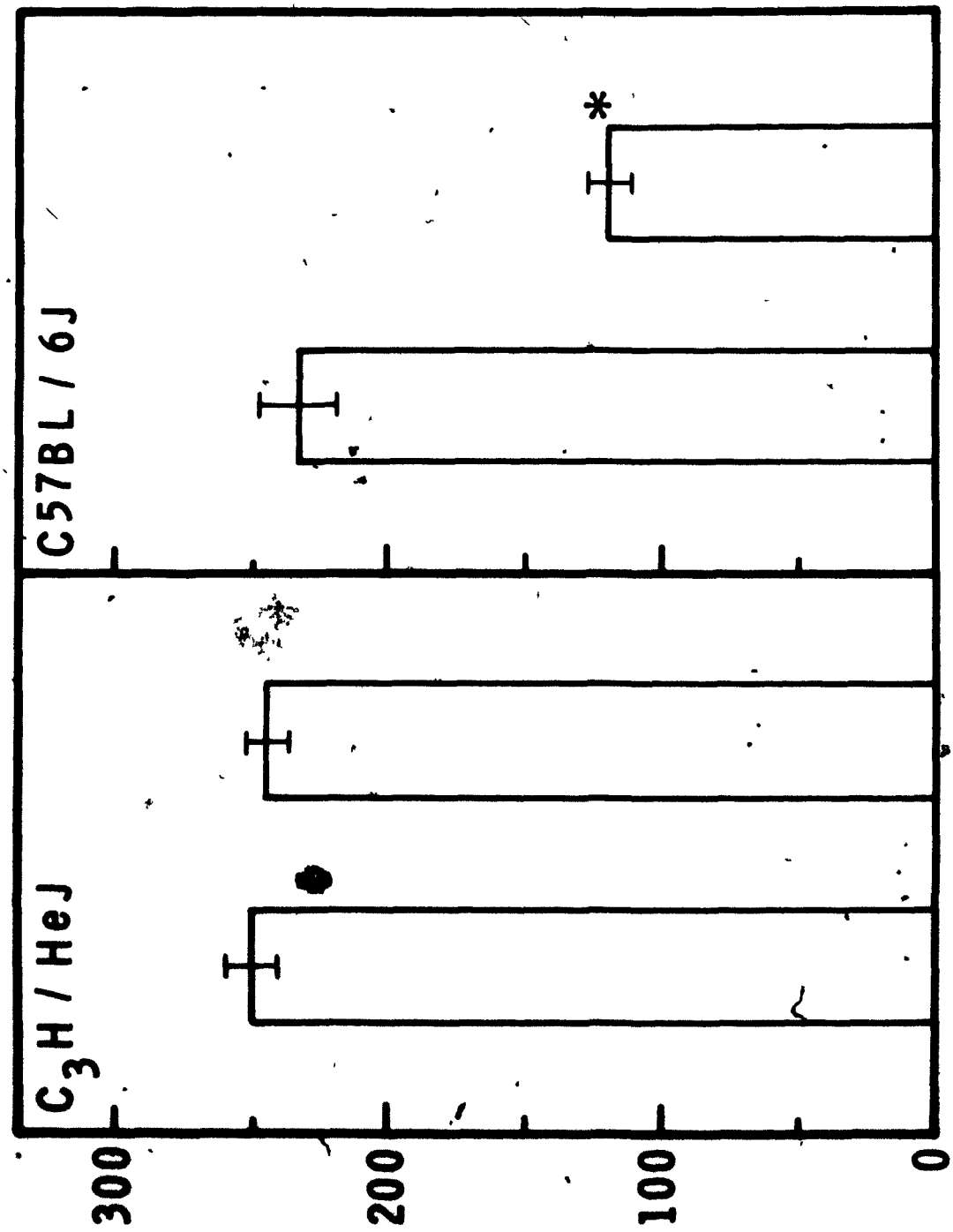

nmoles HCHO / mg Protein / hr
300
200
100
0
C3H / HeJ
C57BL / 6J
Saline
NDV
Saline
NDV
*

FIGURE 8

The effect of Poly r (I.C) on cytochrome P-450 and cytochrome b_5 levels in hepatic microsomes prepared from inbred strains of mice. The animals were killed 24 hours following the administration of Poly r (I.C). The open bars represent cytochrome P-450 levels and the shaded bars the cytochrome b_5 levels. Each value is the mean $\pm$ S.E. of 6 individual mice.

* Significantly different from control, $p < 0.05$.

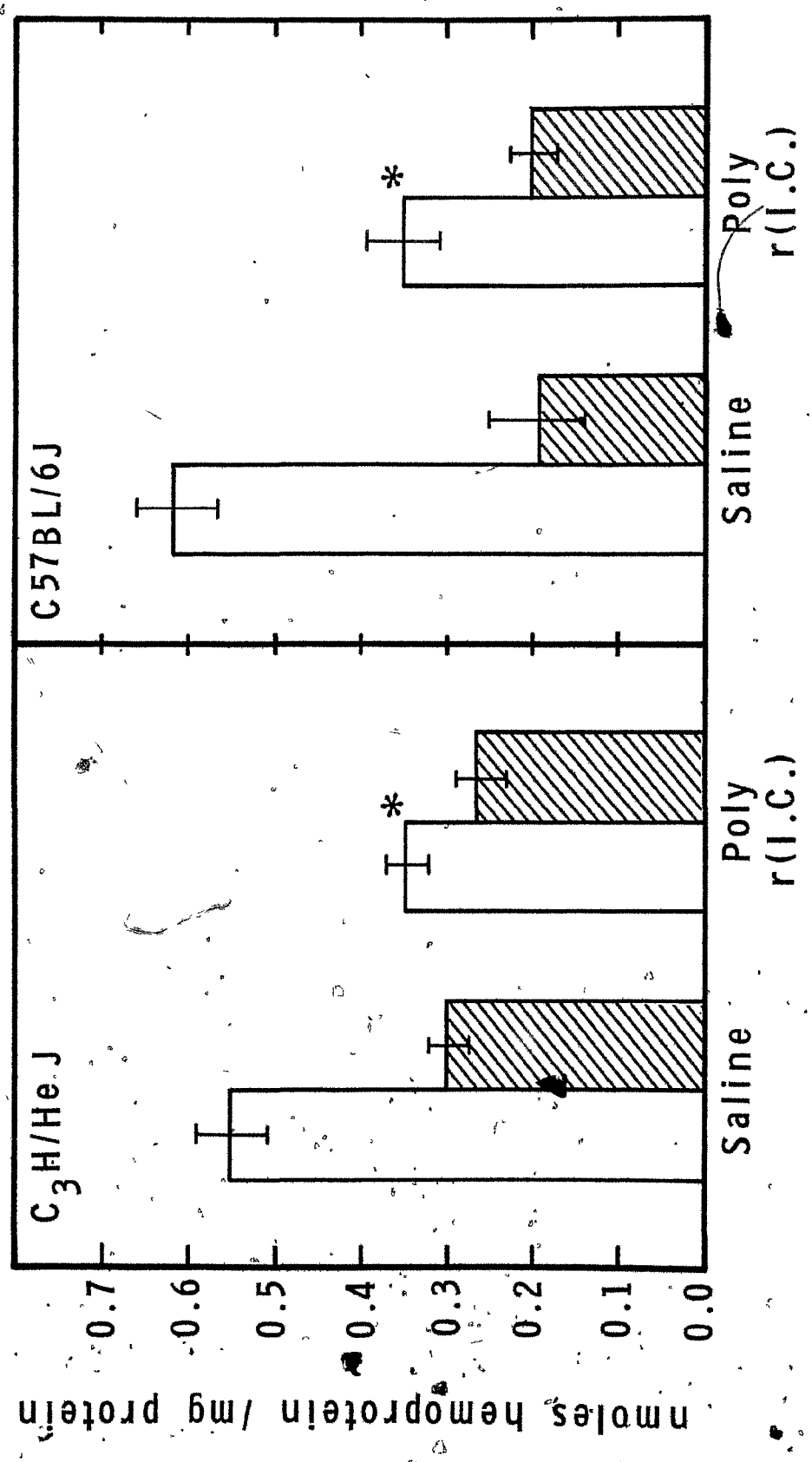
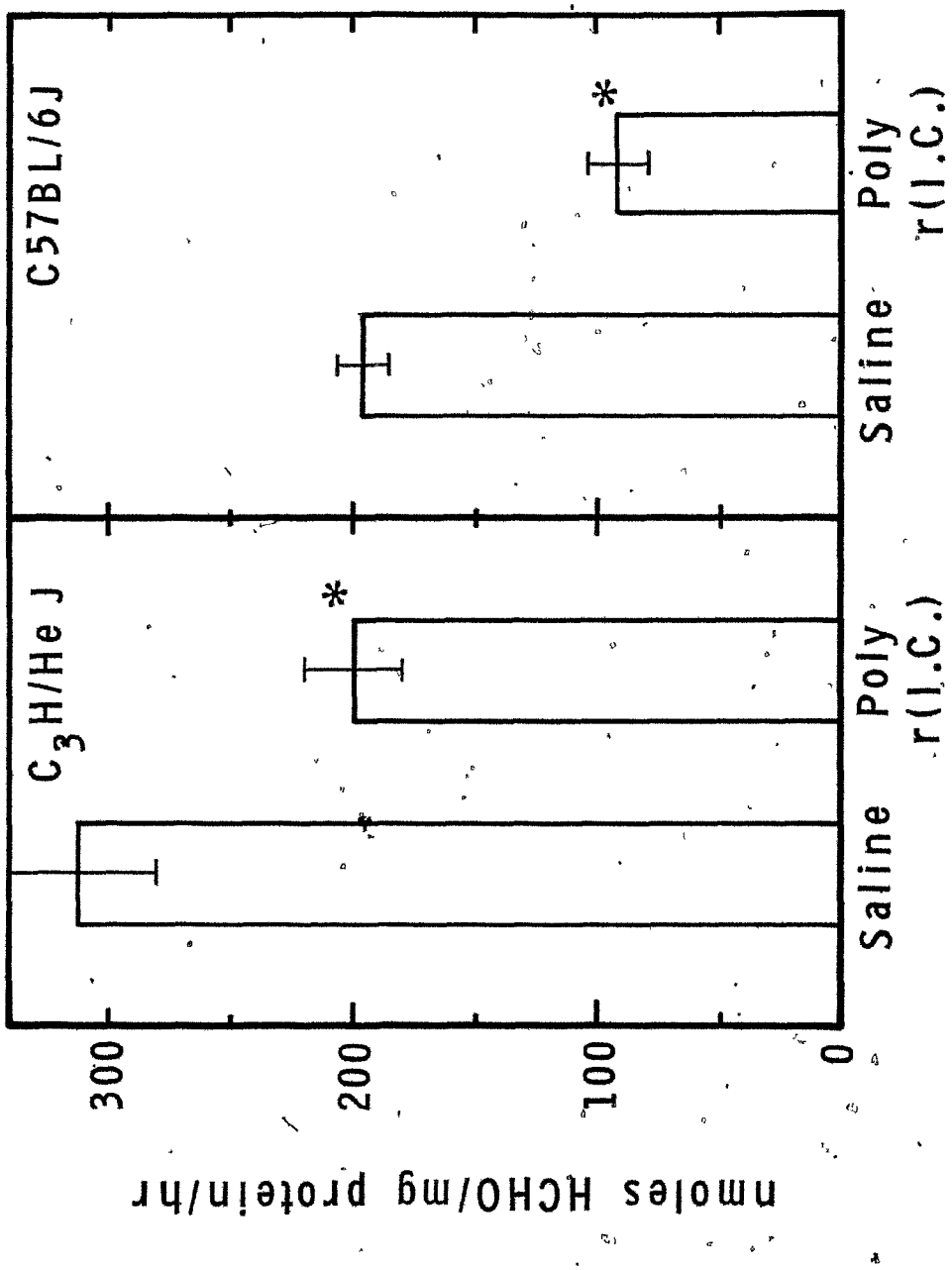

nmoles hemoprotein /mg protein
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
C3H/He J
C57BL/6J
Saline
Poly r(I.C.)
Saline
Poly r(I.C.)
*
*

FIGURE 9

The effect of Poly r (I.C) on aminopyrine N-demethylase activity in hepatic microsomes prepared from inbred strains of mice. The animals were killed 24 hours after the administration of Poly r (I.C). Activity is expressed as the amount of formaldehyde formed/mg protein/hour. Each bar represents the mean $\pm$ S.E. of 6 individual mice.

* Significantly different from control, $p < 0.05$.


nmoles HCHO/mg protein/hr
300
200
100
0
C3H/He J
C57BL/6J
Saline
Poly r(I.C.)
Saline
Poly r(I.C.)
*
*

interferon and depression of Hepatic cytochrome P-450. Twenty-four hours following the administration of fibroblast interferon (Calbiochem.) a significant depression of the mixed function oxidase system was observed in mice. The depression of cytochrome P-450 and aminopyrine N-demethylase activity is summarized in Table 5. In this experiment Poly r (I.C) depressed hepatic cytochrome P-450 to a similar degree as crude interferon. A dose-related decrease in the amount of cytochrome P-450 and aminopyrine-N-demethylase activity was also observed following the administration of fibroblast interferon (Figure 10). Cytochrome b_5 was not affected by fibroblast interferon.

Buffy coat interferon produced from human leukocytes had no effect on hepatic cytochrome P-450, aminopyrine N-demethylase activity or benzo(a)pyrene hydroxylase activity (Table 6). This interferon has no antiviral activity in the mouse.

Crude mouse leukocyte interferon (CD-1) produced from mice given Poly r (I.C) caused a depression in cytochrome P-450, aminopyrine N-demethylase and benzo(a)pyrene hydroxylase activities (Table 7). This preparation of interferon has antiviral activity in the mouse.

D. Depression of Hepatic cytochrome P-450 by homogenous interferon from cloned genes in E. coli.

A homogenous preparation of highly purified cloned human leukocyte interferon (LEIF-AD) which has potent anti-

TABLE 5

Effect of mouse fibroblast interferon (IFN-β) on Hepatic cytochrome P-450

TREATMENT	PROTEIN mg/ml	CYTOCHROME P-450 nmoles/mg Protein	CYTOCHROME b_5 nmoles/mg Protein	AMINOPYRINE N-demethylase Activity nmoles HCHO formed/ mg Protein/hr.	Benzo(a)Pyrene Hydroxylase Activity nmoles 3-OH-BP formed/mg Protein/hr
Saline	5.96 ± 0.93	0.718 ± 0.037	0.269 ± 0.012	380 ± 30	6.68 ± 1.16
Poly r(I.C)	5.15 ± 0.28	0.373 ± 0.038*	0.191 ± 0.023	178 ± 10*	4.36 ± 0.22*
Mouse IFN-β ($5x10^4$ units/ mouse)	6.60 ± 0.21	0.425 ± 0.032*	0.199 ± 0.023	190 ± 17*	6.09 ± 0.30

Mice (BalbC/J) were treated either with Poly r(I.C) (10 mg/kg) or interferon (IFN-β) i.p 24 hours before sacrifice.

Each value is the mean ± S.E.M. of 4 individual animals.

* Significantly different from control $p < 0.05$.

FIGURE 10

Dose-related effect of fibroblast interferon on Hepatic cytochrome P-450, cytochrome b_5 and Aminopyrine N-demethylase activity. The animals were killed 24 hours following the administration of interferon. Each individual point is the mean $\pm$ S.E. of 4 individual mice. (Balb C/J mice were used in this study).

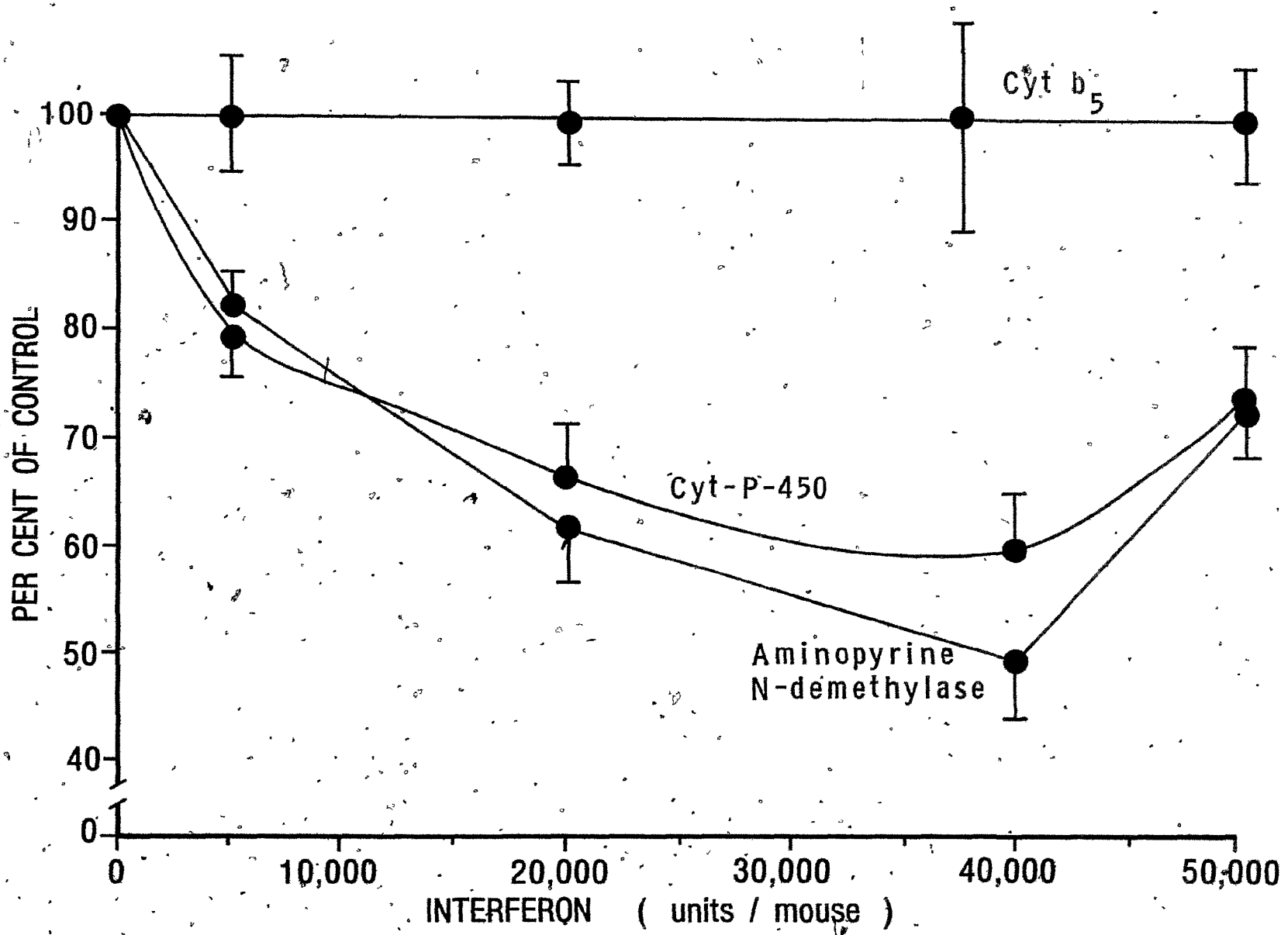

Cyt b_5
Cyt-P-450
Aminopyrine
N-demethylase
PER CENT OF CONTROL
100
90
80
70
60
50
40
0
0
10,000
20,000
30,000
40,000
50,000
INTERFERON (units / mouse)

TABLE 6

Effct of Human Buffy coat-induced (IFN-α) interferon on hepatic cytochrome P-450.

TREATMENT	PROTEIN mg/ml	CYTOCHROME P-450 nmoles/mg Protein	CYTOCHROME b_5 nmoles/mg Protein	AMINOPYRINE N-demethylase Actvity nmoles HCHO formed/ mg Protein/hr.	Benzo(a)Pyrene Hydroxylase Activity nmoles 3-OH-BP formed/mg Protein/hr
Saline	10.68±0.54	0.613 ± 0.041	0.196 ± 0.009	335 ± 10	2.23 ± 0.22
Poly r(I.C)	9.02±0.42	0.439 ± 0.045*	0.183 ± 0.010	243 ± 28*	1.69 ± 0.04*
Buffy coat ($5x10^4$ units/ mouse)	10.62±0.44	0.659 ± 0.015	0.206 ± 0.004	346 ± 10	2.35 ± 0.17

Mice (BalbC/J) were treated either with Poly r(I.C) (10 mg/kg) or interferon (Buffy coat) i.p 24 hours before sacrifice.

Each value is the mean ± S.E.M. of 4 individual animals.

* Significantly different from control $p < 0.05$.

TABLE 7

Effect of mouse Poly r(I.C) induced interferon ((IFN-α) (CD-1)) on hepatic cytochrome P-450.

TREATMENT	PROTEIN mg/ml	CYTOCHROME P-450 nmoles/mg Protein	CYTOCHROME b_5 nmoles/mg Protein	AMINOPYRINE N-demethylase Actvity nmoles HCHO formed/ mg Protein/hr.	Benzo(a)Pyrene Hydroxylase Activity nmoles 3-OH-BP formed/mg Protein/hr
Saline	5.96 ± 0.93	0.718 ± 0.037	0.269 ± 0.012	380 ± 30	6.68 ± 1.16
Poly r(I.C)	5.15 ± 0.28	0.373 ± 0.038*	0.191 ± 0.023	178 ± 10*	4.36 ± 0.22*
CD-1 (5,000 units/ mouse)	6.45 ± 0.48	0.421 ± 0.016*	0.200 ± 0.025	227 ± 8*	5.17 ± 0.99

Mice (BalbC/J) were treated either with Poly r(I.C) (10 mg/kg) or interferon (CD-1) i.p 24 hours before sacrifice.

Each value is the mean ± S.E.M. of 4 individual animals.

* Significantly different from control $p < 0.05$.

viral activity in the mouse depressed the levels of cytochrome P-450 and drug biotransformation in the mouse liver. The administration of 40,000 units of LEIF-AD resulted in a 47% loss of cytochrome P-450 and a 41% loss of cytochrome b_5 in microsomes prepared from the livers of Balbc/J mice (Table 9). This loss of cytochrome P-450 was accompanied by a 55% decrease in the N-demethylation of aminopyrine and a 42% decrease in the hydroxylation of benzo(a)pyrene which are also summaried in Table 9. Similar losses in cytochrome P-450 and drug biotransformations were also observed in C57BL/6J strain mice treated with 40,000 units of LEIF-AD. Even 5,000 units of LEIF-AD had some effect on cytochrome P-450 system but the effect was not significantly different from control (Table 10).

Another highly purified homogenous cloned human leukocyte interferon (LEIF-A) had no depressant effect on cytochrome P-450 or on drug biotransformation in mouse liver (Table 8). This preparation of cloned interferon had no antiviral activity in the mouse. Only qualitative information about the antiviral activities of various interferon preparations is summarized since they were obtained either from Calbiochem. or Genentech. The antiviral activity of various preparations of interferon used in this study are summarized in Table 11. Using two highly purified human leukocyte interferon LEIF-A and LEIF-AD, and several impure preparations of human and mouse

TABLE 8

Effect of human cloned interferon (LEIF-A) on hepatic drug biotransformation

TREATMENT	PROTEIN mg/ml	CYTOCHROME P-450 nmoles/mg Protein	CYTOCHROME b_5 nmoles/mg Protein	AMINOPYRINE N-demethylase Actvity nmoles HCHO formed/ mg Protein/hr.	Benzo(a)Pyrene Hydroxylase Activity nmoles 3-OH-BP formed/mg Protein/hr
Saline	7.23 ± 0.32	0.643 ± 0.011	0.205 ± 0.023	366 ± 36	2.79 ± 0.06
Poly r(I.C)	6.44 ± 0.26	0.491 ± 0.044*	0.230 ± 0.010	264 ± 41*	1.66 ± 0.09
LEIF-A ($5x10^4$ units/ mouse)	8.93 ± 0.43	0.751 ± 0.029	0.227 ± 0.008	392 ± 19	1.96 ± 0.06

Mice (BalbC/J) were treated either with Poly r(I.C) (10 mg/kg) or interferon (LEIF-A) i.p 24 hours before sacrifice.

Each value is the mean ± S.E.M. of 4 individual animals.

* Significantly different from control $p < 0.05$.

TABLE 9

Effect of human cloned leuckocyte interferon (LEIF-AD) on hepatic cytochrome P-450

TREATMENT	PROTEIN mg/ml	CYTOCHROME P-450 nmoles/mg Protein	CYTOCHROME b_5 nmoles/mg Protein	AMINOPYRINE N-demethylase Activity nmoles HCHO formed/ mg Protein/hr.	Benzo(a)Pyrene Hydroxylase Activity nmoles 3-OH-BP formed/mg Protein/hr
Saline	5.30 ± 0.14	0.870 ± 0.064	0.292 ± 0.006	459 ± 35	7.18 ± 0.22
Poly r(I.C)	4.92 ± 0.26	0.589 ± 0.047*	0.246 ± 0.023	366 ± 20*	6.90 ± 0.76
LEIF-AD ($4x10^4$ units/ mouse)	4.44 ± 0.23	0.461 ± 0.042*	0.173 ± 0.013*	250 ± 18*	4.19 ± 0.26*

Mice (BalbC/J) were treated either with Poly r(I.C) (10 mg/kg) or interferon (LEIF-AD) i.p 24 hours before sacrifice.

Each value is the mean ± S.E.M. of 4 individual animals.

* Significantly different from control $p < 0.05$.

TABLE 10

Effect of cloned leuckocyte human interferon (LEIF-AD) on hepatic cytochrome P-450.

TREATMENT	PROTEIN mg/ml	CYTOCHROME P-450 nmoles/mg Protein	CYTOCHROME b_5 nmoles/mg Protein	AMINOPYRINE N-demethylase Actvity nmoles HCHO formed/ mg Protein/hr.	Benzo(a)Pyrene Hydroxylase Activity nmoles 3-OH-BP formed/mg Protein/hr
Saline	6.26 ± 0.39	0.478 ± 0.023	0.184 ± 0.014	406 ± 33	4.63 ± 0.30
Poly r(I.C)	4.89 ± 0.10	0.270 ± 0.027*	0.120 ± 0.015*	161 ± 20*	2.08 ± 0.01*
LEIF-AD ($5x10^3$ units/ mouse)	5.77 ± 0.38	0.380 ± 0.034	0.154 ± 0.003	332 ± 21	4.42 ± 0.61
LEIF-AD ($4x10^4$ units/ mouse)	4.44 ± 0.23	0.278 ± 0.021*	0.133 ± 0.016	159 ± 15*	2.42 ± 0.32*

Mice (C57BL/6J) were treated either with Poly r(I.C) (10 mg/kg) or interferon (LEIF-AD) i.p 24 hours before sacrifice.

Each value is the mean ± S.E.M. of 4 individual animals.

* Significantly different from control $p < 0.05$.

TABLE 11

Antiviral Activity of Interferon in Mouse

TYPE	CELL SOURCE	ANTIVIRAL ACTIVITY IN MOUSE	NOMENCLATURE	SOURCE
Calbiochem	Fibroblast	Yes	IFN-β	Mouse
CD-1	Leukocyte	Yes	IFN-α	Mouse
Buffy Coat	Leukocyte	No	IFN-α	Human
LEIF-A	Cloned Leukocyte	No	IFN-α	Human
LEIF-AD	Cloned Leukocyte	Yes	IFN-α	Human

The antiviral activity of most of the different types of interferon was supplied by the donor i.e. Genentech Inc.

Interferon obtained from calbiochem (IFN-β) was tested for its antiviral activity by Dr. S. Lee (Department of Microbiology, Dalhousie University, Halifax).

interferon we have demonstrated that only interferons with antiviral activity in the mouse appear to depress cytochrome P-450 in that species.

E. Incorporation of ^{14}C-ALA in various subcellular fractions after Poly r (I.C) treatment.

Mice were injected with poly r (I.C) or saline (for controls) for various time intervals. One hour before sacrifice they were injected with ^{14}C-ALA and its incorporation was examined. Marked changes in ^{14}C-ALA incorporation in one hour were observed following the administration of poly r (I.C) for various time intervals. In the 10,000 x g supernatant obtained from whole liver homogenate prepared from mice treated with Poly r (I.C) for 3 hours, the incorporation of ^{14}C-ALA was increased by 60% but was decreased by 40% 9 hours after poly r (I.C) (Fig. 11). In the microsomal fraction obtained by ultracentrifugation of the 10,000 x g supernatant, ^{14}C-ALA incorporation was increased initially 3 hours after poly r (I.C) administration but then gradually decreased over the next 15 hours to 40 percent of that in control mice (Fig. 12).

In the same experiment a general decrease in total microsomal heme was observed after Poly r (I.C) treatment for various time intervals(Fig. 13). A decrease of approximately 50% in total microsomal heme was observed at 24 hours time interval. A parallel decrease in both the

FIGURE 11

^{14}C-ALA incorporation in 10,000 x g supernatant of the liver homogenate. Each point represents mean ± S.E.M. of 3 individual mice. Each sample was assayed in duplicate. The control value for incorporation of ^{14}C-ALA incorporation in the 10,000 x g supernatant was 7,152 ± 464 D.P.M.

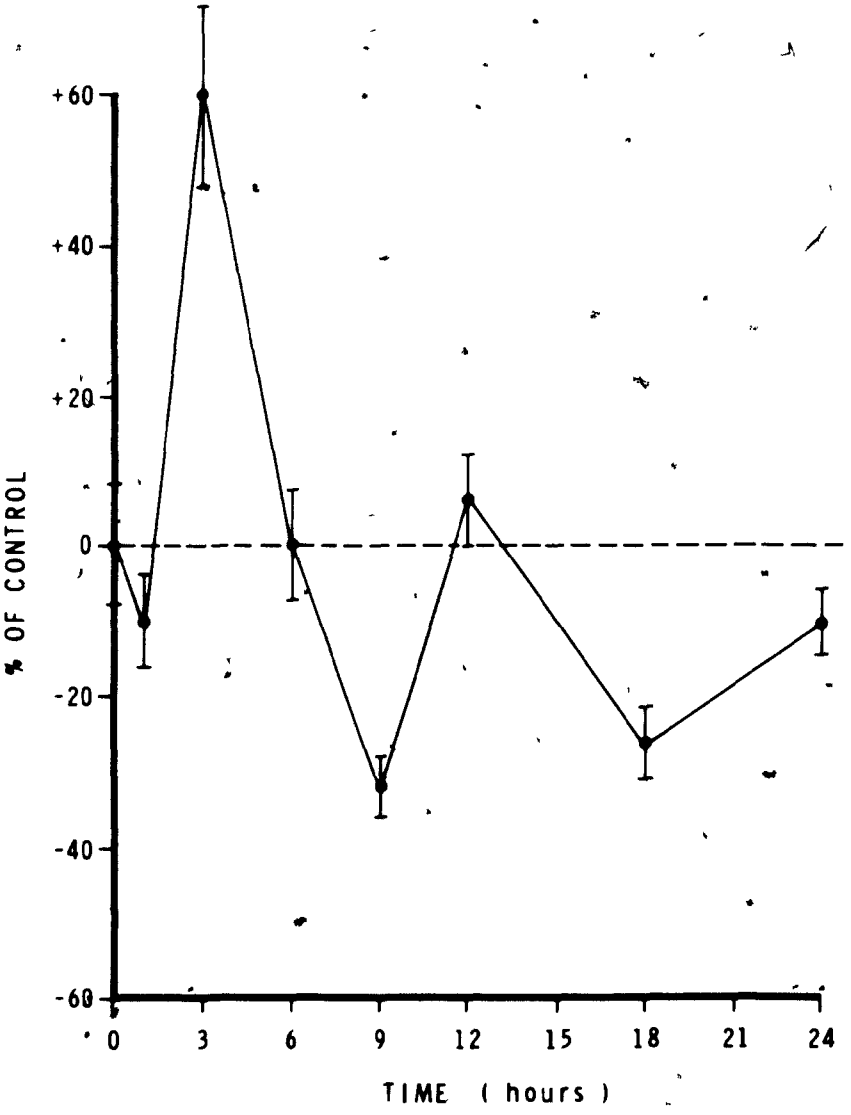

+60
+40
+20
0
-20
-40
-60
% OF CONTROL
0
3
6
9
12
15
18
21
24
TIME (hours)

FIGURE 12

^{14}C-ALA incorporation in microsomes. Each point represents mean ± S.E.M. of 3 individual mice. Each sample was assayed in duplicate. The control value for incorporation of ^{14}C-ALA incorporation in microsomes was 6,949 ± 269 D.P.M.

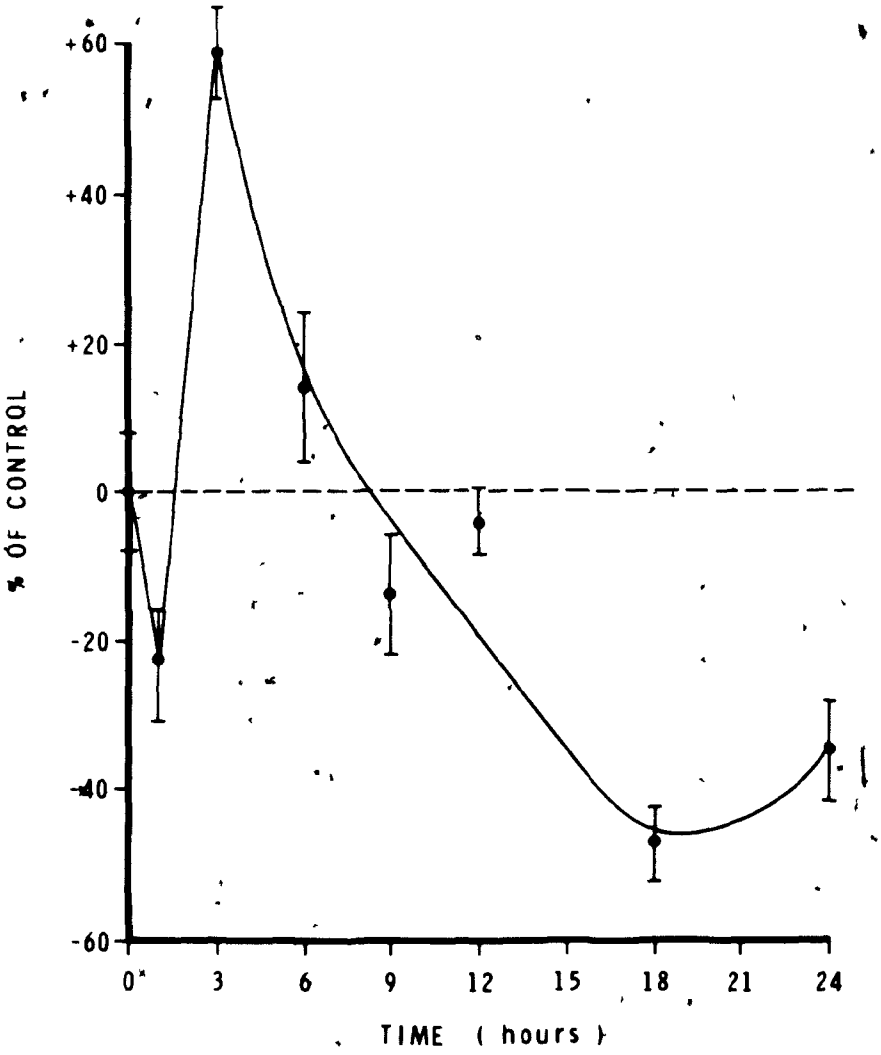

+60
+40
+20
0
-20
-40
-60
% OF CONTROL
0*
3
6
9
12
15
18
21
24
TIME (hours)

FIGURE 13

Effect of Poly r (I.C) at various time intervals on the hepatic total microsomal heme. Each point on the graph represents mean ± S.E.M. of 3 individual animals. Each sample was assayed in duplicate. Total heme was determined by the pyridine hemochromogen method described by Falk (1964).

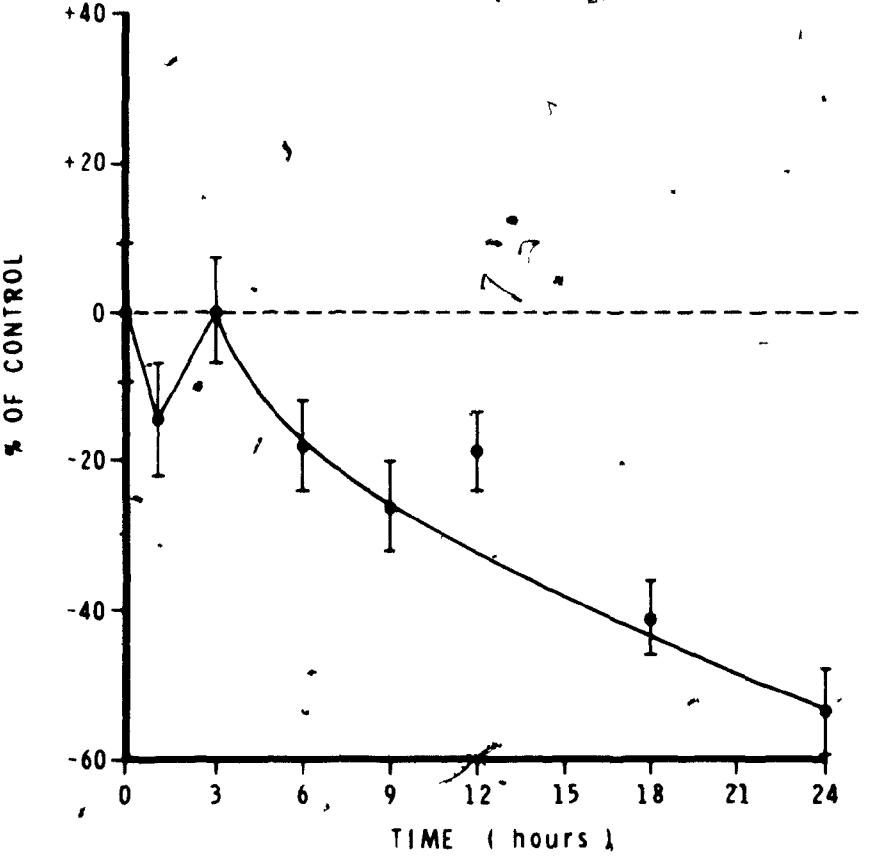

+40
+20
0
-20
-40
-60
% OF CONTROL
0
3
6
9
12
15
18
21
24
TIME (hours)

FIGURE 14

Effect of Poly r (I.C) at various time intervals on Hepatic cytochrome P-450 and cytochrome b_5. Each point on the graph represents mean ± S.E.M. of 3 individual animals.

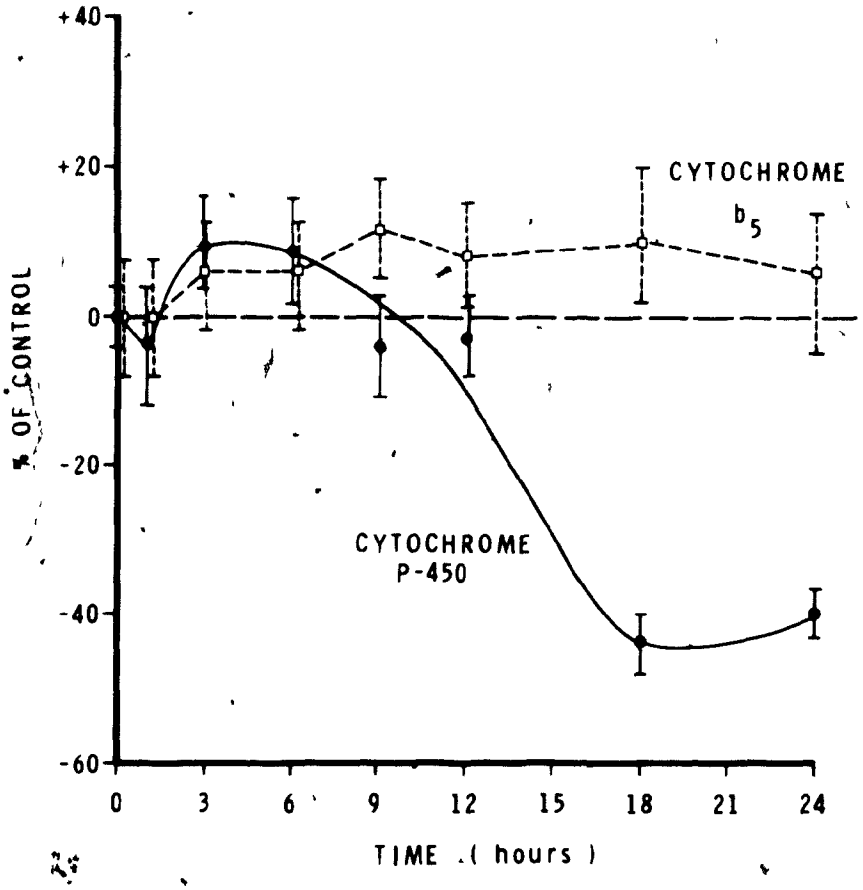
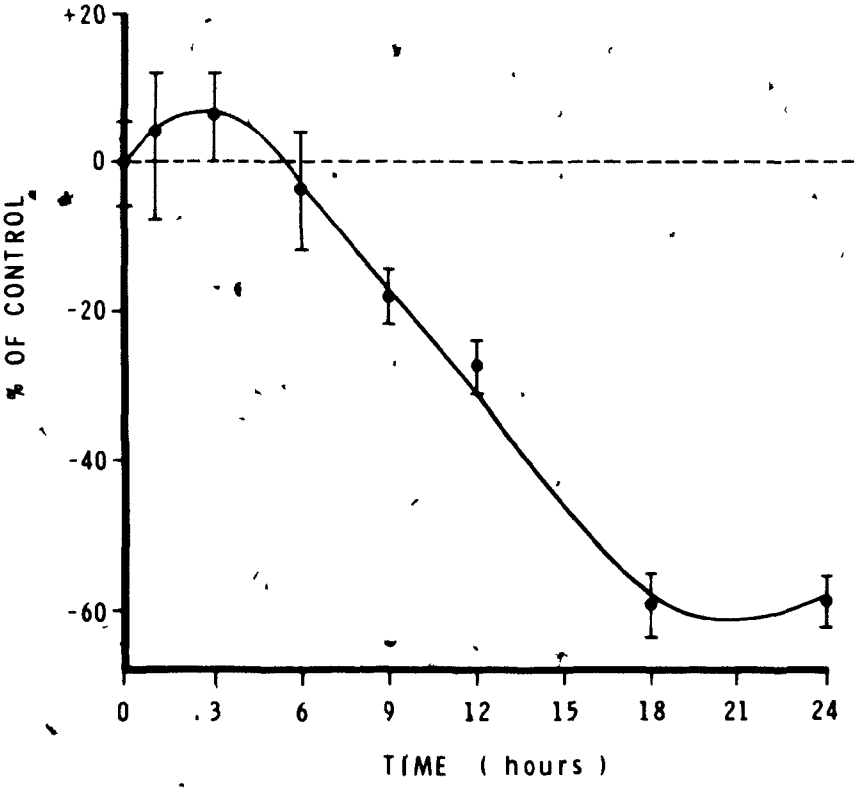

+40
+20
0
-20
-40
-60
% OF CONTROL
CYTOCHROME
b_5
CYTOCHROME
P-450
0
3
6
9
12
15
18
21
24
TIME (hours)

FIGURE 15

Effect of Poly r (I.C) at various time intervals on Hepatic Aminopyrine N-demethylase activity. Each point on the graph represents mean ± S.E.M. of 3 individual animals. Each sample was assayed in duplicate.


+20
0
-20
-40
-60
% OF CONTROL
0
3
6
9
12
15
18
21
24
TIME (hours)

level of cytochrome P-450 and the aminopyrine N-demethylase activity were also observed (Fig. 14 and 15). Microsomal cytochrome b_5 levels were unchanged following Poly r (I.C) treatment (Fig. 14).

F. Effect of Poly r (I.C) on Heme breakdown in vivo.

Cytochrome P-450 degradation was determined by measuring the amount of ^{14}C-carbon monoxide in expired air following the administration of 5-^{14}C-ALA. In mammals carbon monoxide originates solely from the oxidized alpha-methene bridge carbon atom of heme. 5-^{14}C-ALA specifically labels the alpha-methene bridge carbon in the porphyrin ring. On cleavage of the heme ring this carbon is converted to carbon monoxide. Rate of ^{14}CO production from 5-^{14}C-ALA is illustrated in Fig. 16. A biphasic rate of degradation, similar to that obtained by Levin and Kuntzman (1969) is observed.

At different time periods following the administration of Poly r (I.C) mice received 5-^{14}C-ALA and the production of ^{14}CO was measured (Fig. 17). The effect of Poly r (I.C) treatment at various time intervals on slow phase degradation rate is measured using half-life as an index of degradation rate. The degradation rate of slow turnover cytochrome P-450 was increased after 1 hour, 2 hour, 3 hour, 6 hour and 12 hours of Poly r (I.C) treatment. However, 24 hours after Poly r (I.C) treatment normal degradation rates were restored (Fig. 17 and 19).

FIGURE 16

Rate of ^{14}CO production from 5-^{14}C-ALA. Mice received 2.0 Ci/mouse of 5-^{14}C-ALA intraperitoneally at time 0. ^{14}CO was determined at 10 min intervals throughout this experiment. The ^{14}CO exhaled by a single mouse is illustrated in this figure and is the representative pattern produced in all mice studied. PHASE A represents "Apparent net synthesis", PHASE B represents degradation rate of "fast turnover cytochrome P-450" and PHASE C represents the degradation rate of the "Slow turnover cytochrome P-450".

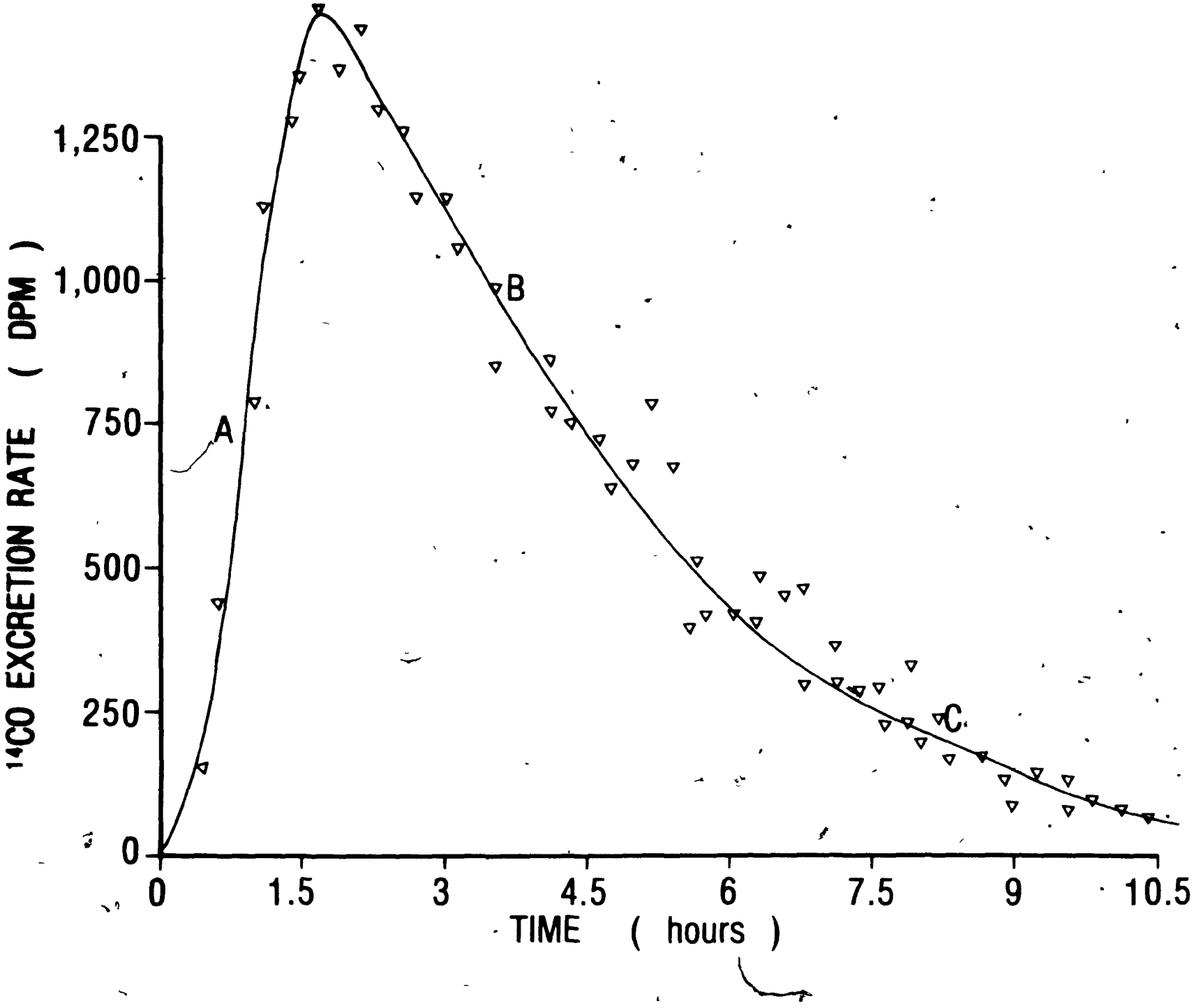

1,250
1,000
750
500
250
0
^{14}CO EXCRETION RATE (DPM)
A
B
C
0
1.5
3
4.5
6
7.5
9
10.5
TIME (hours)

FIGURE 17

Rate of ^{14}CO production from 5-^{14}C-ALA following treatment with Poly r (I.C) for various time intervals. Following Poly r (I.C) treatment for a certain time interval mice received 2.0 μCi/mouse of 5-^{14}C-ALA (i.p) and their ^{14}CO expired in breath was continously monitored.

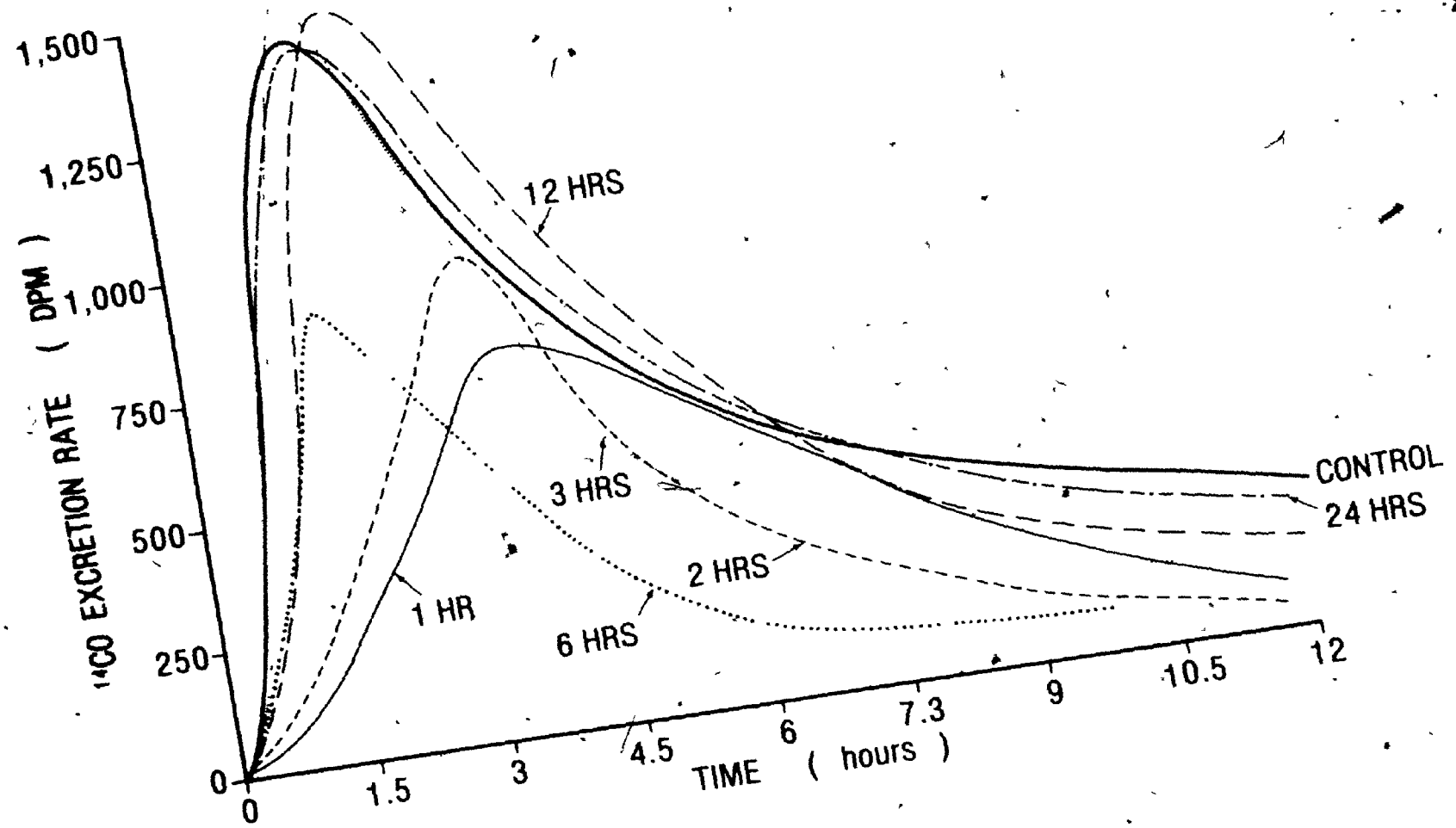
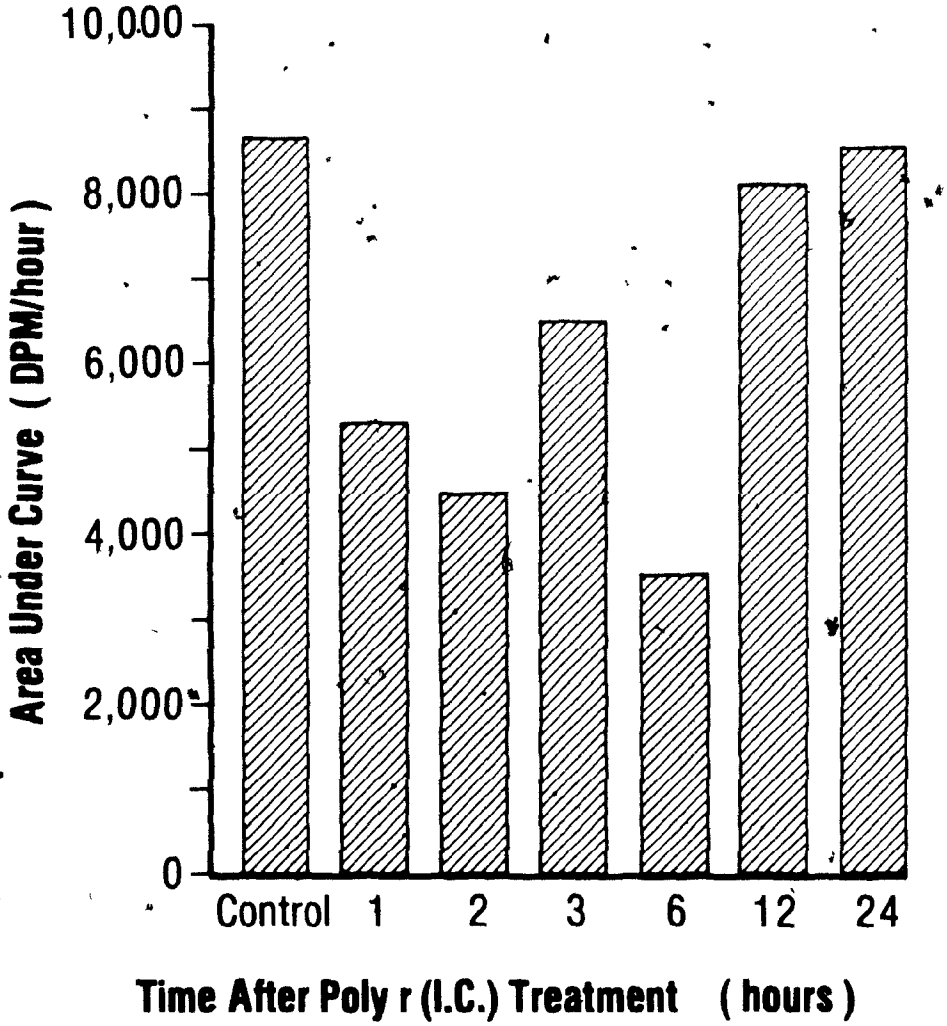

1,500
1,250
1,000
750
500
250
0
^{14}CO EXCRETION RATE (DPM)
12 HRS
CONTROL
24 HRS
3 HRS
2 HRS
1 HR
6 HRS
0
1.5
3
4.5
6
7.3
9
10.5
12
TIME (hours)

FIGURE 18

The effect of Poly r (I.C) at various time intervals on "Net Heme Synthesis" measured by area under the curve. Area's under the curves from Fig. 17 were calculated for each curve and were taken as an index of net heme synthesis.


10,000
8,000
6,000
4,000
2,000
0
Area Under Curve (DPM/hour)
Control
1
2
3
6
12
24
Time After Poly r (I.C.) Treatment (hours)

On the other hand the degradation rate of the fast phase component was only affected after 1 hour and 2 hours of poly r (I.C.) treatment. However 3 hours after poly r (I.C) treatment normal degradation rates of the fast phase were restored.

A defect in the synthesis of heme is also suggested in the experiments involving the elimination of ^{14}CO from the methene bridge of heme (Fig. 17). The area under the curve which represents the total amount of label turning over in the heme moeity is greatly diminished in Poly r (I.C) treated animals (Fig 18).

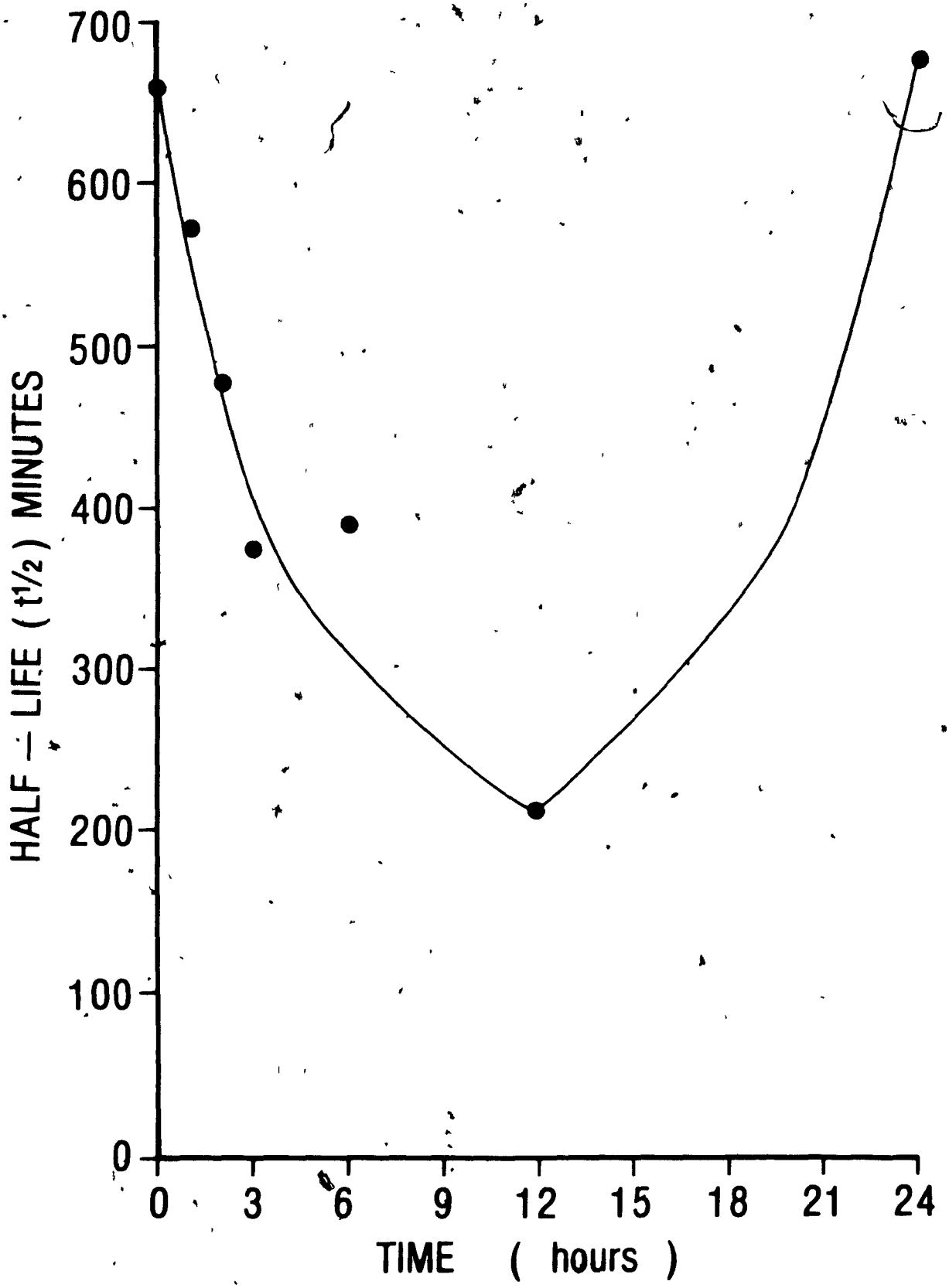
G. Incorporation of ^{3}H-Amino Acids in various subcellular fractions after Poly r (I.C) treatment.

Mice were treated with Poly r (I.C) for various time intervals and one hour before sacrifice a ^{3}H-Amino Acid mixture was administered (i.p.). Whole liver homogenates were prepared using a glass homogenizer and various subcellular fractions were prepared as described earlier. Aliquots of 0.5 ml of the whole liver homogenate or the subcellular fractions were added to 10 mls of scintillation cocktail (Biofluor) to determine the amount of label incorporated.

In whole liver homogenates ^{3}H-Amino Acid incorporation was increased in Poly r (I.C) treated animals compared to controls. A maximum increase of 60% in ^{3}H-amino acid incorporation was observed 6 hours following the treatment

FIGURE 19

Effect of Poly r (I.C)* treatment at various time intervals on degradation rate of slow Phase measured by half-life as an index of degradation. The half-lives of slow phase (Phase C) were calculated from Fig. 17.


700
600
500
400
300
200
100
0
HALF – LIFE (t½) MINUTES
0
3
6
9
12
15
18
21
24
TIME (hours)

with Poly r (I.C). A gradual decline towards control levels as observed after the peak value was achieved (Fig. 20). Similar patterns of ^{3}H-amino acid incorporation were observed in the 10,000 x g supernatant and in microsomes (Fig. 21 and 22). Cytochrome P-450, b_5 and aminopyrine N-demethylase activity were also determined in the microsomal fractions. Both cytochrome P-450 and aminopyrine N-demethylase were depressed in poly r (I.C) treated animals. The results were very similar to those shown in Fig. 14 and 15.

H. Effect of Poly r (I.C) on incorporation of ^{14}C-Amino Acids into Hepatic Cytochrome P-450.

Ten mice were injected with ^{14}C-Amino Acid mixture and sacrificed an hour later. Microsomes prepared from these mice were pooled and solubilized as described in the methods section. An aliquot of 0.5 ml from the solubilized microsomes was added to scintillation cocktail and incorporation of ^{14}C-Amino Acid mixture in the pooled solubilized microsomes was recorded. 10 mls of (1 gm liver/ mL buffer) solubilized microsomes was applied on the sephacryl column for the separation of various proteins on the basis of their molecular weight. A flow rate of 0.5 mls/min was maintained and 4 mL fractions were collected. All the fractions were examined for cytochrome P-450 and cytochrome b_5 content and also monitored for their radio-activity. We were unable to monitor protein at 280 nm

FIGURE 20

^{3}H-Amino Acid mixture incorporation in the liver (whole liver homogenate) following Poly r (I.C) pretreatment. After treatment with Poly r (I.C) for various time intervals ^{3}H-A.A. was injected i.p and 1 hour later the animal was sacrificed and whole liver homogenate prepared. Each point represents mean ± S.E.M. of 3 individual mice. Each sample was counted in duplicate. The control ^{3}H-Amino Acid incorporation was 3,411 ± 155 D.P.M.

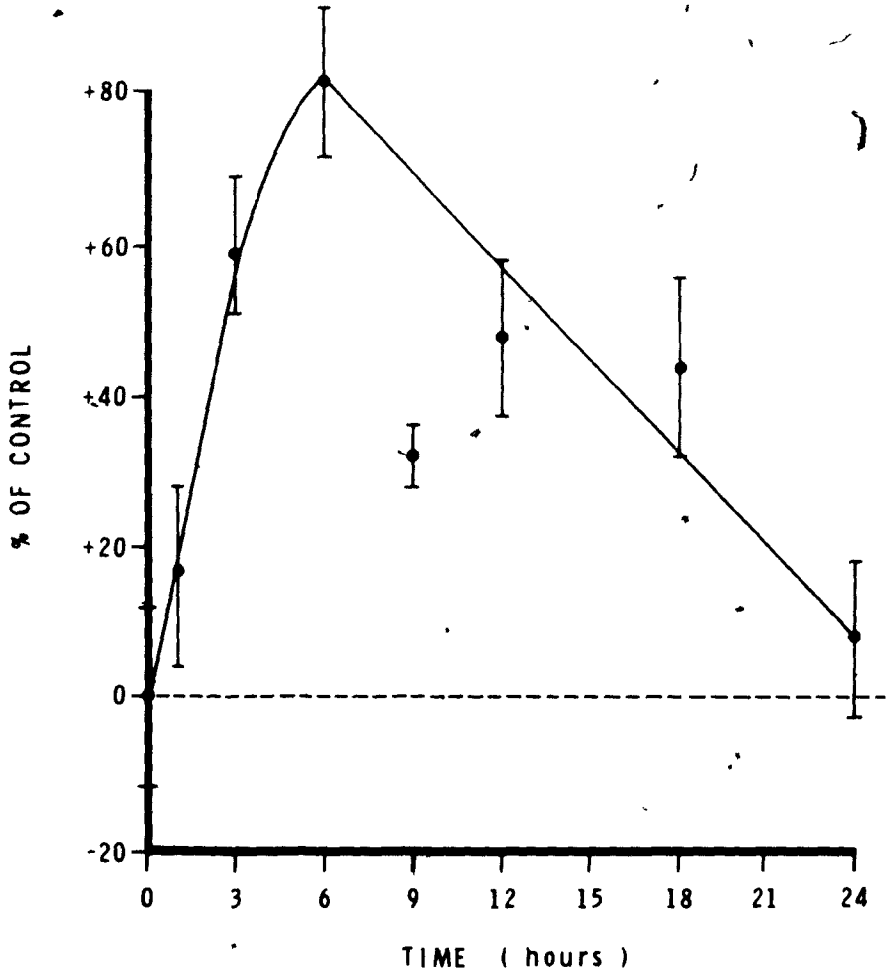

+80
+60
+40
+20
0
-20
% OF CONTROL
0
3
6
9
12
15
18
21
24
TIME (hours)

FIGURE 21

^{3}H-Amino Acid mixture incorporation in 10,000 x G supernatant derived from whole liver homogenate following Poly r (I.C) pretreatment for various time intervals. ^{3}H-A.A. were injected i.p an hour before sacrificing and preparation of supernatant. Each point represents mean $\pm$ S.E.M. of 3 individual mice. Each sample was counted in duplicate. The control ^{3}H-Amino Acid incorporation in supernatant was 2,480 $\pm$ 158 D.P.M.

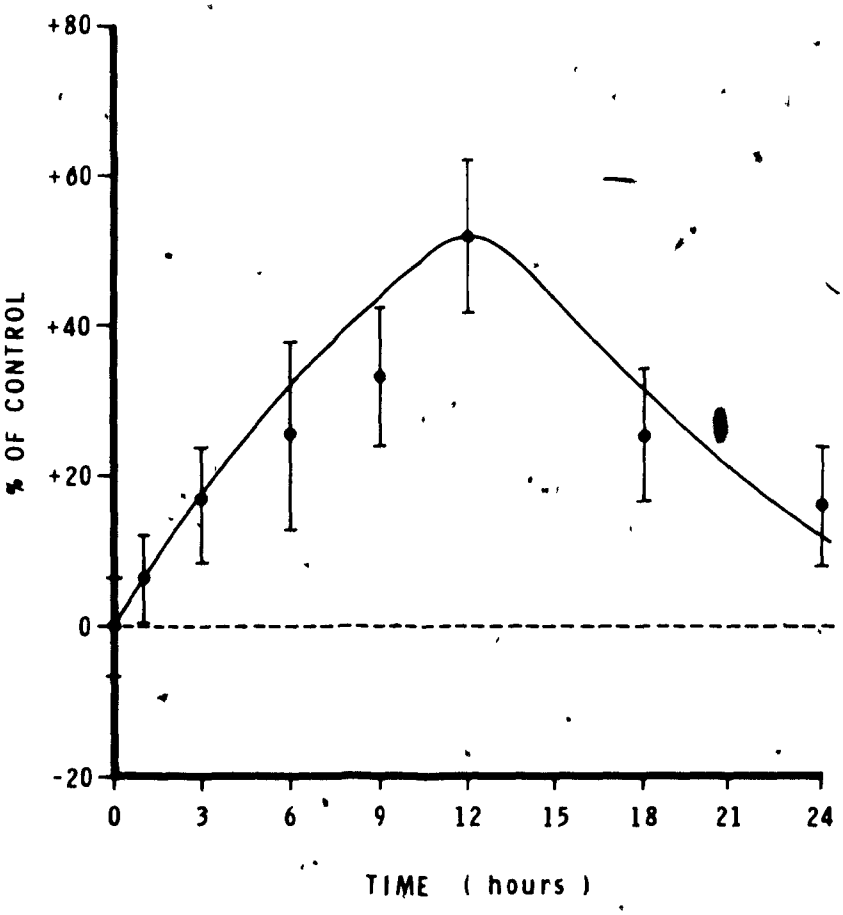
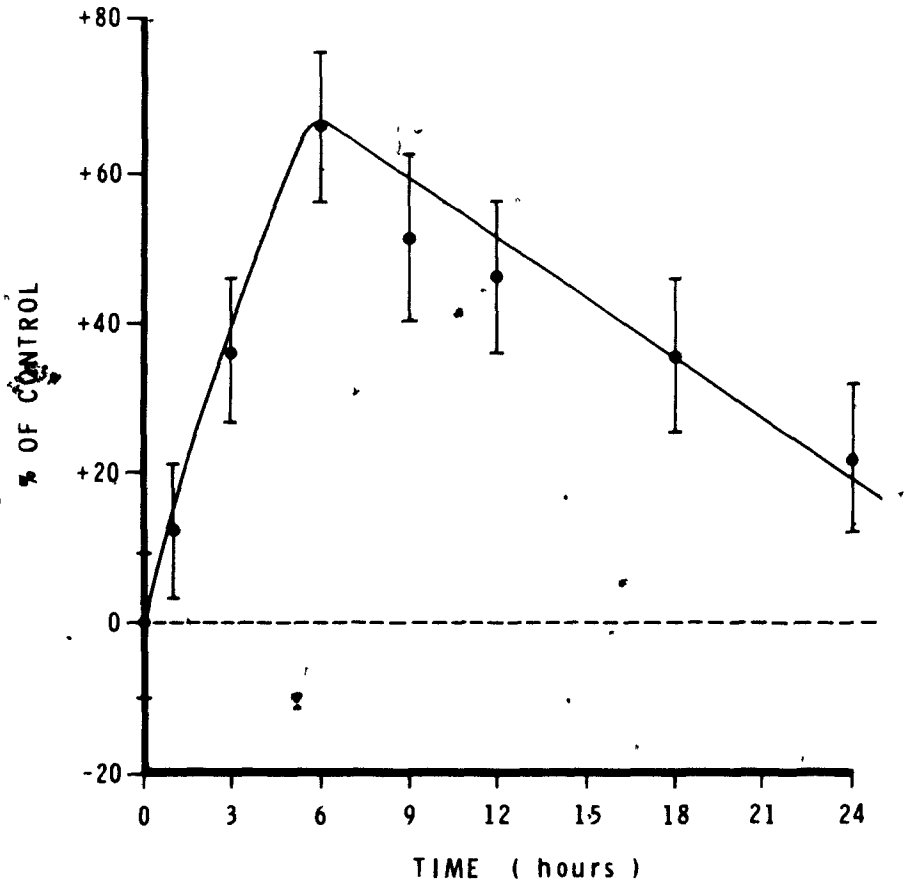

+80
+60
+40
+20
0
-20
% OF CONTROL
0
3
6
9
12
15
18
21
24
TIME (hours)

FIGURE 22

^{3}H-Amino Acid incorporation in Hepatic microsomes prepared by ultracentrifugation at 100,000 x g of the 10;000 x g supernatant derived from whole liver homogenate following Poly r (I.C) pretreatment for various time intervals. Each point represents mean $\pm$ S.E.M. of 3 individual mice. Each sample was counted in duplicate. The control ^{3}H Amino Acid incorporation in microsomes was 942 $\pm$ 162 D.P.M.


+80
+60
+40
+20
0
-20
% OF CONTROL
0
3
6
9
12
15
18
21
24
TIME (hours)

because of the interference with the detergent in the buffer. Both the Lowry assay and the Biuret assay for protein determination were also susceptible to the interference caused by the detergent in the buffer. The radioactivity profile obtained from the fractions is shown in Fig. 23. A rough estimate of the molecular weights of various fractions was obtained by using the calibration profiles of standard protein elution volumes (Fig. 23).

Similarly a group of ten mice pretreated with Poly r (I.C) for 24 hours was injected with ^{14}C-Amino Acid mixture and sacrificed an hour later. Their solubilized microsomes were then eluted through the sephacryl column. The radioactivity profile of the treated solubilized microsomes was expressed as % of the total radioactivity (DPM) ^{14}C-amino acid incorporated into microsomes (Fig. 24). On superimposing the radioactivity profiles of the control group and treated group it is observed that there is increased ^{14}C-amino Acid incorporation in treated animals compared to control animals as shown by the shaded area between the two profiles (Fig. 25).

Finally the fractions which contained cytochrome P-450 were pooled and concentrated to 10 mls. This concentrate was eluted on a DEAE-Anion exchange column. The column was developed first with 100 mls of the same buffered detergent, and then with a 500-ml linear gradient of NaCL from 0 to 0.25 M incorporated in the buffered detergent. The pattern

FIGURE 23

Profile of ^{14}C-Amino Acid mixture incorporated in the microsomal proteins separated on the basis of molecular weight and size on sephacryl S-200 column. Fractions 140 to 240 had detectable amounts of cytochrome P-450. Microsomes obtained from 10 mice were pooled for eluting on the column. The Y-coordinate is expressed as percent of total radioactivity. It is obtained by dividing the radioactivity of each fraction by the total radioactivity in the microsomes that was eluted on the column. The column was calibrated using various standard molecular weight substances as shown in the figure.

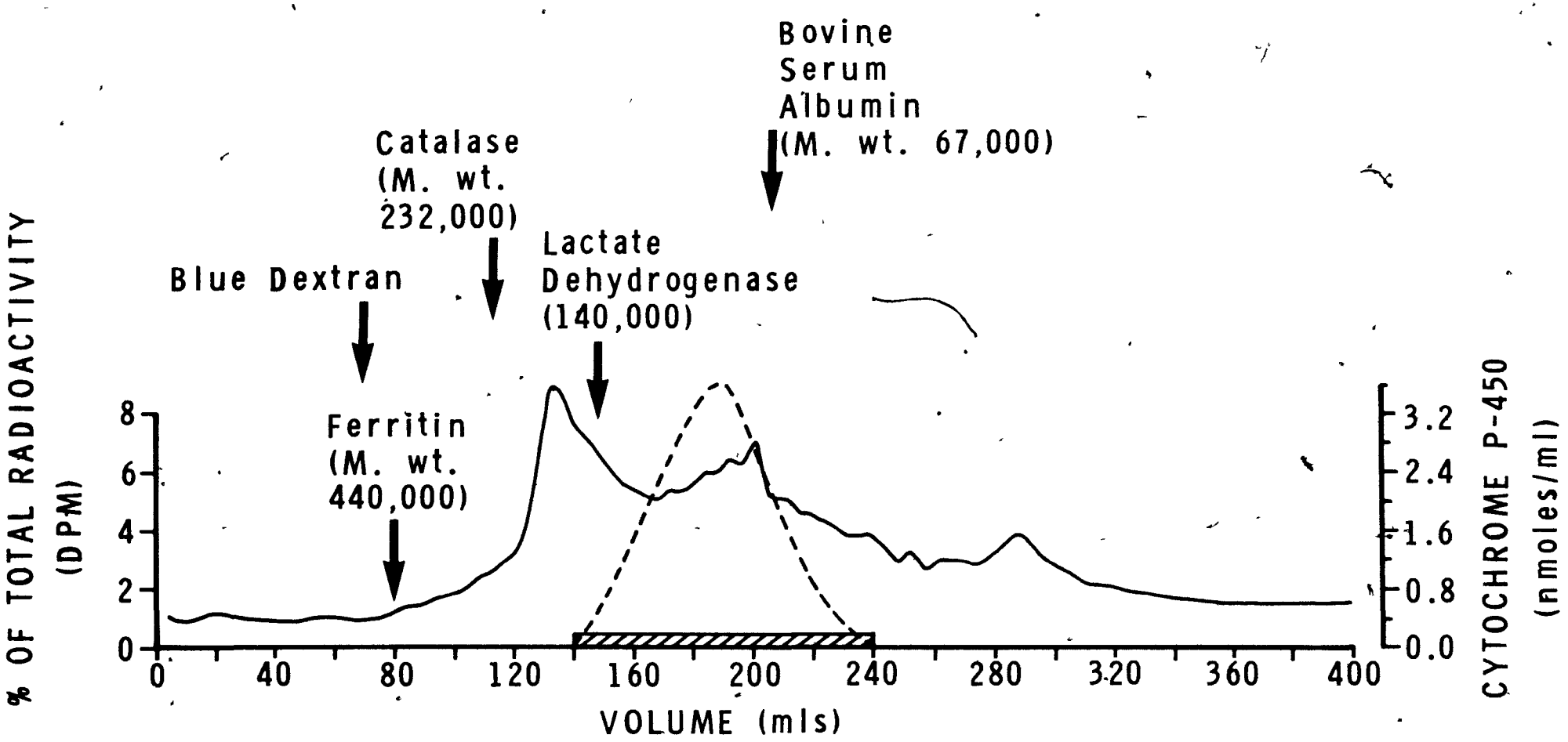
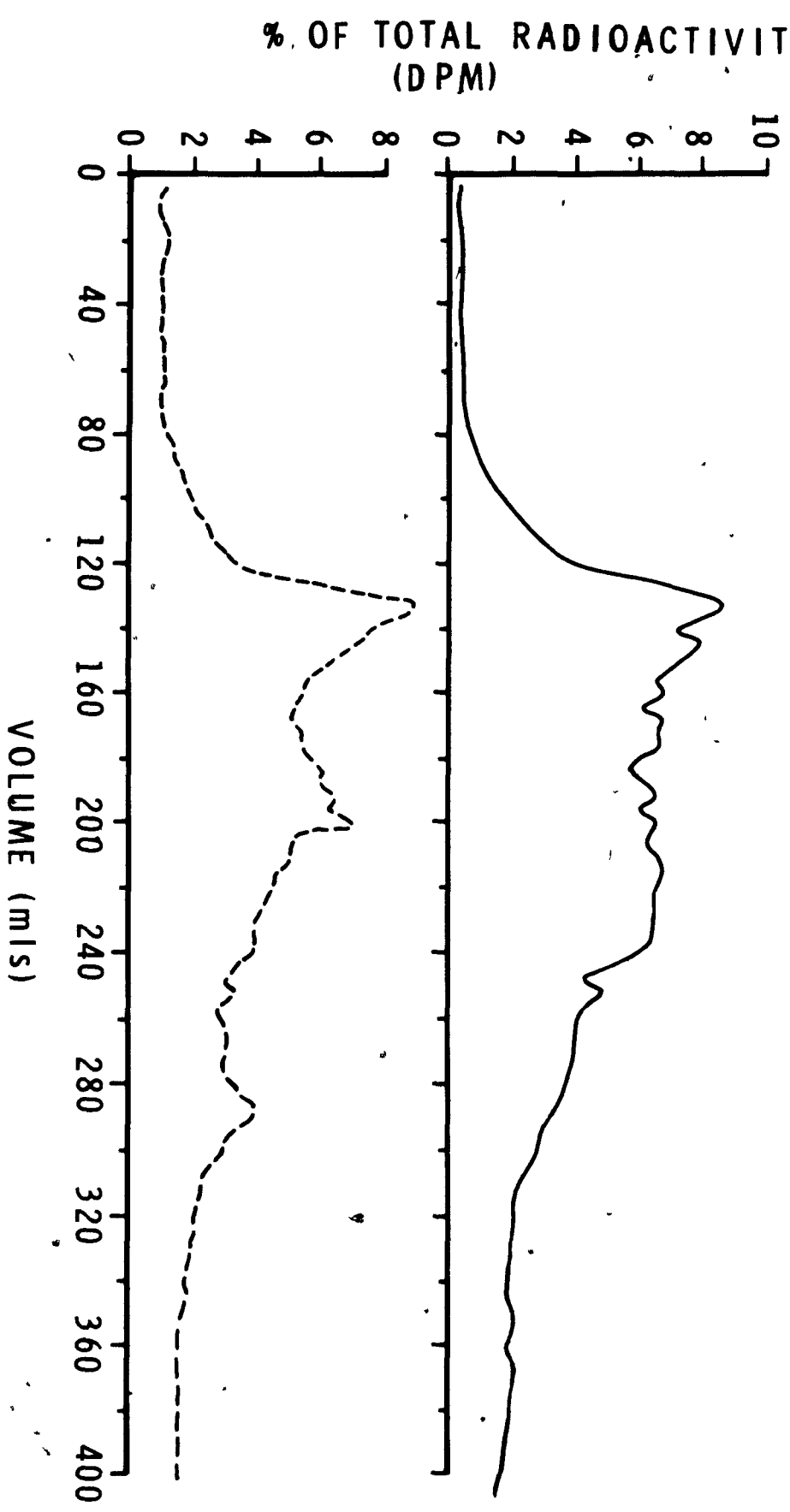

Bovine
Serum
Albumin
(M. wt. 67,000)
Catalase
(M. wt.
232,000)
Lactate
Dehydrogenase
(140,000)
Blue Dextran
Ferritin
(M. wt.
440,000)
% OF TOTAL RADIOACTIVITY
(DPM)
8
6
4
2
0
0
40
80
120
160
200
240
280
320
360
400
VOLUME (mls)
3.2
2.4
1.6
0.8
0.0
CYTOCHROME P-450
(nmoles/ml)

FIGURE 24

Profiles of microsomal proteins separated on sephacryl S-200 column. The upper profile (A) is obtained from animals treated with Poly r (I.C) for 24 hours and the lower profile (B) is obtained from animals treated with saline. These profiles are representative of a single experiment for both saline and poly r (I.C) treated animals. The saline controls were repeated three times and poly r (I.C) treated animals twice, however similar profiles as shown here were obtained respectively for saline and poly r (I.C) treated animals.


% OF TOTAL RADIOACTIVITY
(DPM)
0
2
4
6
8
0
2
4
6
8
10
0
40
80
120
160
200
240
280
320
360
400
VOLUME (mls)

109.

FIGURE 25

Profiles of both Poly r (I.C) treated animals and saline control from Fig. 24 are superimposed. The shaded parts show a difference in ^{14}C-Amino Acid incorporation in Poly r (I.C) animals compared to saline animals.

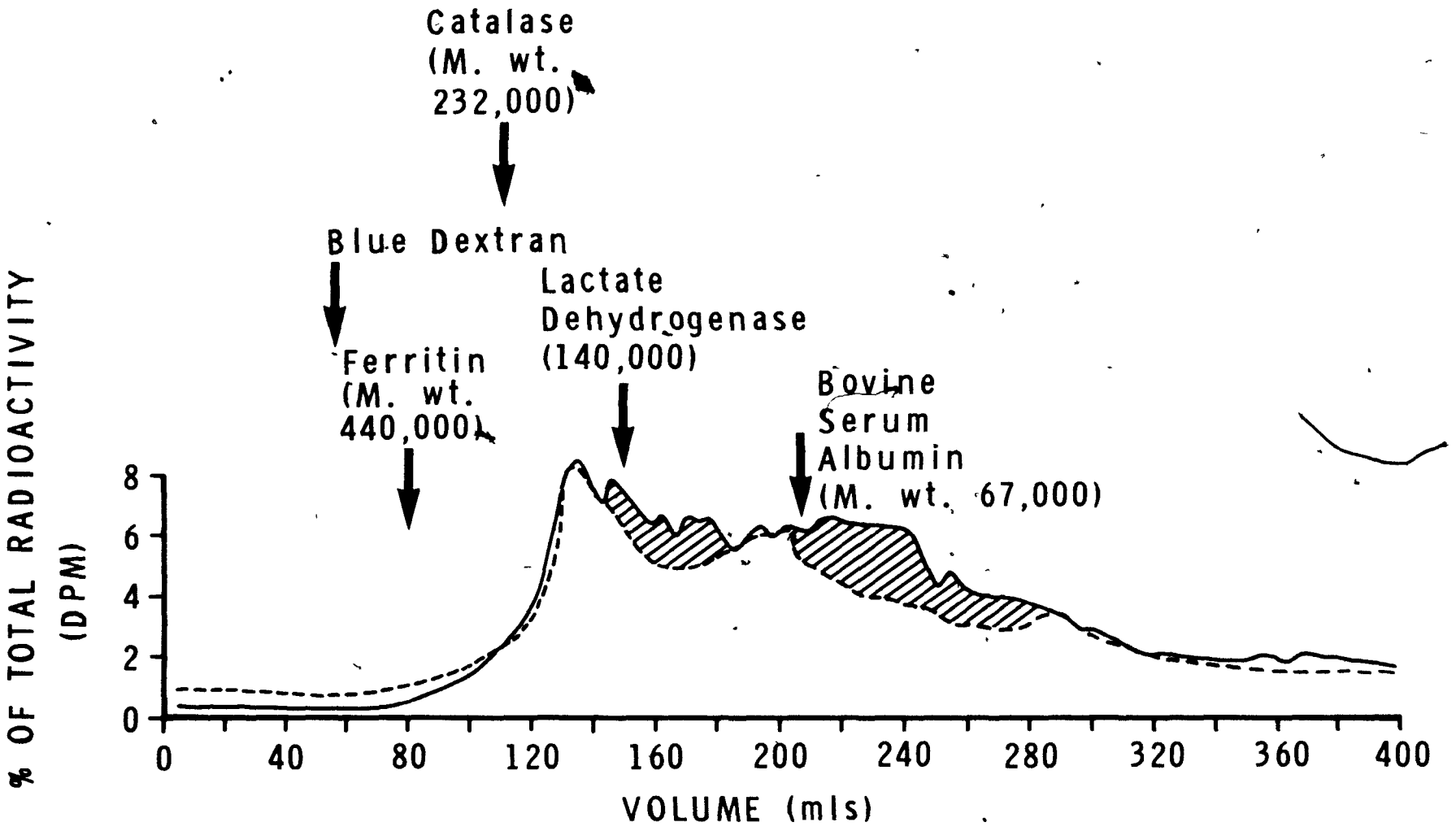
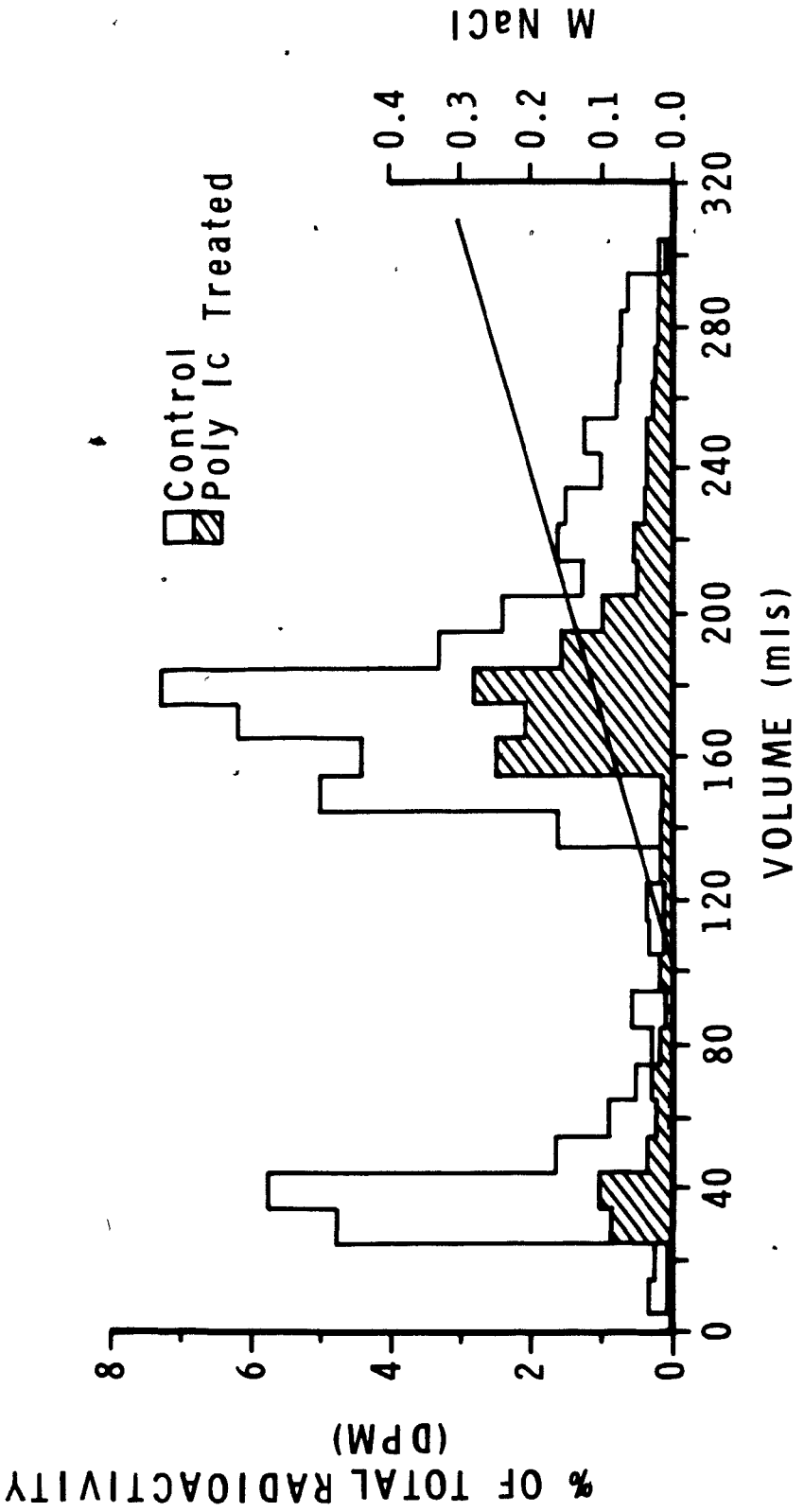

Catalase
(M. wt.
232,000)
Blue Dextran
Ferritin
(M. wt.
440,000)
Lactate
Dehydrogenase
(140,000)
Bovine
Serum
Albumin
(M. wt. 67,000)
% OF TOTAL RADIOACTIVITY
(DPM)
0
2
4
6
8
0
40
80
120
160
200
240
280
320
360
400
VOLUME (mls)

FIGURE 26

Profiles of ^{14}C-Amino Acids incorporated in the hepatic cytochrome P-450 fraction (fractions 140 to 240 from the sephacryl column) separated on DEAE-Anion exchange column. The open bars represent ^{14}C-Amino Acid incorporation in control animals and the shaded bars represent incorporation of ^{14}C-Amino acid in Poly r (I.C) treated animals. The profile for saline control animals was repeated three times and twice for poly r (I.C) treated animals. The patterns obtained in each experiment were similar to those shown in this figure.


% OF TOTAL RADIOACTIVITY
(DPM)
8
6
4
2
0
0
40
80
120
160
200
240
280
320
VOLUME (mls)
0.4
0.3
0.2
0.1
0.0
M NaCl
Control
Poly Ic Treated

of ^{14}C-amino acid incorporation obtained in the effluent was similar to that of various forms of cytochrome P-450 obtained by Warner et al (1978). This indicates that the various forms of cytochrome P-450 are labelled with amino acids. When concentrate obtained from poly r (I.C) treated animals are eluted on DEAE-Anion exchange column, a depression in ^{14}C-Amino acid incorporation was observed compared to the control group. (Fig. 26). A significant drop in ^{14}C-amino acid incorporation was observed in all species of cytochrome P-450 and no single form was completely abolished by poly r (I.C) treatment.

DISCUSSION

A. Involvement of Interferon in causing depression of Hepatic cytochrome P-450.

The results described in this thesis, demonstrate that both the level of hepatic cytochrome P-450 and related drug biotransformation are depressed by interferon. Previously Renton and Mannering (1976) demonstrated that depression of drug metabolism was a common property of interferon inducing agents abut they were unable to clearly identify if this effect was due to interferon itself or to another common property of these agents. Other investigators (Farquar et. al., 1976; Soyka et.al., 1979; Barnes et.al., 1979; Mullen, P.W., 1977) have shown similar effects following the stimulation of host defence mechanisms but it was unclear if individual factors such as immune enhancement, reticulo-endothelial cell stimulation, interferon production or a combination of these was involved in the observed effects.

Further indirect evidence for the involvement of interferon is suggested by the experiments carried out in inbred strains of mice. DeMaeyer and coworkers (1979) recently described four distinct genetic loci which influence the levels of circulating interferon in response to specific viruses. For Newcastle disease virus (NDV) one autosomal locus (IF-1) determines a 10 fold difference in serum interferon levels in different mouse strains. In this study mouse strains carrying the high ($IF\text{-}1^h$) or low ($IF\text{-}1^l$) production allele at the If-1 locus were used to demonstrate

that depression of hepatic cytochrome P-450 by NDV could be correlated with circulating interferon levels. In C57BL/6J mice which carry the 1F-1^h allele and produced high levels of interferon when challenged with NDV, cytochrome P-450 levels and aminopyrine N-demethylase activity was depressed by the virus. In C_3H/HeJ mice which carry the 1F-1^l allele no circulating interferon was detected when challenged with NDV. The virus also had no effect on cytochrome P-450 or drug biotransformation. These experiments suggest that the depression of cytochrome P-450 in response to NDV is dependent on high circulating interferon levels.

In contrast to the results with NDV, treatment of C57BL/6J or C_3H/HeJ mice with Poly r (I.C) produced high concentrations of interferon in the serum of both strains. Hepatic microsomal cytochrome P-450 and aminopyrine N-demethylase activity were both signifcantly depressed in both strains. Poly r (I.C) is a synthetic polynucleotide which induces the formation of interferon at loci other than 1F-1 (DeMaeyer, E. and DeMaeyer-Guignard, J., 1979). This experiment indicates that the cytochrome P-450 system in C_3H/HeJ mice has the ability to respond when high levels of circulating interferon are produced at other gene loci. Therefore by utilizing this genetic model indirect evidence is provided for the involvement of interferon or the process which produces interferon in causing the depression of cytochrome P-450 in the liver. However to provide

unequivocal proof of the involvement of interferon and to resolve whether interferon itself or the induction of interferon causes the lowering of hepatic drug biotransformation, homogenous preparation of interferon would be required to provide direct evidence.

Recent studies by Sonnenfeld et al.(1980) have shown that the administration of crude preparations of immune type interferon (IFN-Gamma, Type II) can depress drug biotransformation in the liver. Crude preparations of fibroblast interferon (IFN-Beta, Type 1) used in this thesis, caused a depression in hepatic cytochrome P-450 too. A dose-related effect of interferon on hepatic microsomal mixed function oxidase system was oberved with fibroblast interferon. Another crude preparation of leukocyte mouse interferon (IFN-alpha, Type I (CD-1)) which was obtained from the serum of mice treated with Poly r (I.C) also caused a marked depression of hepatic cytochrome P-450. However an impure preparation of human leukocyte interferon prepared from buffy-coat cells had no depressant effect on cytochrome P-450 or on drug biotransformation in mouse liver. Although only three impure preparations of interferon were used in this study it appears that only interferons which have antiviral effects against encephalomyocarditis virus in the mouse can depress cytochrome P-450 in that species. This evidence although obtained with crude preparation of interferon indicates that

interferon itself causes depression of hepatic drug biotransformation.

In using crude preparations of interferon the possibility of impurities in the preparation having an effect on cytochrome P-450 rather than interferon itself must be resolved. Impurities such as endotoxin or other biological proteins in the serum, or lipopolysaccharides or some factor in the serum from which interferon is purified could be the primary cause for depression of hepatic cytochrome P-450. In order to have conclusive evidence linking the involvement of interferon in causing the loss of cytochrome P-450, a pure homogenous preparation of interferon is required. The expression of human interferons in Escherchia coli from cloned genes using recombinant DNA technology has provided highly purified homogenous interferon preparations (Wetzel, et al., 1981; Weck et al., 1981a) allowing direct testing of our hypothesis. Human leukocyte interferons are a family of related peptides and each sub-type shows distinct properties (Weck et al., 1981b). A molecular hybrid, LEIf-AD formed between two of the human leukocyte interferon sub-types (LEIf-A and LEIf-D) is remarkable for its pronounced antiviral activity on mouse cells and in mice (Weck et al., 1981).

The administration of 40,000 units of LEIf-AD resulted in a 47 percent loss of cytochrome P-450 and a 41 percent loss of cytochrome b_5 in microsomes prepared from the livers

of BalbC/J mice. This loss of cytochrome P-450 was accompanied by a 55 percent decrease in the N-demethylation of aminopyrine and a 42 percent decrease in the hydroxylation of benzo (a) pyrene which are typical xenobiotic biotransformations carried out by this enzyme system. Similar losses in cytochrome P-450 and drug biotransformation were also observed in C57BL/6J strain mice treated with LEIf-AD. The changes in this enzyme system were of similar magnitude to those produced by the interferon inducer Poly r (I.C) and are equal to the maximum depression which can be caused by this agent. The depression of cytochrome P-450 caused by this highly purified homogenous interferon provides the first indisputable direct evidence that interferon itself causes the depression of the steady-state levels of cytochrome P-450 in the liver and results in a loss in the capacity of the liver to metabolize drugs. Hence the ambiguity of an impurity in interferon causing depression of cytochrome P-450 rather than interferon itself was removed by using highly purified homogenous preparation of interferon.

Using another cloned human leukocyte interferon sub-type, LEIf-A, no depressant effect on cytochrome P-450, or drug biotransformation in mouse liver was observed. However LEIf-A, like human buffy-coat interferon preparation, had no antiviral effect in mouse cells or in mice. An apriori conclusion that antiviral activity of the

interferon is linked to its depressant effect on hepatic cytochrome P-450 can be made from the few types of interferon both crude and pure preparations used in this thesis. However more preparations from a variety of sources are required to confirm this conclusion.

B. Mechanism to explain the depression of Hepatic Cytochrome P-450 mediated by interferon.

In the first part of this thesis I have demonstrated conclusively that interferon causes a marked depression of hepatic cytochrome P-450 in the mouse. It is remarkable that the loss of hepatic cytochrome P-450 by interferon or interferon inducers has never exceeded fifty percent of control. This finding that cytochrome P-450 loss does not exceed fifty percent no matter what dose of interferon or interferon inducer is used has been reported by ourselves and by Mannering (Mannering et.al., 1980). Therefore it appears that hepatic cytochrome P-450 attains a new steady-state level rather than be completely eliminated in a dose dependent fashion as in the case with cobalt chloride or 2-Allyl-2-isopropylacetamide (AIA) (Levin et.al., 1972). Changes in steady-state level of cytochrome P-450 are regulated by the turnover of the enzyme (synthesis and/or degradation). Synthesis requires separate formation of the apocytochrome and the heme moeity which are subsequently

assembled to the holocytochrome (Correia et.al., 1975). A perturbation in any process of holocytochrome synthesis would result in a change in the steady-state level of the enzyme (Kumar and Padmanaban, 1980) . Similarly alteration in the degradation rate would change the steady-state levels of hepatic cytochrome P-450. Levin and Kuntzman (1969) demonstrated that the decay of radioactive hemoprotein labelled with delta-aminolevulinic acid occured in a biphasic fashion. This indicated the existence of at least two hemoprotein fractions with the half-life of the fast phase component of 7 hours and that of the slow phase component of 47 hours.

El Azhary et al. (1980) suggested that interferon inducing agents lowered the concentration of hepatic hemoproteins by increasing their degradation rather than by decreasing their synthesis and showed that only the fast phase of cytochrome P-450 was affected by interferon inducers. On re-evaluating their results in our own laboratory using a computer simulation of cytochrome P-450 turnover it was found that this mechanism could account for less than 10% of the 40-50% total loss. As summarized in the tables below the amount of label remaining at 24 hours in the control group of animals, 72.2% of the cytochrome P-450 must have been resynthesized in order to maintain an unchanged steady-state level of cytochrome P-450. If it is assumed that a similar rate of synthesis occurs in the

treated group then by adding 72.2% (steady-state synthesis) to the amount of labelled ^{3}H-ALA remaining at 24 hours, a loss of 9.5% is calculated. The following table summarizes our calculations made from rate constants obtained from the data of El Azary et al., (1980) to fit a double exponential rate of degradation.

* ^{3}H-ALA remaining at 24 hours	Synthesis to maintain steady-state levels	Calculated loss of cytochrome P-450	Actual loss of cytochrome P-450
(i) control 27.8%	72.2%	0%	0%
(ii) Poly r (I.C) 18.3%	72.2%	9.5%	40-50%

* The following equation was used to calculate the amount of ^{3}H-ALA remaining after 24 hours.

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

α and β were obtained from the fast phase and slow phase Half-lifes.

We conclude that the losses in degradation of cytochrome P-450 found by El Azhary et.al (1980) could not account for the loss of cytochrome P-450 found experimentally. Therefore an entire re-evaluation for the mechanism of hepatic cytochrome P-450 depression by interferon was undertaken in this study.

Degradation of heme was examined by measuring the elimination of ^{14}CO from the methene bridge of heme after 5-^{14}C-ALA was administered to the animal (Fig. 2). Landaw et. al. (1970) demonstrated that endogenously produced carbon monoxide in mammals arises solely from the alpha-methene bridge carbon atom of catabolized heme. Production of ^{14}CO in the first 30 hours after administration of labeled ALA reflects degradation of cytochrome P-450 heme (Bissell, and Hammaker, 1976). This technique for the measurement of degradation of cytochrome P-450 yielded a biphasic degradation of hepatic cytochrome P-450 (Landaw and Winchell, 1966), similar to that described by Levin and Kuntzman (1969). However the half-lives of both the fast phase and slow phase were different than those obtained by Levin and Kuntzman (1969). Half-life of the fast phase in our experiments in mice was observed to be 1 hour compared to 7 hours and that of the slow phase was 11 hours compared to 46 hours obtained by Levin and Kuntzman. Utilizing data from Landaw et.al (1970)) who utilized ^{14}CO expiration method, the half-life of both the fast phase and slow phase in the rat

were comparable to those reported in this thesis. Therefore the discrepancy in the half-lives seem to arise from the methodology used to obtain them rather than species differences.

In the first 12 hours after poly r (I.C) treatment an increased rate of degradation of heme of the slow phase was observed. Unlike ElAzhary (1980) who found no changes in the slow phase turnover of cytochrome P-450 we found a three fold increase in degradation of the slow phase component. The discrepancy in these results can be explained by the different time periods of poly r (I.C) treatment which were used prior to examining the degradation rates. ElAzhary et.al. (1980) measured their degradation rates 24 hours after 4 daily doses of poly r (I.C) treatment whereas we examined degradation rates after shorter periods of single dose treatment (i.e. 1 hour, 2 hours, 3 hours, 6 hours, 12 hours, 24 hours). The increase in degradation rates which we have observed in our experiments are much more in line with the decrease in hepatic cytochrome P-450 observed experimentally following poly r (I.C) treatment.

Heme synthesis was also examined at various times after poly r (I.C) administration by measuring the incorporation of a precursor for heme, ^{14}C-delta-aminolevulinic acid. The incorporation of ALA into microsomes was increased initially at 3 hours followed by a steady decline to 18 hours. Decreased incorporation at later times (between 6 and 18

hours after poly r (I.C) suggest decreased synthesis of heme. These findings are confirmed by evidence that ALA-synthetase activity follows a parallel time course (ElAhzary and Mannering, 1979).

A defect in the synthesis of heme is also suggested in the experiments involving the elimination of ^{14}CO from the methene bridge of heme (Fig. 17). The area under the curve which represents the total amount of label turning over in the heme moeity is greatly diminished in poly r (I.C) treated animals (Fig. 18). If only degradation and not synthesis of heme was affected then the maximum height of the peak of ^{14}CO evolution would increase but the area under the curve would remain unchanged. Our results however indicate that the area under the curve was diminished and that degradation was increased and therefore we can only conclude that both synthesis and degradation were affected by poly r (I.C).

The decrease of hepatic microsomal cytochrome P-450 following poly r (I.C) treatment is paralled by increased incorporation of ^{14}C-labelled amino acids into microsomal proteins. This seemed paradoxical. To resolve this paradox the microsomal proteins were fractionated by use of sephacryl S-200 column, which separates proteins on the basis of their molecular weight. It was observed that the increased incorporation of amino acids was in low molecular weight proteins mostly under 67,000 daltons (Fig. 25).

However these data did not distinguish incorporation of amino acids in hepatic cytochrome P-450 from incorporation in other low molecular weight proteins.

To examine whether the incorporation of amino acids specifically into apoprotein of hepatic cytochrome P-450 was affected by interferon, the fractions of microsomal protein which contained cytocnrome P-450 obtained from the sephacryl S-200 column were separated on an anion exchange column. In the fractions collected from the anion-exchange column we found that amino acid incorporation in poly r (I.C) treated animals was generally depressed. The amino acid incorporation was not depressed in any specific forms of cytochrome P-450 resolved on this column but the reduction in amino acid incorporation occured in all species of cytochrome P-450. This was surprising as previously Zerkle (1980) demonstrated that specific types of cytochrome P-450 were affected more than others in poly r (I.C) and tilorone treated rats when separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis technique.

The results reported here indicated that incorporation of amino acids into cytochrome P-450 was decreased. This is most likely due to inhibition of apocytochrome synthesis by interferon as interferon has been shown to inhibit the synthesis of other cellular proteins (Illinger et.al., 1976). Also another possibility although less likely could explain the decrease in the incorporation of ^{14}C-amino acids

in the isolated cytochrome P-450 fractions. Following normal synthesis of the apoprotein the process involving the attachment of the heme moeity to the protein could be affected by interferon. If these labelled apoproteins (without heme) were retained on the anion exchange column then a decrease in label in the isolated cytochrome P-450 fractions would also be observed.

The inconsistency of increased incorporation of amino acids in the microsomes and decreased amino acid incorporation in apocytochrome (microsomal protein) can be explained by considering that the synthesis of some proteins are induced by interferon (Revel, et.al (1981)) while others are inhibited (Illinger, et.al. (1976)). Apocytochrome P-450 is one of those proteins whose synthesis is inhibited. The increase in ^{14}C-amino acids observed in crude or partially purified microsomes is likely due to the increased incorporation of these amino acids into non-cytochrome P-450 proteins. From this evidence we conclude that interferon inhibits the synthesis of apocytochrome P-450 and thus results in a decrease in the levels of hepatic cytochrome P-450.

The data obtained on heme synthesis and degradation and incorporation of amino acids in apocytochrome P-450 following treatment of mice with poly r (I.C) demonstrate an effect of interferon on hepatic cytochrome P-450. We propose that interferon might decrease formation of the

cytochrome apoprotein which then yields an increased "free" heme concentration. This increased free heme causes a feedback inhibition on ALA-synthetase which is a rate-limiting enzyme and thus results in decreased heme synthesis. Sweeney et al. (1972) demonstrated that exogenous heme causes inhibition of ALA-synthetase and therefore it is reasonable to postulate that increased endogenous heme could cause inhibition, of ALA-synthetase. Heme oxygenase which degrades heme is stimulated by the increased "free heme pool" and this results in an increased degradation rate. Therefore it appears that changes in heme metabolism following poly r (I.C) treatment in mice could be a secondary response to decreased apocytochrome P-450. This proposal is analogous to that proposed by Padmanaban et al. (1973) for the induction of cytochrome P-450 by phenobarbital but reversed. They proposed that the drug stimulates at a translational level increased synthesis of cytochrome apoprotein which combines with free heme to form the holocytochrome. Depletion of "free heme pool" results in increased activity of ALA-synthetase by negative feed-back control. Thus the rise in ALA-synthetase activity is secondary to increased heme requirements and not the result of the drug directly inducing the enzyme. The mechanism suggested in this thesis is not unreasonable since interferon is known to inhibit viral protein synthesis (Friedman, R.M., 1977) and also inhibit some host proteins

(Gressor, I. et.al., 1979). However the molecular mechanism by which interferon depresses apoprotein synthesis was not undertaken in this thesis but it seems to be the logical approach to continue this study and provide evidence for the "apoprotein inhibition" hypothesis by interferon.

C. Clinical Relevance

Various preparations of interferon are being tested throughout the world for their anti-tumor effects. We can therefore predict that impairment of cytochrome P-450 by interferon may lead to changes in the pharmacokinetics and metabolite formation of other drugs used at the same time. Interferon is also likely to be a factor in depressing drug biotransformation during natural infection. Examples have already been reported for the elimination rate of theophylline, which is impaired during influenza infection (Chang et.al., 1978) and following the administration of influenza vaccine (Renton et.al., 1980). Thus an impairment of hepatic cytochrome P-450-mediated drug biotransformation via an interferon-mediated interaction may enhance the toxicity of drugs and exogenously administered chemicals.

CONCLUSIONS

The major significance of this thesis is that it provides conclusive evidence implicating interferon in depressing hepatic cytochrome P-450 and related drug biotransformation. Secondly it postulates that interferon causes inhibition of apoprotein synthesis. This provides one of the first examples of the ability of interferon to affect the synthesis of a major protein in the host cell.

The inhibition of apocytochrome synthesis yields an increased "free heme pool", which causes a change in heme turnover. Both heme synthesis and the rate of heme degradation are affected. Thus the proposed mechanism for the effect of interferon on the depression of hepatic mixed function oxidase system is comprised primarily of an inhibition of apoprotein synthesis and a secondary change in heme synthesis and degradation.

PROPOSAL FOR FUTURE RESEARCH

Further investigation to provide conclusive evidence for the proposed mechanism by which interferon causes depression of hepatic cytochrome P-450 is essential. To obtain this evidence, the molecular events for the synthesis of apocytochrome P-450 have to be considered. The most probable event that leads to inhibition of apocytochrome P-450 by interferon could occur at either the transcription or the translation stage of biosynthesis. Interferon and interferon-inducers inhibit the synthesis of viral proteins and in some cases mammalian cell proteins at the translation stage (Williams and Kerr, 1980). Hence it is plausible to investigate the effect of interferon on the translation of apocytochrome P-450.

Translation of apocytochrome P-450 can be examined by the *in vitro* synthesis of cytochrome P-450 in a cell free system. This involves the isolation of liver polysomes and the *in vitro* translation of the mRNA to apocytochrome P-450 by a reticulocyte lysate translation system (Colbert *et.al.*, 1979). This method has been used in other laboratories to demonstrate an increase in mRNA following treatment of animals with phenobarbital (DiLella *et.al.*, 1981).

Finally the ability and potency of a wider range of interferon types to depress cytochrome P-450 dependent drug biotransformation and their correlation with their ability

to act as anti-viral and anti-tumor agents must be examined. If such a correlation is found, as suggested in this thesis, it could be of great value during trials of interferon for both anti-viral and anti-tumor use in clinical practise.

The major significance of this proposal is that, it would provide a molecular mechanism by which interferon causes depression of hepatic cytochrome P-450. In addition it would provide information on which of the several types of interferon are capable of affecting hepatic drug biotransformation. Thus it would establish whether anti-viral effects of interferon are linked to depressant effects on hepatic cytochrome P-450 or if they are independent of each other.

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