GLUTATHIONE S-TRANSFERASES OF THE RODENT PANCREAS: THEIR RESPONSE TO INDUCING AGENTS AND TO MANIPULATION OF PANCREATIC MASS

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by

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ABSTRACT

Glutathione S-transferases (GSH-T) are a family of enzymes with both detoxifying and transport capacities. Their main role is in the catalysis of detoxification pathways. The enzymes are found in many tissues, with high concentrations in the mammalian liver and the gastrointestinal tract. Significant amounts of the enzyme have also been discovered in the rat pancreas, its function there being currently unknown. Previous studies have demonstrated that GSH-T levels are inducible in the liver with oral administration of certain compounds, such as the dietary antioxidant 2(3)-*tert*-butyl-4-hydroxyanisole (BHA). In the present study, rats, mice, and hamsters were fed a diet containing 0.75% BHA for 14 days. Pancreatic GSH-T activity and levels in response to this were significantly increased over control animals only in hamsters. It is possible that this induction may provide protection against pancreatic carcinogens.

Three other groups of hamsters were given either 0.03 mmol α-angelica lactone (α-AL)/g of diet or 0.03 mmol coumarin (COU)/g of diet, two naturally occurring plant compounds, or 20% green coffee beans (GCB). Although all three of these are known to induce liver and small intestinal mucosal GSH-T in rats and mice, none caused any significant induction of pancreatic GSH-T in the hamster, further exemplifying the difficulty in manipulating these enzymes in the pancreas as compared to other tissues.

In addition to these experiments, groups of rats were fed a diet containing 40% raw soya flour (RSF) for 14 or 21 days. RSF contains trypsin inhibitor (TI) which has been shown to cause pancreatic enlargement, a phenomenon attributed

to increased levels of the gastrointestinal hormone cholecystokinin (CCK). Pancreatic mass increased significantly in response to RSF; however DNA content as related to body weight increased only in the animals on the 3 week feeding schedule, indicating hyperplasia or an increase in cell number in this group. The activity of GSH-T in the enlarged pancreas was not found to be significantly different from that of the control pancreas.

In an additional study, which utilized the compound FOY-305, also a trypsin inhibitor, hamsters were gavaged with 400 mg/kg body weight of FOY-305 once daily for 14 days. Both pancreatic weight and DNA content were significantly increased in these animals with a significant reduction in GSH-T activity. This reduction may be involved in the higher incidence of pancreatic neoplasms observed when trypsin inhibitors are fed for longer periods. Total GSH-T levels in the pancreas remained unchanged in these animals.

Finally, the CCK antagonist L-364,718 was administered orally to hamsters at 1 mg/kg body weight, twice daily for 14 days. This compound was not found to cause a reduction in pancreatic mass or DNA content, thereby indicating that CCK does not seem to be a requirement for normal pancreatic maintenance and growth in this species. Furthermore, no changes in GSH-T activity or levels were observed.

[KEY WORDS]

α-Angelica Lactone

2(3)-*tert*-butyl-4-hydroxyanisole

Coumarin

Detoxification

FOY-305

Glutathione S-transferases

Hypertrophy/Hyperplasia

Induction

Kahweol

L-364,718

Pancreas

Raw Soya Flour

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ABBREVIATIONS

GSH	Glutathione
GSH-T	Glutathione S-transferases
PAH	Polycyclic Aromatic Hydrocarbons
BHA	2(3)-tert-butyl-4-hydroxyanisole
BHT	3,5-di-tert-butyl-4-hydroxytoluene
BP	Benzo[a]pyrene
DNA	Deoxyribonucleic Acid
DMBA	7,12-dimethylbenzanthracene
DSF	Disulfiram
sodium DDC	Sodium Dimethyldithiocarbamate
BEX	Bisethylxanthogen
BITC	Benzyl Isothiocyanate
COU	Coumarin
α-AL	α-Angelica Lactone
CDNB	1-chloro-2,4-dinitrobenzene
CCK	Cholecystokinin
GCB	Green Coffee Beans
RSF	Raw Soya Flour
TI	Trypsin Inhibitor
PCA	Perchloric Acid
DMSO	Dimethyl Sulfoxide
SEM	Standard Error of the Mean

HSF	Heated Soya Flour

Chapter 1

Introduction

1.1. Background Information

1.1.1. Biotransformation of Xenobiotics and Endogenous Compounds

Each day, humans are exposed to a range of environmental chemicals, either those which occur naturally, or those which have been synthesized by man and deposited in the environment. With approximately 60,000 chemicals approved for use, including drugs, food additives, agricultural chemicals, and with human contact with cleaning products and industrial emissions, understanding of the mechanisms by which foreign compounds are eliminated from the body has taken on great importance. Although the immune system is able to deal with some large molecules and particles, small molecules usually undergo metabolism in order that they may be prepared for excretion via the urine or feces. A large proportion of foreign compounds are lipophilic and are not readily excreted directly; therefore they must undergo biotransformation reactions which render them more water-soluble. The resulting hydrophilic metabolites being less membrane-soluble are generally more easily excreted from the body than the original substances (Armstrong, 1987). In addition to lipophilic compounds, there are those which are classified as electrophilic, nucleophilic, reactive, inert, etc., criteria that also affect absorption and distribution.

Most biotransformation reactions are facilitated by families of enzymes which have structures designed for involvement in specific reactions of detoxification. Although most of these reactions lead to a reduction in the toxicity of certain chemicals, activation to more toxic intermediates is not uncommon (Chhabra and Eastin, 1984; Laitinen and Watkins, 1986).

Biotransformation reactions may be divided into two distinct types: (1) Phase I, comprized of oxidation, reduction, and hydrolytic reactions which serve to modify the structure of chemicals (Armstrong, 1987) and, (2) Phase II reactions, such as glucuronidation, glutathione conjugation, sulfate conjugation, methylation, and acetylation (Laitinen and Watkins, 1986), involving the addition of endogenous moities to the chemical in order to enhance water solubility (Armstrong, 1987). The major site of detoxification in the body is the liver; however the gastrointestinal mucosa is also reasonably active (Chhabra and Easton, 1984). The first pass effect, involving the enterocytes and the hepatocytes, is the initial step in the detoxification of non-nutritional compounds. In addition to the liver and intestinal mucosa, the lungs and skin also play an important role in the defense of the body against the actions of xenobiotics.

1.1.2. Phase II Biotransformation

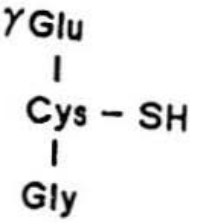
1.1.2.1. Glutathione Conjugation

Only one type of Phase II reaction, specifically glutathione conjugation will be dealt with in detail.

Conjugation of foreign compounds or their metabolites (resulting from Phase I biotransformation reactions) with the tripeptide glutathione, generally gives rise to products which are less toxic than the original chemical. Glutathione (GSH) (Figure 1-1) is a ubiquitous tripeptide which is present mainly in the cytosol, and is probably the most abundant sulfhydryl compound in animal tissues. In mice, GSH is abundant in the liver, spleen, kidneys and pancreas, with lower but significant concentrations in the lungs, heart, adrenals and blood (Chasseaud, 1979). It is synthesized from glutamate, cysteine and glycine requiring two enzymatic reactions; in the first, glutamylcysteine is formed, with the secondary addition of glycine. The rate-limiting feature of these reactions seems to be the availability of the amino acid cysteine, as demonstrated by *in vitro* preparations using cultured hepatocytes (Bidlack, *et al.*, 1986).

The conjugation of GSH with toxic substances is enhanced by the presence of a family of enzymes known as the glutathione S-transferases (GSH-T) (EC 2.5.1.18). Although the GSH-T were not formally identified until 1961, evidence for the involvement of GSH in the conjugation of foreign compounds in the liver was documented as early as 1936 by Boyland and Levi. In the years between 1936 and 1961, further support for glutathione conjugation as well as the presence of certain enzymes involved in the catalysis of these reactions, was recorded. Finally, in 1961, partial purification of a liver enzyme which played an integral role in the formation of glutathione conjugates was reported (Booth, *et al.*, 1961).

Figure 1-1: Glutathione


γGlu
Cys – SH
Gly

We now know that GSH-T are present in relatively large quantities in a variety of animal cells. The family of enzymes represents approximately 10% of the extractable cytosolic protein in rat liver and about 3% in the human liver (Jakoby, 1978). In addition to being present in the liver, the GSH-T are found in all mammalian tissues examined to date. These include the heart, kidney, spleen, adrenal gland, brain, lung, intestine, pancreas, placenta, testis, and erythrocytes (Booth, *et al.*, 1961; Mannervik, *et al.*, 1983).

The major function of the GSH-T is in the catalysis of the Phase II detoxification reactions involving glutathione conjugation (Tahir, *et al.*, 1985). Both endogenous compounds and xenobiotics are conjugated with glutathione. It should be clarified however, that some of these reactions will proceed in the absence of GSH-T (Chasseaud, 1979).

1.1.2.2. Mechanism of Action

The GSH-T catalyze reactions whereby the sulfur atom of GSH makes electrons available for nucleophilic attack on or reduction of an electrophilic substrate (Mannervik, 1985). In this manner, the electrophilic sites are neutralized, making the end products more water-soluble (Habig, *et al.*, 1974). Therefore, the GSH-T serve to increase the nucleophilicity of the sulfhydryl group of GSH. The enzymes are thought to accomplish this by lowering the pK of GSH (Jakoby, 1978). This causes the ionization of the tripeptide at physiological pH, resulting in GSH ---> $GS^- + H^+$. The structure of the enzymes is thought to include a site which has low affinity but broad specificity for lipophilic compounds (Kaplowitz, 1980). In addition, the transferases have a binding site for GSH. *In vitro*, the family of detoxifying enzymes have been shown to catalyze reactions

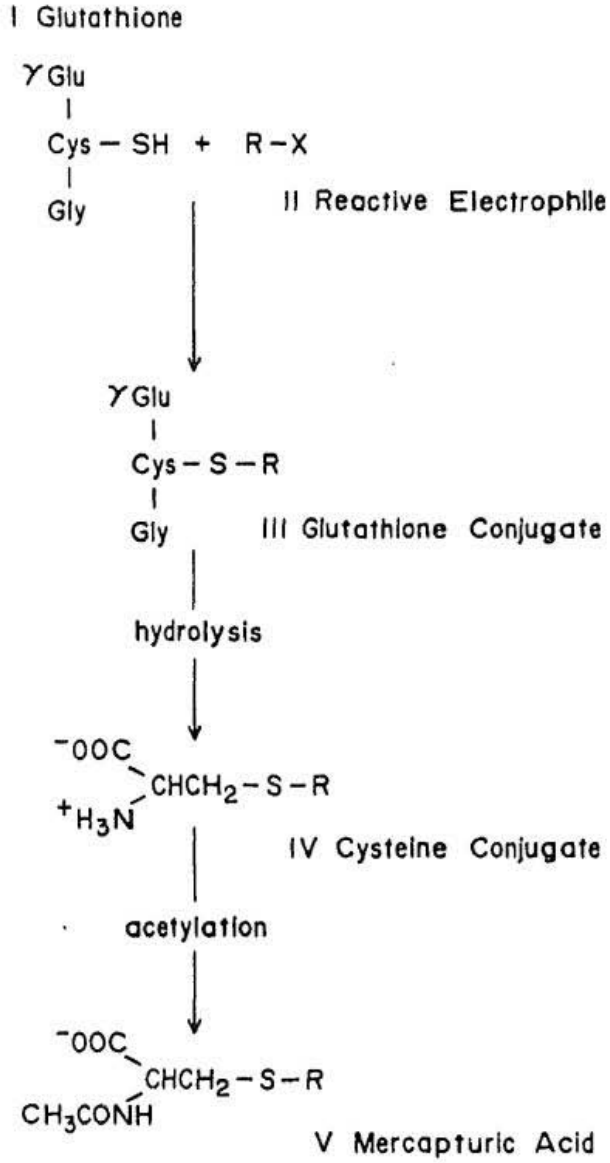
between GSH and electrophilic centres, such as carbon, nitrogroups, halogens, epoxides, and sulfate esters (Kaplowitz, 1980). The group of enzymes, principally of the liver and kidneys, are therefore involved in the catalysis of the first step in the production of mercapturic acids (Booth, *et al.*, 1961; Habig, *et al.*, 1974). The end product of this initial step is most often a thioether, which continues to undergo a series of enzymic reactions ultimately leading to a mercapturic acid (Chasseaud, 1979). A mercapturic acid is a collective term used to name N-acetylated S-substituted cysteines, where the reactive group generally does not undergo further reaction (Wood, 1970). Figure 1-2 shows a series of steps including a reaction catalyzed by GSH-T, leading to a mercapturic acid, a product excretable in urine or bile (Chasseaud, 1979).

1.1.2.3. Substrates for GSH-T

The most common substrates for the GSH-T are synthetic chemicals like halogeno-nitrobenzenes, or, the products of Phase I (cytochrome P-450) reactions, which include arene oxides, formed during the biotransformation of polycyclic aromatic hydrocarbons (PAH). Examples of endogenous substrates include Δ^5-3-ketosteroids, prostaglandin A, sulfate esters, quinones and epoxides (Mannervik, 1985).

In addition to their reactivity with the aforementioned substrates, GSH-T may also have a significant function biologically in protecting living membranes against lipid peroxidation (Chasseaud, 1979; Kaplowitz, 1980; Mannervik, 1985). Several products of lipid peroxidation, such as cholesterol α-oxide, have been shown to be substrates for GSH-T. This function is probably in conjunction with glutathione peroxidase, also a cytosolic enzyme (Mannervik, 1985). Glutathione

Figure 1-2: Steps involved in the formation of mercapturic acids


I Glutathione
γ Glu
Cys – SH + R–X
Gly
II Reactive Electrophile
γ Glu
Cys – S – R
Gly
III Glutathione Conjugate
hydrolysis
⁻OOC
CHCH₂ – S – R
⁺H₃N
IV Cysteine Conjugate
acetylation
⁻OOC
CHCH₂ – S – R
CH₃CONH
V Mercapturic Acid

peroxidase, like catalase,, metabolizes hydrogen peroxide, and is found in various animal tissues. It is believed to provide protection against both endogenously and exogenously induced lipid peroxidation. Glutathione peroxidase contains selenium and is reactive with a variety of organic hydroperoxides in addition to hydrogen peroxide (Wendel, 1981).

1.1.3. GSH-T Role in Storage and Transport

Besides their major role in detoxification reactions, some GSH-T have an intracellular binding function. The first GSH-T to be purified was found to be a binding protein in the liver (Ketterer, *et al.*, 1967; Litwack, *et al.*, 1971). Given the name ligandin or protein Y, it was shown several years later by Habig *et al.* (1974) that this binding protein was one of the GSH-T. The term ligandin was chosen in order to denote the protein's high affinity for a variety of ligands, including heme and its metabolite bilirubin (Jakoby, 1978; Ketterer, 1986). Failure to excrete bilirubin results in jaundice; however in aqueous solution at pH 7, bilirubin is insoluble, so therefore binds extracellularly with albumin in the plasma (Jakoby, 1978). The bilirubin subsequently diffuses into the hepatocytes, where, unless conjugated would sediment out. Therefore, this GSH-T performs a similar function to albumin, except intracellularly. This allows the bilirubin to be stored and remain solubilized within the liver cells until its excretion as soluble glucuronic acid conjugates (Jakoby, 1978).

In general then, the functions of ligandin are threefold in that (1) it may act as a selective transport system for certain chemicals in the liver or kidney;(2) it binds compounds which are insoluble at neutral pH thereby facilitating

intracellular transport and movement to other sites for biotransformation and/or excretion; and,(3) it provides storage of compounds upon binding, thus preventing diffusion back into the plasma (Kaplowitz, 1980).

1.1.4. Structure and Nomenclature of GSH-T

Quite often in nature, organisms may have more than one distinct form of an enzyme, particularly those which function in metabolizing xenobiotics (Jakoby, *et al.*, 1984). These isoenzymes, as they are known, may vary slightly in structure, function, or substrate specificity. The occurrence of multiple forms of GSH-T is a well-researched aspect of this family of enzymes (Mannervik, 1985). The existence of multiple forms of an enzyme, each with slightly different substrate specificities has been suggested as a means of enhancing the metabolizing capacity of the enzyme (Tahir, *et al.*, 1985; Tu and Reddy, 1985). This might be of particular importance in connection with xenobiotic metabolism.

1.1.4.1. Subunit Composition of GSH-T

The isoenzymes of GSH-T are dimers (composed of two subunits) each ranging in M_r from 25,000 - 29,000 in the rat (Jakoby, *et al.*, 1984), and 47,000 - 53,000 in humans (Mannervik, 1985). Thus, isoenzymes may be homo- or hetero-dimers, with the probability of multiple genes and subunit hybridization being responsible for the presence of many different forms (Mannervik, 1985).

1.1.4.2. Rat GSH-T Isoenzymes

Because the GSH-T are dimers and each isoenzyme is unique due to differences in the subunit composition, it became necesary to develop a universally accepted nomenclature for the purpose of identifying the individual forms. Originally it was believed that each isoenzyme was composed of binary combinations of only three distinct subunits, designated Ya, Yb, and Yc, in order of increasing M_r values (Mannervik and Jensson, 1982; Singh and Awasthi, 1986). The isoenzymes were subsequently identified using the letters AA, A, B, C, D and E, in the reverse order of elution using DEAE-cellulose column chromatography (Jakoby, 1978). However, recently, six different subunit structures have been recognized, each designated by an Arabic number from 1 to 6 (Jakoby, *et al.*, 1984). Each isoenzyme is thereby named according to the numbers allocated to its two constituents, such as GSH-T 1-1, 1-2, 2-2 etc. All of the enzymes have been characterized through the use of specific chemicals which a as selective inhibitors to the activities of the various forms of GSH-T (Yalcin, *et al.*, 1983). In addition, the isoenzymes have also been distinguished by their individual substrate specificities and by immunoprecipitation techniques (Mannervik, *et al.*, 1985). In this latter method, the isoenzymes were tested for cross-reactivity with antibodies against rat and human GSH-T.

On these bases, at least six cytosolic isoenzymes have been characterized from rat liver, one major cytosolic isoenzyme from rat testis, as well as a microsomal rat GSH-T (Mannervik, 1985). Table 1-1 summarizes the important features of rat isoenzymes.

TABLE 1-1: MAJOR CHARACTERISTICS OF RAT GSH-T ISOENZYMES (MANNERVIK, 1985)

Nomenclature	Location	Subunit M_r	Major Substrate (other than CDNB)	Major Inducers	Major Inhibitor
GSH-T 1-1 (Ligandin)	liver cytosol	25,000	5-androstene-3,17-dione	phenobarbital, 3-methylcholanthrene, trans-stilbene oxide	hematin
GSH-T 2-2 (Ligandin)	liver cytosol	28,000	cumene hydroperoxide	-	tributyltin acetate
GSH-T 1-2	liver cytosol	25,000; 28,000	cumene hydroperoxide	phenobarbital, 3-methylcholanthrene, trans-stilbene oxide	hematin
GSH-T 3-3	liver cytosol	26,500	bromosulfophthalein	phenobarbital, 3-methylcholanthrene, trans-stilbene oxide	tributyltin chloride
GSH-T 4-4	liver cytosol	26,500	trans-4-phenyl-3-butene-2-one	-	bromosulfophthalein
GSH-T 3-4	liver cytosol	26,500; 26,000	bromosulfophthalein; trans-4-phenyl-3-butene-2-one	-	tributyltin chloride; bromosulfophthalein
GSH-T 5-5	liver cytosol	29,000	1,2-epoxy-3(P-nitrophenoxy propane) (low affinity with CDNB)	-	-
GSH-T 6-6	testis cytosol	26,000	isoenzyme with highest affinity for CDNB	-	cibacron Blue
Microsomal GSH-T	liver cytosol	14,000	N-ethylmaleimide/ tributyltin acetate	-	hematin; bromosulfophthalein

1. CDNB = 1-chloro-2,4-dinitrobenzene

1.1.4.3. Human GSH-T Isoenzymes

Unlike the GSH-T isolated from rat tissues, the isoenzyme pattern for human tissues is generally less intricate (Mannervik, *et al.*, 1983). There is no evidence to support the ability of the individual subunits to hybridize as do those in the rat liver. The human isoenzymes have thus far been categorized into three classes: basic, near-neutral, and acidic GSH-T, based on isoelectric points. Within the basic class, five isoenzymes have been isolated from any one human liver; these have been assigned the Greek letters α, β, γ, δ and ϵ. These five forms were found to be virtually identical in physical and catalytic properties. Originally it was believed that the human GSH-T isoenzymes were the products of a single gene, however evidence demonstrating distinct isoelectric focusing patterns has been documented thus supporting the idea of multiple genes giving rise to multiple forms of the enzyme (Mannervik, *et al.*, 1983; Mannervik, *et al.*, 1985).

There have been no multiple forms of the near-neutral GSH-T discovered, with the single known form denoted as μ. It has been isolated from the liver of only 60% of subjects with no enzyme activity of this specific type in the remaining 40% (Mannervik, 1985).

Two forms of the acidic human GSH-T have been reported. These forms have been denoted by the Greek letters π and ρ (Mannervik, *et al.*, 1983)

Table 1-2 summarizes the major features of human GSH-T isoenzymes.

TABLE 1-2: MAJOR CHARACTERISTICS OF HUMAN GSH-T ISOENZYMES (MANNERVIK, 1985)

Nomenclature	Location	Subunit M_r	Major Substrate (other than CDNB)	Major Inducers	Major Inhibitor
Basic	liver cytosol	51,000	5-androstene-3,17-dione	-	tributyltin acetate
Near-neutral	liver cytosol	53,000	styrene-7,8-oxide	-	Cibacron Blue
Acidic	liver cytosol	47,000	ethacrynic acid	-	Cibacron Blue

1.1.5. Induction of GSH-T

It was first demonstrated that induction of microsomal enzymes, specifically cytochrome P-450 and aryl hydrocarbon hydroxylase, was possible with compounds such as phenobarbital and polycyclic aromatic hydrocarbons (Conney, 1967). Both of these agents have also been shown to increases levels of ligandin and the other GSH-T activities in the rat liver (Habig, *et al.*, 1974; Marnieni and Parkki, 1975). As previously mentioned, GSH-T in rat liver may be induced from 10% of the cytosolic protein to 20% of this total (Jakoby, 1985). This represents a substantial increase in GSH-T molecules, which may be highly significant in the detoxification process. In addition to phenobarbital, an increasing number of other compounds have been found to induce levels of both hepatic and extrahepatic GSH-T in rodents (Sparnins, *et al.*, 1982a; Sparnins, *et al.*, 1982b). Included in this list of inducing agents are certain naturally-occurring dietary constituents as well as certain food additives. Most often, the greatest induction of the enzymes takes place in the liver, although significant enhancement has also been demonstrated in the rat small intestine, kidney, and lung. Furthermore, mouse liver, forestomach, and small intestine GSH-T have also proven to be inducible (Benson, *et al.*, 1979; Sparnins and Wattenberg, 1981).

The nature of the induction signal for GSH-T is thought to involve oxidation-reduction reactions. Chemicals which are susceptible to these reactions may lead to the production of reactive oxygen species or alter the balance of critical intracellular pools of compounds such as glutathione, placing stress on the system. This stress subsequently stimulates the induction of Phase II enzyme systems, like the GSH-T (Prochaska, *et al.*, 1985).

With these increased levels of GSH-T in various tissues, chemically-induced neoplasms are often reduced or inhibited, thereby supporting the notion that a major function of the GSH-T is the detoxification of xenobiotics, many of which are potential carcinogens (Wattenberg, *et al.*, 1979; Cohen, *et al.*, 1986).

1.1.5.1. GSH-T Induction by Antioxidants

Antioxidant chemicals known as antioxidants have been added to foods for many years for the purpose of food preservation. One of the most commonly used dietary antioxidants is the phenolic compound 2(3)-*tert*-butyl-4-hydroxyanisole (BHA), which has been used since 1947 in order to prevent the oxidation of the labile lipid components of food (Lam, *et al.*, 1979). It has been shown that BHA and related antioxidants such as 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT), when administered to rats and mice, counteract a variety of neoplastic, mutagenic and general toxic effects of a wide range of chemicals (DeLong, *et al.*, 1985). There has been no mechanism established for such observations; however several possibilities have been recognized: (1) antioxidants like BHA may act directly with the chemical carcinogen or its metabolites; (2) malignancy is prevented due to the enhancement of certain enzyme activities by the antioxidants - these enzymes serve to inactivate the carcinogens thereby preventing the interaction of the chemicals with critical macromolecules; (3) antioxidants may block metabolic pathways by which procarcinogens become reactive; and (4) the efficiency of DNA repair may be increased by the antioxidants (Benson, *et al.*, 1978). However, evidence leans toward the speculation that in a wide variety of tissues, the major mechanism for protection is via the increased activities of certain detoxifying enzymes, such as the GSH-T (DeLong, *et al.*, 1985; Stewart and Boston, 1987). It

is believed that the elevation in GSH-T activity occurs because of increases in enzyme concentrations and rate of synthesis. Furthermore, glutathione levels in the tissues are also increased (DeLong, *et al.*, 1985).

Increased levels of GSH-T have been found in the cytosol of the mouse liver, upper small intestinal mucosa, lung and forestomach (DeLong, *et al.*, 1985), as well as the rat liver, small intestine, kidney and lung in response to BHA administration (Benson, *et al.*, 1979; Sparnins *et al.*, 1982a; Sato, *et al.*, 1984). Also providing support for the protective nature of BHA, it was found that BHA causes a significant decrease in benzo[a]pyrene (BP) metabolite:DNA adducts in the forestomach, lung and liver of certain strains of mice, a phenomenon thought to be an essential step in the initiation of tumors (Ioannou, *et al.*, 1982). The GSH-T are known to catalyze the conjugation of BP metabolites with glutathione in rat liver, thereby reducing their binding with DNA (Jernstrom *et al.*, 1985).

Wattenberg (1972) found that BHA and BHT, and an antioxidant used in animal feed, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin) were effective in inhibiting the carcinogenicity of BP or 7,12-dimethylbenzanthracene (DMBA) on the mouse forestomach, and also inhibited mammary tumor formation in female rats in response to DMBA. Additionally, Benson, *et, al.* (1978) and Benson, *et al.* (1979) have shown that mice treated with BHA or ethoxyquin had reduced levels of mutagenic metabolites of BP in the urine and peritoneal cavities. Furthermore, short-term exposure of rats to low levels of dietary BHA reduced the incidence of mammary tumors induced by DMBA (Cohen, *et al.*, 1986).

1.1.5.2. Induction of GSH-T with Sulfur Compounds

Several sulfur compounds, namely disulfiram (DSF), sodium dimethyldithiocarbamate (sodium DDC), bisethylxanthogen (BEX), and benzyl isothiocyanate (BITC), when administered in the diet to mice, have been shown to inhibit tumor formation and toxic effects caused by a range of chemical carcinogens (Wattenberg, 1974; Wattenberg and Lam, 1981). There are several proposed mechanisms for this protection; there is evidence that the sulfur compounds may inhibit the oxidation of carcinogens, or increase the inactivation of carcinogenic metabolites (Benson and Barretto, 1985). In addition, there have been studies demonstrating the increase in cytosolic GSH-T of the mouse liver, lung, kidney, urinary bladder, forestomach, proximal small intestine and colon in response to these compounds. Thus, this elevation in the detoxifying enzymes may function in reducing or inhibiting the carcinogenicity of these chemicals. In the liver, BITC and BEX increased GSH-T activities three-fold over controls (Sparnins, *et al.*, 1982a; Benson and Barretto, 1985), whereas DSF was only a mild inducer and sodium DDC had no effect in this organ. Of the tissues examined, the proximal small intestine and the forestomach revealed the most dramatic increases in GSH-T activity in response to the four sulfur compounds (Benson and Barretto, 1985). BITC had the most inducing in these two "portal of entry" organs, however in the other extrahepatic organs, BEX had the greatest effect. In another study by Sparnins, *et al.* (1982b), using BITC, this compound was found to enhance GSH-T activity in the esophagus of mice.

1.1.5.3. Other GSH-T-Inducing Compounds

In addition to the chemicals already discussed, several others have been utilized in the induction of GSH-T in rodents. These include some naturally-occurring compounds such as the coumarins, which are present in many of the fruits and vegetables consumed by humans (Wattenberg, *et al.*, 1979). Coumarin (COU) has been found to induce cytosolic GSH-T in the mouse esophagus (Sparnins, *et al.*, 1982b) and forestomach (Sparnins and Wattenberg, 1981). Coumarin and related compounds, such as umbelliferone (7-hydroxycoumarin), scopoletin (7-hydroxy-6-methoxycoumarin), and limettin (5,7-dimethoxycoumarin) have also been of interest to researchers because of their potential to inhibit tumorigenesis. Of these compounds, COU was the most effective in inhibiting DMBA-induced mammary neoplasia in the rat (Wattenberg, *et al.*, 1979). Coumarin was also effective in alleviating the effects of BP on the forestomach of mice.

Several other inhibitors of BP-induced neoplasia in the mouse forestomach, were also tested for their ability to induce GSH-T. Of these, p-methoxyphenol and α-angelica lactone (α-AL) were effective as inducers of both forestomach and esophageal GSH-T (Sparnins, *et al.*, 1982b). Like the coumarins (however more potently), α-AL decreased BP-induced neoplasia of the mouse forestomach (Wattenberg, *et al.*, 1979).

Finally, two other interesting naturally-occurring compounds found to increase GSH-T were kahweol palmitate and cafestol palmitate (Lam, *et al.*, 1982). Both of these diterpene esters and their corresponding alcohols, kahweol

and cafestol, are active constituents of green coffee beans. When powdered green coffee beans or one of these extracted chemicals were given in the diet to mice, GSH-T activity of the liver and small intestinal mucosa was increased. Kahweol palmitate was found to have the greatest effect. Roasted coffee and instant coffee were weaker inducers; decaffeinated instant coffee gave similar results to instant coffee. In a study by Sparnins, *et al.* (1981), green coffee beans added to the diet of rats, decreased the number of mammary tumors formed in response to DMBA. Thus, the coffee beans by some means enhanced the detoxification of this polycyclic aromatic hydrocarbon.

1.1.6. The Pancreas

1.1.6.1. Background Information

The pancreas is a yellowish-pink colored glandular organ, situated both structurally and functionally in relation to the gastrointestinal tract. It runs in apposition to the duodenum, stretching from the second portion of the duodenum on the right, to the spleen on the left (Jacobson, 1977). The gland is composed of both endocrine and exocrine cells: the endocrine cells, namely the islets of Langerhans, are present in clusters, positioned amongst the acinar cells and intercalated ducts, the latter two comprizing the exocrine component (Gorelick and Jamieson, 1981). Only the exocrine pancreas will be discussed here.

The exocrine pancreas makes up approximately 90% of the gland and is composed of acini, made up of acinar cells, centroacinar cells, and duct cells (Granger, *et al.*, 1985). The acinar cells function mainly in enzyme secretion; acini are drained by the intercalated ducts, which are initially composed of

centroacinar cells but are primarily lined with cuboidal cells within the ducts themselves. The duct system plays an important role in the secretion of water, and electrolytes such as bicarbonate (Gorelick and Jamieson, 1981).

It is the exocrine portion of the pancreas which is concerned in the digestion of food material (Wormsley, 1981). The pancreatic acini secrete enzymes, most of which are hydrolases and play an integral role in protein, carbohydrate and fat digestion in the small intestine. The duct cells, in secreting water and electrolytes, are major contributors to the volume of pancreatic juice and in the maintenance of optimal pH in the upper small intestine. These secretions drain into the duodenum through the pancreatic ducts, and it is here, in the upper small intestine, that most digestion occurs (Wormsley, 1981).

1.1.6.2. Pancreatic Metabolism of Xenobiotics

In addition to the aforementioned secretions, the pancreas has also been found to contain substantial levels of carcinogen-metabolizing enzymes which are not secreted. Pancreatic cancer is a leading cause of death in the western world (Morgan and Wormsley, 1977) although with the exception of some cigarette smoke, and dietary fat (Wynder, 1975) as possible causes, the definite etiological agents in pancreatic cancer remain basically unknown.

Though the liver is the major organ of biotransformation, studies of the rodent pancreas have also indicated the ability of the pancreas to biotransform carcinogens. In two separate studies, it was demonstrated that isolated rat and hamster acinar cells were able to oxidize and conjugate xenobiotics using Phase I cytochrome P-450-dependent enzymes, as well as the Phase II conjugation

enzymes like the sulfotransferases and UDP-glucuronyltransferases (Wiebkin, *et al.*, 1984), and γ-glutamyltransferase (Faribault, *et al.*, 1987).

The duct cells, although making up only a small percentage of the total pancreatic cell mass, must also be considered when assessing the contribution of the organ toward the metabolism of xenobiotics (Wiebkin, *et al.*, 1984).

The GSH-T of the pancreas, although present in fairly substantial levels, have not been studied to any great extent. The complexity of the organ probably accounts for the lack of available information in this area. However in 1984, two simultaneously published studies, one by Black and Howerton and the other by Kawabata, *et al.*, reported their findings concerning the presence of this group of enzymes in the exocrine pancreas.

Using the substrate 1-chloro-2,4-dinitrobenzene (CDNB), Black and Howerton (1984), found that the GSH-T activity in the rat pancreas was about half that of the liver. Furthermore, they demonstrated that the rat pancreatic GSH-T, unlike those of the liver, did not respond to induction attempts with 3-methylcholanthrene or phenobarbital using CDNB. It was suggested that GSH conjugation in the pancreas might be a significant mechanism for xenobiotics in this organ (Black and Howerton, 1984).

In addition to this biochemical study, there have also been immunohistochemical studies using the unlabelled antibody peroxidase - antiperoxidase staining technique done on the pancreas, in order to localize carcinogen-metabolizing enzymes in the various cell components of the exocrine

pancreas (Kawabata, *et al.*, 1984). In four strains of rat and one strain of hamster, the enzymes occur throughout the exocrine component, in the acinar cells, inter- and intra-lobular ducts, and in the epithelial cells of the pancreatic ducts. Both the rat and the hamster pancreas were found to contain two isoenzymes of cytochrome P-450, as well as reduced nicotinamide adenine dinucleotide phosphate-cytochrome P-450 reductase, and epoxide hydrolase. However, in the rat, neither of the isoenzymes of cytochrome P-450 were detected within the epithelial cells of the pancreatic duct system (Baron, *et al.*, 1983). One of these isoenzymes was in fact detected in the duct cells of the hamster. Thus, it was suggested from this investigation, that reactive electrophilic metabolites may be more extensively produced in the rat acinar cells but in both the acinar and duct cells of the hamster. On the other hand, the potential to metabolize these metabolites exists in both acinar and duct cells of the rat and hamster (Kawabata, *et al.*, 1984). Furthermore, GSH-T activity has also been observed in the hepatic bile duct epithelium, which shares a common origin with pancreatic duct epithelial cells. It is possible that the pancreas is exposed to carcinogenic compounds excreted in the bile, which might reflux up the pancreatic ductal system. Thus, because of the presence of GSH-T in the two cell types, biotransformation may well occur in both locations (Kawabata, *et al.*, 1984).

Although it has been demonstrated that BHA and BHT increase GSH-T activity in various tissues, these phenolic antioxidants did not prove to be inducing agents of rat pancreatic GSH-T (Roebuck, *et al.*, 1984). This result was also observed by Ross and Barrowman (unpublished data). On the other hand, the administration of BHA in the diet to rats, did decrease the formation of

atypical acinar cell foci, which represent preneoplastic populations of acinar cells (Roebuck and Longnecker, 1977), in response to the pancreatic carcinogen azaserine (Roebuck, *et al.*, 1984). This suggests that the inhibition of foci by BHA and BHT is not the result of induction of detoxification enzyme systems. Such information parallels the fact that metabolism and activation of azaserine occurs independently of these pathways. Although the antioxidants do seem to reach the pancreas, as evidenced by a reduction in the enzyme glutathione peroxidase in the organ, the inhibition of focus formation may in fact be due to electrophilic scavenging on the part of the antioxidants (Roebuck, *et al.*, 1984). Much work remains to be done in order to elucidate the mechanisms by which the exocrine pancreas metabolizes carcinogens.

1.1.6.3. Experimental Pancreatic Growth

Another area of research concerning the pancreas is the well-studied phenomenon of experimental exocrine pancreatic growth in rodents in response to certain chemicals. It has long been realized that increases in the metabolic activity and turnover of cells make tissues more susceptible to carcinogens (Ryser, 1971). Therefore, agents which have the potential for causing this tissue vulnerability are of great interest to cancer researchers.

In 1967, Rothman and Wells reported that the gastrointestinal hormone cholecystokinin (CCK) and its analogues, when administered exogenously, are potent stimulators of pancreatic growth. Since that time, extensive work has been done in support of this finding (Barrowman and Mayston, 1970; Mayston and Barrowman, 1971; Dembinski and Johnson, 1980; Pfeiffer, *et al.*, 1982). Thus, it followed that substances which increase levels of circulating CCK would also

induce this growth of the pancreas. The most commonly known compounds to do this are the naturally-occurring trypsin inhibitors, such as raw soya flour (Folsch, *et al.*, 1974; Crass and Morgan, 1982; Hasdai and Liener, 1983), and synthetic trypsin inhibitors like Foy-305 (camostate) (Yonezawa, 1983; Goke, *et al.*, 1984; Wereszczynska-Siemiatkowska, *et al.*, 1987).

Under normal physiological conditions, CCK stimulates the secretion of pancreatic enzymes (Naim *et al.*, 1982; Louie, *et al.*, 1986) and probably acts as a trophic hormone for the pancreas under normal circumstances (Dembinski and Johnson, 1986).

The hypothesis used to explain the role of trypsin on pancreatic secretion was proposed by Green and Lyman (1972). The presence of free trypsin in the small intestine results in a decrease in the secretion of pancreatic enzymes due to the inhibition of CCK release. On the other hand, agents which contain trypsin inhibitors cause trypsin in the small intestine to be irreversibly bound, therefore leading to a stimulation of CCK release, and subsequent pancreatic secretion (Louie, *et al.*, 1986). During the feeding of diets containing raw soya flour to rats, the sustained release of CCK which occurs is thought to be responsible for the increases in pancreas size and weight (Oates and Morgan, 1984). This enlargement of the pancreas has been reported to be the result of hypertrophy (an increase in cell size) and/or hyperplasia (an increase in cell number) of pancreatic acinar cells (Oates and Morgan, 1984; Roebuck, 1986).

Concomitant with pancreatic growth, it has been shown that the feeding of

these trypsin inhibitors also causes an increase in pancreatic neoplasms with the concurrent or subsequent administration of pancreatic carcinogens (McGuinness, *et al.*, 1981; Roebuck, 1986; Roebuck, 1987). A recent study which involved the effect of pancreatic growth on certain detoxification enzymes in the pancreas, revealed that a reduction in some of these enzymes, like the GSH-T, may be involved in the finding of a greater incidence of pancreatic neoplasia in conjunction with raw soya flour feeding (Ross and Barrowman, 1987). However, this remains to be proved.

In addition to growth, experimental pancreatic atrophy has also been studied. Trophic factors affecting the pancreas are not yet fully understood, however as previously described, the gastrointestinal hormone CCK seems to be involved. Pancreatic atrophy, it would seem, could be mediated by the administration of CCK antagonists; until recently, the only CCK receptor antagonists available were compounds such as proglumide and benzotript, which are nonspecific in this action (Hahne, *et al.*, 1981). In the last few years new, more specific compounds have been discovered to block more specifically the effects of CCK. These include certain benzodiazepines such as L-364,718, which has been found to block the trophic effects of trypsin inhibitors in rats (Wisner, *et al.*, 1988). Administered on its own, L-364,718 has also been demonstrated to cause a significant reduction in pancreatic weight (Wisner, *et al.*, 1988).

Studies on the effects of CCK antagonists on levels of detoxification enzymes like GSH-T have not yet been published, leaving this field open for extensive research.

1.1.7. Objectives

1. To determine the inducibility of pancreatic GSH-T in the rat, mouse, and hamster using the phenolic antioxidant 2(3)-*tert*-butyl-4-hydroxyanisole.

2. To determine the inducibility of pancreatic GSH-T in the hamster in response to α-angelica lactone, green coffee beans, and coumarin, three naturally-occurring plant compounds.

3. To examine the effects of a raw soya flour diet on pancreatic weight, DNA content, and GSH-T levels in the rat.

4. To examine the effects of a serine protease inhibitor, FOY-305 (camostate) on pancreatic weight, DNA content, and GSH-T levels in the hamster.

5. To examine the effects of a CCK antagonist, L-364,718, on pancreatic weight, DNA content, and GSH-T levels in the hamster.

Chapter 2

Materials and Methods

2.1. Glutathione S-transferase Induction

2.1.1. Animals

Male Sprague-Dawley rats (100-150 g) and male Syrian Golden hamsters (90 - 100 g) were purchased from Canadian Hybrid Farms (NS, Canada). Female CD-1 mice (13 - 14 g) were purchased from Charles River Canada, Incorporated (PQ, Canada). All animals were maintained under standard conditions of 12 hour photoperiod, 23 ° C and 40% relative humidity. Animals were housed in Shoe-box cages (3 rats/cage, 4 mice/cage, 1 hamster/cage), which consist of a plastic rectangular box with sawdust bedding equipped with a rod removable lid containing the food tray. Food and tap water were provided *ad libitum* until the day of sacrifice.

2.1.2. Chemical Treatment

Four chemicals were used in the attempt to induce pancreatic GSH-T. The dietary antioxidant 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) was obtained from Sigma (MO, USA). The naturally occurring plant lactone α-angelica lactone (α-AL), was purchased from the Aldrich Chemical Company (WI, USA), as was coumarin (COU) a compound known for its anticoagulant properties and found

naturally in many plants. Green coffee beans (GCB), naturally containing kahweol, were purchased from Mary Jane's Specialty Foods Ltd. (NF, Canada).

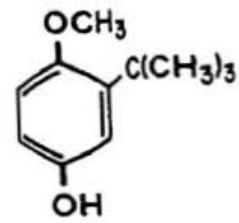
2.1.2.1. BHA Diet (Figure 2-1)

The BHA was obtained by Ralston Purina Company (IN, USA), incorporated into a basal purified diet (Ralston Purina Company) at a level of 0.75% BHA as recommended by Benson, *et al.* (1978) and pelleted. Eleven rats were maintained in staggered groups on the BHA diet while six control animals received the pelleted basal purified diet without BHA. Mice (9 BHA, 6 basal purified diet) and hamsters (7 BHA, 7 basal purified diet) all in staggered groups were also provided with these diets on a similar schedule. All animals were sacrificed on the fifteenth day and the pancreas removed for cell fractionation.

2.1.2.2. Intragastric α-AL (Figure 2-2)

The α-AL was administered at a level of 0.03 mmol/g diet, as recommended by Sparnins, *et al.* (1982). Because the α-AL was in a liquid form, it was suspended in distilled water, with aliquots of 0.5 mL delivered intragastrically using a 5French infant naso-gastric feeding tube (Medi-Craft Limited, ON, Canada). A group of eight hamsters in staggered groups received the α-AL at 10:30 AM every day for 14 days whereas the eight control animals received 0.5 mL of distilled water using the same procedure. All of these animals were lightly anesthetized using diethyl ether during the treatment periods. Animals were sacrificed on the fifteenth day and the pancreas removed for cell fractionation.

Figure 2-1: 2(3)-*tert*-butyl--4-hydroxyanisole (BHA)


OCH3
C(CH3)3
OH

2.1.2.3. Subcutaneous α-AL

In another experiment studying α-AL, the compound was suspended in 0.9% NaCl solution at a concentration of 0.03 mmol/g diet. Seven hamsters separated into staggered groups were injected subcutaneously with 0.5 mL aliquots using 1 cc tuberculin syringes. The injections were made on the anterior portion of the back twice a day (8:30-9:30 AM and 4:30-5:30 PM) for a period of seven days. A group of eight hamsters were injected with 0.5 mL of 0.9% NaCl solution on the same schedule. Animals were sacrificed on the eighth day and the pancreas removed for cell fractionation.

2.1.2.4. GCB Diet (Figure 2-3)

A group of eight hamsters divided into staggered groups were fed a diet consisting of powdered standard Purina Rat Chow (Ralston Purina Company) containing 20% powdered GCB beans as recommended by Sparnins, *et al.* (1982a). Both the chow and the coffee beans were powdered using a Waring Commercial Blendor (CT, USA). The eight control animals were fed the powdered rat chow without the GCB. Animals were sacrificed on the fifteenth day and the pancreas removed for cell fractionation.

2.1.2.5. COU Diet (Figure 2-4)

Coumarin was added to powdered Purina Rat Chow at a concentration of 0.03 mmol/g diet, the level suggested by Sparnins, *et al.* (1982a). Eight hamsters in staggered groups were maintained on this diet for 14 days, while eight control animals received only the powdered chow. Animals were sacrificed on the fifteenth day and the pancreas removed for cell fractionation.

Figure 2-2: α-Angelica Lactone (α-AL)

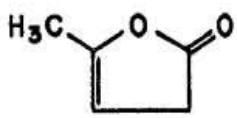

H3C
O
O

Figure 2-3: Kahweol

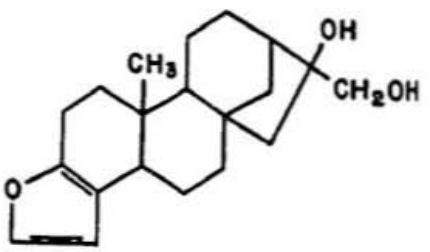
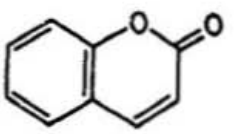

OH
CH3
CH2OH
O

Figure 2-4: Coumarin


O
O

2.1.3. Cell Fractionation

Animals were allowed free access to food and water until the time of sacrifice. Rats were killed by decapitation using a Whamann guillotine (MD, USA) and blood was allowed to drain by inverting the animal. Hamsters and mice were killed by cervical dislocation. The abdomen was opened and a midline incision was made beginning just above the penis and continuing anteriorly above the sternum. The cavity was flooded with chilled 0.9% NaCl solution, the pancreas excised and concurrently trimmed of most fat. The organ was then transferred to iced saline and trimmed of remaining fat, major blood vessels, and connective tissue. The surface saline was removed by pressing the pancreas between two layers of F-2402 filter paper (Canlab) under a 200 g weight for 20 seconds. After weighing, it was immediately transferred into a beaker containing 25 mL of ice cold Tris buffer (50 mM Tris with 0.25 M sucrose, pH 7.5) which was then added to a 30 mL glass homogenization tube (Wheaton, USA).

Homogenization of the tissue was performed at 0 - 4° C using a teflon pestle attached to an electric 3/8" drill (Black and Decker). The tube was moved up and down nine times at a pestle speed of 1200 r.p.m. This caused the mechanical breakdown of the tissue and disruption of the cells to yield an homogenate consisting of diluted cell cytoplasm, intracellular particles and some unbroken cells.

The homogenates were subsequently transferred into polycarbonate 1" x 3 1/2" centrifuge bottles (Beckman) and centrifuged for 60 minutes (Beckman L5-65 type/H ultracentrifuge, 50.2Ti rotor, 4° C). The 105,000g supernatant was

decanted and three 10 μL samples taken for protein determination and three 2 mL samples transferred to 7 mL glass scintillation vials, frozen using liquid nitrogen, and stored at -70 ° C in a Revco freezer until assayed for GSH-T.

2.1.4. Protein Assay

Protein estimation of samples was performed using the Bio-Rad protein assay kit which is based on the differential color change of a dye in response to various protein concentrations. The dye involved is Coomassie brilliant blue G-250, which on binding to protein, causes a shift in the absorption maximum of the dye from a wavelength of 465 nm to 595 nm with this increase in absorption monitored.

2.1.4.1. Preparation of Dye Reagent

The dye reagent concentrate was freshly diluted and filtered just prior to use; this was a five-fold dilution of one volume of dye concentrate diluted with four volumes of deionized water and filtered though Whatman no. 1 filter paper into a glass beaker, where it was kept at room temperature until used.

2.1.4.2. Protein Standard

The protein standard used was the Bio-Rad protein standard I, one of the standards recommended for use with the dye concentrate. It consisted of a lyophilized bovine gamma globulin which was reconstituted with 20 mL of deionized water to achieve a final protein concentration of 1.4 mg/mL. The rehydrated protein was kept at 4 ° C for up to 60 days.

2.1.5. Protein Estimation

Standard solutions which contained 26.4, 39.6, 52.8, 66.0, 79.2, 92.4, 105.6, 118.8 and 132.0 μg protein/mL were placed in 4.5 mL polystyrene disposable cuvettes with 2.5 mL diluted Bio-Rad reagent; mixing of the samples was achieved with gentle sucking and expelling using a pasteur pipette. Samples were read at 595 nm with a Gilford 250 spectrophotometer. Absorbance readings were used to plot a standard curve of protein concentration against absorbance. This standard curve was then used to determine the protein concentration of the unknown samples.

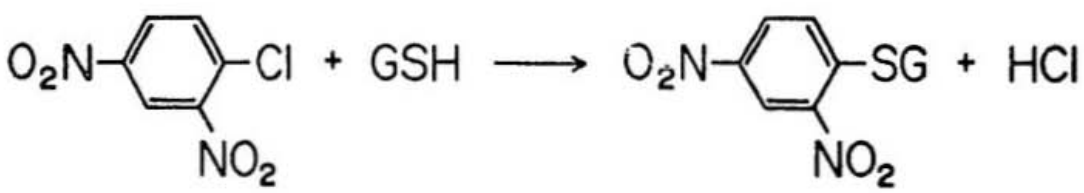
2.1.6. Glutathione S-transferase Assay

The method used to assay for GSH-T activity was that of Habig *et al.* (1974) as modified by Kraus and Kloft (1980). The principle of the assay is based on the formation of a glutathione/substrate conjugate over time.

The substrate utilized was 1-chloro-2,4-dinitrobenzene (CDNB) which is the most widely used general substrate for GSH-T. The reaction involves CDNB reacting with the peptide glutathione (GSH), with catalysis by GSH-T. The product of this is a GSH conjugate and hydrochloric acid (figure 2-5). CDNB was dissolved in 10 mL of absolute ethanol and was made up fresh daily for each set of assays.

A Gilford 250 spectrophotometer equipped with a water - jacketed cuvette holder maintained at 37 ° C was used for all GSH-T assays. The holder was designed to read four cuvettes at a time; assays were done in 4.5 mL polystyrene

Figure 2-5: The reaction of 1-chloro-2,4-dinitrobenzene (CDNB) with GSH to produce a glutathione conjugate


O2N
Cl
NO2
+ GSH
O2N
SG
NO2
+ HCl

disposable cuvettes. For each assay, one cuvette served as the blank containing only diluted Bio-Rad reagent, two contained 10 - 20 μL of 105,000g cytosol, and the other contained 300 μL of lyophilized rat liver GSH-T standard (Sigma). One unit of the GSH-T standard conjugated 1.0 μM of CDNB with reduced glutathione per minute. All cuvettes also contained 100 μL of glutathione (GSH) to give a final concentration of 1 mM GSH.

The final incubation volume was made up to 3 mL with 10 mM potassium phosphate buffer with 1.1 mM $MgCl_2$ at a pH of 7.4. The cuvette contents were quickly stirred and allowed to incubate at 37 ° C for 5 minutes. The reaction was started by the addition of 50 μL CDNB solution (final concentration of 1.1 mM). The GSH-T activity was measured spectrophotometrically at a wavelength of 340 nm and recorded on a Gilford 6050 Chart Recorder for at least 3 minutes after the addition of CDNB. Assays were always carried out in quadruplicate with average enzyme activity expressed as nmol of GSH conjugated/min/mg protein.

2.2. Experimental Pancreatic Growth in Rats

2.2.1. Animals

Male Sprague-Dawley rats (225 - 340 g at the time of sacrifice) purchased from Canadian Hybrid Farms (NS, Canada) were used. They were housed under the same standard conditions as previously described.

2.2.2. Treatment

A group of seven rats (in staggered groups) were fed a basal purified diet containing 40% raw defatted soya flour (RSF) which was pelleted by Ralston Purina (IN, USA). Raw soya flour contains a trypsin inhibitor (TI) at a level of 29.5 mg/g which is responsible for the increase in pancreatic weight associated with its administration. During the same period, another group of seven rats (staggered) were maintained on pellets of the same composition except that the soya flour had been heated in order to inactivate most of the trypsin inhibitor activity (reduced from 29.5 mg to 5 mg TI/g) (data from supplier).

This regimen was implemented in fourteen rats for a period of fourteen days, and in another group of fourteen rats for twenty-one days. Animals were killed by decapitation on the fifteenth or twenty second day and the pancreas removed for cell fractionation.

2.2.3. Cell Fractionation

The pancreas was processed as previously described for protein estimation and GSH-T assay. However, before homogenization, approximately 250 mg pancreas were removed and frozen at -15° C for later use in the assay for deoxyribonucleic acid (DNA).

2.2.4. Deoxyribonucleic Acid (DNA) Assay

The procedures employed in assaying for the amount of DNA present in a rat pancreas involves the inital extraction of DNA, and subsequent DNA estimation as a function of color development. The extraction methods were those of Schmidt and Thannhauser (1945) as modified by Steele, *et al.* (1964). The subsequent DNA estimation as a function of color development were the techniques of Burton (1956).

2.2.5. DNA Extraction

Approximately 250 mg of pancreas were placed in 5 mL of 2.5% perchloric acid (PCA) in 10 mL glass homogenization tubes (Wheaton,USA). The tissue was homogenized using a teflon pestle attached to an electric 3/8" drill (Black and Decker). The tube was moved up and down three times at a pestle speed of 1200 r.p.m. The homogenates were diluted to 10 mL with 2.5% PCA and centrifuged using a Clay Adams (NJ, USA) Dynac II Centrifuge (1500g for 5 minutes using a 6-place 15 mL Angle Rotor in 15 mL Pyrex conical centrifuge tubes). The resulting pellet was washed three times with 10 mL of 2.5% PCA followed by one washing with 5 mL of 90% ethanol containing 2% sodium acetate, and, finally, two washes with 5 mL of a 3:1 mixture of 90% ethanol/sodium acetate:ether made fresh each day.

The pellet was then resuspended in 3 mL 0.5N KOH and incubated in a 37 ° C water bath for one hour to allow protein digestion to occur. Following digestion, the contents of the tube were neutralized by the addition of 0.25 mL of 5N HCl and an equal volume of 2.5% PCA. The resuspended pellet was

centrifuged at 1500g for 5 minutes and the resulting pellet washed with 3 mL of 2.5% PCA. DNA was then extracted by heating the pellet with 3 mL of 5% PCA in a 70° C water bath for 20 minutes.

2.2.6. DNA Estimation

Highly polymerized calf thymus DNA (Sigma) dissolved in 5mM NaOH to give a concentration of 0.4 mg/mL was used as the standard. This stock solution was stored at 4° C with working standards produced from this every three weeks. The working standards were prepared by mixing equal volumes of stock solution and 1N PCA and heating at 70° C for 15 minutes. This was stored at 4° C. Duplicate standards containing final concentrations of 4.4 - 31.0 μg/mL were run with each DNA assay. A standard curve was plotted of DNA concentration against optical density, and used to calculate the DNA concentration of the unknown samples. Two 1 mL samples (duplicates) of the DNA extract were each diluted with 0.5 mL 0.5N PCA in 5 mL culture tubes. To each of these 1.5 mL volumes was added two volumes (3 mL) of diphenylamine reagent (1.5 g diphenylamine dissolved in 100 mL of glacial acetic acid and 1.5 mL of concentrated sulfuric acid with 0.10 mL of 1.6% acetaldehyde/20 mL of reagent added just prior to use). Blanks containing 1N PCA, 0.5N PCA and diphenylamine reagent were also set up with each assay. After mixing by inverting the tubes several times, the samples, standards, and blanks were incubated in a 25 - 35° C water bath for 20 hours to allow color development. The contents of the tubes were then mixed by inverting several times and aliquots transferred to 4.5 mL polystyrene disposable cuvettes. Samples were read against the described blank using a Gilford 250 spectrophotometer at a wavelength of 600 nm, and compared with the standards.

2.3. Experimental Pancreatic Growth in Hamsters

2.3.1. Animals

Male Syrian Golden hamsters (85 - 115 g) at the time of sacrifice were purchased from Canadian Hybrid Farms (Nova Scotia, Canada). The animals were housed under the usual standard conditions.

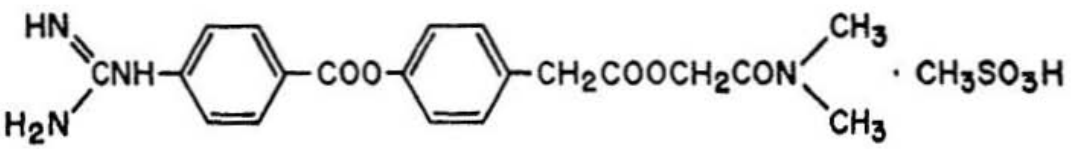
2.3.2. Treatment

Eight hamsters divided into staggered groups were gavaged once a day (5:00 - 6:00 PM) with 400 mg/kg body weight of N,N-dimethylcarbamoylmethyl p-(p-guanidino-benzoyloxy)phenylacetate methanesulfonate (FOY-305) (Figure 2-6) (Ono Pharmaceutical Co., Ltd., Japan), a potent inhibitor of serine proteases, including trypsin. The compound was suspended in deionized water and administered in 0.5 mL volumes using a 19G gavage needle attached to a 3 cc syringe. Another group of eight hamsters was used as the control, and received 0.5 mL of deionized water once daily. Animals were lightly anesthetized with diethyl ether during the gavaging. All animals were killed on the fifteenth day for cell fractionation of the pancreas.

2.3.3. Cell Fractionation

The pancreas was processed as noted earlier for protein estimation and GSH-T assay. Approximately 150 mg of pancreas were removed and frozen for use in the DNA assay.

Figure 2-6: FOY-305


HN
CNH
H2N
COO
CH2COOCH2CON
CH3
CH3
· CH3SO3H

2.4. Experimental Pancreatic Atrophy

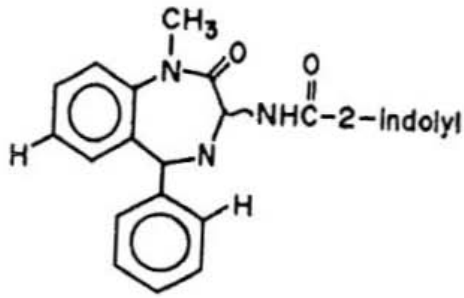
2.4.1. Animals

Male Syrian Golden hamsters (85 - 115 g) at the time of sacrifice were purchased from Canadian Hybrid Farms (Nova Scotia, Canada). The animals were housed under the standard conditions previously described.

2.4.2. Treatment

A group of eight hamsters (in staggered groups) were gavaged twice daily (10:30 - 11:30 AM and 5:00 - 6:00 PM) with 1 mg/kg body weight of 3S(-)-N(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-yl)-1*H*-indole-2-carboxamide (L-364,718) (Figure 2-7) (Merck Sharp & Dohme Research Laboratories, N.J.), a test drug which acts a cholecystokinin antagonist. The drug is insoluble in water and several other solvents so was therefore dissolved in 4% dimethyl sulfoxide (DMSO). The total volume of drug and DMSO administered was 0.5 mL using a 19G gavage needle attached to a 3 cc syringe. An additional group of eight hamsters in staggered groups served as controls and received 0.5 mL of 4% DMSO twice daily by the same method. Animals were lightly anesthetized during dosing sessions. All animals were sacrificed on the fifteenth day for cell fractionation of the pancreas.

Figure 2-7: L-364.718


CH3
N
O
O
NHC-2-Indolyl
H
N
H

2.4.3. Cell Fractionation

The pancreas was processed as described for both protein estimation and GSH-T assay. However, as in experiments involving pancreatic growth, approximately 150 mg of pancreas were removed and frozen for future use in the DNA assay using the methods already discussed.

2.5. Statistical Analysis

All results were expressed as mean ± standard error of the mean (SE).

Control and experimental groups were compared using the Student's t-test with significance levels established at $p<0.05$ unless otherwise stated.

Chapter 3

Results

3.1. Effect of Certain Chemicals on Pancreatic GSH-T Activity and Total Pancreatic GSH-T of Rats, Mice, and Hamsters

This study was undertaken in order to observe the effects of a dietary antioxidant and certain naturally-occurring compounds on pancreatic GSH-T in the rat, mouse, and hamster.

3.1.1. BHA Treatment(Table 3-1)

The results showed significant interspecies differences in pancreatic GSH-T activity in response to the dietary antioxidant 2(3)- *tert*-butyl-4-hydroxyanisole (BHA).

In the rat (Figures 3-1 and 3-2) and mouse (Figures 3-3 and 3-4), there was no significant induction of pancreatic GSH-T activity, or in total pancreatic GSH-T after the animals had been fed 0.75% BHA for two weeks. However, in the case of the hamster, there was a significant increase in the GSH-T activity of the pancreas ($p<0.05$) by 30% over controls (Figure 3-5). Total levels of GSH-T in the hamster pancreas were also increased significantly ($p<0.001$) by 23% as compared with controls (Figure 3-6).

TABLE 3-1: GLUTATHIONE S-TRANSFERASE (GSH-T) ACTIVITY AND TOTAL PANCREATIC GSH-T IN THE RAT, MOUSE, AND HAMSTER PANCREAS AFTER TREATMENT WITH BHA

Animal	Pancreatic GSH-T Activity		Total Pancreatic GSH-T	
	BHA	Control	BHA	Control
Rat	89.51 ± 10.73	72.08 ± 5.88	8.57 ± 0.50	7.40 ± 0.41
Mouse	980.56 ± 37.11	901.77 ± 42.82	24.60 ± 2.31	23.40 ± 1.28
Hamster	169.01 ± 12.81*	129.74 ± 7.06	7.12 ± 0.18**	5.79 ± 0.30

1. GSH-T activity expressed as nmol glutathione conjugated/mg protein/min.
2. Total GSH-T per pancreas expressed as μmol glutathione conjugated/min.
3. Each value represents mean ± SEM.
4. * = significant difference ($p<0.05$)
   ** = significant difference ($p<0.001$)

Figure 3-1: Effect of BHA on pancreatic GSH-T activity in the rat
Control: white
BHA treated: dotted
Each bar represents the mean ± SE.

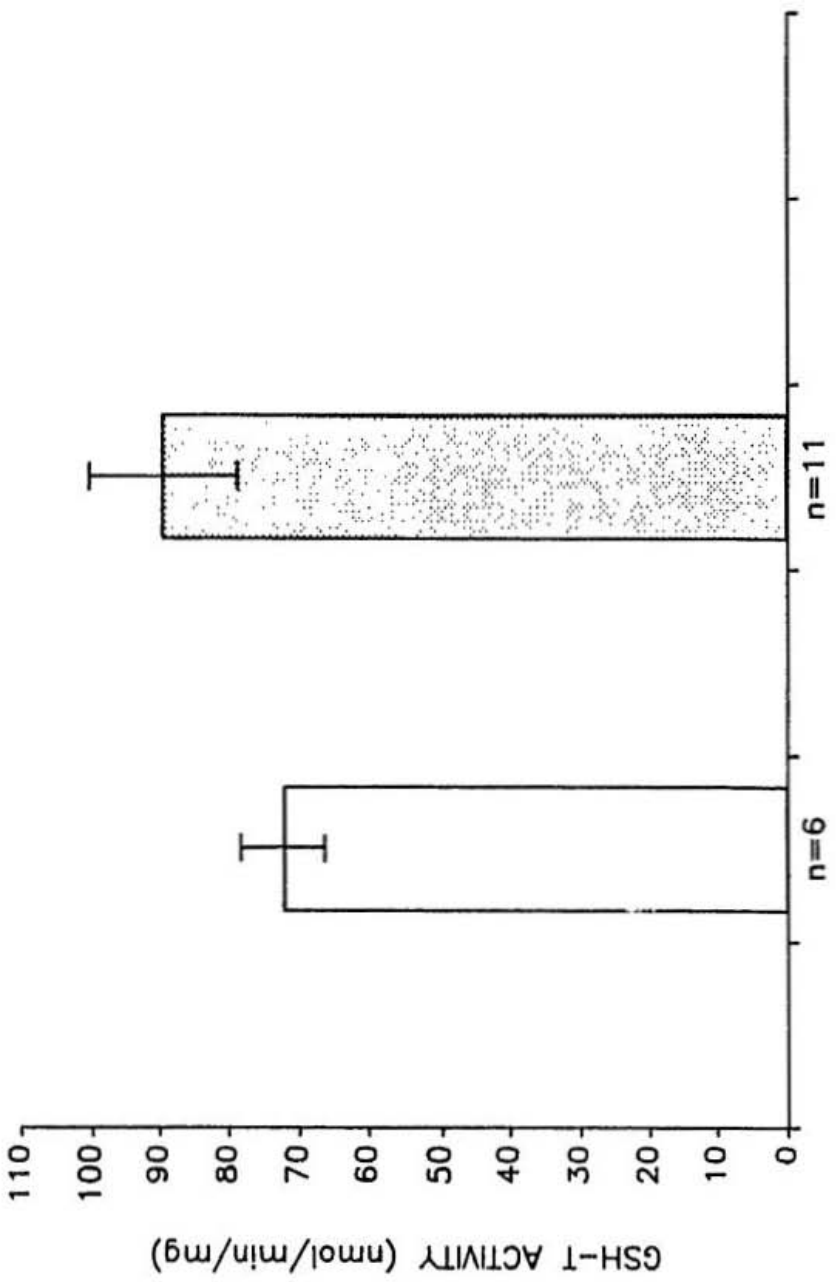

GSH-T ACTIVITY (nmol/min/mg)
0
10
20
30
40
50
60
70
80
90
100
110
n=6
n=11

Figure 3-2: Effect of BHA on total pancreatic GSH-T in the rat

Control: white

BHA treated: dotted

Each bar represents mean ± SE.

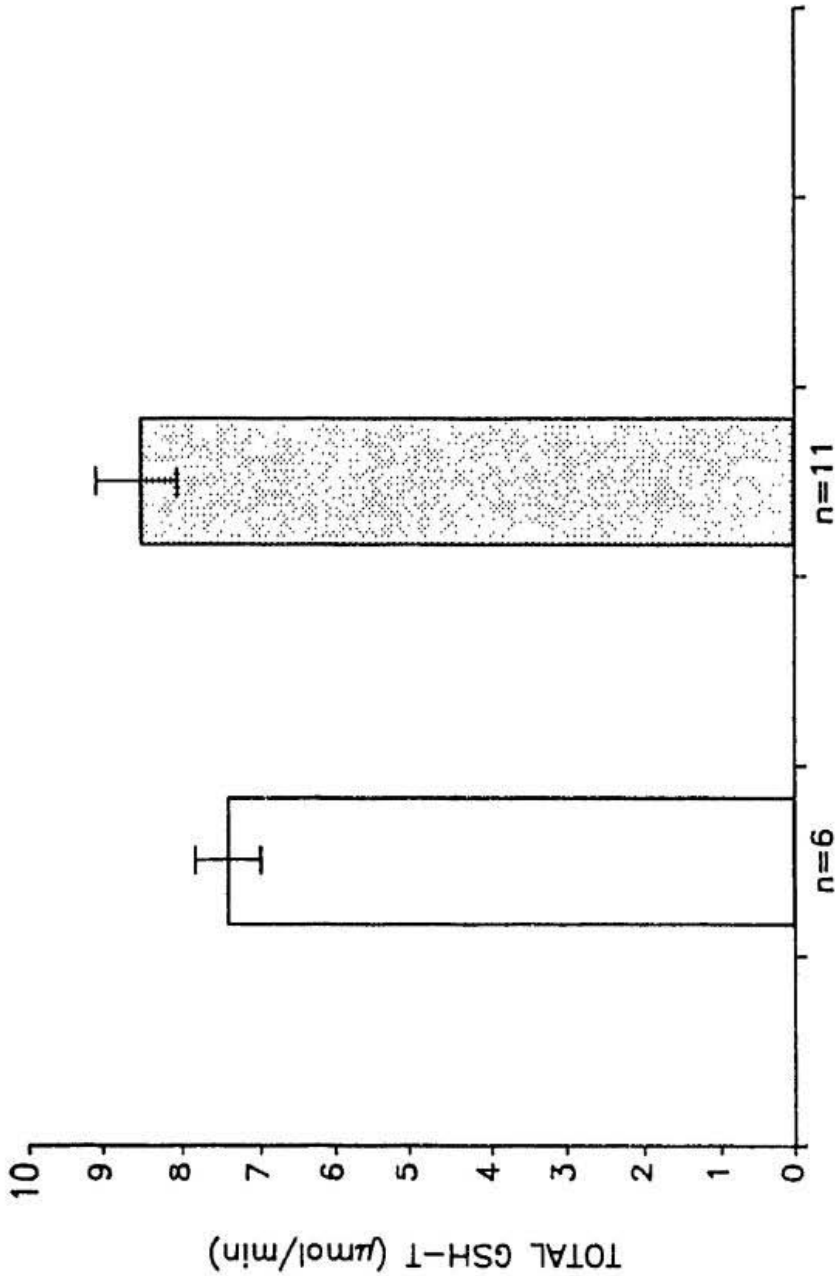

TOTAL GSH-T (μmol/min)
0
1
2
3
4
5
6
7
8
9
10
n=6
n=11

Figure 3-3: Effect of BHA on pancreatic GSH-T activity in the mouse
Control: white
BHA treated: dotted
Each bar represents mean ± SE.

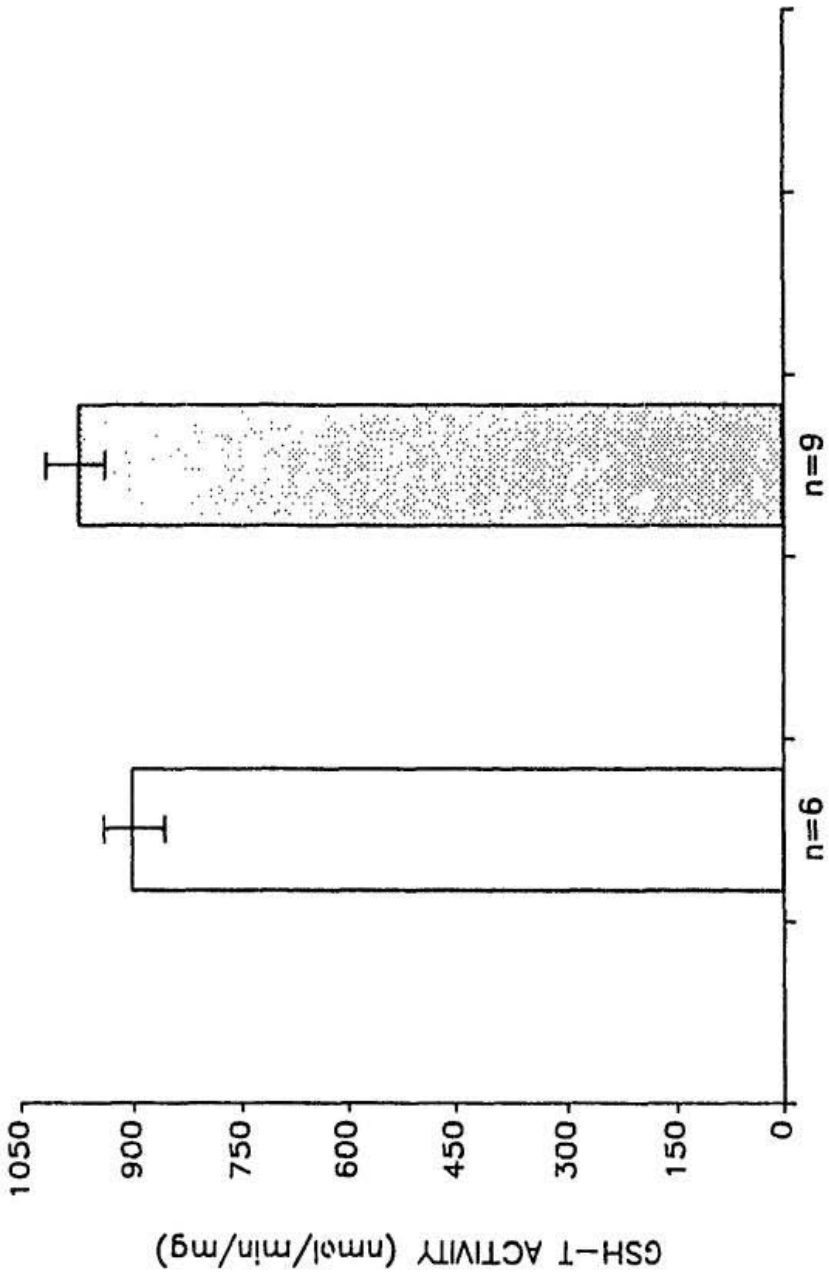

GSH-T ACTIVITY (nmol/min/mg)
1050
900
750
600
450
300
150
0
n=6
n=9

Figure 3-4: Effect of BHA on total pancreatic GSH-T in the mouse
Control: white
BHA treated: dotted
Each bar represents mean ± SE.

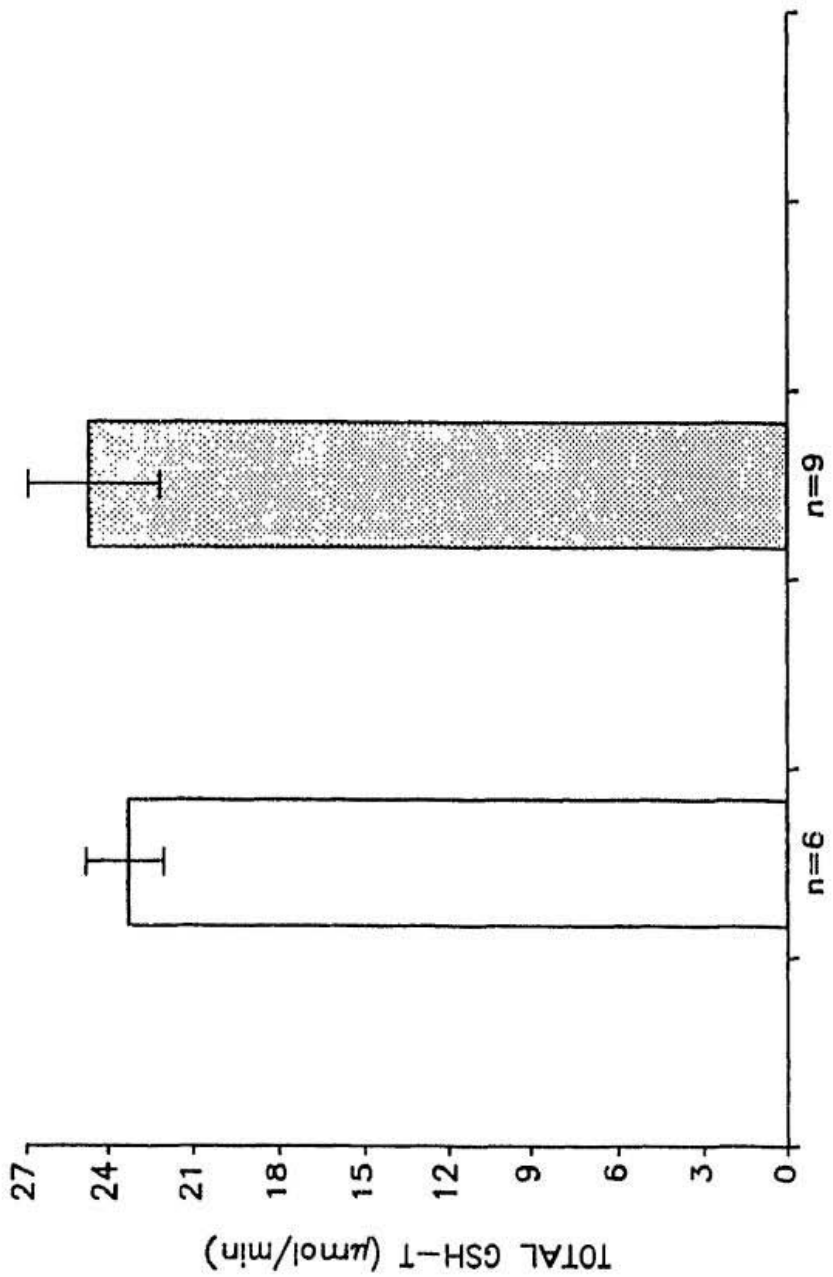

TOTAL GSH-T (μmol/min)
27
24
21
18
15
12
9
6
3
0
n=6
n=9

Figure 3-5: Effect of BHA on pancreatic GSH-T activity in the hamster

Control: white

BHA treated: dotted

Each bar represents mean ± SE.

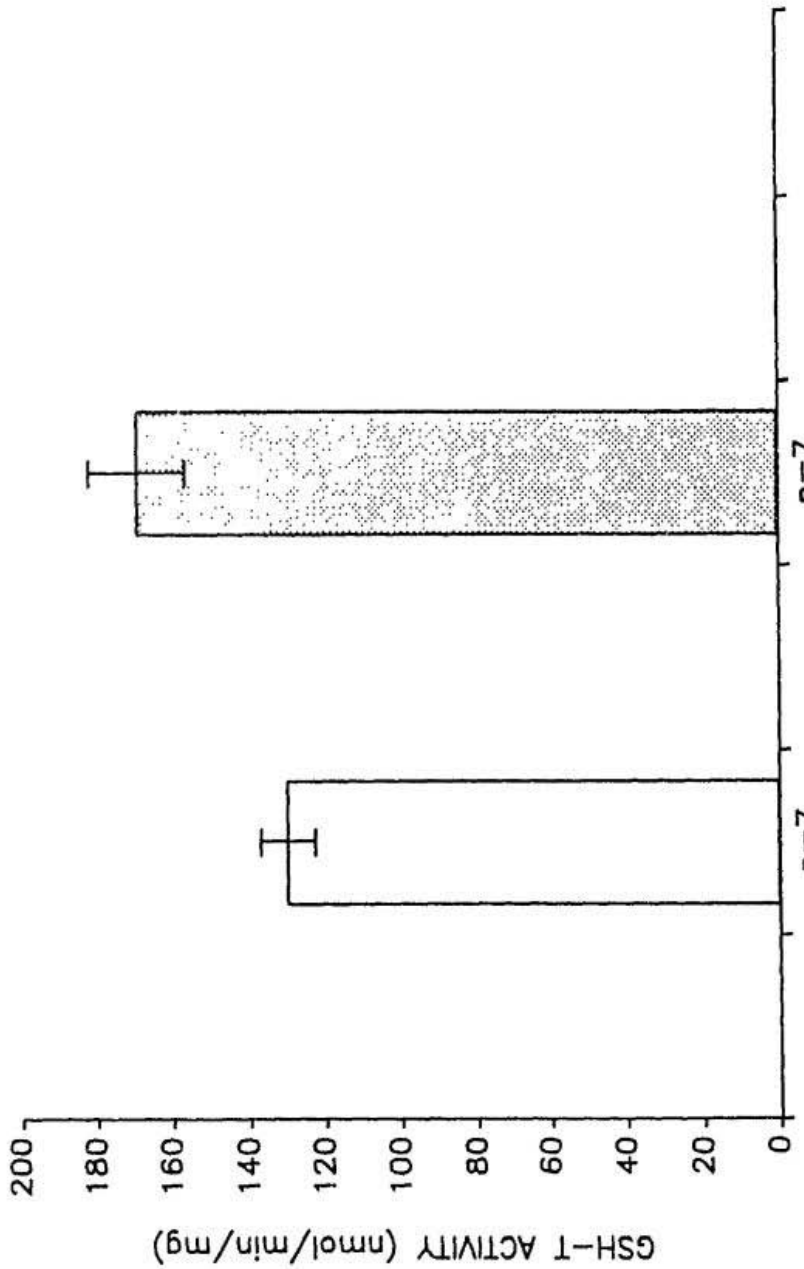
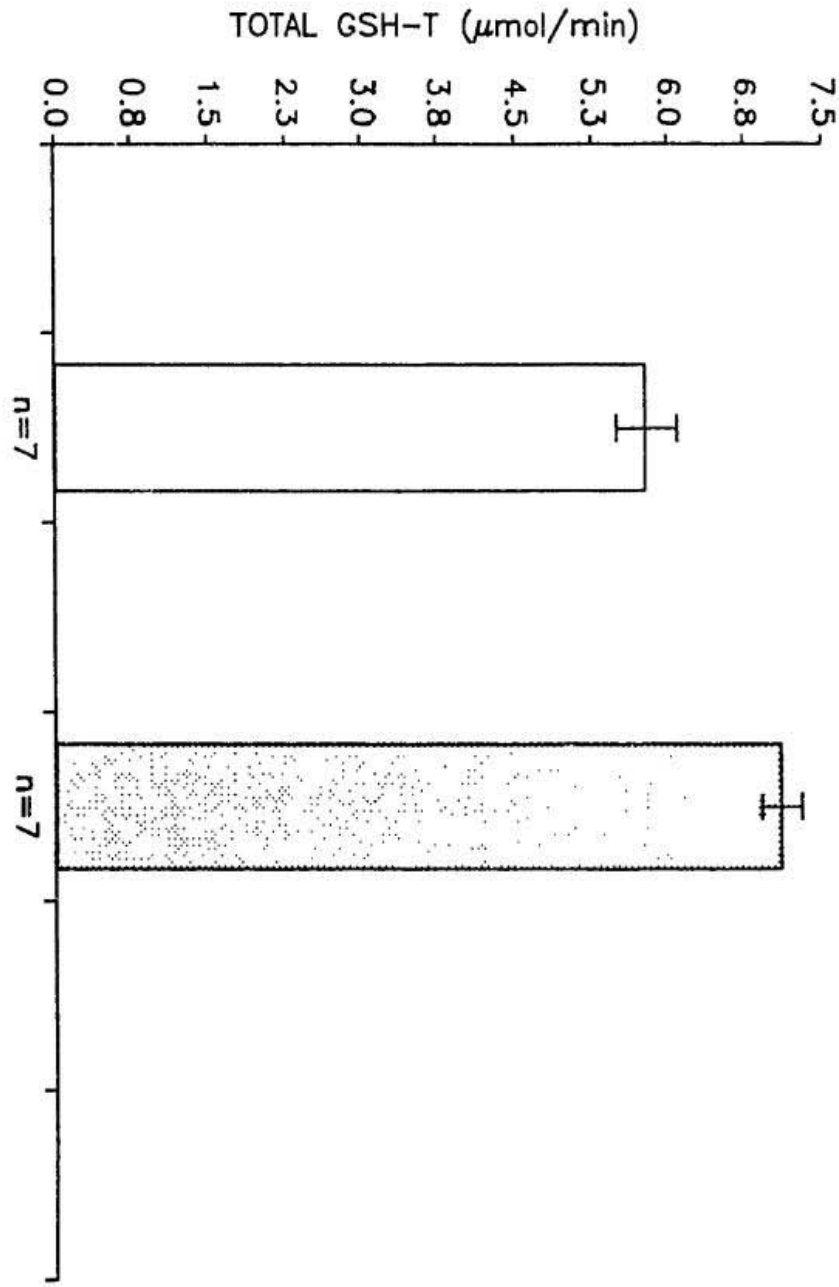

GSH-T ACTIVITY (nmol/min/mg)
0
20
40
60
80
100
120
140
160
180
200
n=7
n=7

Figure 3-6: Effect of BHA on total pancreatic GSH-T in the hamster

Control: white

BHA treated: dotted

Each bar represents mean ± SE


TOTAL GSH-T (μmol/min)
0.0
0.8
1.5
2.3
3.0
3.8
4.5
5.3
6.0
6.8
7.5
n=7
n=7

3.1.2. a-Angelica Lactone Treatment (Table 3-2)

The five-membered ring compound naturally found in many edible fruits and vegetables, a-angelica lactone (a-AL), was used in two separate experiments.

In one experiment, a-AL was given to hamsters by gavage and in the other, by subcutaneous injection. Neither pancreatic GSH-T activity nor total pancreatic GSH-T was induced as compared with controls when the compound was given by the intragastric route (Figures 3-7 and 3-8). Similarly, there was no effect on GSH-T activity when a-AL was administered by subcutaneous injection (Figure 3-9). On the other hand, subcutaneous dosing of a-AL to hamsters did cause a significant reduction in total pancreatic GSH-T ($p<0.025$). This was a 17% reduction as compared with the control group (Figure 3-10).

3.1.3. Green Coffee Bean Treatment (Table 3-2)

In this experiment, the effects of powdered green coffee beans (GCB) containing the active ingredients kahweol palmitate and cafestol palmitate in the diet, on hamster pancreatic GSH-T activity and total pancreatic GSH-T were studied.

As in the experiment using a-AL, no significant induction in either GSH-T activity (Figure 3-11) or in total levels pancreatic GSH-T (Figure 3-12) was observed in the hamster pancreas.

TABLE 3-2: PANCREATIC GLUTATHIONE S-TRANSFERASE (GSH-T) ACTIVITY AND TOTAL PANCREATIC GSH-T IN HAMSTERS TREATED WITH α-AL, GCB, OR COU

Compound	Pancreatic GSH-T Activity		Total Pancreatic GSH-T	
	Treated	Control	Treated	Control
α-AL (Intragastric)	375.61 ± 16.64	356.39 ± 15.58	11.89 ± 0.50	11.79 ± 0.50
α-AL (Subcutaneous)	413.41 ± 38.89	429.76 ± 17.93	9.72 ± 0.50*	11.37 ± 0.38
GCB	416.93 ± 32.27	400.31 ± 16.80	12.72 ± 0.56	12.91 ± 0.36
COU	384.01 ± 24.33	379.33 ± 24.56	12.08 ± 0.43	12.14 ± 0.59

1. GSH-T activity expressed as nmol glutathione conjugated/mg protein/min.
2. Total GSH-T expressed as μmol glutathione conjugated/min.
3. Each value represents mean ± SEM.
4. * = significant difference ($p<0.05$)

Figure 3-7: Effect of intragastric α-AL on pancreatic GSH-T activity in the hamster

Control: white

α-AL treated: dotted

Each bar represents mean ± SE.

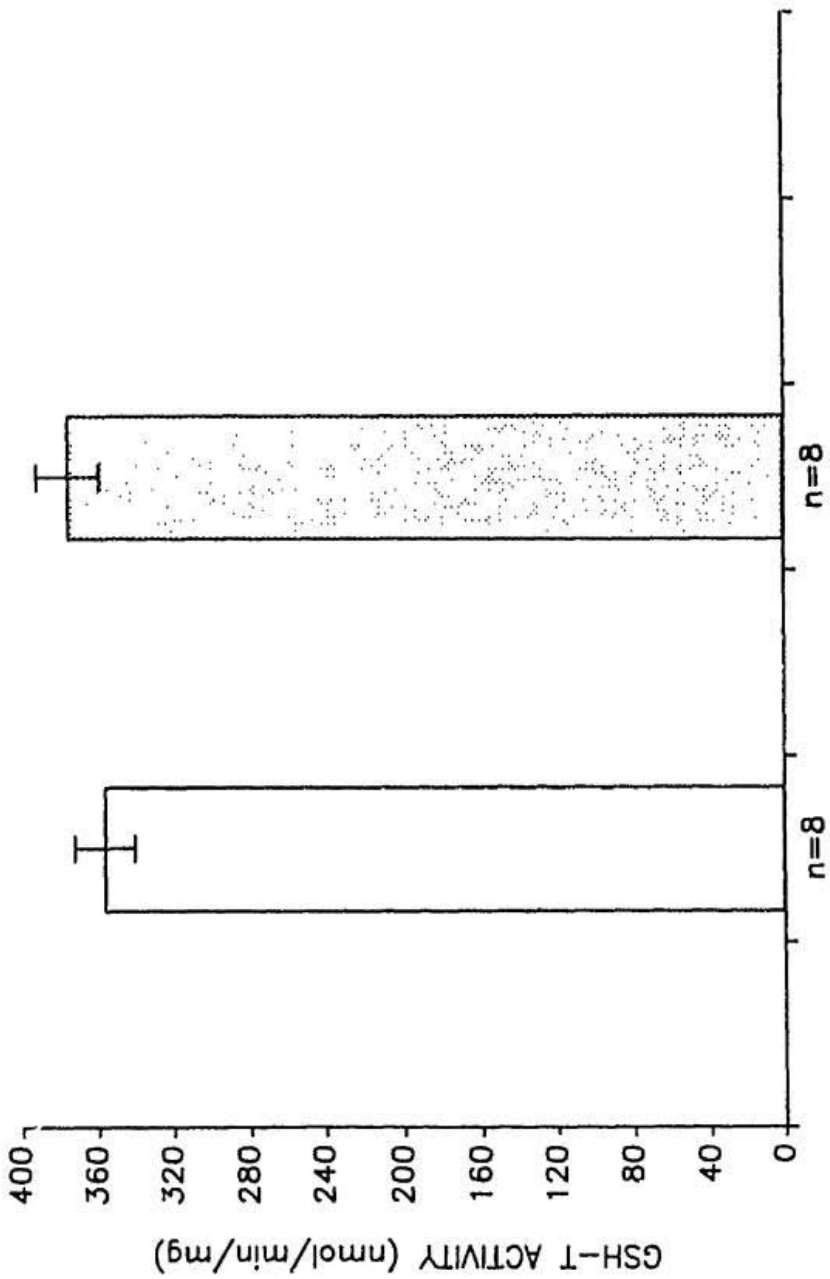

GSH-T ACTIVITY (nmol/min/mg)
400
360
320
280
240
200
160
120
80
40
0
n=8
n=8

Figure 3-8: Effect of intragastric α-AL on total pancreatic GSH-T in the hamster

Control: white

α-AL treated: dotted

Each bar represents mean ± SE.

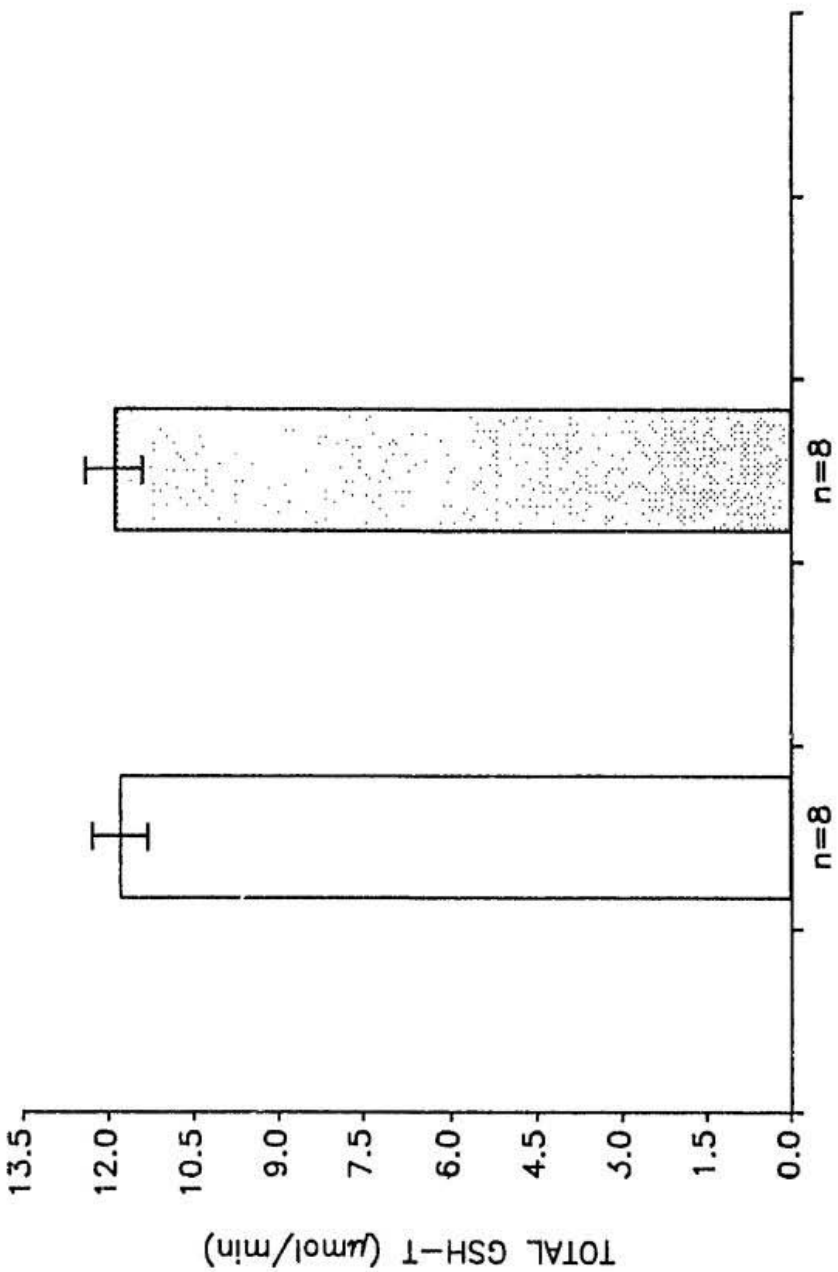

TOTAL GSH-T (μmol/min)
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0.0
n=8
n=8

Figure 3-9: Effect of subcutaneous α-AL on pancreatic GSH-T activity in the hamster

Control: white

α-AL treated: dotted

Each bar represents mean ± SE.

GSH-T ACTIVITY (nmol/min/mg)
480
420
360
300
240
180
120
60
0
n=7
n=8

Figure 3-10: Effect of subcutaneous α-AL on total pancreatic GSH-T in the hamster

Control: white

α-AL treated: dotted

Each bar represents mean ± SE.

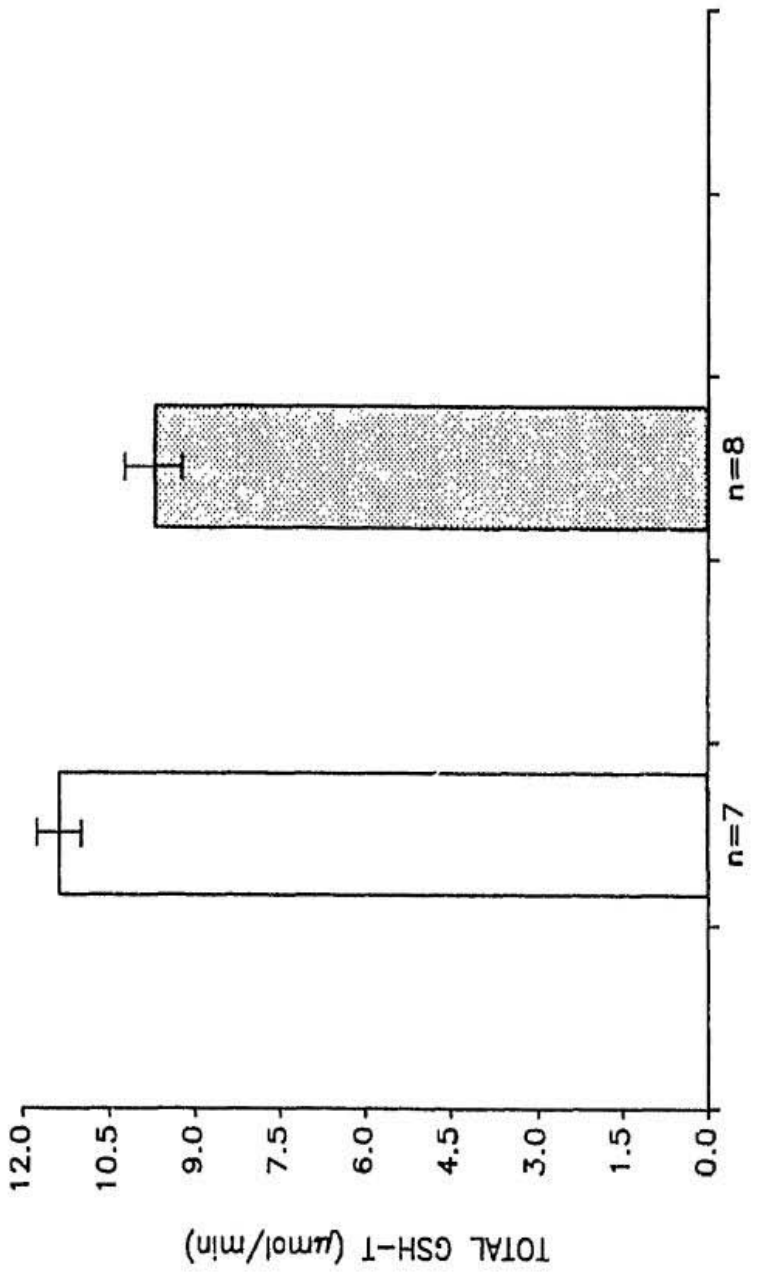

TOTAL GSH-T (μmol/min)
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0.0
n=7
n=8

Figure 3-11: Effect of GCB on pancreatic GSH-T activity in the hamster
Control: white
GCB treated: dotted
Each bar represents mean ± SE.

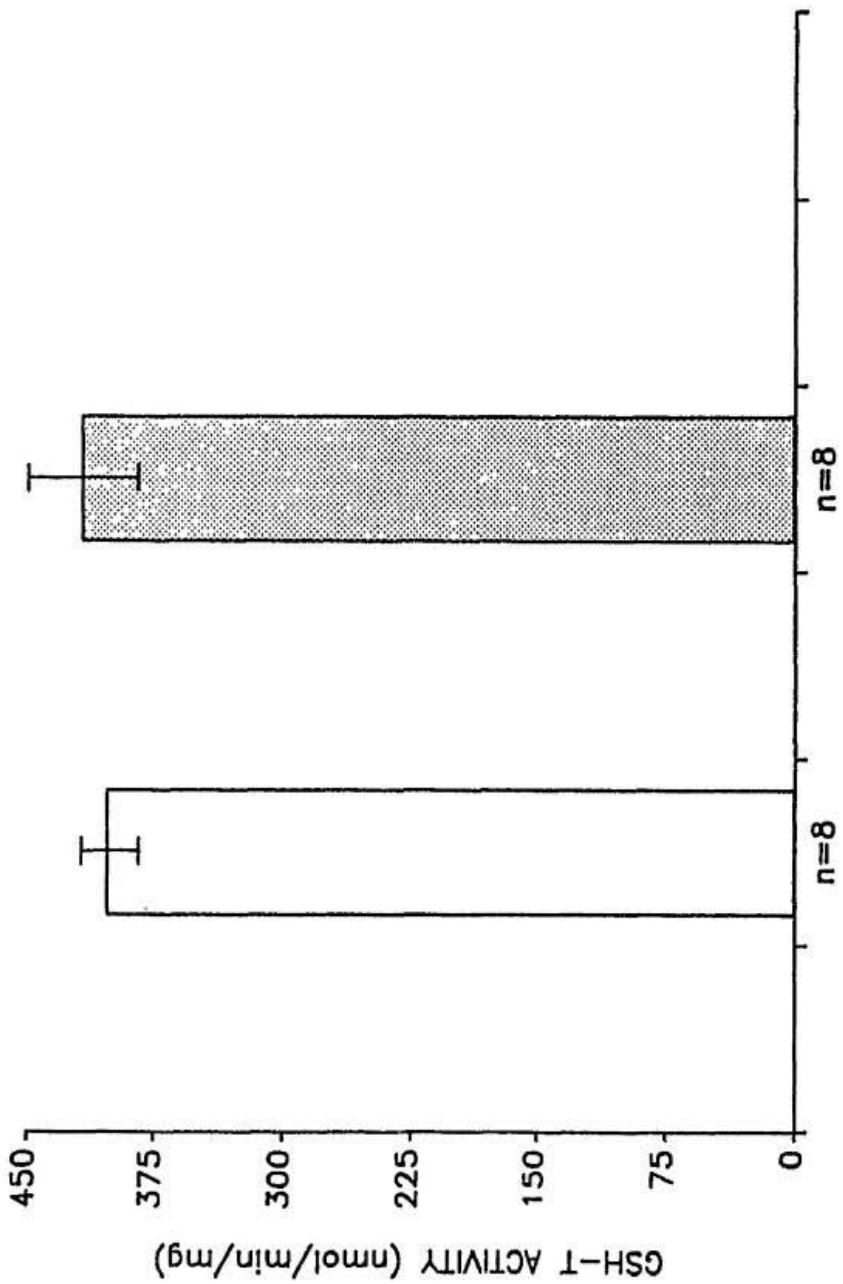
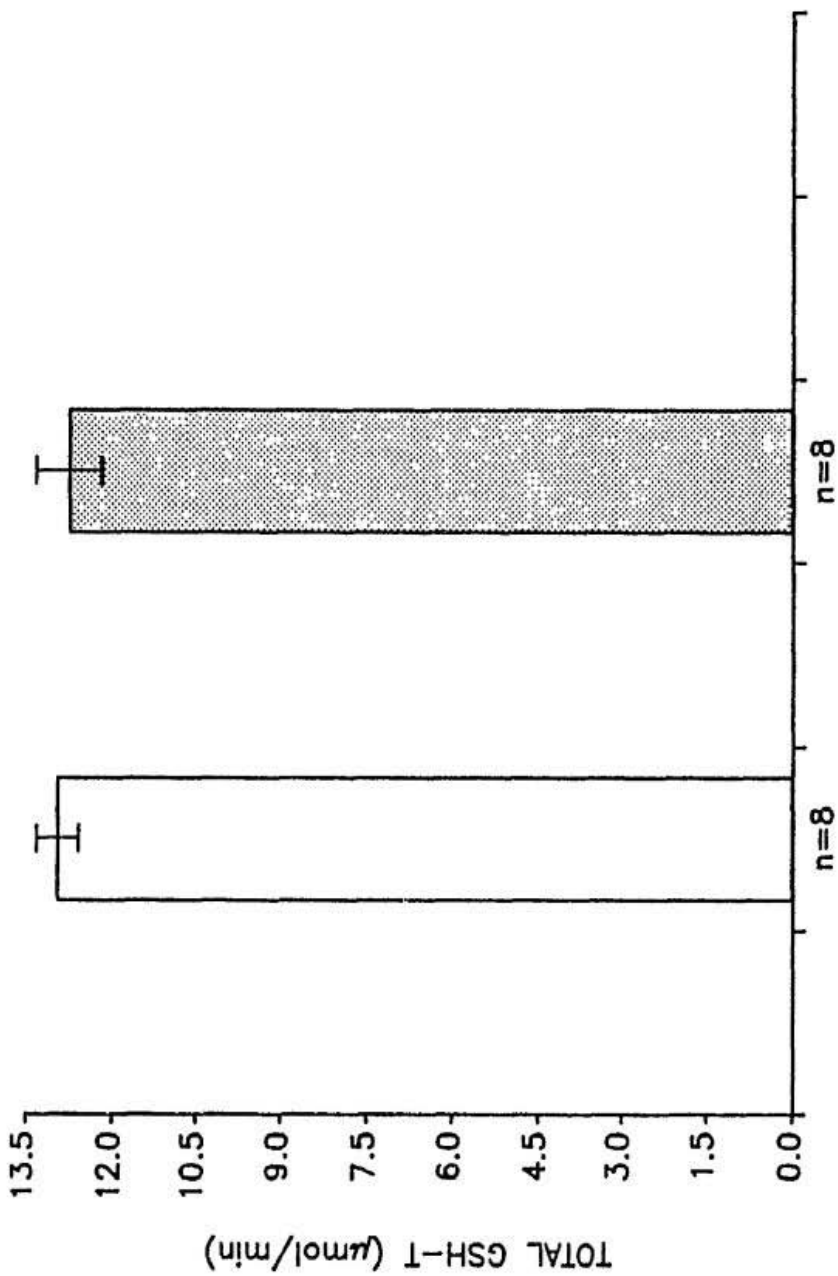

GSH-T ACTIVITY (nmol/min/mg)
450
375
300
225
150
75
0
n=8
n=8

Figure 3-12: Effect of GCB on total pancreatic GSH-T in the hamster
Control: white
GCB treated: dotted
Each bar represents mean ± SE.


TOTAL GSH-T (μmol/min)
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0.0
n=8
n=8

3.1.4. Coumarin Treatment (Table 3-2)

Coumarin, a compound commonly present in many edible fruits and vegetables, was also tested for its effects on pancreatic GSH-T activity and on the total levels of the enzymes in this organ.

Once again, the activity of the GSH-T was unaffected by the inclusion of this chemical in the hamster diet (Figure 3-13). Total levels of pancreatic GSH-T also remained unchanged (Figure 3-14).

3.2. Effect of Raw Soya Flour on Pancreatic Weight, DNA Content, Pancreatic GSH-T Activity, Total Pancreatic GSH-T, and GSH-T as Related to DNA Content in the Hamster (Tables 3-3 and 3-4)

Two experiments involving raw soya flour (RSF) feeding were performed; the first studied the effects of a diet containing RSF for 14 days on the rat pancreas, and the other involved looking at these same criteria after a 21 day feeding schedule of the RSF.

3.2.1. Raw Soya Flour Diet for Two Weeks (Table 3-3)

3.2.1.1. Pancreas Weight

As previously reported by others, the pancreases of RSF-fed rats were significantly larger ($p<0.001$) than those of rats fed the same diet containing heated soya flour (HSF) (Figure 3-15). The mean pancreas weight of those animals receiving the RSF was 54% greater than controls.

Figure 3-13: Effect of COU on pancreatic GSH-T activity in the hamster
Control: white
COU treated: dotted
Each bar represents mean ± SE.

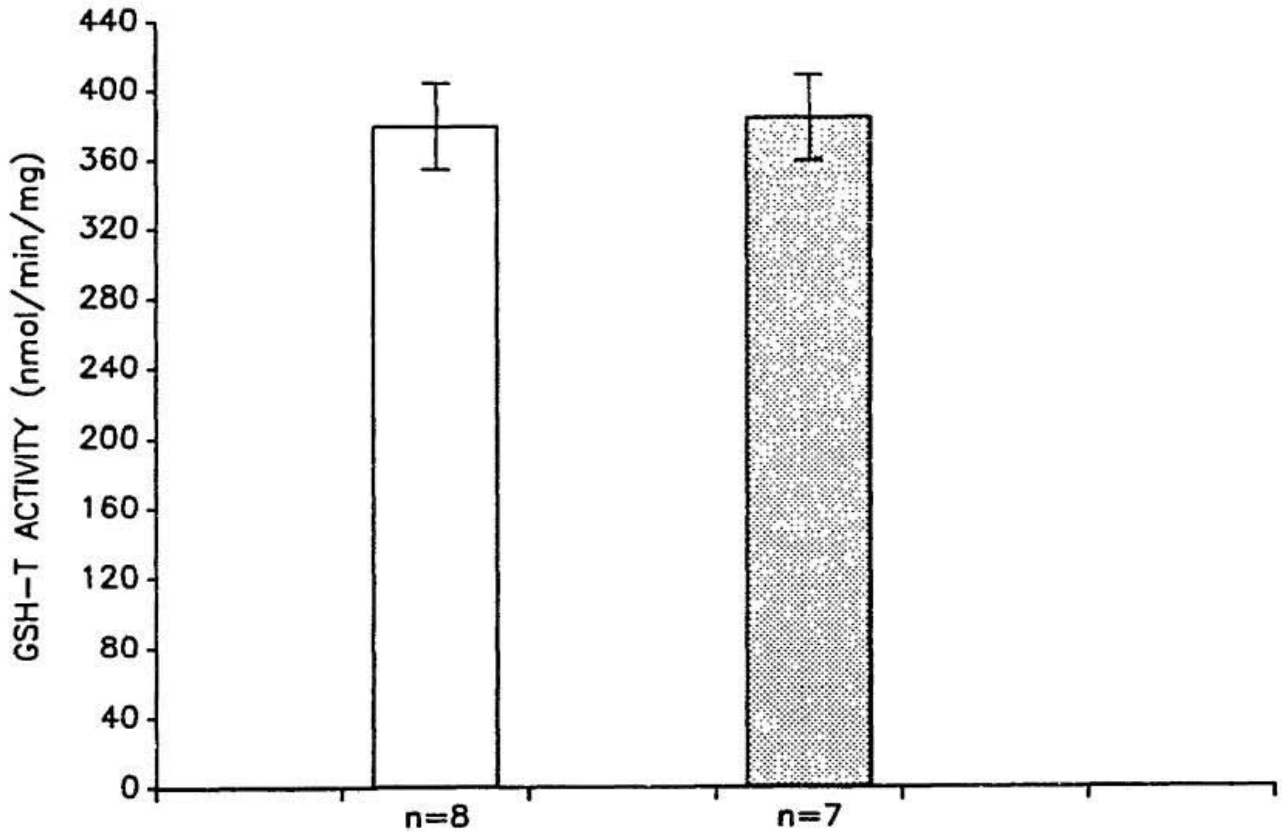

GSH-T ACTIVITY (nmol/min/mg)
440
400
360
320
280
240
200
160
120
80
40
0
n=8
n=7

Figure 3-14: Effect of COU on total pancreatic GSH-T in the hamster
Control: white
COU treated: dotted
Each bar represents mean ± SE.

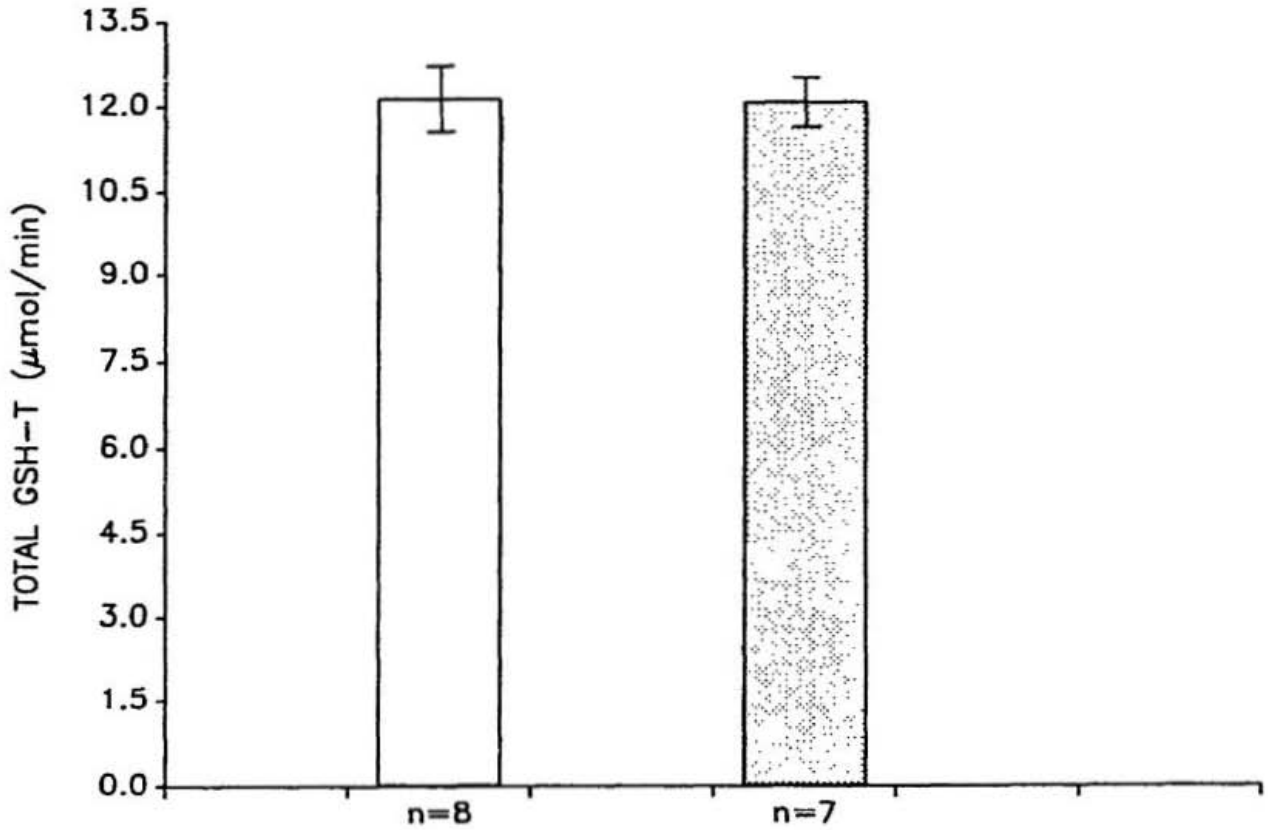

13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0.0
TOTAL GSH-T (μmol/min)
n=8
n=7

TABLE 3-3: EFFECT OF 14 DAY RAW SOYA FLOUR (RSF) FEEDING ON PANCREATIC WEIGHT, DNA CONTENT, GSH-T ACTIVITY, TOTAL GSH-T, AND TOTAL GSH-T AS RELATED TO DNA CONTENT IN THE RAT

	TREATMENT		Significance[4]
	RSF	HSF	
Pancreas Weight (mg/100g body weight)	806.26 ± 41.79	522.81 ± 19.82	p<0.001
DNA Content (mg/pancreas)	2.02 ± 0.13	2.12 ± 0.18	NS
DNA Content (mg/100g body weight)	0.73 ± 0.06	0.68 ± 0.07	NS
Pancreatic protein (mg)/ mg DNA	81.53 ± 9.13	65.21 ± 4.20	p<0.01
GSH-T Activity[1]	80.52 ± 2.17	83.80 ± 2.51	NS
Total Pancreatic[2] GSH-T	11.96 ± 1.18	10.01 ± 0.45	NS
GSH-T as Related[3] to DNA Content	6.02 ± 0.66	4.91 ± 0.42	NS

1. GSH-T activity expressed as nmol glutathione conjugated/mg protein/min.
2. Total GSH-T expressed as µmol glutathione conjugated/min.
3. Total GSH-T as related to DNA content expressed as µmol glutathione conjugated/min/mg DNA.
4. NS = Not Significant.

TABLE 3-4: EFFECT OF 21 DAY RAW SOYA FLOUR (RSF) FEEDING ON PANCREATIC WEIGHT, DNA CONTENT, GSH-T ACTIVITY, TOTAL GSH-T, AND TOTAL GSH-T AS RELATED TO DNA CONTENT IN THE RAT

	T R E A T M E N T RSF	HSF	Significance[4]
Pancreas Weight (mg/100g body weight)	841.20 ± 35.38	498.66 ± 13.10	p<0.001
DNA Content (mg/pancreas)	2.62 ± 0.06	2.70 ± 0.17	NS
DNA Content (mg/100g body weight)	1.02 ± 0.06	0.85 ± 0.13	p<0.025
Pancreatic protein (mg)/ mg DNA	58.30 ± 3.81	47.77 ± 1.59	p<0.001
GSH-T Activity[1]	90.67 ± 6.23	90.20 ± 6.04	NS
Total Pancreatic[2] GSH-T	13.51 ± 0.53	11.36 ± 0.41	p<0.01
GSH-T as Related[3] to DNA Content	5.18 ± 0.24	4.29 ± 0.28	p<0.025

1. GSH-T activity expressed as nmol glutathione conjugated/mg protein/min.
2. Total GSH-T expressed as µmol glutathione conjugated/min.
3. Total GSH-T as related to DNA content expressed as µmol glutathione conjugated/min/mg DNA.
4. NS = Not Significant.

Figure 3-15: Pancreatic weight in rats after receiving RSF diet for 14 days
Control (HSF): white
RSF treated: dotted
Each bar represents mean ± SE.

PANCREATIC WEIGHT (mg/100 g body weight)
900
750
600
450
300
150
0
n=7
n=7

3.2.1.2. Pancreatic DNA Content

Unlike the observations of several previous researchers, pancreatic DNA content and pancreatic DNA content as related to body weight was not increased by the 14 day feeding regime of RSF in the diet, as detected by colorimetric techniques (Figure 3-16 and Figure 3-17).

3.2.1.3. Pancreatic GSH-T Activity and Total Pancreatic GSH-T

The GSH-T activity in the pancreas of RSF-fed rats was similar to that observed in the pancreas of those which received the HSF in the diet (Figure 3-18). Total levels of pancreatic GSH-T were also not significantly affected as compared to controls (Figure 3-19).

Pancreatic GSH-T as related to DNA content of the pancreas was similar in animals receiving either the RSF or the HSF diet (Figure 3-20).

3.2.2. Raw Soya Flour Diet for Three Weeks (Table 3-4)

Certain results of the 21 day feeding of RSF were similar to those of the 14 day experiment.

3.2.2.1. Pancreas Weight

Once again, the pancreases of rats fed a diet containing RSF were significantly increased in weight ($p<0.001$) over those of rats fed a diet with HSF (Figure 3-21). The increase in pancreatic weight in the experimental animals was approximately 69% over controls.

Figure 3-16: Pancreatic DNA content in rats after receiving RSF diet for 14 days

Control (HSF): white

RSF treated: dotted

Each bar represents mean ± SE.

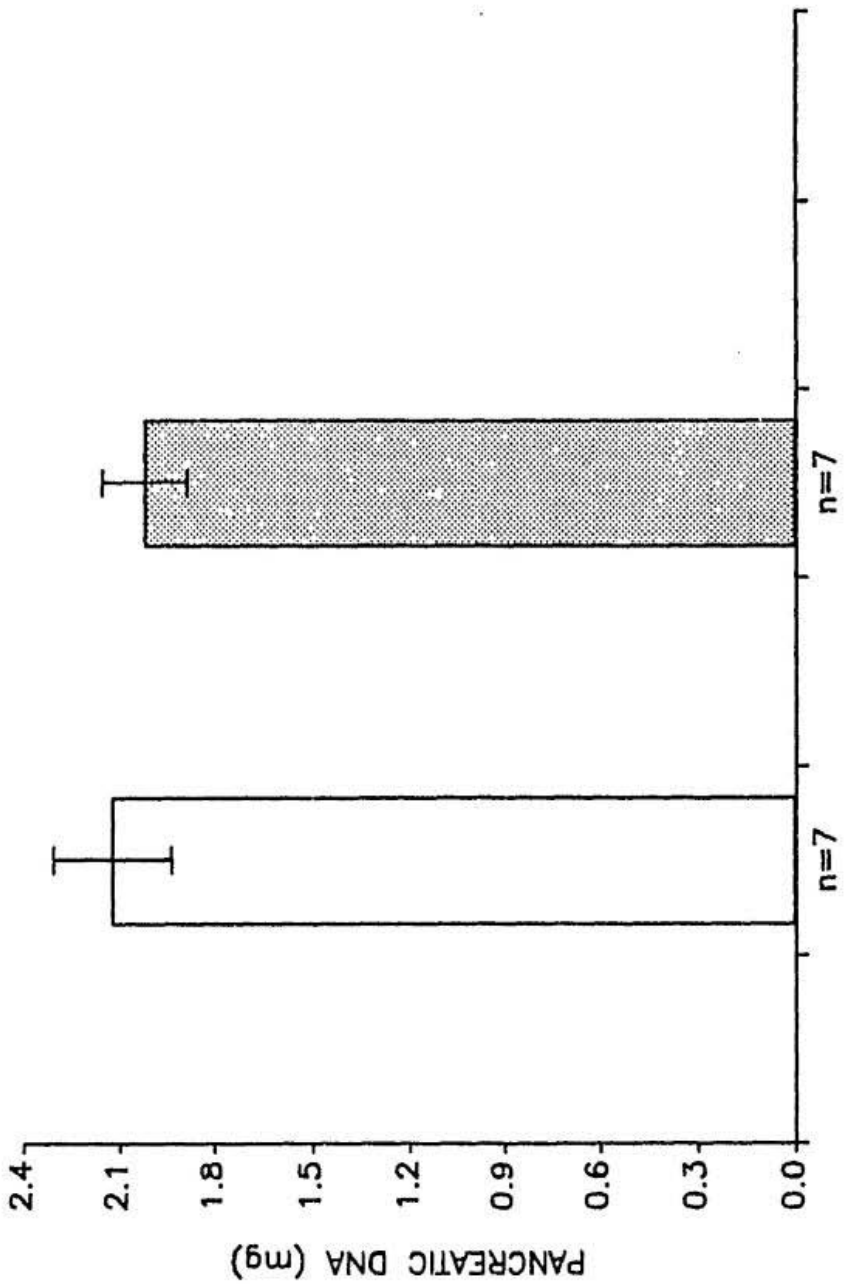

PANCREATIC DNA (mg)
2.4
2.1
1.8
1.5
1.2
0.9
0.6
0.3
0.0
n=7
n=7

Figure 3-17: Pancreatic DNA content in rats as related to body weight after receiving RSF diet for 14 days

Control (HSF): white

RSF treated: dotted

Each bar represents mean ± SE.

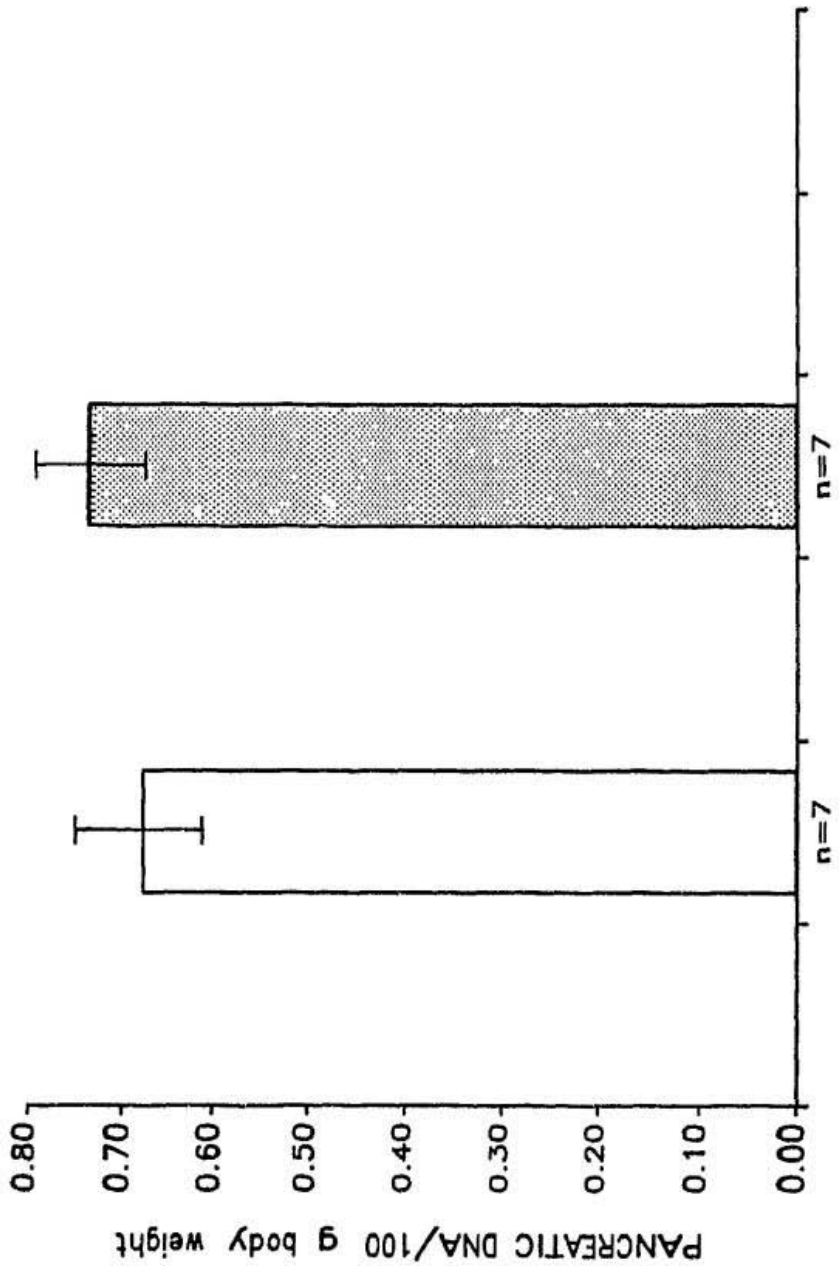

PANCREATIC DNA/100 g body weight
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
n=7
n=7

Figure 3-18: Pancreatic GSH-T activity in rats after receiving RSF diet for 14 days
Control (HSF): white
RSF treated: dotted
Each bar represents mean ± SE.

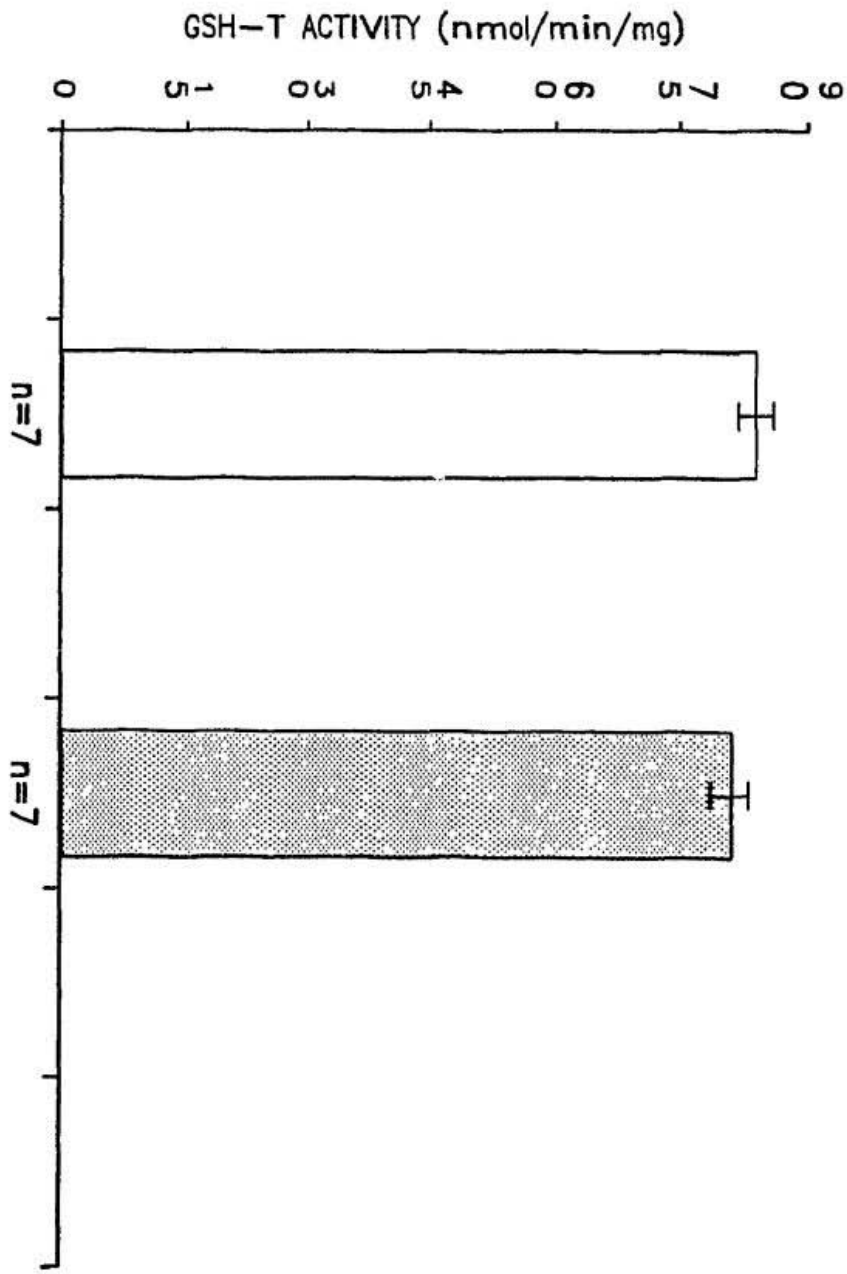

GSH—T ACTIVITY (nmol/min/mg)
0
15
30
45
60
75
90
n=7
n=7

Figure 3-19: Total pancreatic GSH-T in rats after receiving RSF diet for 14 days
Control (HSF): white
RSF treated: dotted
Each bar represents mean ± SE.

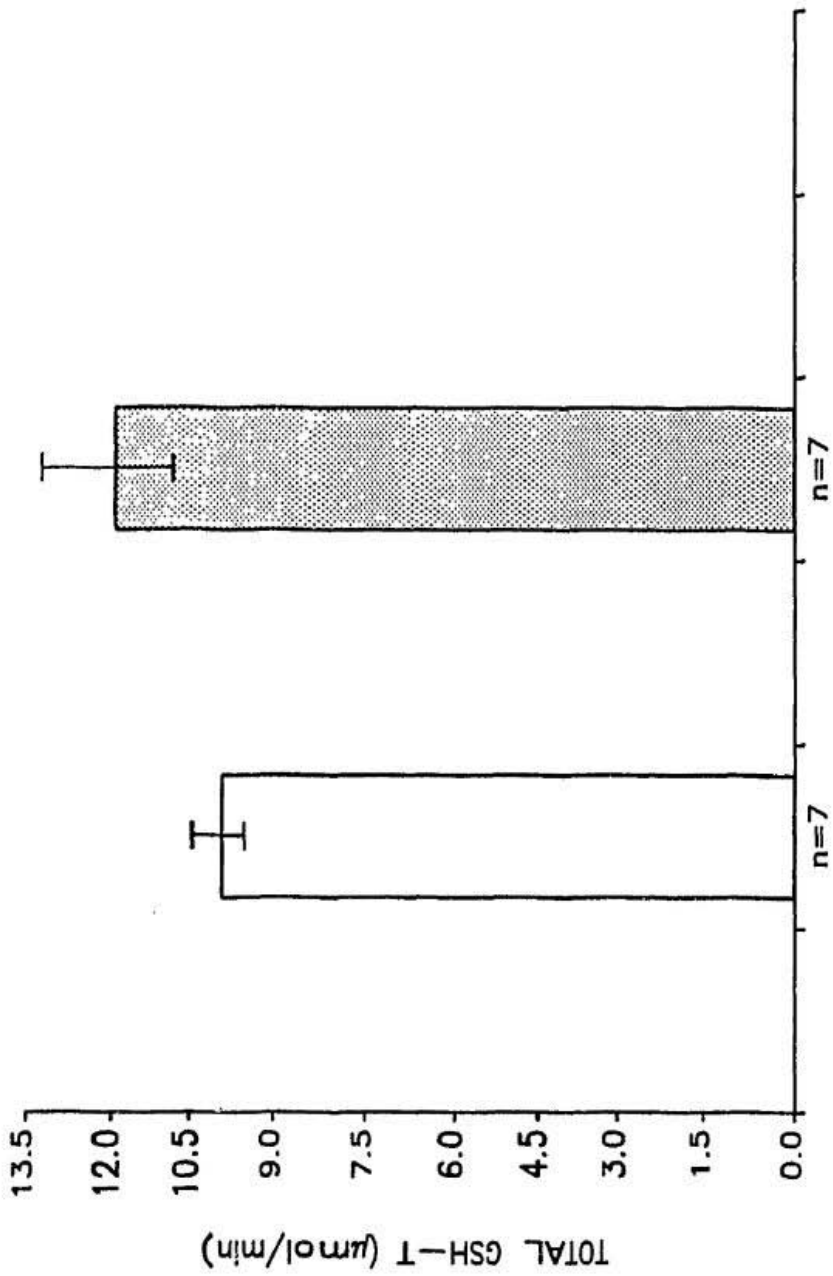

TOTAL GSH–T (μmol/min)
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0.0
n=7
n=7

Figure 3-20: Total pancreatic GSH-T as compared to DNA content in rats receiving RSF for 14 days
Control (HSF): white
RSF treated: dotted
Each bar represents mean ± SE.

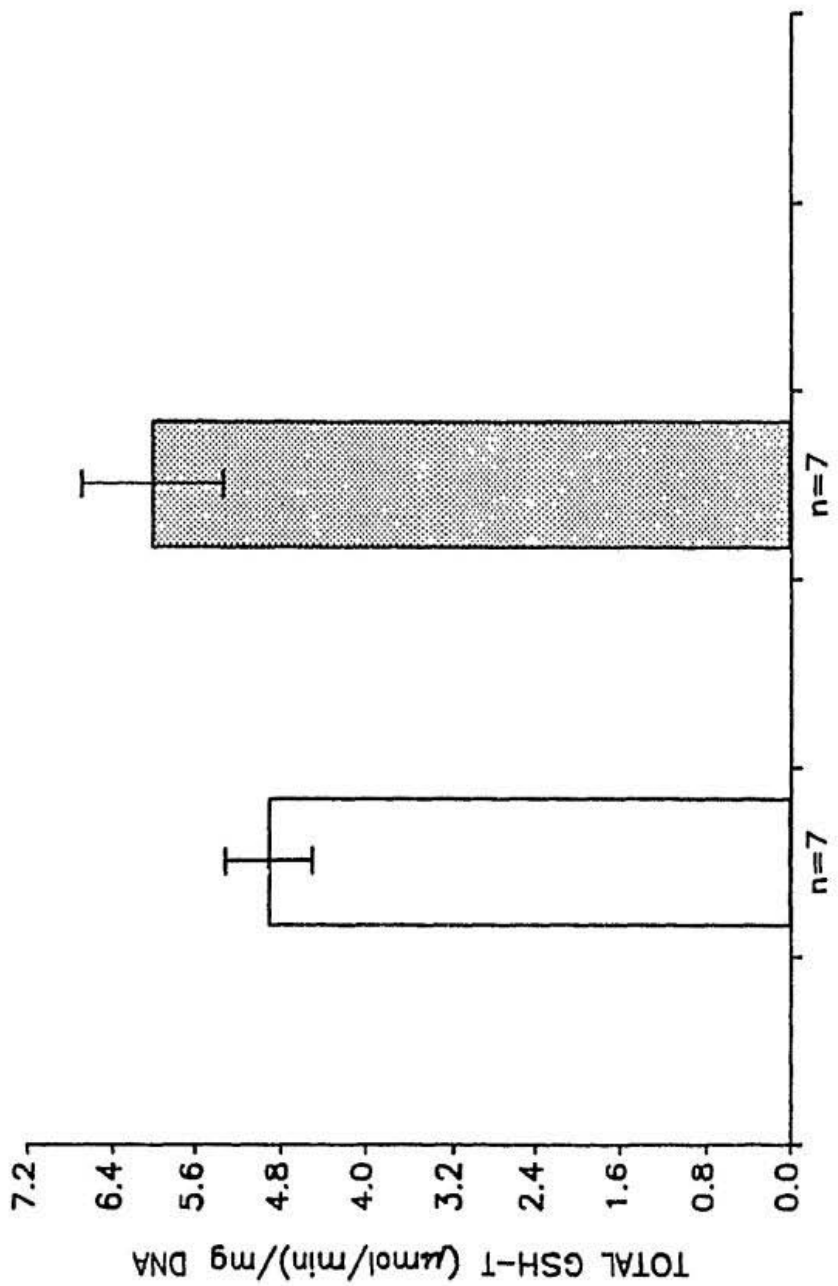

TOTAL GSH-T (μmol/min)/mg DNA
0.0
0.8
1.6
2.4
3.2
4.0
4.8
5.6
6.4
7.2
n=7
n=7

Figure 3-21: Pancreatic weight in rats after receiving RSF diet for 21 days
Control (HSF): white
RSF treated: dotted
Each bar represents mean ± SE.

PANCREATIC WEIGHT (mg/100 g body weight)
900
750
600
450
300
150
0
n=7
n=7

3.2.2.2. Pancreatic DNA Content

Pancreatic DNA content remained unchanged from control values in rats receiving the RSF diet (Figure 3-22). However, DNA content as related to body weight was significantly increased over controls, with $p<0.025$ (Figure 3-23).

3.2.2.3. Pancreatic GSH-T Activity and Total Pancreatic GSH-T

The GSH-T activity in the pancreas of rats fed RSF in the diet diet for 21 days was unaffected by this inclusion and was virtually identical to the activity observed in the HSF-fed animals (Figure 3-24). However, total levels of GSH-T in the hamster pancreas were significantly increased ($p<0.01$) after a 21 day feeding of RSF as compared with controls (Figure 3-25). This amounted to a 19% increase in the experimental pancreas.

Finally, total pancreatic GSH-T as related to DNA content of the pancreas was significantly increased ($p<0.025$) in hamsters which had received the RSF diet for 21 days (Figure 3-26). This was a 21% increase over the control pancreas.

3.3. Effect of FOY-305 on Pancreatic Weight, DNA Content, Pancreatic GSH-T Activity, Total Pancreatic GSH-T, and GSH-T as Related to DNA Content in the Hamster (Table 3-5)

Figure 3-22: Pancreatic DNA content in rats after receiving RSF diet for 21 days
Control (HSF): white
RSF treated: dotted
Each bar represents mean ± SE.

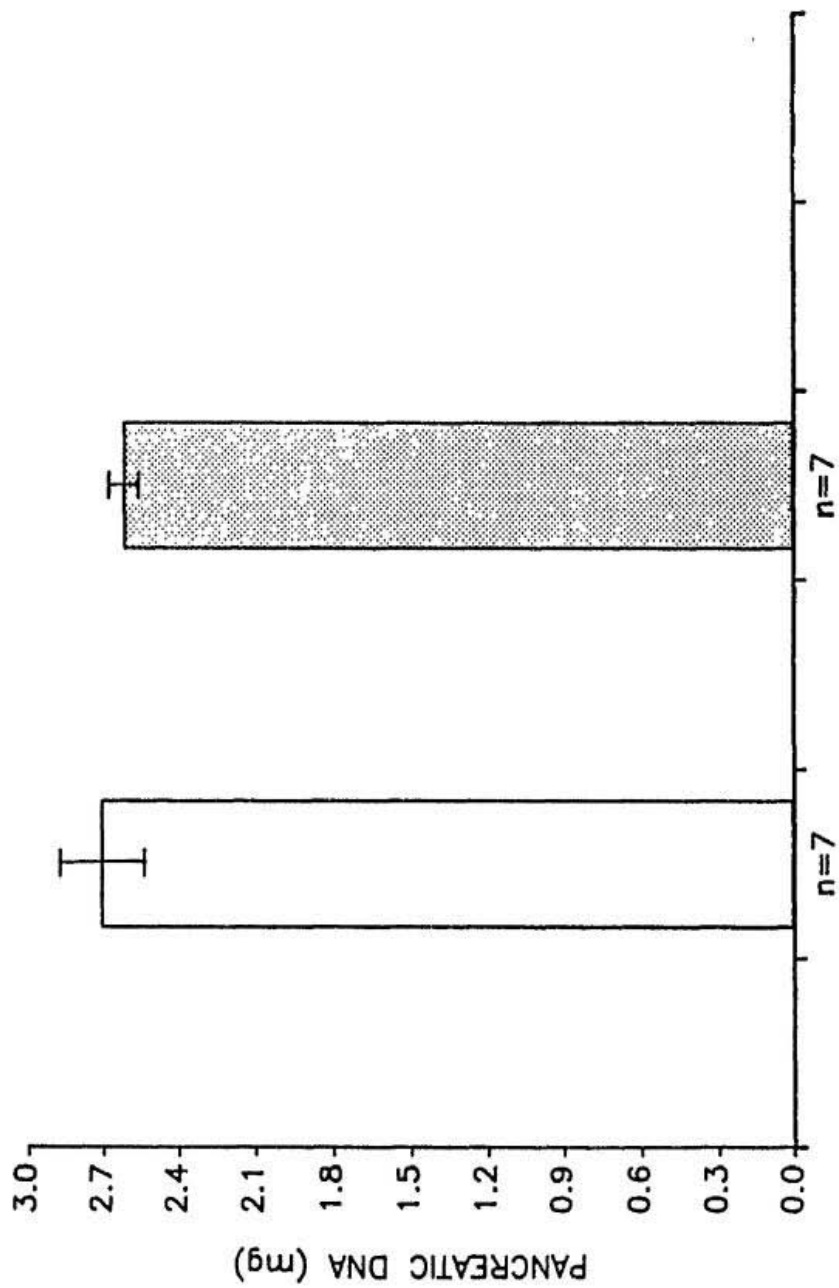

PANCREATIC DNA (mg)
3.0
2.7
2.4
2.1
1.8
1.5
1.2
0.9
0.6
0.3
0.0
n=7
n=7

Figure 3-23: Pancreatic DNA content in rats as related to body weight after receiving RSF diet for 21 days

Control (HSF): white

RSF treated: dotted

Each bar represents mean ± SE.

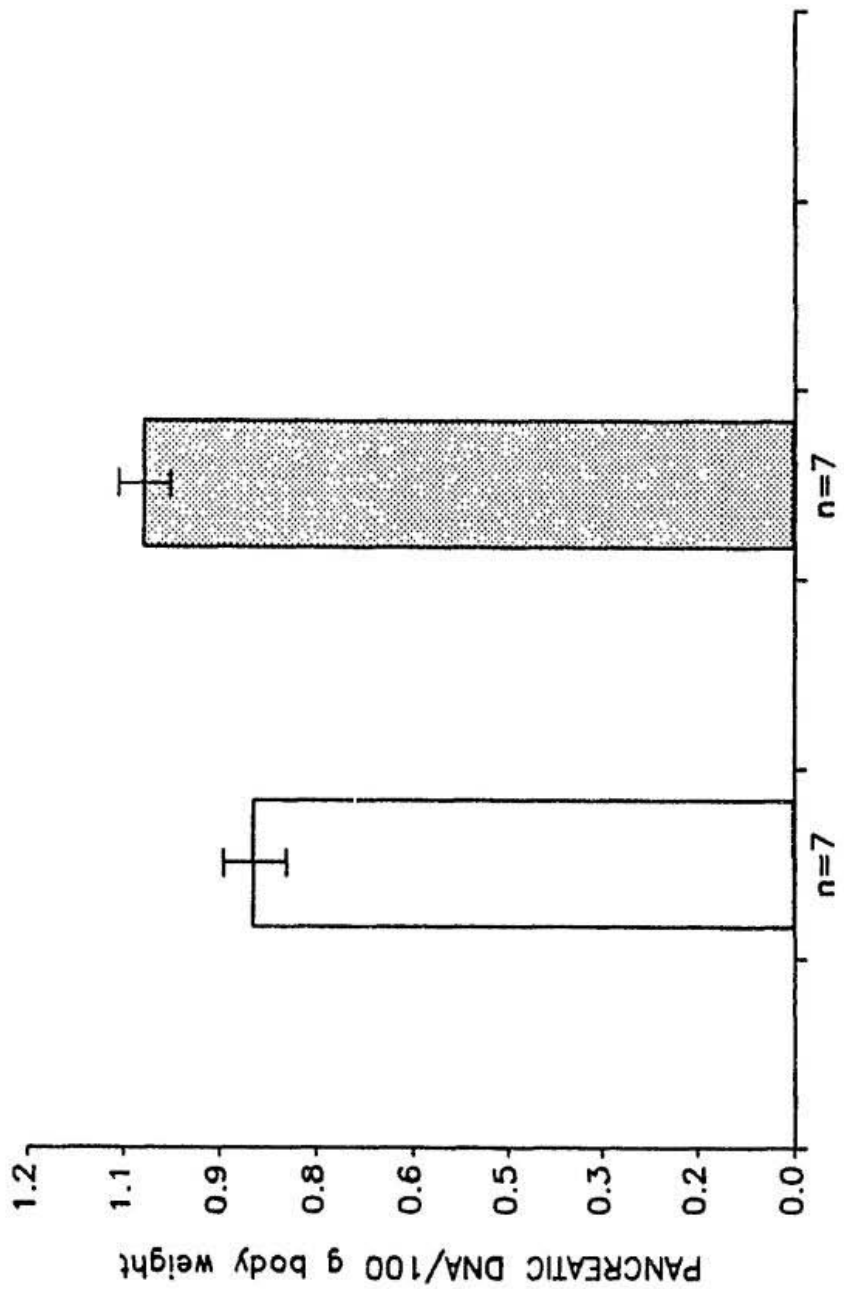

PANCREATIC DNA/100 g body weight
1.2
1.1
0.9
0.8
0.6
0.5
0.3
0.2
0.0
n=7
n=7

Figure 3-24: Pancreatic GSH-T activity in rats after receiving RSF diet for 21 days
Control (HSF): white
RSF treated: dotted
Each bar represents mean ± SE.

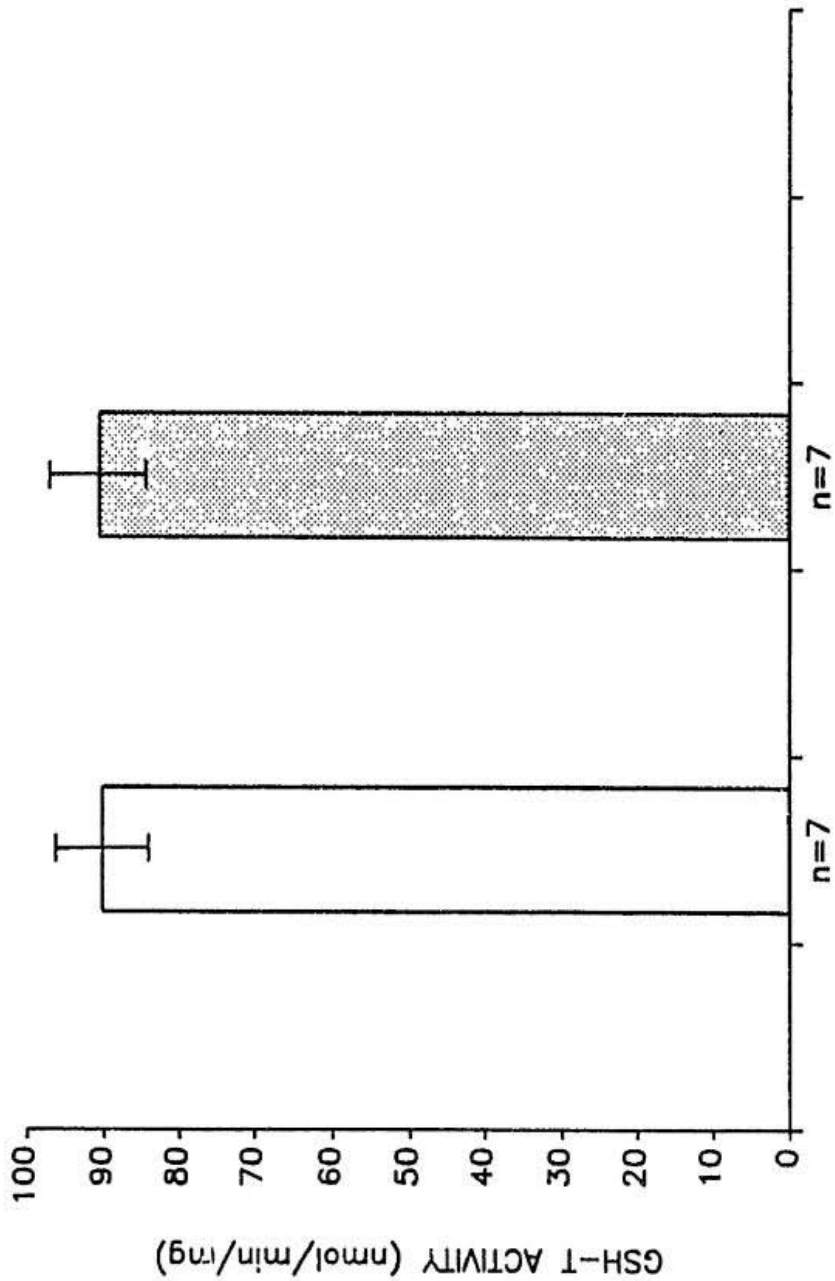

GSH-T ACTIVITY (nmol/min/mg)
100
90
80
70
60
50
40
30
20
10
0
n=7
n=7

Figure 3-25: Total pancreatic GSH-T in rats after receiving RSF diet for 21 days
Control (HSF): white
RSF treated: dotted
Each bar represents mean ± SE.

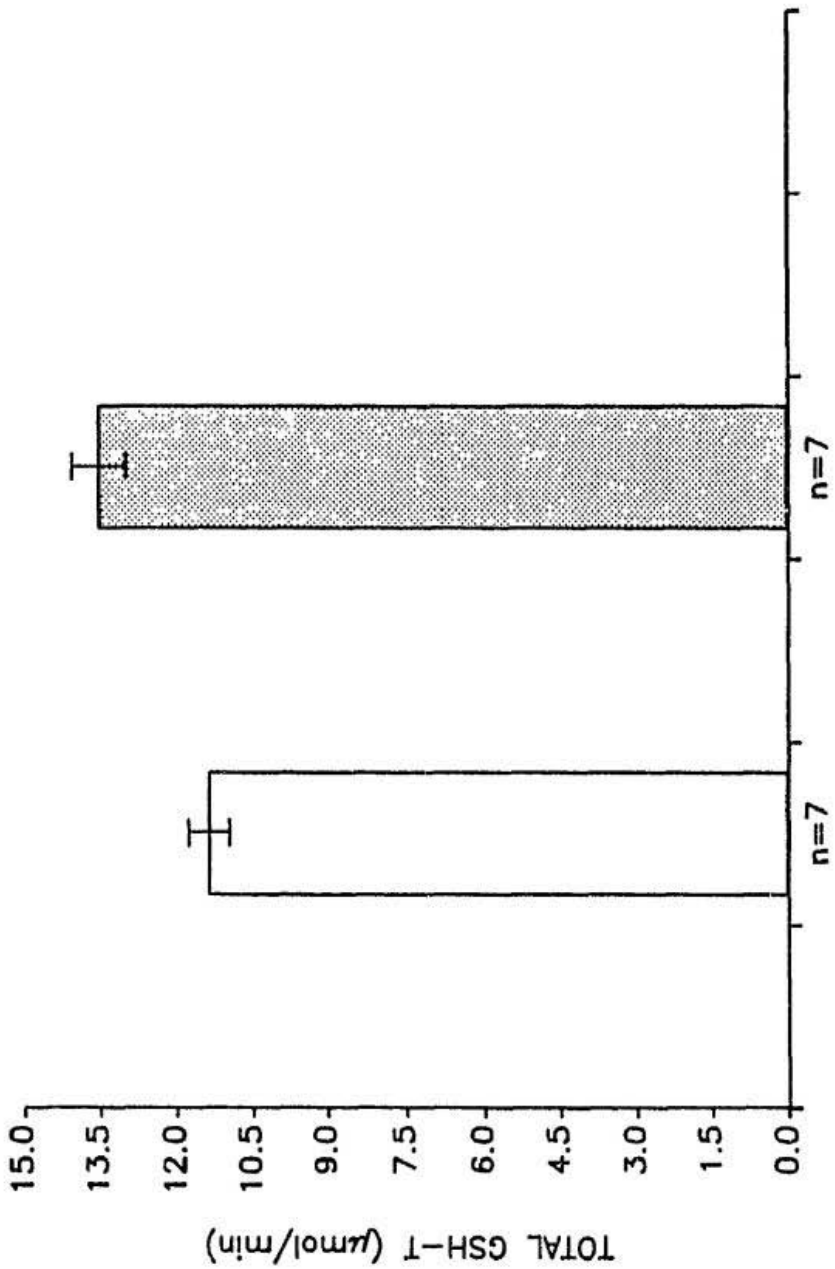

TOTAL GSH-T (μmol/min)
15.0
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0.0
n=7
n=7

Figure 3-26: Total pancreatic GSH-T as compared to DNA content in rats receiving RSF diet for 21 days

Control (HSF): white
RSF treated: dotted
Each bar represents mean ± SE.

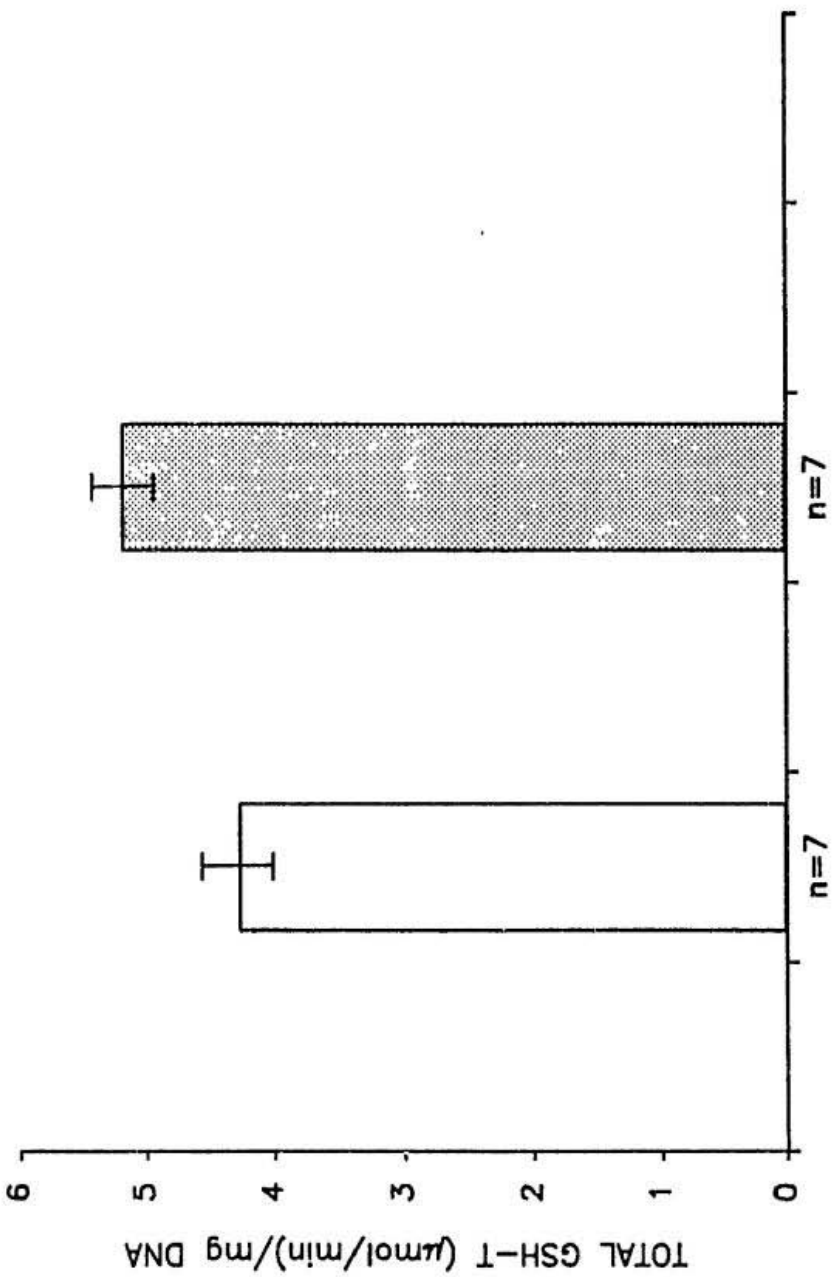

TOTAL GSH-T (μmol/min)/mg DNA
0
1
2
3
4
5
6
n=7
n=7

TABLE 3-5: EFFECT OF FOY-305 ON PANCREATIC WEIGHT, DNA CONTENT, GSH-T ACTIVITY, TOTAL GSH-T, AND TOTAL GSH-T AS RELATED TO DNA CONTENT IN THE HAMSTER

	TREATMENT		Significance[4]
	FOY-305	WATER	
Pancreas Weight (mg/100g body weight)	609.96 ± 11.36	465.63 ± 17.95	p<0.001
DNA Content (mg/pancreas)	1.26 ± 0.06	1.07 ± 0.06	p<0.05
GSH-T Activity[1]	244.38 ± 9.26	302.58 ± 24.45	p<0.025
Total Pancreatic[2] GSH-T	6.70 ± 0.37	7.53 ± 0.51	NS
GSH-T as Related[3] to DNA Content	6.04 ± 0.41	6.36 ± 0.33	NS

1. GSH-T activity expressed as nmol glutathione conjugated/mg protein/min.
2. Total GSH-T expressed as µmol glutathione conjugated/min.
3. Total GSH-T as related to DNA content expressed as µmol glutathione conjugated/min/mg DNA.
4. NS = Not Significant.

3.3.0.1. Pancreas Weight

As documented by previous researchers (Yonezawa, 1983; Goke, *et al.*, 1986; Wereszczynska-Siemiatkowska, *et al.*, 1987), pancreas weight in hamsters receiving FOY-305 (suspended in deionized water) by gavage, was significantly increased ($p<0.001$) over those animals receiving only water (Figure 3-27). This represented a 31% increase in pancreatic weight in the Foy-305 group.

3.3.0.2. Pancreatic DNA Content

The amount of DNA in the pancreases of hamsters gavaged with FOY-305 was significantly increased ($p<0.05$) as compared with those animals which got only water (Figure 3-28). This was a 17% increase over the control group. DNA content as related to body weight was also significantly increased over controls with $p<0.001$ (Figure 3-29).

3.3.0.3. Pancreatic GSH-T Activity and Total Pancreatic GSH-T

The GSH-T activity in the pancreas of hamsters administered FOY-305 was significantly reduced ($p<0.025$) as compared with the activity of the enzymes in the control pancreas (Figure 3-30). This was a 24% reduction from control values. However, the total amount of GSH-T in the hamster pancreas remained constant in both the FOY-305 group and in the control group (Figure 3-31).

Total levels of pancreatic GSH-T as related to DNA content of the pancreas was similar for both groups of hamsters (Figure 3-32).

Figure 3-27: Pancreatic weight in hamsters after receiving FOY-305 for 14 days
Control: white
FOY-305 treated: dotted
Each bar represents mean ± SE.

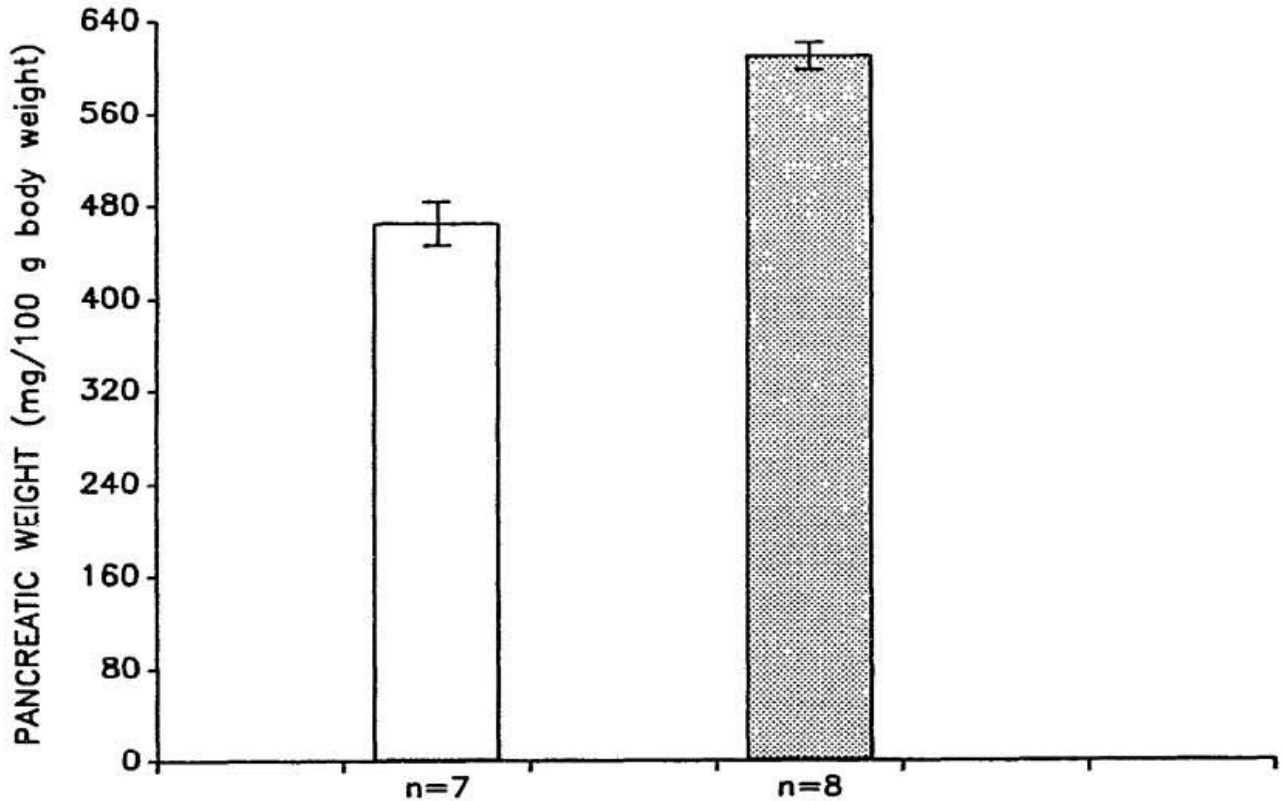

PANCREATIC WEIGHT (mg/100 g body weight)
640
560
480
400
320
240
160
80
0
n=7
n=8

Figure 3-28: Pancreatic DNA content in hamsters after receiving FOY-305 for 14 days
Control: white
FOY-305 treated: dotted
Each bar represents mean ± SE.

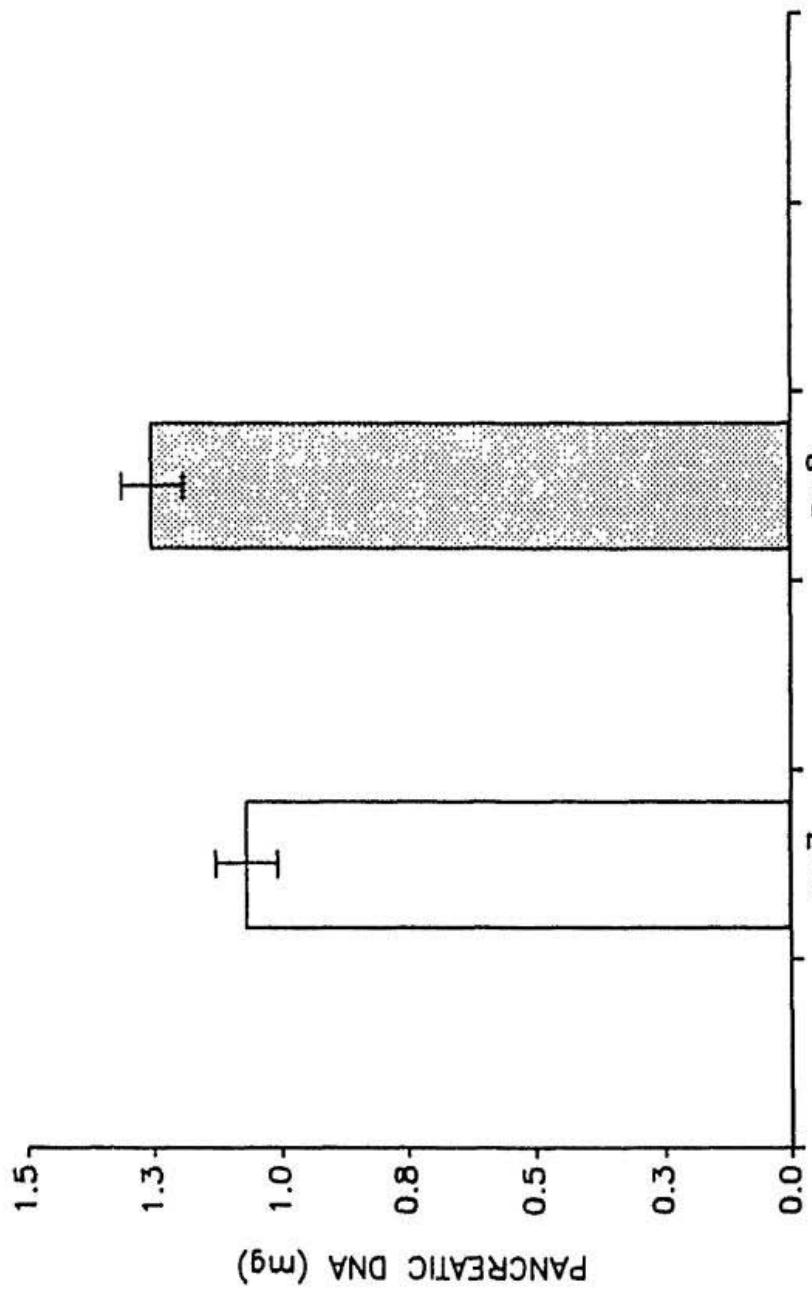

PANCREATIC DNA (mg)
1.5
1.3
1.0
0.8
0.5
0.3
0.0
n=7
n=8

Figure 3-29: Pancreatic DNA content in hamsters as related to body weight after receiving FOY-305 for 14 days

Control: white

Foy-305 treated: dotted

Each bar represents mean ± SE.

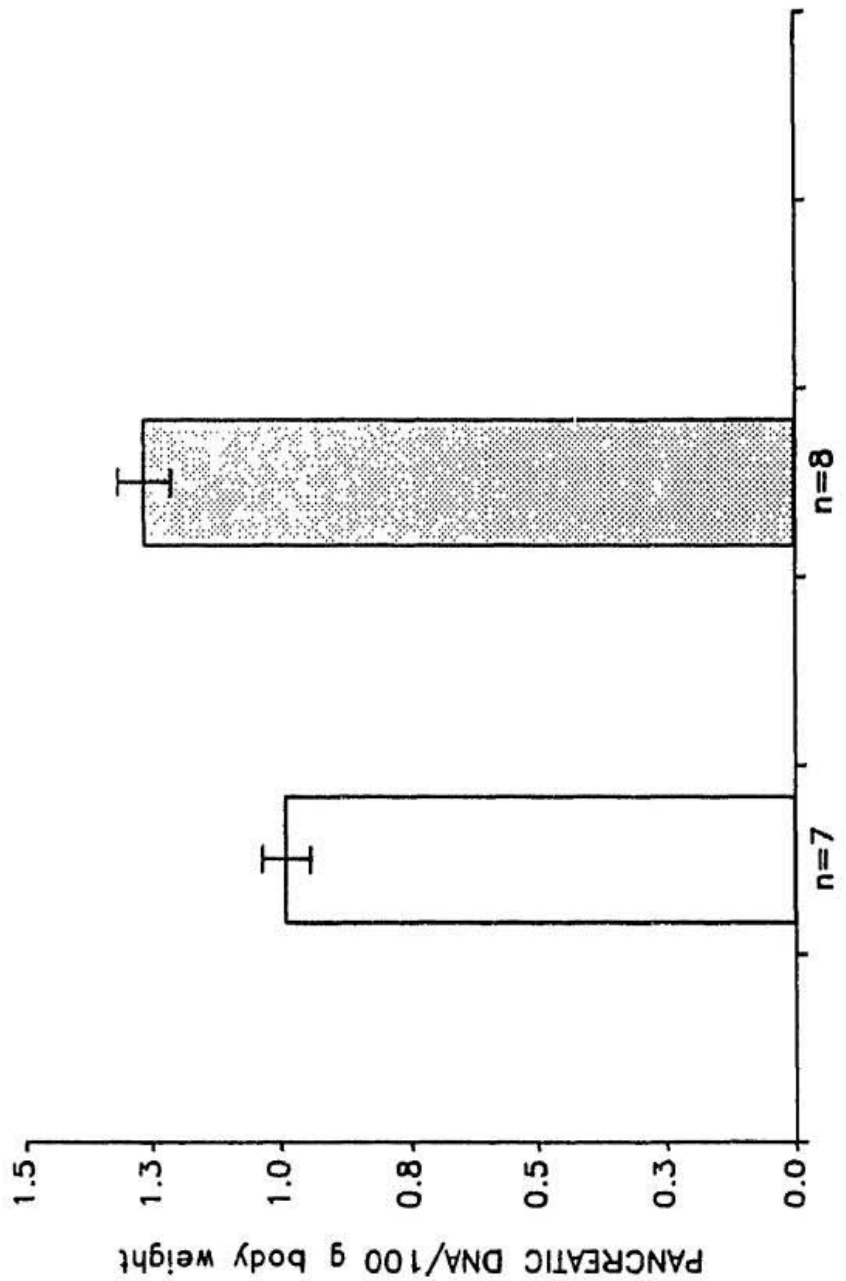

PANCREATIC DNA/100 g body weight
1.5
1.3
1.0
0.8
0.5
0.3
0.0
n=7
n=8

Figure 3-30: Pancreatic GSH-T activity in hamsters after receiving FOY-305 for 14 days
Control: white
FOY-305 treated: dotted
Each bar represents mean ± SE.

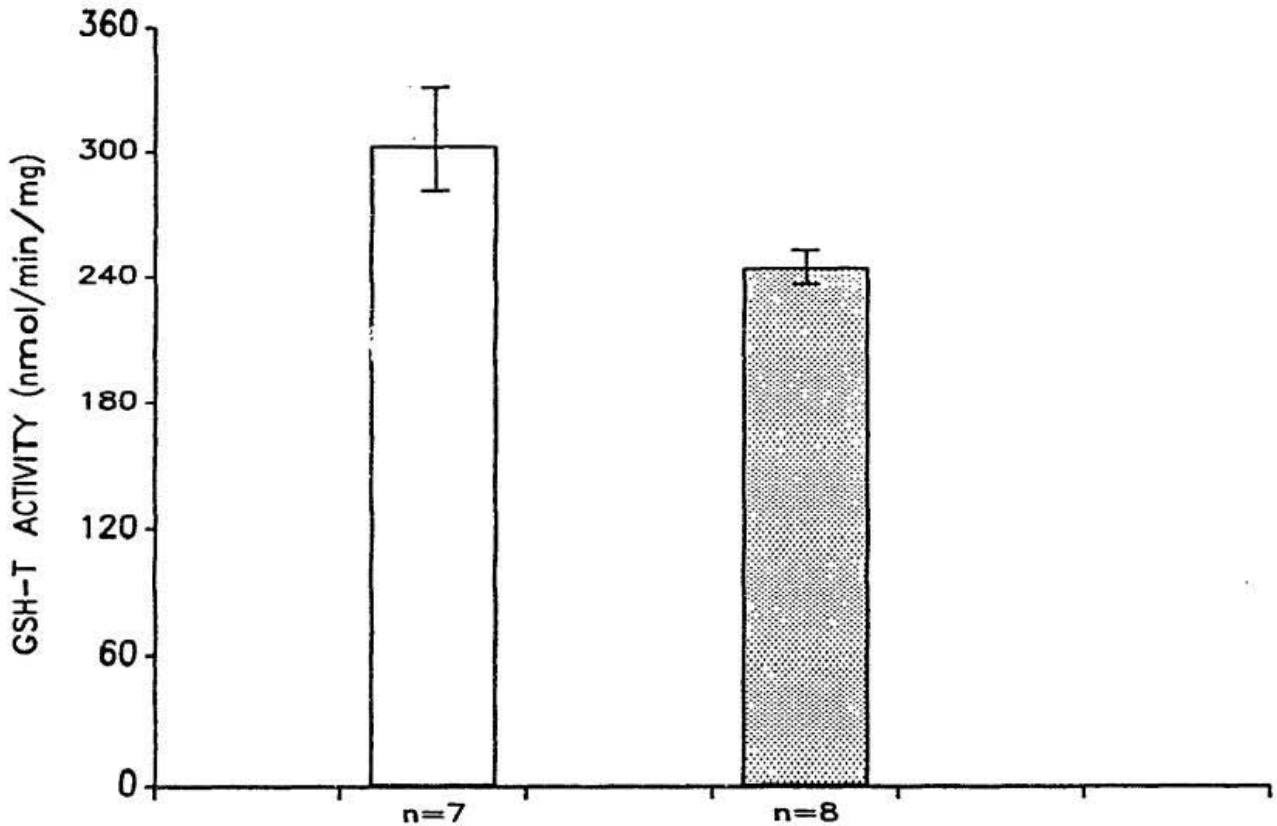

360
300
240
180
120
60
0
GSH-T ACTIVITY (nmol/min/mg)
n=7
n=8

Figure 3-31: Total pancreatic GSH-T in hamsters after receiving FOY-305 for 14 days
Control: white
FOY-305 treated: dotted
Each bar represents mean ± SE.

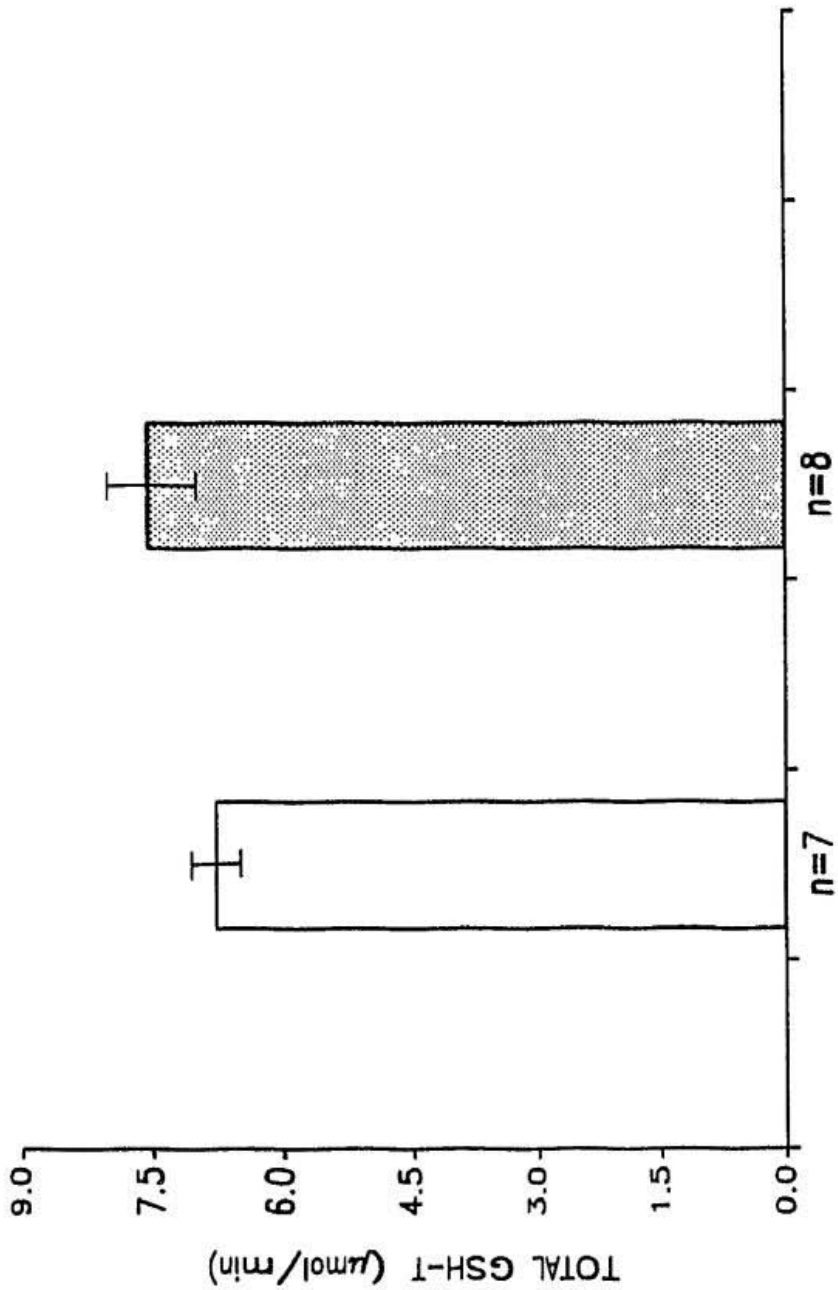
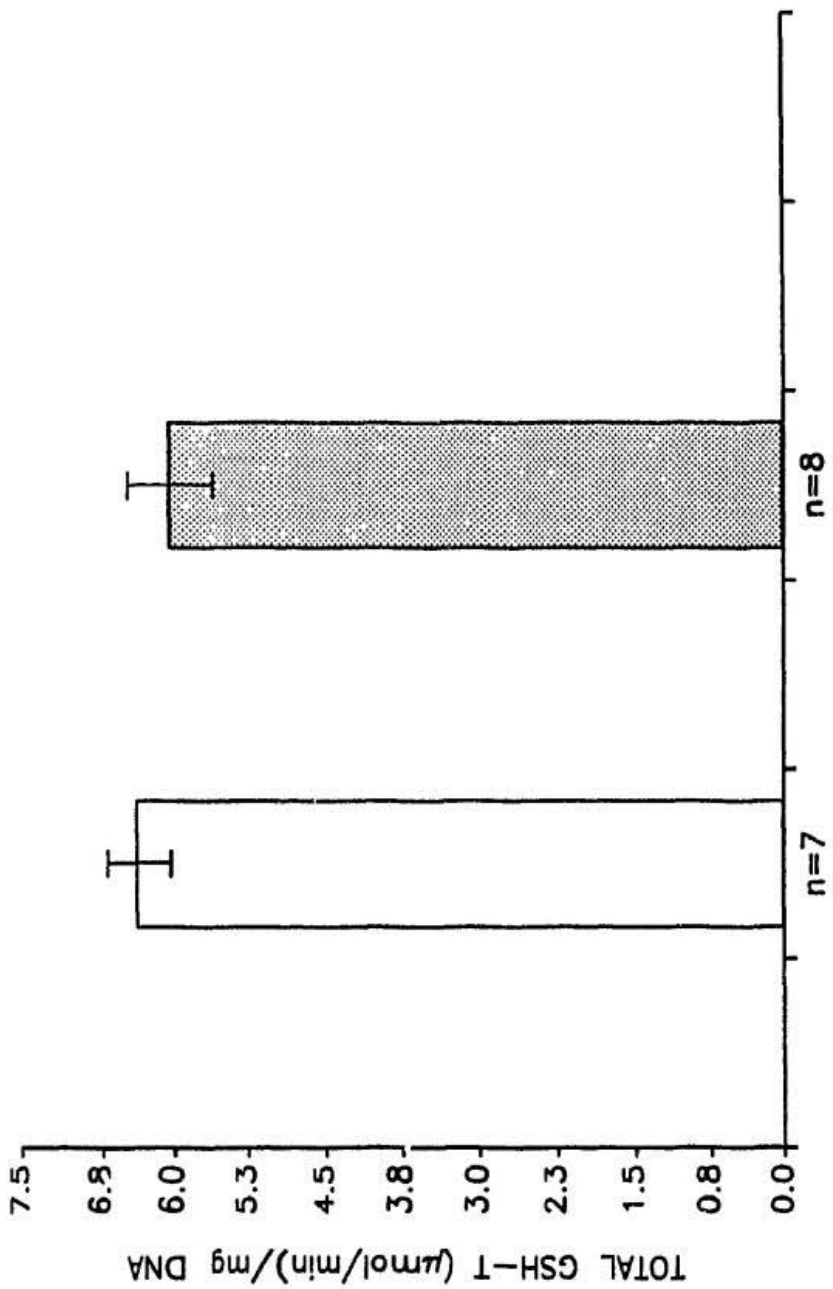

TOTAL GSH-T (μmol/min)
9.0
7.5
6.0
4.5
3.0
1.5
0.0
n=7
n=8

Figure 3-32: Total pancreatic GSH-T as compared to DNA content in hamsters after receiving FOY-305 for 14 days

Control: white

FOY-305 treated: dotted

Each bar represents mean ± SE.


TOTAL GSH-T (μmol/min)/mg DNA
7.5
6.8
6.0
5.3
4.5
3.8
3.0
2.3
1.5
0.8
0.0
n=7
n=8

3.4. Effect of L-364,718 on Pancreatic Weight, DNA Content, Pancreatic GSH-T Activity, Total Pancreatic GSH-T, and GSH-T as Related to DNA Content of the Hamster (Table 3-6)

3.4.0.1. Pancreas Weight

Unlike one previous report (Zucker, *et al.*, 1988), there was no significant decrease in pancreatic weight in those animals which had received L-364,718 (dissolved in 4% DMSO) by gavage, as compared with the control pancreas weights (Figure 3-33).

3.4.0.2. Pancreatic DNA Content

Pancreatic DNA content of both L-364,718 animals and those animals which were gavaged with 4% DMSO was identical (Figure 3-34), as was pancreatic DNA as related to body weight (Figure 3-35).

3.4.0.3. Pancreatic GSH-T Activity and Total Pancreatic GSH-T

Once again, there was no difference on pancreatic GSH-T activity of animals which received L-364,718 as compared with controls (Figure 3-36). This same observation held true for the total GSH-T levels in the pancreas of the two groups (Figure 3-37).

Like the preceeding results concerning L-364,718, pancreatic GSH-T levels as related to DNA content of the pancreas were also not significantly reduced from the levels present in animals gavaged with 4% DMSO (Figure 3-38).

TABLE 3-6: EFFECT OF L-364,718 ON PANCREATIC WEIGHT, DNA CONTENT, GSH-T ACTIVITY, TOTAL GSH-T, AND TOTAL GSH CONTENT AS RELATED TO DNA CONTENT IN THE HAMSTER

	TREATMENT		Significance[4]
	L-364,718	4% DMSO	
Pancreas Weight (mg/100g body weight)	486.80 ± 12.13	497.36 ± 15.15	NS
DNA Content (mg/pancreas)	0.92 ± 0.05	0.92 ± 0.05	NS
GSH-T Activity[1]	511.90 ± 79.31	535.28 ± 45.54	NS
Total Pancreatic[2] GSH-T	6.17 ± 0.46	7.28 ± 0.35	NS
GSH-T as Related[3] to DNA Content	7.23 ± 0.69	8.03 ± 1.17	NS

1. GSH-T activity expressed as nmol glutathione conjugated/mg protein/min.
2. Total GSH-T expressed as μmol glutathione conjugated/min.
3. Total GSH-T as related to DNA content expressed as μmol glutathione conjugated/min/mg DNA.
4. NS = Not Significant.

Figure 3-33: Pancreatic weight in hamsters after receiving L-364,718 for 14 days
Control: white
L-364,718 treated: dotted
Each bar represents mean ± SE.

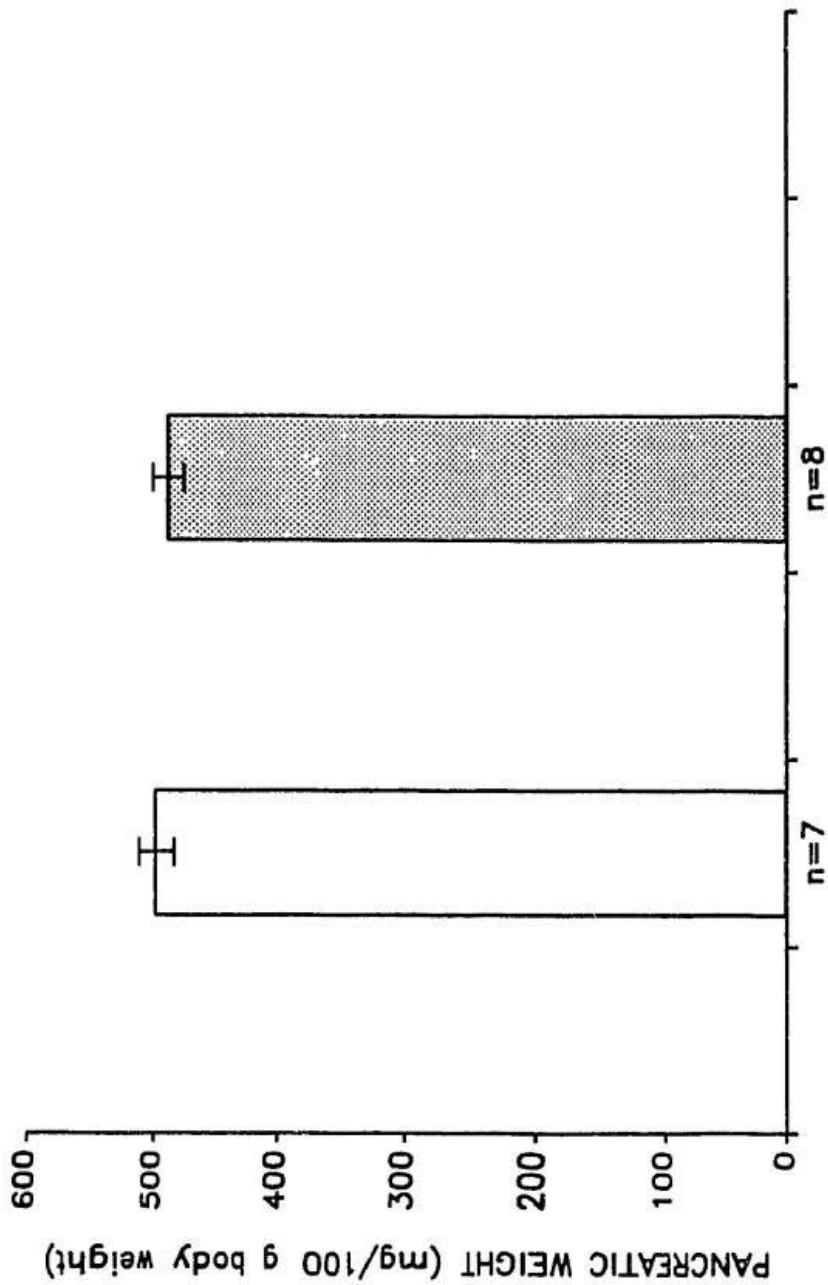

PANCREATIC WEIGHT (mg/100 g body weight)
600
500
400
300
200
100
0
n=7
n=8

Figure 3-34: Pancreatic DNA content in hamsters after receiving L-364,718 for 14 days
Control: white
L-364,718 treated: dotted
Each bar represents mean ± SE.

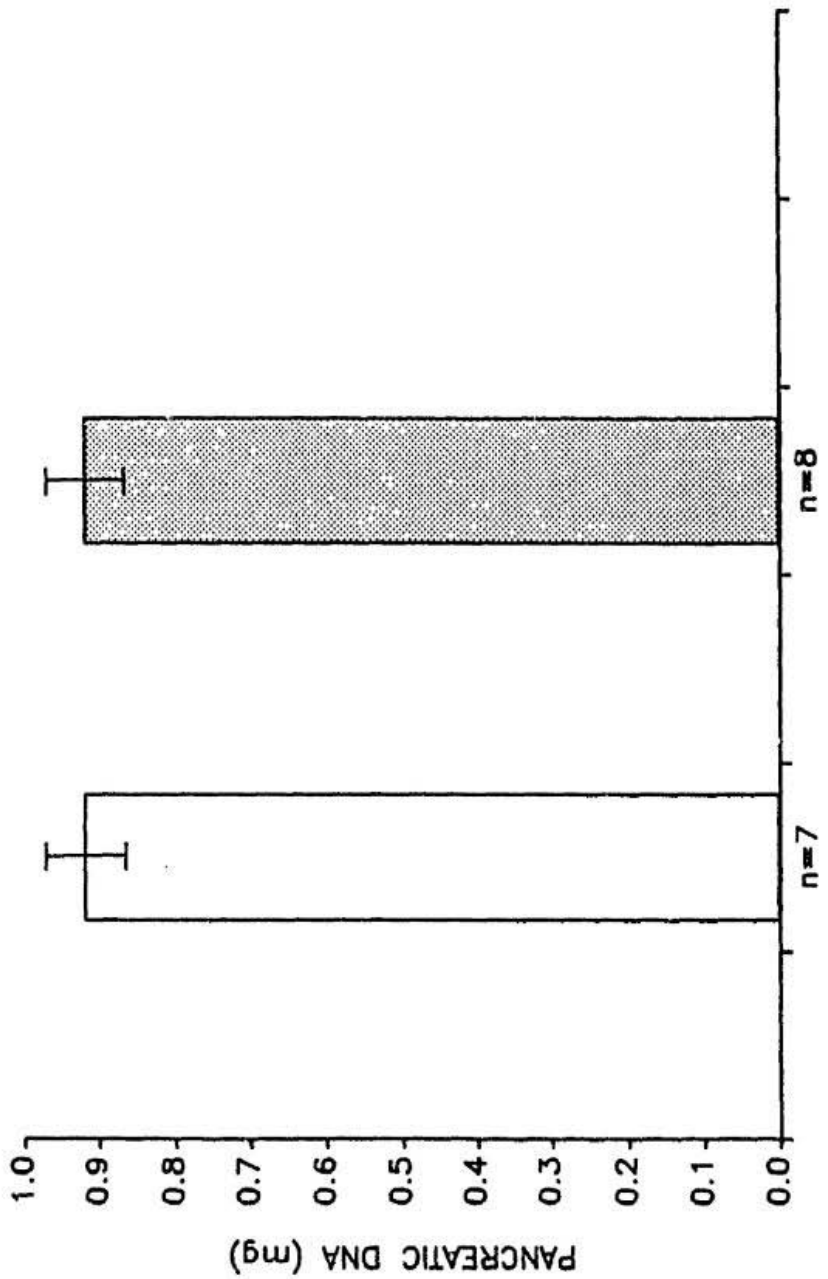

PANCREATIC DNA (mg)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
n=7
n=8

Figure 3-35: Pancreatic DNA content in hamsters as related to body weight after receiving L-364,718 for 14 days

Control: white

L-364,718 treated: dotted

Each bar represents mean ± SE.

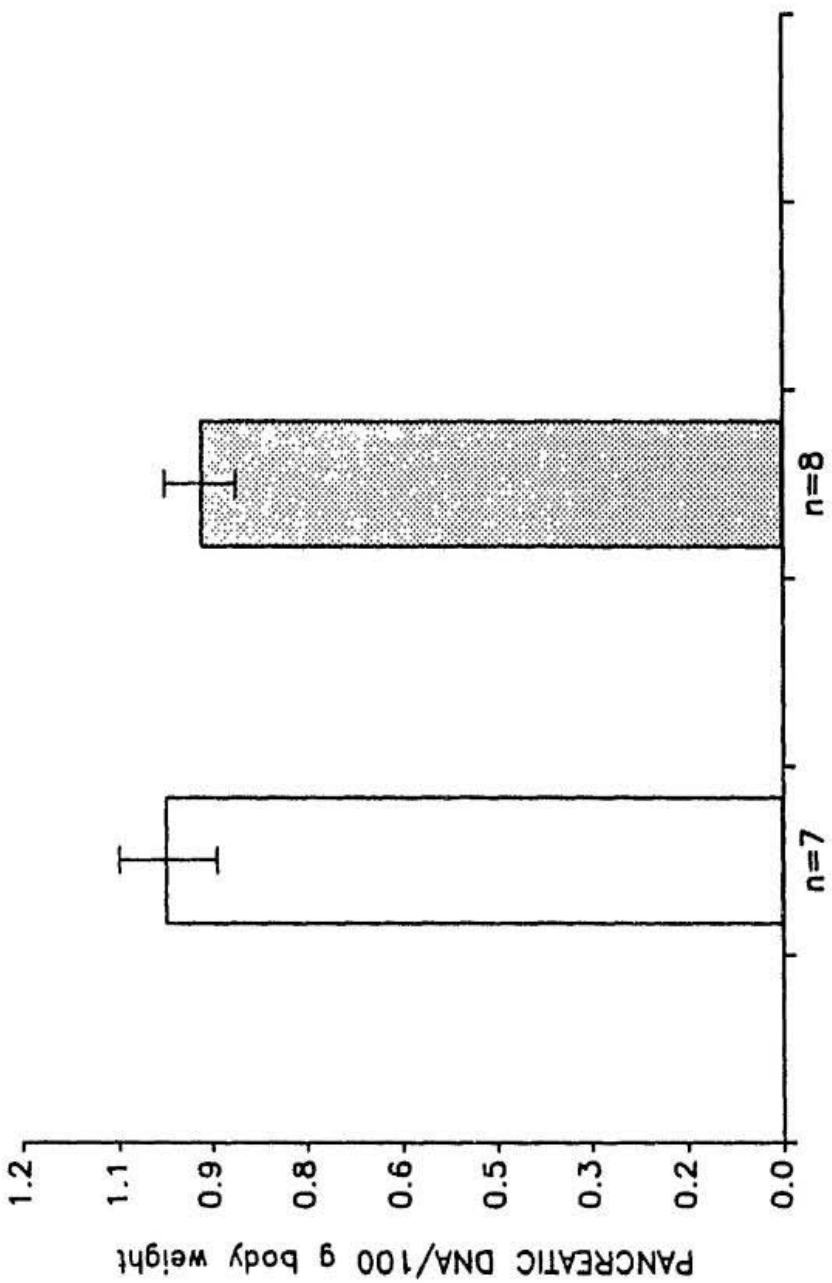

PANCREATIC DNA/100 g body weight
1.2
1.1
0.9
0.8
0.6
0.5
0.3
0.2
0.0
n=7
n=8

Figure 3-36: Pancreatic GSH-T activity in hamsters after receiving L-364,718 for 14 days
Control: white
L-364,718 treated: dotted
Each bar represents mean ± SE.

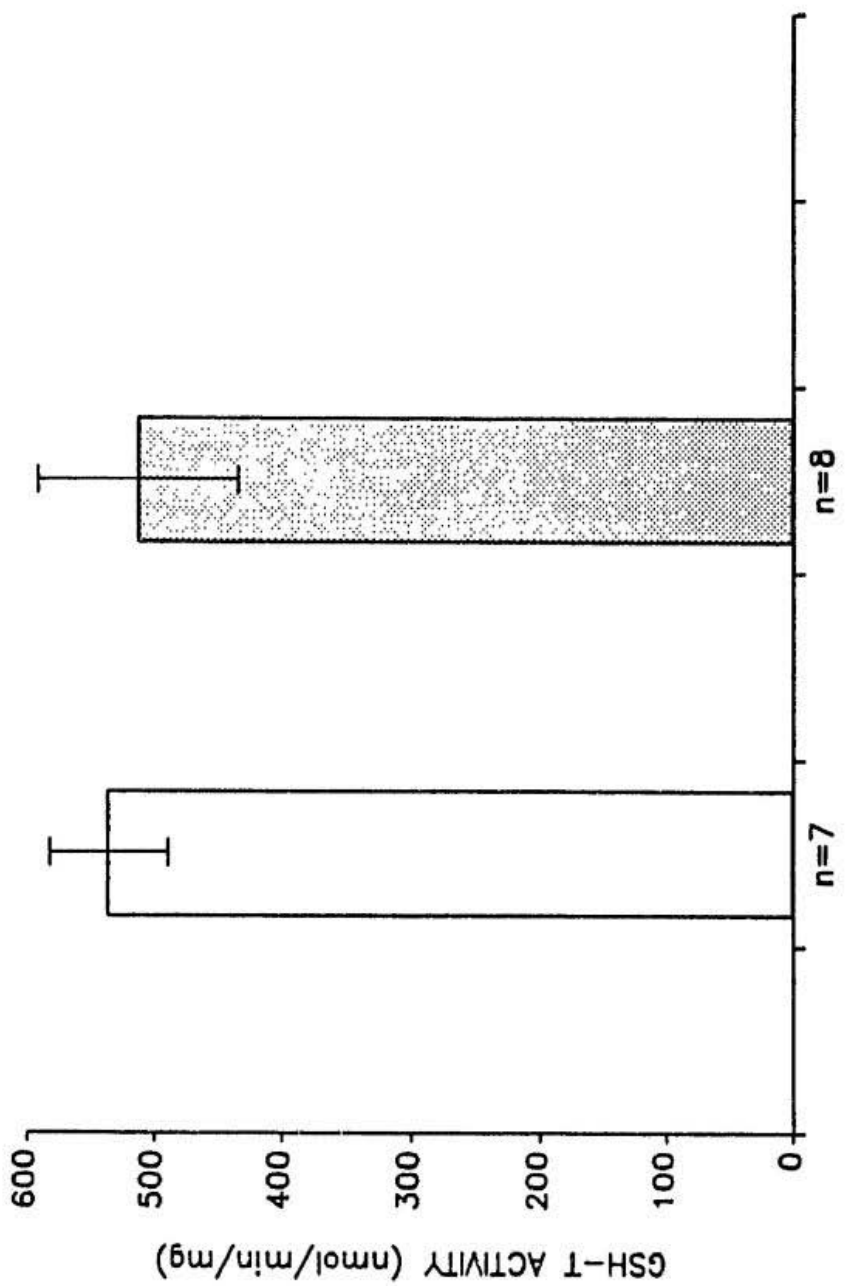

GSH-T ACTIVITY (nmol/min/mg)
600
500
400
300
200
100
0
n=7
n=8

Figure 3-37: Total pancreatic GSH-T in hamsters after receiving L-364,718 for 14 days
Control: white
L-364,718 treated: dotted
Each bar represents mean ± SE.

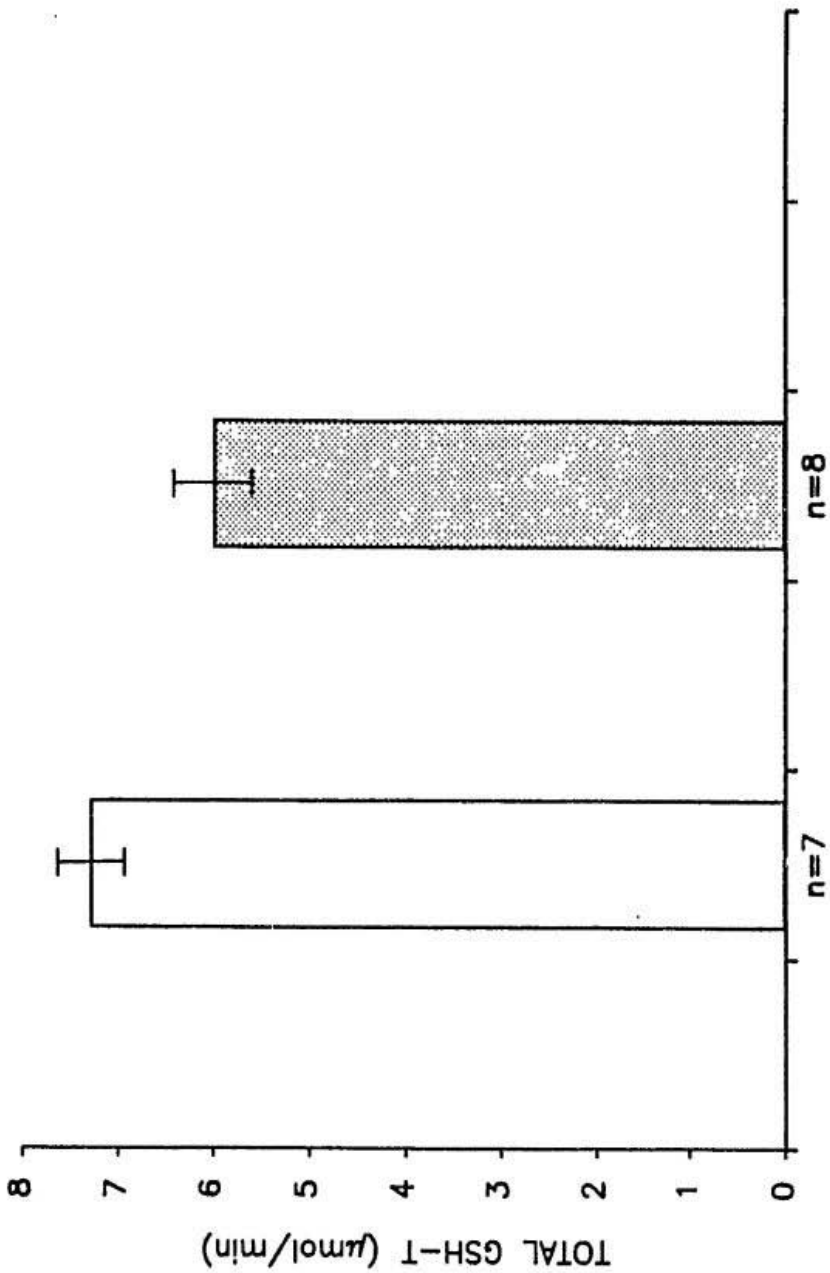
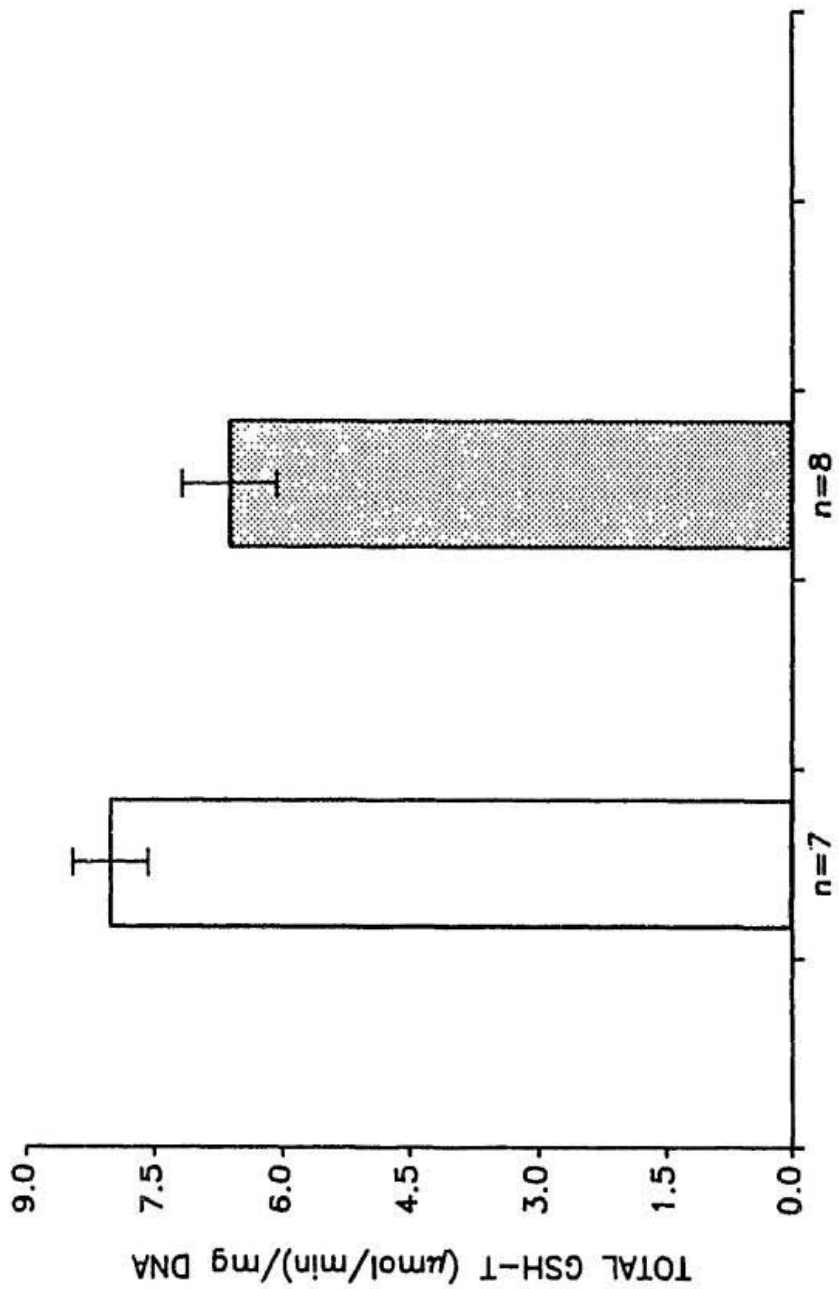

TOTAL GSH-T (μmol/min)
0
1
2
3
4
5
6
7
8
n=7
n=8

Figure 3-38: Total pancreatic GSH-T as compared to DNA content in hamsters after receiving L-364,718 for 14 days

Control: white

L-364,718 treated: dotted

Each bar represents mean ± SE.


TOTAL GSH-T (μmol/min)/mg DNA
9.0
7.5
6.0
4.5
3.0
1.5
0.0
n=7
n=8

Chapter 4

Discussion

4.1. Pancreatic GSH-T Induction

4.1.1. BHA Diet

Before a rodent model for use in GSH-T induction experiments was chosen, a preliminary study was undertaken using the phenolic antioxidant BHA. As described earlier, BHA is an excellent inducer of liver (and other tissues) cytosolic GSH-T; however, with the exception of the rat, no previous work was found in which rodent e.g. hamster and mouse pancreatic GSH-T inducibility was studied. In a previous study in which rats were employed, GSH-T activity in the pancreas was not induced in response to 0.45% BHA inclusion in the diet (Roebuck, *et al.*, 1984). In the current study, our results for the rat using 0.75% BHA (Figures 3-1 and 3-2) agree with those of Roebuck, *et al.* (1984). This also held true for the mouse, where pancreatic GSH-T were not significantly responsive to treatment with BHA (Figures 3-3 and 3-4). Although there were no significant inductions in these cases, as seen in Table 3-1, there is a general trend towards induction in both GSH-T activity and in total levels of GSH-T in the pancreas of the rat and mouse. It is possible that with a longer feeding period or with an increased dosage of BHA in the diet, a significant effect may have been observed in the rat and/or mouse.

In addition, it may be suggested that although BHA does induce liver GSH-T in certain rodent species, the gene involved in coding for these enzymes in the pancreas may not be turned on by the BHA metabolites which it encounters; in the liver, the BHA molecule is probably presented to the organ, unless it is significantly metabolized by intestinal epithelial cells, whereas the pancreas receives blood after the first pass through the liver. Furthermore, in the rodents used, foreign compounds may be sequestered by many other tissues, and thus never reach the pancreas in sufficient amounts.

The data observed in the hamster were somewhat different than those of the mouse and rat. In the hamster, there was a significant increase in pancreatic GSH-T (Figures 3-5 and 3-6) and in total pancreatic GSH-T whereas there was no significant induction in the other two species. Two major reasons may be suggested for this: (1) species differences in the handling of chemicals, and; (2) the gene(s) coding for GSH-T in the hamster may be more responsive to treatment with BHA than those of the rat and mouse.

Obviously, the system is quite complex, and whatever the exact reason for induction in the hamster, these increased levels may ultimately provide a protective mechanism against xenobiotics and pancreatic carcinogens.

Once induction of pancreatic GSH-T had been established in the hamster pancreas, this model was chosen for use in further research involving attempts to modify levels of this group of enzymes in response to other previously tested compounds. These compounds were shown by various investigators to influence levels of GSH-T in tissues other than the pancreas.

4.1.2. Intragastric a-AL, GCB and COU

All three of these compounds may be addressed in one section as none had any affect on GSH-T activity or on total GSH-T in the hamster pancreas, as can be seen in Table 3-2, Figures 3-7, 3-8, 3-11, 3-12, 3-13, and 3-14.

Although α-AL given orally was found to be a good inducer of GSH-T in the mouse forestomach, lung, and esophagus (Sparnins, *et al.*, 1982a; Sparnins, *et al.*, 1982b), this did not hold true for pancreatic GSH-T. The same was the case for GCB and COU which are known to cause increases in rat liver and small intestinal mucosal GSH-T.

Once again, as in the BHA experiments, it may be suggested that, like the liver, the esophagus and forestomach are exposed to the compound or major amounts of it in the same form as it was ingested. Thus, these tissue types require mechanisms by which to protect themselves against foreign compounds. Therefore, the gene or genes which code for GSH-T in tissues most vulnerable to the actions of xenobiotics and/or potential carcinogens, may be activated by the presence of such chemicals. However, the pancreas is less likely to be exposed to the intact molecules of orally-administered α-AL, GCB, or COU; therefore only basal levels of GSH-T are found and no induction occurs. On the other hand, sequestering of the compound and its metabolites by other tissues is also a possibility, and thus the pancreas never has to deal with significant amounts of the chemical in question.

4.1.3. Subcutaneous a-AL

This experiment was carried out in an effort to present an unmetabolized compound to the pancreas. Given by subcutaneous injection, a compound reaches tissues without first being handled by the liver, although not at the full dosage. The results of this experiment demonstrated that only the total levels of GSH-T in the hamster pancreas were affected by this treatment (Table 3-2, Figures 3-9 and 3-10). This effect involved a significant reduction in total pancreatic GSH-T as compared with control animals. The reduction was the result of a decrease in cytosolic protein which was also observed in these animals. Thus, it may be concluded that subcutaneous presentation of α-AL to the pancreas in some way caused a decrease in protein synthesis by the pancreas, ultimately leading to a reduction in levels of pancreatic GSH-T.

In observing Tables 3-1 and 3-2, it may be noticed that control hamsters in the BHA experiment show significantly reduced levels of pancreatic GSH-T as compared with controls in the α-AL, GCB, or COU experiments. When an explanation for this was being contemplated, it was found that the composition of the diets may have been the important issue. The hamsters in the BHA experiment were fed a basal purified diet, alone or with BHA. However, in the other induction experiments, hamsters received Purina Rat Chow, a nonpurified feed. It has been found by other researchers, that this Rat Chow has the ability to induce GSH-T activity in both the small intestinal mucosa and the liver (Sparnins, *et al.*, 1982a). These inductions are in the order of 59% in the small intestine and 21% in the liver. Interestingly enough then, the constituents present in the feed could in fact be an answer to the question of pancreatic GSH-T

induction. One problem is however, that the actual component of the chow responsible for this effect is not known, and it would be a time consuming undertaking in order to make any conclusions.

Finally, it may be concluded that the area of pancreatic GSH-T induction still remains somewhat of a mystery. With such a complex system, where many variables are involved, numerous factors must be considered. Therefore with much more research, it remains to be seen as to why the GSH-T are actually present in the pancreas and their biological role in this organ. The present data and those of others suggest that unlike the GSH-T of the liver and tissues such as the esophagus and stomach (Sparnins, *et al.*, 1982a; Sparnins, *et al.*, 1982b), the GSH-T of the pancreas is rather resistant to agents known to have powerful inducing capacity on the above organs (Roebuck, *et al.*, 1984)

4.2. Experimental Pancreatic Growth in the Rat

4.2.1. Raw Soya Flour Diet

Many reports have been published concerning the dramatic response of the exocrine pancreas to a diet containing raw soya flour. All of the work agrees on the fact that RSF, containing trypsin inhibitor, causes a significant increase in pancreas weight (Folsch, *et al.*, 1974; Crass and Morgan, 1982; Hasdai, *et al.*, 1983; McGuinness, *et al.*, 1981).

In the current study, after both 14 and 21 days (Figures 3-15 and 3-21), the pancreas of RSF-fed rats had significantly increased in weight, by 54% after 14 day and 69% after 21 days. These values are similar to percent increases found in

most studies (Folsch, *et al.*, 1974; Oates and Morgan, 1984; Temler, *et al.*, 1984). However in another experiment by Oates and Morgan (1982), after 2 weeks they found a 128% increase in pancreatic weight in RSF animals as compared with controls. As described in Chapter 1, this increase in pancreas size is thought to be the result of trypsin inhibition in the duodenum, thus allowing continuous release of CCK, a hormone thought to play a major role in pancreatic growth (Green and Lyman, 1972). This proposed mechanism depends on the notion of a feedback regulation of CCK synthesis and/or release by intraduodenal tryptic activity.

By extracting and measuring DNA content of the pancreas, the basis for such growth may be determined. Two possible reasons may be used to explain the increase in pancreatic size: hypertrophy, or an increase in size of already present cells; or hyperplasia, an increase in the number of cells which occurs through cell division. Eventually, these conditions can be discriminated by DNA determination, incorporation of ^{3}H-thymidine into DNA, and/or through microscopic techniques. In numerous studies of this nature, DNA determination is a commonly utilized approach to this question. This procedure is straightforward and takes into account that with cell division (hyperplasia) there is an increase in the mass of DNA.

In ^{3}H-thymidine techniques, actively dividing cells will incorporate radiolabelled thymidine into their DNA. If incorporation is noted, this is evidence for cell division.

On the other hand, sections of pancreas can be taken for observation with

the microscope, with cells and nuclei counted over standard areas and increases in number and/or size noted.

In this study involving the feeding of RSF to rats, the DNA was extracted and the amount present determined colorimetrically. Although a large proportion of literature shows evidence for increased amounts of DNA, thereby indicating hyperplasia in the pancreas of those rats receiving RSF for 2 weeks, our study showed no difference in DNA content after this time period (Figure 3-16). Expressing DNA content in terms of rat body weight (as is pancreas weight) also shows no significant difference (Figure 3-17). Thus, we are able to conclude that in this study, the increase in pancreas size was the result of cellular hypertrophy and not hyperplasia.

Most though not all of the previous work in this field has been done using male Wistar rats with levels of trypsin inhibitor present at 40 mg/g of food (Crass and Morgan, 1982; Oates and Morgan, 1984). In this laboratory, male Sprague-Dawley rats were utilized, with only 29.5 mg trypsin inhibitor/g of diet. Thus, both strain differences and the concentration of active trypsin inhibitor may be used to explain our finding of no hyperplasia after the 14 day feeding of RSF.

It must also be stressed that the areas of both normal and experimental pancreatic growth are still heavily debated. The system is quite a complex one, and it is not possible to state that CCK is totally responsible for normal pancreas maintenance and growth. Several other factors must be considered, such as the roles of other hormones e.g. neurotensin, bombesin, gastrin, the pituitary gland

hormones (Mayston and Barrowman, 1973), the nerves which innervate the pancreas, and combined effects of all of these. Such factors and other interactions are extremely difficult to study, and thus a complete understanding of exactly how they all function in the maintenance of normal pancreatic mass will probably not be available for some time.

In addition to pancreas weight and DNA content, GSH-T activity and total GSH-T of the pancreas were also studied. Although it was recently found that in rats fed RSF for 28 days pancreatic GSH-T activity was reduced (Ross and Barrowman, 1987), in our 14 day experiment, there was no significant difference in GSH-T activity (Figure 3-18), total GSH-T (Figure 3-19), or GSH-T as related to DNA content (Figure 3-20). Perhaps then, the shorter feeding regime was insufficient to affect the levels of these enzymes in the pancreas.

With regards to the 21 day feeding of RSF to rats, a somewhat different set of results were observed. As in the shorter experiment, the pancreas weight was once again significantly increased in the RSF fed rats, about 69% (Figure 3-21). Similarly, there was no difference in the DNA content of the pancreas (Figure 3-22). However, if the DNA content was related to animal body weight, as was the pancreas, there was indeed a significant increase in DNA in the 21 day RSF fed animals (Figure 3-23). This was the case in an experiment by Struthers, *et al.* (1983), in which male Sprague-Dawley rats were also used. Although this group of researchers found no significant difference in pancreatic DNA content after 28 days of receiving RSF, there was a significant increase in DNA if calculated in relation to body weight. Therefore, we may conclude that after 21 days of

receiving RSF, there was both hypertrophy and hyperplasia of the pancreas, thus causing a weight increase.

GSH-T activity in these rats was, as in the 14 day experiment, unaffected by the RSF (Figure 3-24). As previously mentioned, 21 days may not have been sufficient to affect pancreatic GSH-T activity. On the other hand, total levels of GSH-T in the rat pancreas were significantly increased (Figure 3-25), a result that coincides with previous observations in this laboratory (Ross and Barrowman, 1987). These results may be expected in that it has been shown that these larger pancreases do in fact have more zymogen granules and probably also synthesize more cytosolic protein (Folsch, *et al.*, 1974). In accordance with this, there was also found to be more GSH-T as related to the DNA content of the pancreas (Figure 3-26).

4.2.2. FOY-305 Treatment

The oral administration of FOY-305 (camostate), a synthetic inhibitor of proteases including trypsin, has been found to affect the rat exocrine pancreas in a similar manner to RSF, in that enlargement of the pancreas is observed (Yonezawa, 1983; Goke, *et al.*, 1986; Wereszczynska-Siemiatkowska, *et al.*, 1987). The mechanism of FOY-305 is thought to be similar as for RSF, and believed to be mediated by CCK, as an increase in endogenous CCK levels was observed by Goke, *et al.* (1986).

In this study, 400mg/kg FOY-305 (comparable to the dose used in other studies) were administered to hamsters by gavage for 2 weeks. After this time, it was found that as in the previously mentioned studies there was a significant

increase in pancreas weight in the FOY-305 animals as compared with the control animals which were dosed each day with distilled water. The increase in pancreas weight over controls was 31% (Figure 3-27).

The issue of whether the increase in pancreatic weight with the FOY-305 administration is due to hypertrophy and/or hyperplasia is unsettled. In one study, there was a significant increase in DNA content of the rat pancreas after 2 weeks of FOY-305 treatment (Goke, *et al.*, 1986). However, in two other studies there was no significant difference in pancreatic DNA content in rats or mice (Yonezawa, 1986; Wereszczynska-Siemiatkowska, *et al.*, 1987). In our study, there was a significant increase in pancreatic DNA in the hamster after 14 days of receiving FOY-305 (Figure 3-29). DNA content as related to body weight was also increased in the FOY-305 animals. This is indicative of hyperplasia; hypertrophy is probably also involved. The results of these present studies utilizing the trypsin inhibitors FOY-305 or RSF are somewhat different from much of the data presented in the literature. As stated previously, most investigators have found an increase in pancreatic DNA in rats when RSF was administered. However, in this study, such was not the case. Furthermore, unlike the two studies which found that FOY-305 had no effect on the mass of pancreatic DNA in rodents, our study shows a definite increase in this parameter. Thus, it may be said that the factors which determine the DNA response to these two similar stimuli of pancreatic growth are unclear. Presumably, CCK is involved to some extent, however the conflicting results probably indicate that other factors such as nervous stimulation and other hormones are also important. In agreement with the fact that trypsin inhibitors cause a reduction in the

activities of xenobiotic-metabolizing enzymes in the pancreas as determined by Ross and Barrowman (1987), the GSH-T activity in the pancreas of FOY-305 treated animals was significantly reduced (Figure 3-30). Thus, this reduction may be one of the factors responsible for the increased incidence of neoplasia when trypsin inhibitors are administered long-term (Morgan, *et al.*, 1977; Roebuck, *et al.*, 1987), although much research is necessary to prove this hypothesis correct.

Total levels of GSH-T (Figure 3-31) and GSH-T as related to DNA in the hamster pancreas (Figure 3-32) were unchanged by the FOY treatment. Total pancreatic GSH-T was slightly but not significantly reduced, possibly because there was a slight increase in protein content of the pancreas in response to the FOY, thereby partially blocking the effect of reduced GSH-T activity. This is similar to the RSF study, where there was also a greater amount of protein in the pancreas in response to RSF. As reported earlier, trypsin inhibitors have been shown to cause such an increase (Folsch, *et al.*, 1974; Yonezawa, 1983).

4.2.3. L-364,718 Treatment

In Chapter 1, it was reported that recently the benzodiazepine derivative L-364,718, had been found to specifically block CCK receptors (Chang and Lotti, 1986; Chang *et al.*, 1987). The finding was of particular importance because previous to this, effective blockage of CCK receptors was not possible. With the ability to totally abolish the effects of CCK, the implications of this involving the pancreas were obvious. The prospects of revealing whether or not CCK is responsible for normal maintenance and growth of the pancreas would be more likely.

The literature involving experiments using L-364,718, like FOY-305, has become controversial. Because the compound is new, there nave been few published reports concerning its effects on the exocrine pancreas and those which are available are not in agreement with each other. In one previous report by Wisner, *et al.* (1988). it was found that L-364,718 given orally to rats along with FOY-305, completely eliminated the effects of the latter compound. In this same experiment, when L-364,718 was given alone, there were significant reductions in both pancreas weight (25%) and DNA content (30%), indicating pancreatic atrophy. However, in another report, by Zucker, *et al.* (1988), L-364,718 given subcutaneously had no affect on pancreas weight or DNA content in several species.

In the present study, L-364,718 at 1 mg/kg body weight, comparable to the dose used in other studies, there was no significant difference in pancreas weight (Figure 3-33) or DNA content (Figures 3-34 and 3-35) in the pancreas of hamsters in response to oral L-364,718 compared to animals getting only the vehicle DMSO. These results therefore agree with those of Zucker *et al.* (1988), thereby indicating that although the pancreas may respond to exogenous CCK with cell division and increased mass this hormone may not be necessary for normal maintenance or growth of the pancreas, at least in the hamster. Another possibility, however, is that a class of CCK receptors in the pancreas which initiates growth responses is not blocked by L-364,718.

In addition, the pancreatic GSH-T of the hamster were not affected by L-364,718 (Figure 3-36, 3-37, and 3-38). It might be noted that control hamster

GSH-T activity in this study was significantly elevated over all other hamster controls in the other experiments. The reason for this is not known; however, because there were no internal controls which received only water, the possibility of contributions from the solvent DMSO cannot be ruled out. However, the pancreatic weights of animals receiving DMSO were not significantly different from animals receiving only saline in other experiments. Thus, it is unlikely that DMSO had any significant trophic or antitrophic effects on the pancreas. Also, the results within the L-361,718 group were somewhat variable, the causes of which are difficult to deduce, as in the other experiments the SEM were quite low.

Conclusions

The purpose of this thesis project was to try and establish a better understanding of the roles of the pancreas in xenobiotic metabolism. Because such limited information is available in this area, and because phase II enzymes such as the GSH-T are present in the pancreas, the importance of knowledge in this field is important. Furthermore, the pancreas is probably responsive to a number of environmental carcinogens in man, which is evidenced in epidemiological studies linking tobacco smoke with pancreatic cancer (Wynder, 1975). Because of the high death-rate attributed to pancreatic cancer in the western world, the ability of the pancreas to effectively rid itself of reactive chemicals would be of considerable benefit.

These experiments showed that the pancreatic GSH-T of rodent species seem to be somewhat different functionally, than those of the liver, forestomach, lung, and intestine. Although certain chemicals will induce GSH-T in these

tissues, induction in the pancreas seems to be more difficult to achieve experimentally. It is possible that these enzymes in the pancreas have other and perhaps more important roles, such as intracellular transport and binding. Although the GSH-T are present in a variety of tissues, the roles of the enzymes in any one of the tissues may be entirely different. For example, the gene or genes responsible for coding for GSH-T in an organ such as the pancreas may not be activated by the same chemicals or stimuli as are the genes in the liver. Thus, the genes are supressed, and do not respond with increased production of GSH-T in the presence of the chemicals used in this study. Another area of uncertainty is interspecies variation in the handling of these chemicals.

Nonetheless, the fact that 0.75% BHA in the diet induced hamster pancreatic GSH-T is encouraging and suggests further experiments. For example, a study using the other antioxidants such as BHT and ethoxyquin might be carried out in the hamster. In addition, an investigation of the ability of BHA to inhibit pancreatic cancer in the hamster in response to the well-studied hamster pancreatic carcinogen N-nitosobis(2-oxopropyl)amine (BOP) is needed. More information concerning the characteristics of the gene or genes which code for pancreatic GSH-T is required. In this case, gene mapping techniques could be employed in the hamster, and the results compared to such information already available for the liver and/or other tissues. Furthermore, the hamster pancreas enzymes themselves should be characterized, the GSH-T of the pancreas purified, and techniques used in order to deduce which and how many isoenzymes are actually present in the pancreas. For instance, the GSH-T in the pancreas may be of the ligandin type, and function primarily in transport and storage, with

suppression of the genes responsible for coding for those GSH-T involved in detoxification.

In conclusion, it has been shown that GSH-T are present in the rat pancreas, and demonstrated that they are also found in the pancreas of mice and hamsters. With some manipulation, hamster GSH-T levels can be increased, as in the response to dietary BHA. Also, these enzymes in the hamster pancreas are affected by trypsin inhibitors like FOY-305, and respond with a reduction in activity. It must be pointed out however, that since there was no significant reduction in total pancreatic GSH-T levels in response to FOY-305 treatment, this decrease in GSH-T activity may simply be a dilution effect as the mass of the pancreas increases. Thus, the reports of higher incidence of pancreatic neoplasms in response to trypsin inhibitors, may be partially or totally because of the reduction in these detoxification enzymes. However, overall, these enzymes are difficult to induce in the pancreas of rodent species in reponse to the spectrum of chemicals known to have this capacity in other tissues. Much more research in the biochemical characterization of pancreatic GSH-T must be done before their function in this organ can be fully understood.

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