THE EFFECT OF α-SOLANINE ON ACETYLCHOLINESTERASE ACTIVITY *IN VIVO*: AN EXAMINATION OF UNDOCUMENTED BELIEFS

by

Airdrie M. Walker

A Thesis
Submitted to the Faculty of Graduate Studies
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Dedication

Dedicated to the memory of my loving and much beloved parents.

ABSTRACT

Solanaceous plants, such as potato, contain glycosidic steroidal alkaloids. The potato glycoalkaloids, principally α-solanine and α-chaconine, have been implicated in numerous cases of toxicity to humans and animals. The mechanism of action for the toxic effect is poorly defined but toxicity has been attributed to their anti-cholinesterase property. Much of the support for this notion has derived from *in vitro* studies, limited animal studies in which potato peels were fed, and retrospective case studies of humans. The evidence for glycoalkaloid toxicity is weak and anecdotal in nature.

The present study examined the effect of α-solanine *in vivo* under controlled experimental conditions. Since dietary protein can alter the rate of biotransformation lipophilic xenobiotics to hydrophilic metabolites and the excretion of xenobiotics, the study examined the effect of α-solanine on cholinesterase enzyme activity at two levels of dietary protein. Eighty-six (86) weanling male Sprague-Dawley rats were fed a low (7.5%) or standard (15%) protein diet *ad libitum* for four weeks. At the end of the dietary treatment period they were injected intraperitoneally with α-solanine suspended in 8% ethyl alcolhol at a dose level of 1 mg/Kg B.W. Enzyme activity was measured in erythrocytes, plasma, liver and brain at three time periods: 3, 8 and 12 hours post-injection.

The study showed that α-solanine at this dose level inhibited the cholinesterase activity in the liver but only at 12 hours post-injection in the animals fed the standard protein diet. In the other tissues the enzyme activity was reduced due to the glycoalkaloid

treatment but the difference was not statistically significant. For example, in the erythrocytes at 8 hours post-injection the enzyme activity was 2.3±1.2 I.U.B. in the control group compared to 1.5±0.6 I.U.B. in the treated group but this difference was not significant statistically.

The study examined the relationship between dietary factors and the anti-cholinesterase effect which has not been studied previously. The effect of low dietary protein was evident in all tissues but was only significantly different in the liver. In the liver, a significant difference in enzyme activity was observed in both the low and standard protein groups at all three time points. In other tissues there was a reduction in activity due to the low protein diet but it was not statistically significant. For example, in brain at 3 hours post-injection the activity was 12.0±4.4 in the standard protein control group compared to 9.3±2.7 in the low protein control group. This may be explained by differences in unique tissue response to dietary protein.

This study suggests that the anti-cholinesterase effect of α-solanine is a factor in the toxicity of animals resulting from the ingestion of potato glyoalkaloids and toxicity may be greater in animals with inadequate nutritional status.

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LIST OF ABBREVIATIONS

ACh	acetylcholine
AChE	acetylcholinesterase
BBB	blood brain barrier
BuCh	butyrylcholine
BuChE	butyrylcholinesterase
B.W.	body weight
CHO	carbohydrate
CNS	central nervous system
cyt-P450	cytochrome P-450
DMSO	dimethyl sulfoxide
DTNB	dithionotrobenzoate
EC_{50}	concentration causing malformation in 50% of the of the surviving embryos
FAO	Food and Agriculture Organization
FETAX	frog embryo teratogenisis assay-Xenopus
FWB	fresh weight basis
GA	glycoalkaloid(s)
g.i.	gastrointestinal
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
LDH	lactate dehydrogenase

LC_{50}	median concentration causing 50% embryo lethality
MAS	metabolic activation system
MFO	mixed function oxidase
NOAEL	no observed adverse effect level
NTD	neural tube defect
ODC	ornithine decarboxylase
PEM	protein energy malnutrition
SGOT	aspartate transaminase
SGPT	gamma glutamyl transminase
TGA	total glycoalkaloid
TI	teratogenic index

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Chapter I

INTRODUCTION

1.1 Thesis

This thesis is an empirical analysis of the effect of α-solanine on acetylcholinesterase activity in erythrocytes, plasma, liver and brain. In addition, it examines the effect of dietary protein levels on this enzyme activity.

1.2 Background

Glycoalkaloids (GA), a class of nitrogen-containing steroid glycosides, occur naturally in Solanaceae plants such as potatoes, tomatoes, eggplant and nightshade berries. When ingested, glycoalkaloids may be toxic to humans and animals. Numerous cases of poisonings (2000) and deaths (30) have been attributed to the consumption of potato glycoalkaloids (Jadhav, 1991; Morris and Lee, 1984). Whether glycoalkaloids alone are responsible for the toxic actions observed remains unclear, but potatoes associated with cases of human poisoning show abnormally high glycoalkaloid concentration. Although there are many different glycoalkaloids, the only documented cases of glycoalkaloid poisoning in humans involved the ingestion of potatoes in which the principal glycoalkaloids are α-chaconine and α-solanine (Harvey et. al., 1985; Slanina, 1990; McMillan and Thompson, 1979). This information must be cautiously interpreted as there are few analytical standards and the chemical methods for glycoalkaloid determination are suspect at best (Przybylski, R., unpublished data).

1.3 Relevance of Research

It has been estimated that more than 99% of all toxins consumed by individuals are naturally occurring (Ames, 1990a,b). Although, an exhaustive toxicological evaluation, employing established procedures, is done to ensure the safety of our diet when synthetic chemicals are to be introduced into our foods, it is only recently that attention has focused on components of the diet which occur naturally. To date, we have adopted a much less critical approach for evaluating and regulating natural constituents. Although reports of cases of potato glycoalkaloid poisoning have been recorded for most of this century (Rothe, 1918; Harris and Cockburn, 1918; Bomer and Mattis, 1924; Willimott, 1933; McMillan and Thompson, 1979) these reports are anecdotal in nature and there is a paucity of scientific data on this potential problem.

There is a critical need for continuing surveillance over our food supply and an urgent need to be aware of the dangers of natural products that may be contained therein. The GA have been described on a quantitative basis as the most highly consumed natural toxins in the North American diet (Hall, 1992) due to the high consumption of potatoes (70Kg/person/year).

The toxicological status of the glycoalkaloids present in this important dietary staple is surprisingly poorly defined. Because α-chaconine and α-solanine are ingested by large numbers of people on a routine basis over a lifetime, and because no appropriately designed animal studies have been conducted, it was deemed essential that these toxicological consequences of their ingestion be examined. In fact, the GA may be a more serious threat to the health of the individual than contaminants arising from environmental and industrial pollution by virtue of man's exposure to low concentration

over long periods of time. In addition, there is widespread occurrence of processed potato in the diet. The decline in the consumption of fresh potatoes has been matched by a significant increase in intake of processed products so that overall consumption has not decreased. This change in eating habits reflects increased demand for convenience foods, fast foods and snacks including canned, dried and frozen potato forms, patties, puffs, hashbrowns, mashed and dehydration products such as potato flour, starch, granules, flakes and diced potatoes.

Potato is an extremely valuable food source and makes a significant contribution to the nutrition of people in most parts of the world (FAO, 1994). Potatoes can be grown under a wide variety of climatic conditions relatively easily, quickly and economically. Because of their relatively heavy yield, ease of storage, food versatility and low cost, potatoes represent one of the world's major agricultural food products. The worldwide production of potatoes is estimated at nearly three hundred million tonnes (FAO, 1994) ranking fourth by total yield per acre after rice, wheat and maize and first by protein yield per acre (Rhoades and Roger, 1982). Potatoes provide more than energy. They provide an excellent source of carbohydrate and vitamin C, as well as a good quality of protein. Potato protein has an adequate ratio of total essential amino acids to total amino acids and balance in the concentration of essential amino acids (Lopez de Romana et. al., 1981). Its importance lies in the fact that it is usually consumed daily in many different forms.

When plants are subjected to physiological stress, most commonly due to fungal invasion or mechanical damage, they undergo metabolic changes which can result in the production and accumulation of abnormal and sometimes toxic secondary metabolites.

There is little definitive information on the toxic effect of these metabolites and even less is known about the interaction of these toxins with nutrients. Therefore, it was the objective of the research to explore the anti-cholinesterase activity of α-solanine and also the effect of these glycoalkaloids in cases of dietary protein inadequacy in living animals.

The potato is an essential and primary food source for the indigenous peoples of South America. (Kubo and Fukuhara, 1996). These people are often engaged in strenuous physical work resulting in very high energy requirements. These demands are most often met by consuming potatoes several times each day and frequently the meal consists solely of potatoes. Although their energy requirements may be satisfied, the protein levels in the diets are usually insufficient. Protein deficiency is a common state in undernourished peoples causing increased susceptibility to disease, illness and toxic insult. Great care must be taken to limit the intake of glycoalkloids becaues potatoes are such an essential constituent in the diets of these people.

Studies have demonstrated that high levels of glycoalkaloids in potatoes, whether caused by genetic, environmental or storage and processing factors present a significant risk to human health.

The Andean people in the highlands of southern Peru and Bolivia, for centuries, have utilized a type of freeze drying called *chunification* to reduce the GA content of bitter potatoes (Woolfe, 1987; Kubo and Fukuhara, 1996). Bitter potatoes with high GA levels are grown plentifully in the high altitude zones of the Andes (>3600m). Potatoes are spread evenly on the ground and allowed to freeze overnight. The frozen tubers thaw during the day as the temperature rises. Successive freezing and thawing causes the tuber cells to separate and destruction of the differential permeability of the cell membrane

allows cell sap to diffuse from the cells into the intercellular spaces. This released liquid is squeezed out of the tubers by trampling which also removes the tuber skin. The trampled tubers are then transferred to a running stream and immersed covered with straw for one to three weeks. After removal from the water they are spread in the sun to dry. The white dust which forms on the tuber gives it its name *chuno blanco*. Production of *chuno negro* is similar except that the skins are not removed during trampling and the trampled tubers are not soaked in water. After trampling they are immediately sun dried which makes the product dark brown to black. Christiansen (1977) found that the GA level was reduced from 30 mg/100grams on fresh weight basis (FWB) to 4 mg/100grams FWB and 16 mg/100grams FWB in *chuno blanco* and *chuno negro* respectively. These products may be stored unchanged for several years and frequently form the basis of soups and stews. However, many of the nutrients are also substantially reduced by this process.

Protein-energy malnutrition (PEM) is the most common form of malnutrition in developing countries. PEM is associated with low birth weight infants and child mortality. It is the most important nutritional disease in developing countries. It affects over 512 million persons in Africa (32%), Latin America (15%), Near East (15%) and the Far East (30%), (Torun and Chew, 1994). Although no actual food consumption data exists, the relative amounts of energy and protein available in the food supply provide a strong indication of dietary adequacy. For example, as seen in Table 1, the amount of food energy available in Peru is roughly half of that in Canada on a per capita basis. Similarly, protein intake is 49.3 grams per capita whereas in Canada it is 96.1 grams per capita. It appears that both energy and protein are insufficient.

1.4 Objectives and Scope

There are few reports on the toxicity of GA and those that have been published are inconsistent and uninformative. A systematic examination of the physiological effects of the principle potato GA would provide new information on the potential hazard of potato consumption. This work will focus on the toxicity produce by α-solanine because the glycoalkaloids from potatoes are a highly consumed natural toxin in the diet.

Glycoalkaloids produce several adverse effects, but it is the anti-cholinesterase activity which is thought to be most deleterious. A decrease in acetylcholinesterase enzyme activity results in an accumulation of acetylcholine. The excess acetylcholine causes depression of the central nervous system (CNS) due to loss of neural transmission followed by cessation of respiration and terminal loss of heart function due to ventricular fibrillation. Although these neurological symptoms of solanine intoxication have been explained by their anti-cholinesterase activity (Morris and Lee, 1984) this notion has not been supported by experimental data. Therefore, it was the objective of this research to explore the anti-cholinesterase activity of α-solanine on butyrylcholinesterase and acetylcholinesterase. In addition, it examines the effect of α-solanine toxicity in animals which consume a low protein diet, a common condition in regions where potatoes with high GA content are used extensively.

A better understanding of the biochemical basis of the adverse effects is needed to devise appropriate preventive measures to lower the toxic potential of glycoalkaloids in potatoes. This research was designed to provide preliminary information in this respect.

1.5 Methodological Considerations

Much of the literature concerning cases of GA poisoning in man and animals has been based on solanine as a part of a mixture of GA from berries, shoots and tubers. There is very little pharmacologic information on the *in vivo* toxicity of the pure alkaloid. For example, Bushway et. al., (1987) used the pure chemical, but their work was done on bovine and eel cholinesterase *in vitro*. In the present study, the pure chemical was used (98% pure, Sigma Chemical) and the studies were conducted *in vivo*.

Many examples of the anti-cholinesterase activity of α-solanine have been drawn from experiments conducted *in vitro*. These do not consider the effects of biotransformation or other body processes which significantly alter the xenobiotic toxicity at the sites of action studied (liver, brain, plasma and erythrocytes). In this study the effect of α-solanine on living animals was examined.

In order to investigate the adverse effect of interest, the anti-cholinesterase effect, the i.p route of administration was used to circumvent the delay and variability of absorption and gastric emptying in the gastrointestinal tract.

Large species differences in sensitivity to α-solanine poisoning occur. Humans are believed to be much more susceptible than many animals to this type of poisoning. Ideally, animal models would resemble man as closely as possible with respect to rates and routes of excretion, the metabolic intermediates formed in tissues, the metabolites excreted in urine and bile and the pharmacokinetic profile. This is not usually attainable due to practical considerations and availability (Caldwell, 1992). The rat was deemed to be an acceptable model for the research carried out in this thesis.(Groen et. al., 1993).

Table 1

Food Supply per Caput per Day

	Energy	Protein	Fat
World	2718 Kcal	70.8 gms	68.7 gms
Canada	3094 Kcal	96.1 gms	132.8 gms
Peru	1882 Kcal	49.3 gms	36.2 gms

FAO, 1994

Chapter II

REVIEW OF LITERATURE

2.1 Toxicology

Toxicology is the study of the adverse effects of chemicals on living organisms. As a result of the wide diversity of chemicals to which man and animals are exposed, and the variety of adverse reactions which occur, this is a very broad science. Chemicals foreign to the body include food additives, pesticides, industrial chemicals and naturally occurring toxicants which collectively are called xenobiotics.

2.2 Nutritional Toxicology

Nutritional toxicology is the study of food-borne xenobiotics and the interaction of these with nutrients and the nutritional status of the affected individual. This includes the diet as a source of the toxicant, the effects of toxicants on nutrients, the effects of nutrition on the toxicant as well as regulatory decisions to establish safety guidelines and risk or hazard assessment (Hathcock, 1982; Hathcock, 1987).

The adverse effects elicited by a toxicant occur when the toxicant or a metabolite formed by its biotransformation reaches the site of action at a threshold concentration and for a sufficient duration to produce the toxic manifestations. The major factors which determine toxicity therefore are the chemical and physical properties of the toxicant, the route of administration, the concentration at the site of action, the duration and frequency of the exposure, the form of the toxicant and susceptibility of the biological system.

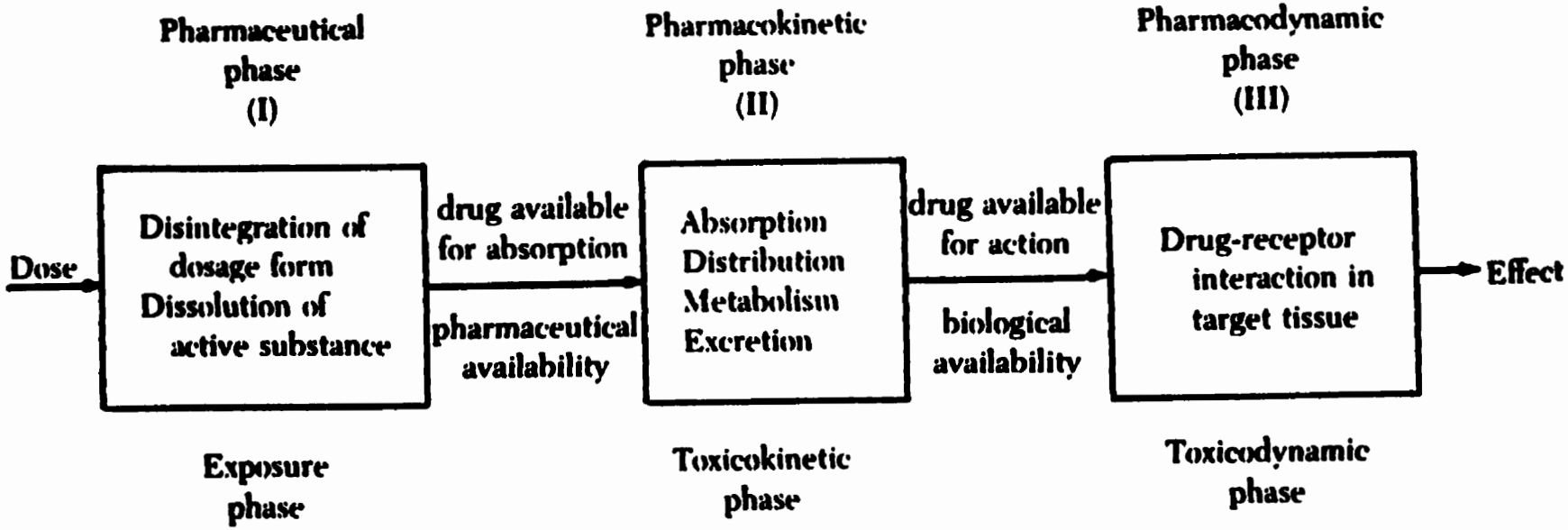
2.2.1 Dose-Response

The dose-response relationship, a correlation between the characteristics of exposure and toxic events that follow, is the most fundamental tenent in the study of toxicology. For this to be valid there must be convincing evidence that there is a causal relationship between the dose and the toxic effect observed. The generation of toxicological effects is a complex process but in this complex sequence of events there are three phases which can be identified (Figure 1):

1) The Exposure Phase includes factors which determine the concentration of the toxicant that comes into contact with the biological system. For any xenobiotic to be toxic there must be dissolution of the dosage form and solubilization of the active substance to make the toxicant available for absorption.

2) The Toxicokinetic Phase comprises the processes determining the concentration of the active agent at the molecular sites of action. The toxicity of any substance depends on the dose and its concentration at the site of action. The concentration at the site of action is dependent in part on the dose, but differences in disposition of the xenobiotic may lead to vastly different concentrations at any particular site. Toxicokinetics is the study of the movement of drugs or xenobiotics within the body. It is comprised of four elements: absorption, distribution, metabolism or biotransformation and excretion. The physiological response is influenced by these processes. Absorption is the process by which toxicants cross body membranes and enter the systemic system. This is accomplished by passive diffusion, active transport, facilitated diffusion or pinocytosis

Figure 1



The various phases of toxic action.

primarily in the gastrointestinal tract but also in the lungs and skin.

Distribution occurs rapidly and is dependent on the blood flow through the organ, the ease with which the chemical passes through the cell membrane (for example, hydrophilicity, lipophilicity, size of molecule) and the affinity of the compound for various tissues. Distribution and the storage of the toxicant in tissues has a major impact on the concentration of the toxicant at its site of action. Binding or dissolving in body components (fluids, bone etc.) alters the distribution of the xenobiotic. Toxicants are often concentrated in specific tissues, which may or may not be its site of action. Four major storage sites exist:

a) plasma proteins - Serum albumins have a high capacity to bind many compounds and because of their combined high molecular weight they are unable to cross capillary walls. Other plasma proteins such as transferrin, ceruloplasmin, the α-lipoproteins and ß-lipoproteins also bind chemical substances as well as xenobiotics. The bound form then is unavailable.

b) liver and kidneys - These organs have a high capacity to bind xenobiotics and so are important in biotransformation and excretion.

c) adipose tissue - Highly lipophilic compounds rapidly penetrate cell membranes and are stored in adipose tissue. Large amounts of toxicants with a high lipid/water partition coefficient may be stored in body fat. This does not inactivate them, rather it sequesters them in the tissue and these xenobiotics may be passed down the food chain.

d) bone - The process of skeletal uptake of a xenobiotic is essentially a surface chemistry phenomenon between the hydroxyapatate crystals of bone matrix and the extra-cellular fluid.

3) The Toxicodynamic Phase comprises the processes involved in the interaction between the toxicant and its molecular site of action and the sequences of biochemical and biophysical events which are induced and finally lead to the toxic effect observed.

2.2.2 Blood Brain Barrier

The blood brain barrier (BBB) is a combination of structures and transport systems which closely control the type and the rate of chemicals entering the CNS. Structurally the brain endothelial cells are different from endothelial cells in capillaries and other organs. The nonneural capillary wall is comprised of flat endothelial cells joined loosely with gaps between the cells. In the brain there is a continous tight junction which prevents transcapillary movement of polar molecules. Substances which pass from blood plasma to the brain must first pass through these membranes and the cytoplasm of the endothelial cells. These two membranes and the interposed cytoplasm constitute the BBB (Oldendorf, 1990). The BBB is selectively permeable and operates to maintain an optimum environment for CNS function. Its permeability depends on lipid solubility, polarity, molecular size and the existence of specific transport systems. Lipid soluble compounds readily cross the BBB whereas the BBB is almost impermeable to hydrophilic and polar molecules. Small molecules such as water readily enter the brain by diffusion. Some substances such as glucose, monocarboxylic acids (e.g., pyruvate, lactate, and ketone bodies) and amino acids which have low lipid solubility gain access mediated by specific transport proteins in the plasma membranes of the endothelial cells of the BBB.

There are two main mechanisms by which circulating xenobiotics may be reduced; metabolism or biotransformation and excretion.

2.2.3 Biotransformation

After ingestion, xenobiotics are subjected to a number of biochemical processes called biotransformation. They are usually enzymatic in nature, which makes the compound more water soluble, changes or increases its polarity and may destroy the biological activity. Biotransformation reactions are crucial for lipophilic xenobiotics. They readily cross the cell membranes and so are readily absorbed but poorly excreted.

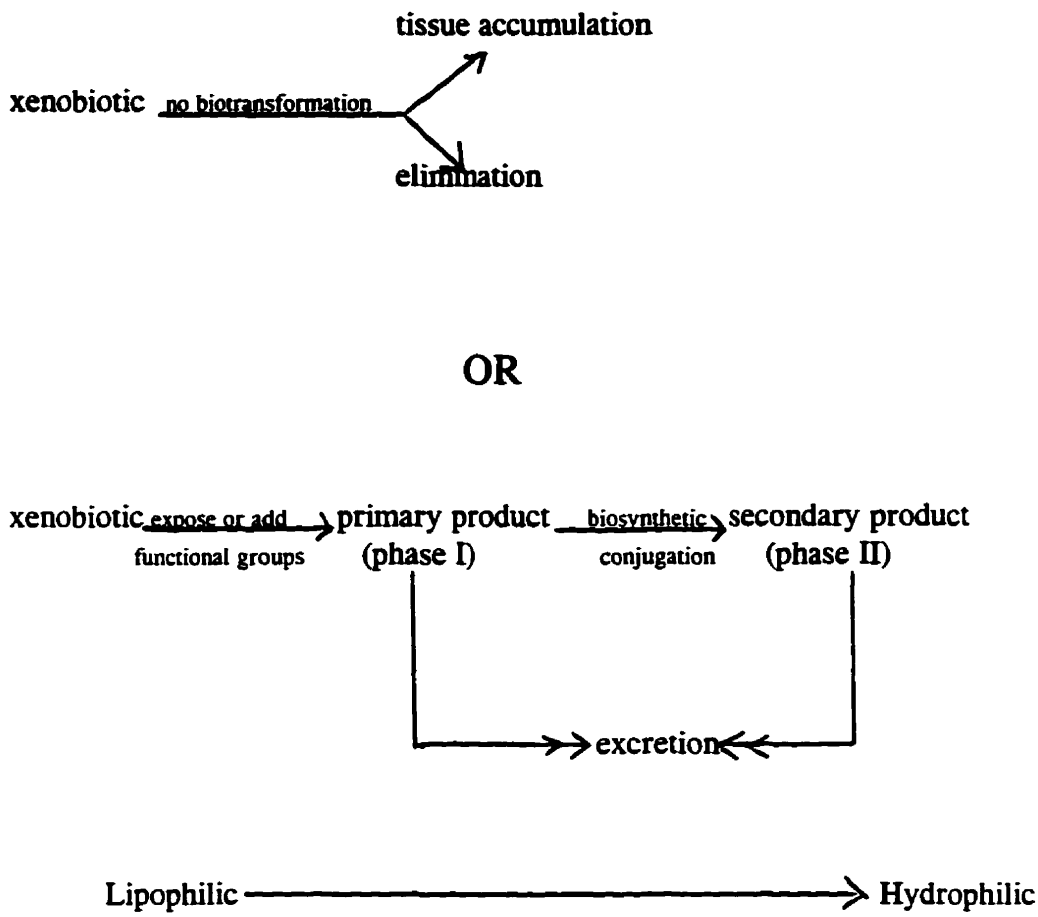
These enzymic reactions are of two types: Phase I reactions which involve oxidation, reduction and hydrolysis, and phase II reactions which consist of conjugation or synthetic reactions. (Figure 2.)

Phase I enzymes, the mixed function oxidases (MFO) and cytochrome P-450 (cyt P-450) convert xenobiotics to more water soluble derivatives by adding or exposing functional groups such as OH, SH, NH and COOH. Phase I reactions allow the compounds to further undergo phase II reactions. The phase II enzymes are synthetic in nature. Xenobiotics and phase I derived metabolites are linked to an endogenous molecule such as glucuronic acid, sulphate or glutathione to increase their water solubility, increase their ability to ionize at physiological pH, transfer across hepatic, renal and intestinal membranes thus increasing their excretion.

2.2.4 Factors Affecting Biotransformation

The biotransformation of xenobiotics is affected by many factors such as dose,

Figure 2.



Integration of phase I and phase II biotransformation reactions

(Sipes and Gandolfi, 1991)

frequency of administration, diet and nutritional status, species and strain, age, sex, body weight, route of administration, time of administration, the interaction of anti-nutrients, drugs and endogenous metabolites, pregnancy and physiological abnormalities and enzyme induction.

Foetuses and neonates have limited ability to detoxify xenobiotics. For example, in rats, the low activity of cyt P-450 enzyme observed at birth increases steadily to reach a maximal level by 30 days of age and then decreases gradually (Sipes and Gandolfi, 1991).

Variations in metabolism between species may be qualitative or quantitative. In rats, amphetamines are primarily biotransformed by aromatic hydroxylation while in rabbits amphetamines are primarily deaminated (Sipes and Gandolfi, 1991). Species variation is also apparent in phase II reactions. These differences stem from differences in the ability to synthesize or activate metabolic co-factors, the nature and amount of the transferase enzyme, the presence of the endogenous conjugation agents and the nature of the xenobiotic. Thus the toxicity of a xenobiotic cannot be extrapolated from one species to another. Strain differences in biotransformation are also under genetic control. For example, marked strain differences in the rates of oxidation of hexobarbital among Holtzman, Sprague-Dawley and Wistar rats have been noted. There are also significant differences in enzyme activities between individuals (Sipes and Gandolfi, 1991).

The toxicity of a chemical usually depends on the route of administration. When administered intra-peritoneally (i.p.) xenobiotics enter the liver via the portal circulation (first pass circulation) and they may be extracted and/or completely biotransformed prior to gaining access to the systemic circulation and target tissue. Therefore, a xenobiotic

which undergoes significant first pass deactivation may be far more toxic when given intra-venously (i.v.), intra-muscularly (i.m.) or sub-cutaneously (s.c.). If a toxin is given i.v., it circulates to all tissues prior to reaching the liver. When there is no hepatic biotransformation in the liver the toxicity should be independent of the route of administration if the rates of absorption are the same. Comparison of the toxicity by different routes of administration will then provide some information on biotransformation and excretion.

The time of administration is a factor which relates primarily to animals. Circadian rhythms also influence the rate of biotransformation. This variation is related to variations in endocrine function which is influenced by the light/dark cycle. Most rodents are nocturnal and therefore more biotransformation occurs at night.

Also, there are differences in biotransformation between male and female animals of the same species. The organophosphate insecticide parathion is only half as toxic to male rats as it is to female rats. The ratio of male and female sex hormones is an important determinant of cytochrome P-450 enzyme activity.

2.2.5 Effect of Diet on Biotransformation

There is an increasing awareness that biotransformation of xenobiotics is markedly affected by nutritional status. Deficiencies of nutrients such as protein, essential fatty acids, minerals and vitamins can reduce detoxification, thereby increasing the toxic manifestation of xenobiotics.

Mineral deficiencies (Ca, Zn, Cu, Fe, Mg) decrease both cytochrome P-450 catalyzed oxidation and reduction reactions. These minerals are essential co-factors to

enzyme systems which generate NADPH for the phase I microsomal cytochrome P-450 dependent MFO (Sipes and Gandolfi, 1991).

Vitamin deficiencies reduce the rate of detoxification by altering the energy and redox state of the cells. This hinders the production of metabolic co-factors.

Dietary lipids affect cyt P-450 concentration and the rate of oxidative demethylation in rat liver. Dietary linoleic acid is important is determining normal levels of hepatic microsomal enzyme activities (Crawford and Wheeler, 1983). The increased susceptibility of unsaturated fatty acids to undergo peroxidation causes microsomal membrane degradation with loss of membrane bound cyt P-450.

Food deprivation such as fasting and starvation also change the toxicokinetics of xenobiotic metabolism (Kato, 1977; Wattenburg, 1971).

Natural xenobiotics, such as indoles, flavones, xanthines in vegetables such as broccoli and cauliflower are strong inducers of the MFO (Parke and Ioannides, 1981). Also, polycyclic hydrocarbons in charcoal-broiled meat stimulate rat placental and intestinal drug metabolism, and increases hepatic metabolism of anti-pyrine and theophylline and the intestinal metabolism of phenacetin in humans (Conney, 1976; Kappas, 1978).

2.2.6 Effect of Dietary Protein

Nutritional deficiencies alter the primary susceptibility of cells to toxicants. This may occur through decreases in the rates of cellular replacement, decreased enzyme synthesis and activation, as well as decreased coenzyme synthesis. By altering the biochemical makeup or structural integrity of the cells they are more susceptible to

damage by toxicants. The maintenance and stability of excitable membranes are essential for normal physiology.

In protein deficiency the biotransformation of many chemicals is impaired. It has been demonstrated that the toxicity of pesticides such as chlorinated hydrocarbons and other acetylcholinesterase inhibitors, is significantly increased by protein deficiency (Boyd and Krupa, 1970). Protein exerts this protective effect by increasing the activity of the drug metabolizing enzyme system. Kato et. al. (1980) observed that increasing dietary protein up to 35% increased the MFO enzyme activity. Conversely, protein deficiency decreases the activity of the MFO system in the liver, this results in an increased half life of numerous xenobiotics. This effect has been attributed to a decrease in the biosynthesis of microsomal protein, changes in the membrane environment and a decrease in the activity of the existing enzyme systems (Campbell and Hayes, 1976).

One of the four major storage sites for toxicants is plasma proteins. Intra and extra cellular proteins, particularly serum albumin, control the absorption and distribution of xenobiotics to intra cellular biotransformation enzymes. Serum albumins have a high capacity to bind many compounds and because of their high molecular weight (albumin and toxicant) they cannot cross capillary walls. Protein deficiency decreases the albumin binding capacity. The increase in serum albumin in Kwashiorkor-rehabilitated children has been found to correlate with an increased rate of metabolism and decreased half-life of acetanilide (Buchanan, 1986).

Protein calorie deficiencies have been correlated with a decrease in glomerular filtration and renal plasma flow. This results in an increase in the half life of some xenobiotics making them more toxic (Buchanan, 1979).

Protein deficiency alters the action of xenobiotics. Depending on the compound, whether the parent compound or its metabolite produces the toxicity, protein deficiency may exacerbate or alleviate the toxic effect (Hathcock, 1987). When the parent compound is biotransformed to a less active metabolite, protein deficiency by reducing the body metabolism and clearance, would markedly increase the toxicity. For example, chlorinated hydrocarbons have an increased metabolic half life and greater toxicity during protein deficiency. In other cases, where the parent compound is metabolized to a more toxic product, protein deficiency would have the effect of decreasing the toxicity. For example, parathion is bioactivated to paraoxon which is much more potent. In protein deficiency this process is impeded, therefore, the end result is decreased toxicity.

2.2.7 Excretion

Excretion, a physical mechanism which removes xenobiotics from the blood and returns them to the external environment, is a function of kidney, liver, biliary systems, the g.i. tract, lungs, sweat and mammary glands. The toxic manifestation of a xenobiotic is critically influenced by the rate of excretion. The route and speed of elimination is largely dependent on the physico-chemical properties of the xenobiotic. The major excretory organs, liver and kidney, remove only hydrophilic (usually ionized) chemicals.

Urinary Excretion The kidney is a major excretory organ which removes toxicants by glomerular filtration, tubular excretion by passive diffusion and active tubular secretion. In the kidney, toxicants with high lipid/water coefficient will be passively reabsorbed whereas polar compounds and ions will be unable to diffuse accross the membranes and

will be excreted in the urine. Nutritional deficiencies, such as protein and calorie restrictions have been correlated with reductions in glomerular filtration and renal plasma flow. For example, an increased half life of gentamicin has resulted when these nutrients are deficient (Buchanan et. al., 1979).

Fecal Excretion Fecal excretion is another major pathway for elimination of xenobiotics.

a) Non-absorbed ingested material -- macromolecules and some essentially completely ionized compounds of high molecular weight, are eliminated in feces.

b) Liver can extract toxic agents from blood after their absorption from g.i. tract. Because the liver is the main site of biotransformation, the metabolites formed may be excreted directly in bile. In circumstances, first pass circulation may completely biotransform xenobiotics prior to reaching target tissue. Toxic agents bound to plasma proteins are fully available for active biliary excretion. Having been excreted with bile into the intestine, xenobiotics can be reabsorbed or excreted in feces. Reabsorption of a xenobiotic completes an enterohepatic cycle which may lead to very long half-lives of xenobiotics in the body. The hepatic excretory system is not fully developed in newborns which causes some xenobiotics to be more toxic to very young animals than adults (Klassen, 1972).

Intestinal Excretion Some chemicals are excreted into feces by a direct transfer from blood into the intestinal contents. This is thought to occur by passive diffusion. In some cases rapid exfoliation of the cells of the intestinal wall may increase fecal excretion of some compounds.

Xenobiotics which exist in gaseous form are eliminated by exhalation. Other routes of elimination are by milk, sweat, saliva and cerebrospinal fluid.

2.3 Glycoalkaloids

Glycoalkaloids are nitrogen-containing glycosidic steroidal alkaloids naturally occurring in various members of the *Solanaceae* family, including *Solanum Tuberosum* (potato). They consist of the steroid aglycone, solanidine and a carbohydrate side chain attached at the 3-OH group.

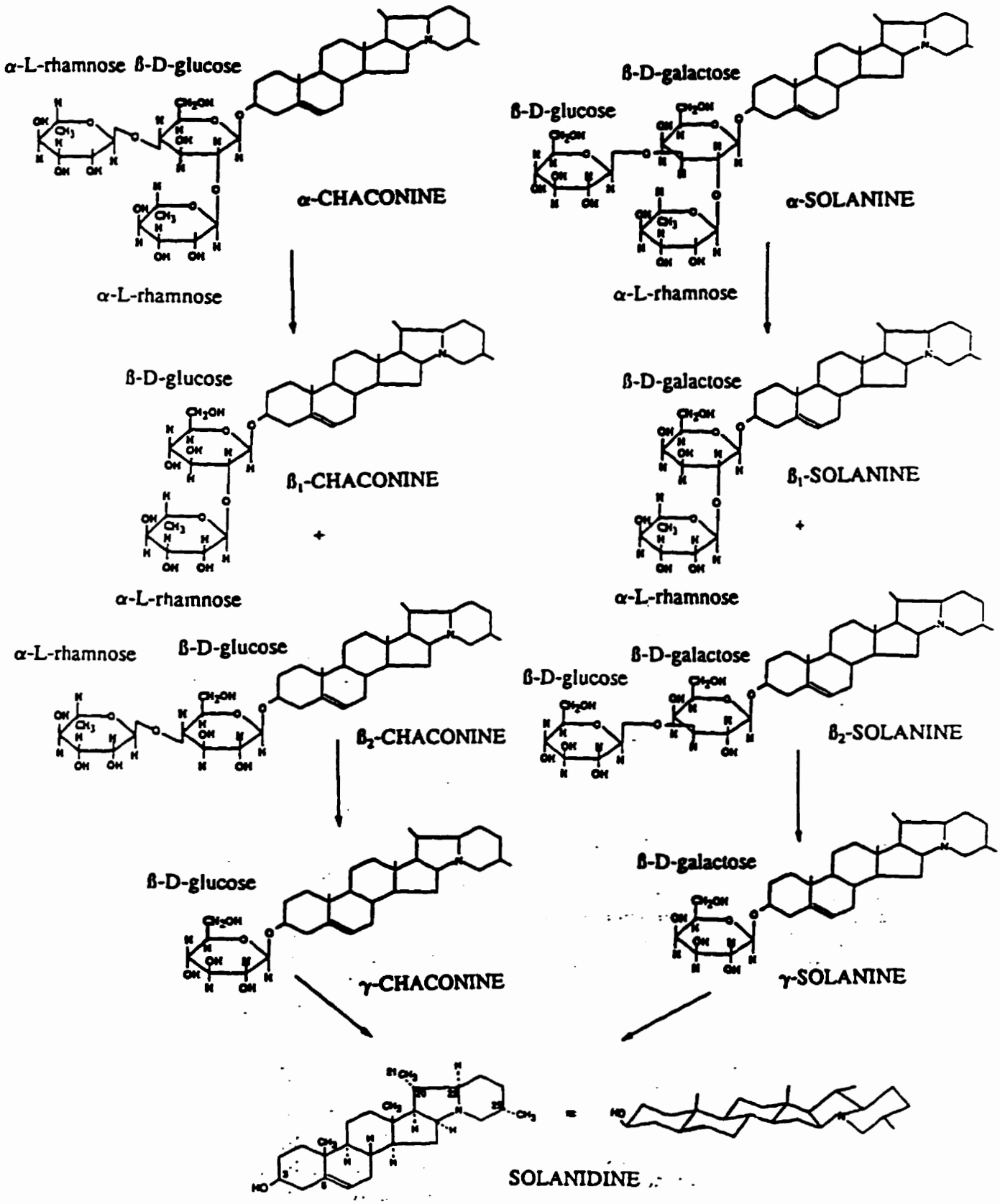
2.3.1 Structure and Identification

At least 20 structurally different alkaloids have been identified in potatoes, tomatoes and eggplant and about 300 more in other Solanaceae species (Schreiber, 1979). Of these, there are approximately 60 which are of pharmacological and toxicological interest, all derived from the C-27 steroidal skeleton of cholestane (Heftman, 1983).

2.3.2 Principle Glycoalkaloids in Potatoes

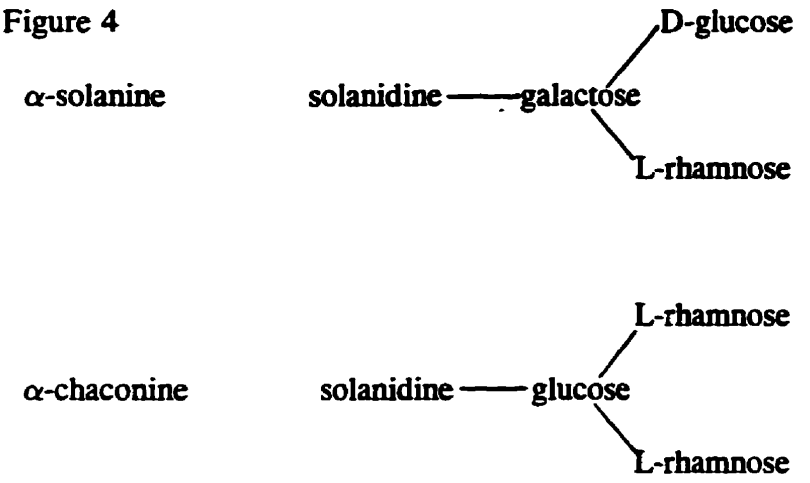
The two principal GA (95%) in cultivated potato species commercially available are α-solanine and α-chaconine. They are present at concentration ratios of between 26:74 and 40:60 (α-chaconine:α-solanine) (Paseshnichenko, 1956; Friedman and Dao, 1992; Sharma and Salunkhe, 1989). Molecules of both are derived from the same alkaloidal aglycone, solanidine, but differ in the trisaccharide moiety (Jadhav, 1981). The structural relationships of these compounds are shown in Figure 3. The common sugar in α-solanine is galactose whereas in α-chaconine it is glucose (Figure 4). The remainder of the total glycoalkaloid (TGA) fraction consists of other glycosides of solanidine (beta and gamma forms), other solanidine alkaloids and a second type of alkaloid, the spirosolanes (Osman, 1980). Instead of a trisaccharide side chain, the beta and gamma

Figure 3.


α-L-rhamnose ß-D-glucose
α-CHACONINE
α-L-rhamnose
ß-D-glucose
ß1-CHACONINE
+
α-L-rhamnose
α-L-rhamnose ß-D-glucose
ß2-CHACONINE
ß-D-glucose
γ-CHACONINE
ß-D-galactose
ß-D-glucose
α-SOLANINE
α-L-rhamnose
ß-D-galactose
ß1-SOLANINE
+
α-L-rhamnose
ß-D-galactose
ß-D-glucose
ß2-SOLANINE
ß-D-galactose
γ-SOLANINE
SOLANIDINE

forms of solanine and chaconine have a disaccharide or monosaccharide moiety.

Figure 4



2.3.2.1 Distribution

Glycoalkaloids are present in most tissues of the potato, except the pith, with the greatest concentration in the areas of highest metabolic activity (sprouts, blossoms, foliage) followed by the peel and the tuber (stolon). Within the tuber the concentration of GA are greatest in the outermost region (Table 2). As well, there is considerable variation in GA content in potato products (Table 3).

Table 2

Distribution of solanine in the potato plant

Solanine concentration in mg/100 grams fresh weight

tubers	0.5
main stems	1.2
small stems	1.89
roots	4.2
leaves	6.05
sprouts	47.6

Table 3

Glycoalkaloid content in various potato products

GA content in mg per 100 grams of product

potato skins	1.74--8.35
pancake powder	1.94--2.41
mashed flakes	3.29
commercial chips	1.05--5.02

2.3.2.2 Physiological Function

GA confer resistance to insect infestation and frost, and exhibit antimicrobial and fungitoxic activity (Sanford, 1990; Deahl, 1991; Tingey, 1984; Osman, 1991). They are thought to be involved in protecting the plant against late blight. Fewell and Roddick, 1993 observed that α-chaconine and α-solanine act synergistically over a wide range of GA ratios with as little as 10-20% of either GA causing a pronounced increase.

Low levels of GA, less than 10 mg/100g FWB are considered necessary for the proper flavouring of potatoes (Ross et. al., 1978). However, potatoes containing elevated GA concentration, above 10 mg/100g FWB, are considered bitter by most individuals (Sinden and Deahl, 1976). The immediate bitter taste (30 sec -1 min) is followed by a longer lasting burning sensation in the mouth and throat (3-5 mins). When the GA content exceeds 20 mg/100g FWB, the burning sensation is immediate and the tuber is considered unfit for human consumption.

2.3.2.3 Tolerances

The potential for toxicity from GA has led to establishment of guidelines limiting the GA content in potato cultivars. The normal concentration of GA in potatoes accepted for human consumption range from 1-15 mg/ 100 grams FWB. At these levels human health and culinary quality are not compromised.

There is general agreement that potatoes can contain up to 20 mg/100 grams FWB of potato and be safe for human consumption (Jadhav and Salunkhe, 1975). Not all scientists support this postition. Morris and Lee (1984) and Ross et. al. (1978) reviewed the existing data and concluded that this value is too high. They recommend that 10 mg/100 grams of fresh weight be accepted as the safe upper limit.

2.3.2.4 Factors Affecting GA Levels

Genetic:

The GA content of potato varieties can vary quite widely from 1.8 mg/100 grams FWB to 30 mg/100 grams FWB (Woolfe, 1987). (Table 4). Initial GA levels are highly heritable (Sanford and Sinden, 1972). Although there are significant interactions between variety and the environment, those cultivars which have a high average GA level are predisposed to synthesize extremely high levels when subjected to stresses or improper handling.

Cultivation Factors:

GA content varies quantitatively and qualitatively in response to soil, moisture, stage of plant growth, location, and seasonal variation. Within a cultivar, smaller tubers have a higher concentration of GA. Also, immature tubers, irrespective of size, have a higher GA content. Cool dull weather and a short growing season produced tubers with a higher GA content (Bomer and Mattis, 1924; Sinden and Webb, 1974; Patchett et. al., 1976; Jadhav et. al., 1981).

There are conflicting reports regarding the influence of fertilization on GA levels. Sinden and Webb (1974) reported that fertilization was not a factor, whereas Cronk et.al., (1974) reported increased GA levels following fertilization.

A food crop which is not protected from insect damage may produce increased levels of natural toxins. GA content in tubers of plants subjected to defoliation damage by a common pest, the Colorado potato beetle, were consistently higher than those found in tubers from undamaged plants (Hlywka, et. al., 1994). The mechanism responsible for

Table 4 Glycoalkaloid content of some common North American and European commercial potato varieties.

Variety	Glycoalkaloid content mg/100 grams (FWB)
Columbian russet	1.8
Russet rural	2.1
Sebago	2.1
McCormick	2.6
Jubel	3.2
Triumph	3.6
King	3.7
Brown Beauty	3.7
Earlaine	3.9
Green Mountain	4.0
Wee McGregor	4.2
Jossing	4.2
Red Pontiac	4.3
Russet Burbank	4.7
Early Ohio	4.8
Mesaba	5.2
Hundred-day cobbler	5.3
Arnica	5.4
Katahdin	5.5
Irish cobbler	5.6
Ackersegen	5.8
Cobbler	6.4
Houma	6.4
Chippewa	6.6
Libertas	6.8
Warba	6.9

Blue Salad	7.0
Early Rose	7.2
Netted Gem	7.9
Golden	8.8
Early epicure	8.9
Furore	9.2
White-blossomed cobbler	9.4
As	9.6
Gineke	9.6
Kennebec	9.7
Pioneer rural	10.0
Erstling	10.0
Hindenger	11.6
Rural New Yorker	13.0
Kerr's pink	13.6
Pimpernell	15.3
King George	18.7
Prestkvern	34.5

Woolfe, 1987

the increased synthesis of GA resulting from pest interaction with foliage has not been determined (Bergenstrahle, 1992a).

Post-harvest Treatment:

The exposure to natural or artificial light stimulates glycoalkloid synthesis in potato tubers (Dale et. al., 1993; Jadhav and Salunkhe, 1975; Deahl et. al., 1991). Tubers exposed to various forms and durations of illumination including daylight, ultra-violet light, fluorescent and incandescent light during harvest, transport and marketing show greatly increased levels of GA and a decreased ratio of α-chaconine to α-solanine irrespective of the source of the illumination or the age of the tubers (Baerug, 1962; Percival and Dixon, 1994).

Physical stress such as bruising, cutting, and slicing results in GA synthesis (Sinden, 1972; Salunkhe et. al., 1972; Wu and Salunkhe, 1976). Levels of wound induced GA as high as 200 mg/100 grams FWB, ten times higher than the level generally regarded as safe, have been reported (Wu and Salunkhe, 1976). Cut potatoes stored at ambient temperatures can have significant amounts of GA synthesized. During frying it was found that the level of GA was increased due to dehydration. Therefore, it is important that bulk raw potato fries prepared for fast food and the catering trade are handled correctly.

2.3.2.5 Wild Species

There is increasing interest in the use of wild potato species in breeding programs (Flanders, 1992). They are a source of desirable genes and may be used to improve frost and pest resistance and improve yield and quality (Deahl and Sinden, 1987; Ross, 1966;

Sinden et. al., 1986a,b).

These wild species contain a much greater GA concentration (upwards of 134 mg/100g FWB) than are found in Solanum Tuberosum (Schreiber, 1963; Osman et.al., 1978; Van Gelder et. al., 1988), and this high GA content may pose a threat to breeding porgrams. Gregory et. al. (1981), analyzed the GA from 16 wild, tuber bearing Solanum species and found a wide quantitative and qualitative variation in GA contents. It must be emphasized that evaluation of GA as a potato quality factor is required when wild species are to be used in breeding programs.

In both developed and developing countries, the use of a wide range of chemicals to destroy pests and weeds is an important agricultural practice. Although this practice undoubtedly increases crop yield and reduces losses, the extensive use of chemicals results in residues in foods. There is widespread concern over the adverse effects of pesticides and herbicides on human health. Paradoxically, avoiding the use of synthetic pesticides, although desirable, may in fact produce higher levels of natural pesticides due to the pest related stresses. Some authorities argue, the judicious use of synthetic pesticides to alleviate a pest stress may in fact, result in a higher degree of food safety as the production of natural toxins to combat the pest stress may be reduced (Hall, 1992).

2.3.2.6 Effects of Processing and Cooking

Potato GA are stable under typical food processing and cooking conditions. GA are insoluble in water and have a very high melting point--286°C (Chaube and Swinyard, 1976). Boiling, baking, frying or freezing do not reduce TGA concentration (Zitnak, 1970; Baker and Lampitt, 1955; Bushway and Ponnampalam, 1981).

New potato cultivars with superior processing characteristics have not been put into production due to unacceptable GA content. Most notably, the netted gem and Lenape in North America and Magnum Bovum in Sweden have been withdrawn from commerce or had restrictions placed on their sales or excluded from seedling certification, because of GA concentration. In the case of the netted gem, the GA content a few hours post harvest was 5 mg/100 g FWB. However, 6-8 hours of exposure to sunlight at near freezing temperatures caused a threefold increase in TGA content. Once this high level was reached the GA diffused throughout the tuber. The cultivar Lenape has a high solid content and low levels of reducing sugars resulting in a potato with excellent chipping and storage properties. However, its high GA content made it unacceptable for commerce and it was withdrawn from the market (Sinden and Webb, 1974).

In Sweden in 1986, the variety Magnum Bovum ranked third in annual production, but because of its high GA content (mean 25.4 ± 10.6 mg/100 grams FWB upwards to an extreme value above 60 mg/100 grams FWB) a conditional sales ban was imposed. The usual GA content for this variety ranged from 6.0-8.5 mg/100 grams FWB. Persons who ingested a single intake of these peeled, boiled potatoes reported solanine intoxication (Hellenas, 1995).

Maga (1980) reported reductions of TGA in potato flakes extruded in a small screw extruder under a wide range of barrel temperatures and moisture contents. However, Camire et. al. (1993) found that extrusion with a lab scale turn extruder did not significantly reduce GA in potato peels.

In the USA, french fry production generates nearly two million tonnes of potato

peels as a by product. Potato processors are examining the feasibility of using potato peels as a concentrated source of dietary fibre since potato peels contain 45% total dietary fibre on a dry weight basis (Camire, 1993). The presence of GA may severely limit their use as food additives.

The only efficient and effective way to control GA in potatoes is to develop cultivars which have a low GA content and to inhibit further synthesis during growth, harvest and post-harvest.

2.3.2.7 Bioavailability

Bioavailability is an index of the extent of systemic absorption of a xenobiotic. It is expressed as a proportion of plasma xenobiotic concentration after intravenous and oral dosing. Because the concentration of the toxicant at the site of action is the most critical factor in toxicity to most organs, the fraction of a chemical reaching systemic circulation is of importance. Several factors affect this systemic availability: a) absorption after oral dosing, b) intestinal first pass effect, c) hepatic first pass effect, and d) the mode of formulation, which affects the dissolution rate or incorporation into micelles. Also, the rate at which a xenobiotic becomes available systemically is important. The time (t_{max}) to reach peak plasma concentration (C_{max}) will be longer for a slowly, than for a rapidly absorbed compound. Since toxic effects occur only above a critical plasma/organ concentration, a slowly absorbed versus rapidly absorbed xenobiotic may not attain a concentration great enough to produce toxicity.

Studies using tritiated α-solanine and tritiated α-chaconine indicate consideravle differences between animal species (Slanina, 1990). Nishie, (1971) using tritiated α-

solanine in rats showed that the GA:

1) are poorly absorbed from the g.i. tract,

2) are rapidly eliminated from urine and feces,

3) reach maximum tissue concentration in 12 hours, and that

4) hydrolysis of α-solanine to the less toxic solanidine occurs in the g.i. tract.

After p.o. exposure to α-solanine Groen (1993) observed that hamsters had a higher concentration than rats but only 3% of the dose was present in the systemic circulation. The higher bioavailability was reflected in relatively lower amount excreted in urine and feces 7 days after administration. The elimination half-life after i.v administration was significantly longer than after p.o. administration which may indicate that even at low dose levels α-solanine inhibits the activity of the enzymes involved in its metabolism -- an hypothesis supported also by Nishie, (1971). Also, there is evidence that uptake and retention of α-solanine in tissues increases disproportionately with increasing doses.

Clearance, is the overall efficiency of removal of a xenobiotic from the body. Total body clearance is the sum of clearances from each organ. Systemic and metabolic clearance varies between species. Groen et. al. (1993) found that the systemic and metabolic clearance of ^{3}H solanine were 1.6 and 2.7 times higher respectively in rats than hamsters.

When administered i.p., fecal and urinary excretion rates were similar over a range of doses 5-16 mg/Kg B.W. There was an abrupt decrease in excretion rate when the dose was increased to 25 mg/Kg B.W. and corresponding increase in tissue concentration in liver, spleen, kidney and intestine.

Gull (1961) demonstrated species differences in sensitivity to α-solanine. Man

appears to be more sensitive to GA poisoning after p.o. intake than animals (Slanina, 1990) (Tables 5 & 6). However, it is extremely difficult to compare GA toxicity data because human data is retrospective in nature and largely based on case studies. Rodent studies are prospective, use pure compounds, use straight forward designs and the experiments are not compromised by the fact that the toxin came in a mixture of foods.

2.3.2.8 Toxicity

The toxicity of GA has been documented (Ruhl, 1951; Tingey, 1984; Morris and Lee, 1984; Wilson, 1959; McMillan and Thompson, 1979; Hellenas, 1992; Harvey et. al., 1985; Slanina, 1990.) The most consistent symptom of toxicity is gastroenteritis; severe gastric pain with nausea, vomiting and diarrhea. More severe toxicity produces bradychardia along with neurological symptoms of CNS depression -- drowsiness, apathy, restlessness, shaking, confusion, weakness, vision disturbances and unconsciousness. Symptoms usually appear 8-12 hours after ingestion, much longer than the 15 min-2 hours typical of most plant toxins and shorter than the 18-24 hours typical for most bacterial food poisoning. Toxicity signs include fever, headache, rapid weak pulse, low blood pressure and rapid, shallow respiration. However, the mechanisms underlying these physiological manifestations are not well understood.(McMillan and Thompson, 1979).

In the outbreak of potato poisoning among school children in London, U.K. (McMillan and Thompson, 1979) the ingested peeled potatoes contained 330 mg GA per Kg of potato. This is consistent with other cases of poisoning. Harris and Cockburn (1918) reported that 410 mg/Kg of potato caused toxicity, and Rothe (1918) reported GA

Table 5. Toxic and lethal doses of glycoalkaloids in man.

AUTHOR	Cases	Route of administration	Toxic dose mg/Kg	Lethal dose mg/Kg
Schroff (1865)	adult man	p.o.	2.5	
Swinyard & Chaube (1973)	adult man	p.o.	2.5	
Pfuhl (1899)	56 adults	p.o.	2.5	
Rothe (1918)	41 persons	p.o.	2.5	
Harris & Cockburn (1918)	61 persons 1 child died	p.o.	2.5	5.0
Bomer & Mattis (1924)	large no. of persons	p.o.	3.0	
Griebel (1924)	large no. of persons	p.o.	5.0	
Ruhl (1951)	adults	p.o.	2.2	
Wilson (1959)	4 persons	p.o.	20-25 mg*	
McMillan & Thompson (1979)	78 children	p.o.	2.0	3 critically ill

*The figure used by Wilson was for total consumed dose--not mg/Kg B.W.

Table 6. Effect of α-solanine administered to different species

SHEEP	p.o.	225mg/Kg B.W.	poisoning
	p.o.	500mg/Kg B.W.	lethal
	i.v.	17mg/Kg B.W.	poisoning
	i.v.	50mg/Kg B.W.	lethal
RABBIT	i.p.	20mg/Kg B.W.	death in < 24 hrs
	i.p.	30mg/Kg B.W.	death in < 7 hrs
	i.v.	10mg/Kg B.W.	death in 2 minutes
RAT	gastric intubation p.o.	590mg/Kg B.W.	50% death in 24 hours LD_{50}
	i.p.	75 mg/Kg B.W.	death in a few hours
	i.p.	20 mg/Kg B.W.	none out of 15 died
	i.p.	40 mg/Kg B.W.	1 out of 15 died
	i.p.	60 mg/Kg B.W.	3 out of 15 died
	i.p.	85 mg/Kg B.W.	6 out of 6 died
MOUSE	p.o.	1000mg/KgB.W.	non-toxic (no effect)
	i.p.	10mg/Kg B.W.	poisoning
	i.p.	32mg/Kg B.W.	50% death
	i.p.	> 50mg/Kg B.W.	lethal

Maga, 1980

poisoning after consumption of potatoes containing 430 mg/Kg. At an average consumption of 140 grams of potato per person this would give a dose of approximately 1-1.5 mg/Kg B.W. This is considerably lower than the published toxic dose of 2-5 mg/Kg B.W. or the lethal dose of 3-6 mg/Kg B.W. indicating a much smaller dose induces toxicity in humans (Hellenas, 1992). It should be noted that potatoes with greater than 20 mg of GA /100 grams FWB are extremely bitter and that TGA in the range of 40 mg/Kg FWB burn the mouth leaving a severe aftertaste.

a) Dose-response

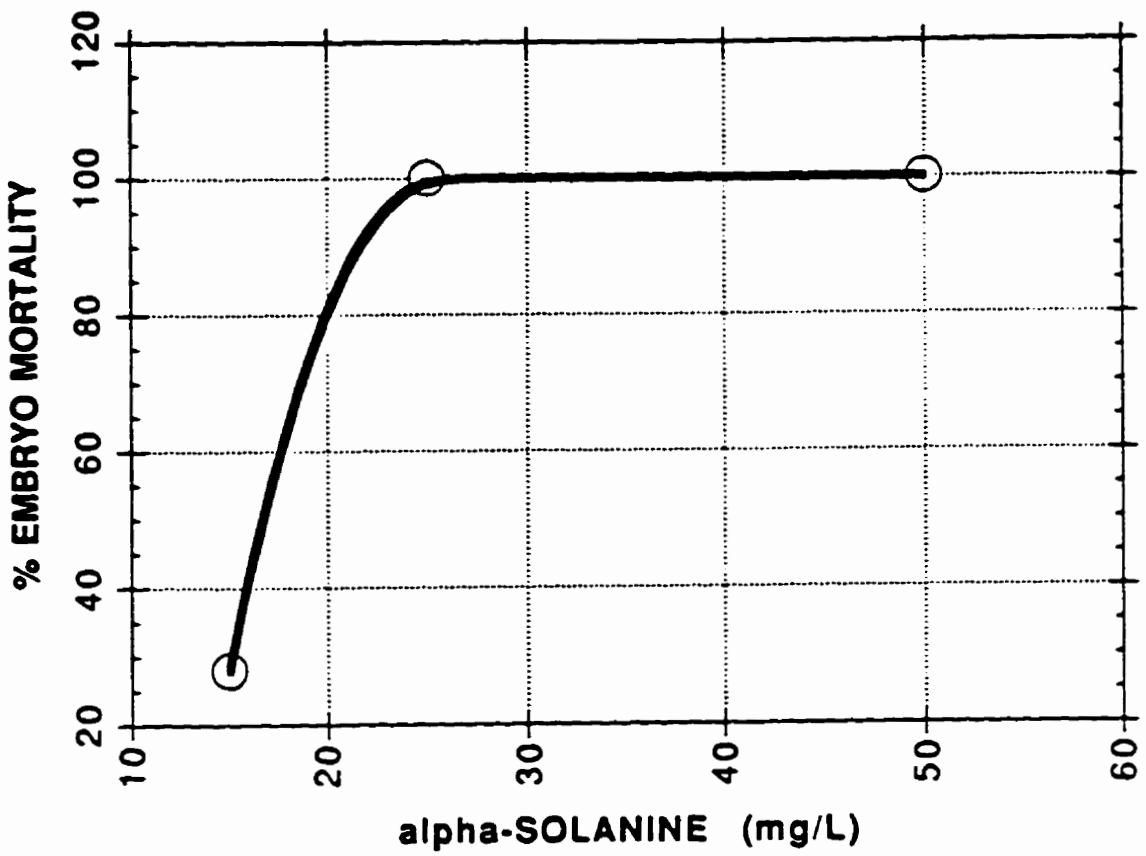
The toxicity curve for α-solanine rises steeply over a relatively small increase in dose. Studies on the embryos of rainbow trout and madaka showed that the toxicity curve for α-solanine was quite steep between 15-25 ug/ml of the vehicle DMSO. Mortality increased from 30% to 100% over this mere 10 ug difference (Crawford and Kocan, 1993); (Figure 5).

b) Structural Features Governing Toxicity

Two factors which affect developmental toxicity are the nature of the carbohydrate side chain and the synergistic action between α-chaconine and α-solanine.

The chemical structure of the carbohydrate side chain, the number of carbohydrate groups attached to solanidine and the stereo chemical orientation of the glycosides has an effect on the developmental toxicity of the glycolakloids (Rayburn et. al., 1994) Friedman, 1994). Removal of the carbohydrate side chain from α-solanine and α-

Figure 5


% EMBRYO MORTALITY
120
100
80
60
40
20
10
20
30
40
50
60
alpha-SOLANINE (mg/L)

chaconine greatly reduced their biological activity. The mechanism of action is unclear but it may be that the removal of the sugars alters the transport across the cell membranes. These changes in toxicity may be a result of changes in molecular size and polarity. The relative potency of α-solanine (with glucose, galactose and rhamnose) is significantly lower than α-chaconine (with two rhamnose molecules and one glucose) attached to the same aglycone. One or more carbohydrate groups may be cleaved either by HCl in the stomach or by enzymatic hydrolysis to beta and gamma chaconine and solanine which reduces the toxicities (Bushway, 1988, 1990).

c) Adverse Effects:

Toxicity from GA produce the following physiologically adverse effects: anti-cholinesterase activity in the CNS, disruption of cell membranes, induction of liver enzymes, teratogenicity, embryotoxicity and genotoxicity.

1) Inhibition of Acetylcholinesterase

The inhibitory action of α-solanine on AChE has been confirmed by a number of investigators *in vitro* (Orgell, 1958; Harris and Whittaker, 1962; Orgell, 1963; Alozie et. al., 1979; Bushway, 1987; Patil, 1958, 1972). However, the results of these studies are variable. Given the differences in experimental design including the purity of the chemicals used, the type of cholinesterase tested, the concentration of solanine administered and the conditions of the test (*in vitro or in vivo*), the lack of consensus is understandable.

Harris and Whittaker (1962) studied the effects of α-solanine at a concentration

of 3uM on the sera of humans *in vitro* and reported enzyme inhibition of 86.2%. Orgell (1963) recorded 50% AChE inhibition by 5 uM solanine. Roddick et. al. (1989) used the pure chemical to test α-solanine and α-chaconine on bovine cholinesterase *in vitro*. At a dose level of 10uM there was a 44.3% inhibition of the cholinesterase. At 100uM concentration the inhibition was of the order of 75%. The inhibitory effect of α-solanine and α-chaconine were similar (27.7% and 25.95, respectively) and combining them did not provide evidence of synergism. Roddick concluded that if anything, they were slightly antagonistic or non-interactive (37.3%).

Bushway et. al., (1987) tested several pure glycoalkaloids for cholinesterase inhibition using an eel enzyme preparation. Of the seven glycoalkaloids tested, solanidine glycosides with 2 or 3 sugar residues were the most potent *in vitro* inhibitors. At a concentration of 40 uM α-solanine produced a 26% inhibition of eel cholinesterase. Tomatine, a glycoalkaloid with 4 sugar residues, was much less potent producing 4.2% inhibition. At 33uM Demissine was almost as potent an inhibitor as α-solanine (21.6%). The low enzyme inhibition of tomatine indicates both the number of sugar residues and the alkaloid are important in determining the degree of inhibition. Tomatine has the same sugar residues as demissine but a different aglycone.

Patil et. al., (1972) injected New Zealand white rabbits intraperitoneally with doses of 20 or 30 mg/Kg B.W. of α-solanine. At both dose levels, weak to moderate inhibition of both butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) was observed but the effect was less marked in the erythrocytes than in plasma. The effect of cumulative doses of solanine on AChE activity was also studied. Solanne was injected i.v. into and anaesthetized dog in doses of 6 mg/Kg B.W. at ten minute intervals five

times (30 mg/Kg B.W. An initial inhibition of AChE was followed by rapid reversal of plasma cholinesterase. The erythrocytes were unaffected.

2) Disruption of Cell Membranes

The biological mechanism by which GA cause toxic effects is unknown but may be due to saponin-like properties leading to haemolytic and hemorrhagic damage followed by septicemia and death (Morris and Lee, 1984). Keukens (1992) studied the interaction of GA on membranes and found that GA bind irreversibly to sterol containing membranes causing rapid loss of barrier function and bilayer integrity. This interaction depends on the composition of the sugar moiety of the GA and the type and content of the sterol present in the membrane. The presence of sterols is necessary for membrane disruption and the presence of cholesterol in the membrane increases this interaction. They concluded that sterol mediated membrane disruption is one of the important mechanisms of GA induced intoxication. At a dose level of 170 ug/Kg B.W. p.o., macroscopic examination showed severe damage to the wall of duodenum. Groen (1993) and Roddick (1988) also reported disruption of cell membranes of the g.i. tract. Blankenmeyer et. al. (1992) demonstrated that potato glycoalkaloids alter the integrity of the membranes of frog embryos. Membrane potential, as measured by fluorescence increased by 1600% for α-chaconine and 400% for α-solanine. Blankenmeyer suggested that the mechanism of action for membrane potential is the same mechanism of action for teratogenicity and lethality.

3) Induction of Liver Enzymes

A characteristic of biotransformation enzymes is that their activity may be enhanced in response to an animal's exposure to xenobiotics. Ornithine decarboxylase (ODC) induction may be used as an index of the trophic effects of a xenobiotic on specific target tissue (Janne, 1978; Russel, 1980).

Hepatotoxicity is one effect of GA exposure. To measure hepatotoxicity Caldwell, et.al. (1991), measured the ODC activity *in vivo*. Enzyme activity increased as early as 3 hours after GA administration and peaked 5 hours post-injection. This effect was transient. ODC activity decreased to one third the maximal level at 8 hours and declined to control levels 24 hours post-injection. At doses of 7.5, 15 and 30 mg/Kg B.W. the hepatic enzyme response was linear. This observation was confirmed by Dalvi, (1985) who demonstrated an increase of SGOT, SPGT and the inhibition of cholinesterase following i.p. injection of α-solanine.

4) Teratogenicity

A great deal of the concern with respect to GA poisoning has centred around their possible teratogenic effects. A teratogen is a chemical which significantly increases the incidence of structural or functional anomalies in live offspring after it is administered to either parent before conception, to the female during pregnancy or directly to the developing embryo. In order to be classified as a developmental toxicant a xenobiotic must produce adverse effects on the developing embryo at levels which do not produce severe maternal toxicity.

Environmental factors, including diet appear important in the etiology of teratogenicity (Milunsky, 1989). Steroids are known to cause changes in tissue levels of

certain nutrients such as retinol, ascorbate and other water soluble vitamins (Millan and Woollam, 1957; Warhany, 1971). Since potatoes containing GA are widely consumed (Friedman and Dao, 1992) and since high levels of GA in potatoes may be teratogenic. potato consumption during pregnancy is a concern.

Renwick (1972), using epidemiological evidence, suggested that maternal ingestion of late blighted potatoes with high GA content was causally related to an increased incidence of the human neural tube defects (NTD'S) anencephaly and spina bifida. Swinyard and Chaube, (1973) tested this hypothesis, feeding blighted potatoes by gavage or i.p. injection of α-solanine or GA to rats and rhesus monkeys. They failed to produce neural tube defects but did produce a high incidence of minor abnormalities. At 10 mg/kg/ B.W. per day basis they found a naturally occurring mixture of GA to be seven times more toxic to fetuses than solanine and produced similar foetal abnormalities.

There is evidence that GA greatly suppress fertility. In 1976, Chaube and Swinyard found that feeding either α-solanine or α-chaconine or crude GA extract did not cause foetal abnormalities, but did increase resorption of fetuses. However, the research is inconsistent. The literature contains reports of both GA induced developmental toxicity and the absence of teratogenic effects. These contradictory results may be attributed to different routes of administration, the animal species studied, as well as the teratological endpoints used. A substantial difference in absoption and retention of potato GA between hamsters and rats has been indicated in earlier studies (Norred, 1976; Alozie, 1979). In these studies the route of administration is a key factor. The ratio po:ip LD_{50} for solanine in rats is 8:1 (Gull, 1961). There appears to be a large variation in susceptibility to GA induced teratogenicity among different animal species

that may be due also to different mother to foetus transport among the species, differences in inherent genetic susceptibility, bioavailability and metabolism (Watanabe and Endo, 1989). A substantial difference in absorption and retention of potato GA between hamsters and rats has been indicated in earlier studies (Norred, 1976; Alozie, 1979). In these studies the route of administration is a key factor.

More recently Renwick (1984) reported foetal NTD'S in offspring of Syrian hamsters given a single gavage of α-solanine during gestation. However, the dose was non physiological (exceeded 100 mg/Kg B.W). The intake of GA from potato meals for the majority of consumers is estimated to be <1 mg/Kg B.W. Renwick's study, using 100 mg/Kg B.W. provides little direct evidence of a threat from GA. therefore this is not a real threat.

In a study of Kunming pregnant mice, Wang (1993), showed that potato GA have teratogenic effects on the embryos and could induce NTD's. GA caused death of embryos, absorption and death of foetuses, as well as intrauterine growth retardation. When GA were given to pregnant mice on the 5^{th} and 6^{th} day of gestation, a miscarriage would occur. The limited human data does not provide a direct link between GA consumption and NTD's. When Harvey et.al. (1986) measured the serum GA in two groups of women: one group pregnant with a foetus affected by NTD and the other group pregnant with a healthy foetus they found a lower GA concentration in the group who subsequently delivered babies with NTD's.

Also, it has been shown that elevated amniotic fluid levels of alpha fetoprotein and AChE are reliable biochemical markers to detect AWD's and NTD's in the second tirmester of pregnancy in human (Jorgensen et.al., 1995; Loft, 1993; Van Gelder, 1989).

However, Hellenas et. al. (1992), basing their approach on blood concentration rather than dose, Hellenas et.al., (1992) concluded that potato GA at levels normally found in potatoes do not cause teratogenesis in humans.

In GA risk assessment studies consideration must be given to chemical interaction among potential toxicants and dietary components. To date, this has not been done. Fewell and Roddick (1993) reported that as little as 10-20% of either glycoalkaloid in the system initiated a marked synergistic action. Because potatoes contain GA in varying ratios depending on variety, Rayburn et.al. (1995) examined possible synergism in developmental toxicity of α-chaconine and α-solanine. They found that α-chaconine and α-solanine acted synergistically in terms of mortality and malformation over a wide range of chaconine:solanine ratios (3:1, 1:1, 1:3) in the frog embryo teratogenesis assay - Xenopus (FETAX). The abnormalities seen with α-chaconine and α-solanine at low concentration were mostly abnormal gut coiling and facial abnormalities. Concentrations that produced 40% mortality caused severe facial malformations, microencephaly and anencephaly. All ratios showed synergism for mortality and malformation. The teratogenic index (TI) did not change from that observed for individual GA, therefore, GA seem to be more embrytoxic than teratogenic.

At this time it is not known whether heat labile lectins (haemagglutinins), digestive enzyme inhibitors, which are present in potatoes modulate the biological effects of glycoalkaloids when they are consumed as part of a potato containing diet. (Friedman, 1992; Lisinska, 1989).

5) Embryotoxicity

The *in vitro* frog embryo assay (FETAX), a time and cost effective method which allows rapid evaluation of developmental toxicity, correlates well with evaluations done in live animals (Bantle et. al.,1990). Data from this test suggest that α-solanine is modestly teratogenic and embryotoxic (Friedman et.al., 1992). However, a significant number of severe malformations such as gut coiling, misshapen eyes, cranio-facial malformations, microencephaly and growth inhibition were observed. The teratogenic index is a measure of the teratogenicity of a chemical relative to its lethal concentration. The teratogenic index was greater than 1.5, indicating significant teratogenic risk to xenopus embryos.

$$TI = LC_{50}/EC_{50}$$

LC_{50}----survival

EC_{50}----malformation

Their data showed that:

GA are more toxic than the corresponding aglycone, for GA toxicity, the nature of the CHO side chain strongly influences potency, the nitrogen of the steroid is required for teratogenicity and the orientation of the unshared electron pair associated with the nitrogen atom does not affect potency.

Microsomes used for metabolic activation (MAS) in FETAX has no effect on the developmental toxicity of potato GA (Friedman, 1991; Ford et. al., 1988).

6) Genotoxicity

Recently, it has been demonstrated that the study of gene mutations *in vivo* can

be done by using transgenic mice (Gossen, 1989; Hoorn, 1993; Myrh, 1991). When a maximum tolerated dose (110 mg/Kg B.W. of α-solanine) was administered i.p. to impregnated transgenic mice, mutation frequencies were 3-4x higher in fetuses and dam livers than normal in this mouse strain (Crawford and Myrh, 1995).

By contrast, Friedman and Henika (1992), showed no genotoxicity using the Ames Salmonella typhimurium assay supporting the notion that GA do not induce damage to intracellular DNA. However, we should interpret this data cautiously since these investigators used only two strains of the bacterium (TA98 and TA100) whereas regulatory guidelines recommend the use of four or five strains (Ames et. al., 1975). GA were also negative in the foetal and adult erythrocyte micronucleus test suggesting that potato GA do not induce chromosomal damage *in vivo*.

2.3.2.9 Pharmacokinetics of Solanine

There is very little information of the pharmacokinetics of the glycoalkaloids. Studies using tritiated solanine at a dose of 5 mg/Kg B.W. (Nishie et.al.,1971) demonstrated that solanine is poorly absorbed from the intestinal tract as evidenced by the low blood concentration. After p.o. administration the largest concentration occurred in the kidneys, spleen, liver and lungs. Lower concentrations occurred in adipose tissue, heart, brain and blood. Peak tissue concentration for all tissues occurred at 12 hours. At 24 hours considerably greater proportion of the dose was present after i.p. injection. Specific activity in liver, kidneys, spleen and lungs was more than twice the activity in fat, heart , brain and blood and remained elevated longer. By 96 hours, the activity decreased to almost nil. The increasing specific activity in the kidney, liver, and spleen

with increasing doses indicates the impaired ability to metabolize or excrete α-solanine at higher doses.

The ability of the rat to excrete radioactivity from increasing doses of labeled solanine differed according to the route of administration. At 24 hours following p.o. administration, excretion of tritium in urine and feces was 6% and 72% respectively. When administered i.p. excretion rates were 15% and 20% for urine and feces respectively over dose ranges 5-15 mg/Kg B.W.

With doses up to 15 mg/Kg B.W. urinary and fecal excretion showed very little impairment but at doses between 15-25 mg/Kg B.W.the urinary excretion of labeled α-solanine dropped dramatically and fecal excretion was essentially nil. There was a corresponding increase in concentration in the liver, spleen, kidneys and intestines. This was accompanied by build up of radioactivity in the g.i. tract accompanied by moderate (10-15 mg/Kg) or marked (25 mg/Kg) ascites. There was not a proportionate increase in concentration of solanine in lung, heart, blood and brain over the dose range of 5-25 mg/Kg.

When a single meal of mashed potatoes with a GA content corresponding to 1mg GA/Kg B.W. was consumed the blood serum levels peaked at 4-8 hours (Hellenas, 1992).

Groen, et. al. (1993), also studied the toxicokinetics of tritiated solanine following p.o. (170 ug/Kg) and i.v. (54 ug/Kg) administration in rats and hamsters. The elimination half-life of the unchanged solanine after i.v. administration was significantly longer than after p.o. administration. Groen speculated that the activity of enzymes involved in the metabolism of this compound are inhibited by α-solanine. This is a

common notion in the literature, however, we could find no experimental to support this idea. Data show that α-solanine may be hepatotoxic (Caldwell, 1991) and that it accumulates in the liver (Norred et. al., 1976) possibly due to reduced enzymatic activity. Claringbold et. al., (1982) observed that solanidine is sequestered in the body for long periods of time.

In the rat, excretion of total radioactivity after p.o. administration was 3.3% in urine and 86% in feces (89%) whereas after i.v. administration it was 26.3% in urine and 36.6% in feces (69%) In the hamster, after p.o. administration it was 9.91% in urine and 29.0 % in feces (38.9%) and after i.v. it was 27.7% and 23.2% respectively.

2.3.2.10 Acceptable Daily Intake

Normally, food tolerance is calculated beginning with the NOAEL (no observed adverse effect level). A tenfold uncertainty factor is added to allow for extrapolation from animal data to human data. A second tenfold uncertainty factor is added to account for variations in susceptibility in humans. Also, inherited variations in human serum cholinesterase activity has long been recognized (Sidell and Kaminskis, 1975).

With regard to α-solanine, there seems to be no adequate data to calculate the acceptable daily intake. However, 20 mg/100grams FWB of potato is most frequently cited in the literature as safe. If the GA level is 20 mg/100 grams, the average 60Kg person consuming 300 grams of potato (daily average in Sweden, higher in South America) would be consuming 1 mg/Kg B.W. Human epidemiological investigations suggest that 2 mg/Kg B.W. can be toxic. Therefore, this recommendation of 10 mg/100

g FWB allows for a safety factor of only two. Given, that GA levels often exceed 20 mg/100grams FWB, and that higher than average consumption often occurs, this safety margin is not satisfactory. Risk assessment for pesticides used on potatoes have a safety factor of 100 or more. Collectively these observations illustrate the discrepancy between risk assessment of naturally occurring toxins and synthetic chemicals.

Blood concentration rather than the dose of GA should be used as a basis for extrapolating the results of animal toxicity studies to man to compensate for species differences (Slanina, 1990).

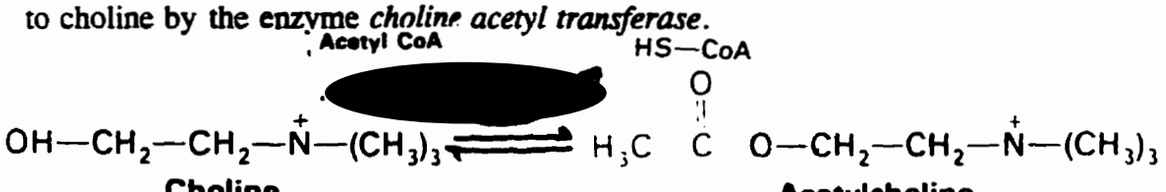
2.4 Acetylcholine

The concept of chemically mediated neurotransmission was first introduced by Elliot in 1904. He observed that an extract of adrenal gland could mimic the action of neurotransmission in the sympathetic nervous system. Later, acetylcholine was established as a primary chemical messenger. Results of pharmacological experiments led Dale (1914) to propose the existence of an enzyme capable of metabolizing the hydrolysis of this neurotransmitter in nerve tissue. In 1939, Cole and Curtiss confirmed that acetylcholine produced a change in permeability of the nerve cell membrane, allowing acetylcholine to pass through and act as a trigger for the excitation of adjacent nerve or muscle cells. The hydrolysis of ACh into its inactive components acetic acid and choline, restored the resting condition of the membrane.

With the development of theories of chemical transmission, Dale recognized the need to classify cells on a functional rather than an anatomical basis. He designated neurons which produce acetylcholine as cholinergic and those producing adrenaline as adrenergic. Acetylcholine is also found in non-cholinergic tissue such as erythrocytes. liver and plasma where its function is unknown.

2.4.1 Biosynthesis of Acetylcholine

Acetylcholine is synthesized by the transfer of an acetyl group from acetyl CoA to choline by the enzyme *choline acetyl transferase*.



Availability of choline, regulated by the high affinity choline uptake mechanism is the

rate limiting step in the synthesis of acetylcholine. It is stored in synaptic vesicles -- small membrane bound particles.It is generally accepted that acetylcholine is synthesized in the liver and usually compromised by acute hepatitis, acute cirrhosis and liver metastases, i.e. those conditions where hepatic synthesis of protein is impaired.

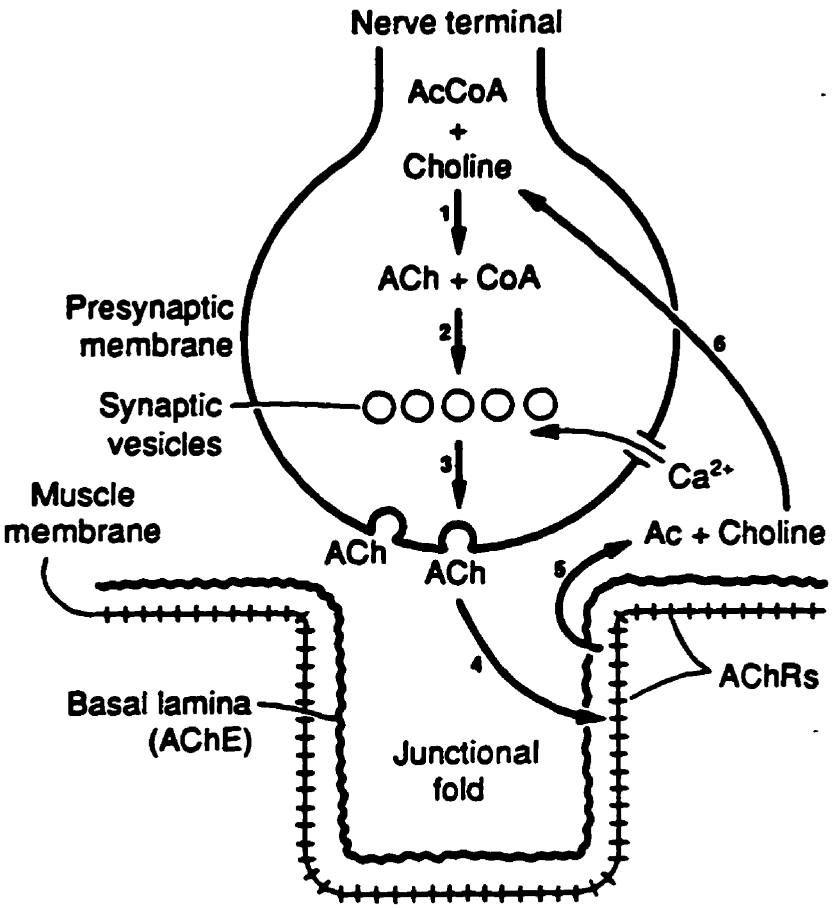
2.4.2 Biological Significance of Acetylcholine

Acetylcholine is essential for the generation of bioelectric currents which propagate impulses along nerve and muscle fibres. This transmission occurs by means of an electrochemical mechanism that depends on changes in ion distribution.

Acetylcholine is released from the synaptic vesicles spontaneously by exocytosis which results in small miniature end plate potentials. The depolarization of a nerve ending by transmission of a nerve impulse opens voltage sensitive Ca^{++} channels which permits an influx of Ca^{++} from the synaptic space into the nerve terminal. The Ca^{++} is essential for the exocytosis that releases acetycholine into the synaptic space. The released ACh diffuses rapidly across the synaptic cleft to the receptors in the junctional folds. Two molecules of ACh bind to a receptor causing a conformational change (Figure 6). This change allows a channel in the receptor to open resulting in the entry of Na^{++} ions.

In the resting neuron, the inner surface of the cell membrane is negatively charged compared with the outer surface. Sodium is actively pumped out of the cell and potassium is pumped into the cell. Sodium is unable to diffuse back but the potassium does diffuse outward along its concentration gradient. Because of this unequal distribution of ions, the inside of the axon is negatively charged with respect to the outer interstitial

Figure 6.



Schematic representation of some of the events at the neuromuscular junction.

AcCoA ------acetyl CoA

ACh ----------acetylcholine

AChRs --------acetylcholine receptors

AChE -------- acetylcholinesterase

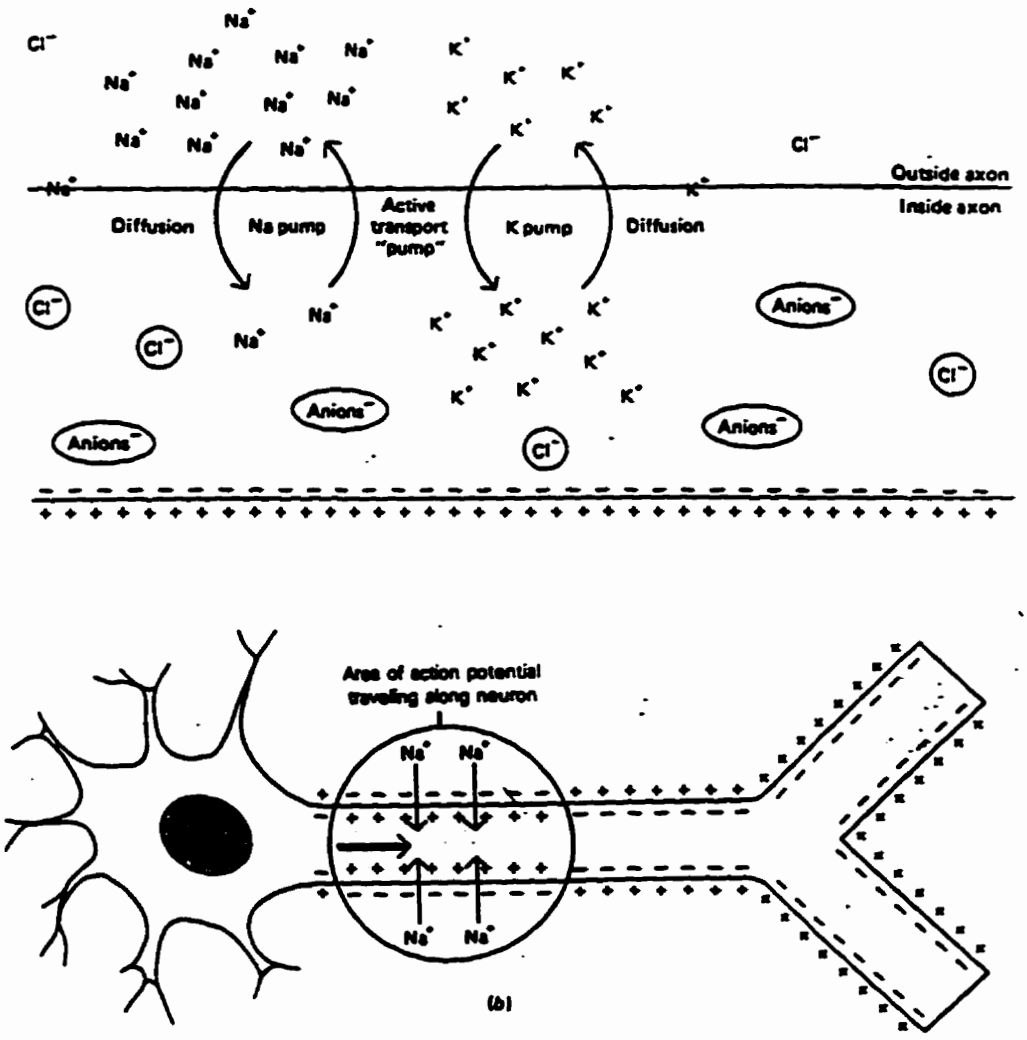
Ac ---------- acetate

fluid and electrically polarized with a resting potential of -70mV. During stimulation, the membrane becomes permeable to sodium ions and tremendous numbers of sodium ions flow into the interior of the axon. Acetylcholine facilitates the opening of the sodium gates which allow this sudden influx. The more sodium ions that diffuse into the cell, the less negative is the inner surface of the membrane, and the lower the resting potential. The membrane depolarizes up to +35mV and the neural impulses are transmitted as waves of depolarization. (Figure 7). Normally when the acetylcholine is released from the synaptic cleft, it is rapidly hydrolyzed by the enzyme acetylcholinesterase to its inactive components acetate and choline. The resting condition is thus restored and the next impulse is able to pass. When toxins or drugs inactivate or inhibit acetylcholinesterase, the acetylcholine accumulates and this excess repetitively stimulates the muscle or nerve causing overstimulation with acute cholinergic crisis with fasciculations and muscle weakness. This muscle spasm or respiratory paralysis leads to subsequent degeneration: the acute myopathy. The activity of this enzyme is absolutely essential for the elementary process of conduction. Orgell (1958) demonstrated that cholinesterase inhibition was one of the acute effects of solanaceous glycoalkaloid intoxication. Glycolakaloids are potent inhibitors of acetylcholinesterase ranking fourth out of 134 known inhibitors (Merck Index). The organophosphate insecticides and carbamates are only slightly more potent.

2.4.3 Acetylcholinesterase and Butyrylcholinesterase

In 1940, Alles and Hawes observed that two types of cholinesterases occur in tissues and biological fluids. More recently it has been demonstrated by McQueen (1995)

Figure 7.


Cl⁻
Na⁺
K⁺
Outside axon
Inside axon
Diffusion
Na pump
Active transport "pump"
K pump
Diffusion
Anions⁻
Area of action potential traveling along neuron
(b)

that AChE and BuChE are two distinct enzymes encoded by two different but related genes. True acetylcholinesterse, which is found in nerve and muscle, was named acetylcholinesterase (AChE 3.1.1.7) and the other was called either pseudo, serum or butyrylcholinesterase (BuChE 3.1.1.8). Although both of these enzymes hydrolyze their substrate at exceptionally high rates, they display differences in substrate specificity and sensitivity to inhibitors. In order to be classified as AChE, the enzyme must have a high affinity for acetylcholinesterase, show substrate inhibition and substrate preference whereby the rate of hydrolysis declines with respect to an increase in length of acyl chain (Rosenberry, 1963).

acetylcholine $>$ propionylcholine $>$ butyrylcholine

They were differentiated by the observation that acetylcholinesterase had a well defined optimum substrate concentration. Excess substrate caused a sharp decrease in acetylcholinesterase activity. In contrast, serum choline esterase followed typical Michaelis-Menten kinetics and did not display substrate inhibition.

For BuChE, the rate of hydrolysis increases in order

acetylcholine $<$ propionylcholine $<$ butyrylcholine

Brain and erythrocyte acetylcholinesterase forms possess a large common catalytic domain but differ in their C-terminal part. The erythrocyte acetylcholinesterase is 26 amino acids shorter than the brain acetylcholinesterase (Boschetti et. al., 1996). The

acetylcholinesterase from torpedo mamorata has different peptide sequence than brain AChE.

The discovery of increased concentration of AChE in muscle, especially at the endplate regions, led Marnay and Nachmansohn (1938) to examine the activity of the electric organs of eel and Torpedo ray -- these are essentially modified neuromuscular junctions. Due to the relatively large quantities of enzyme available from this source, much of the work on AChE has involved chemical, structural, kinetic and pharmacological studies on this enzyme *in vitro*.

Erythrocyte acetycholinesterase activity is relatively constant and depends upon the rate of erythopoiesis. Newly formed erythrocytes have a high level of enzyme activity, and the activity diminishes with age. Therefore, enzyme activity is largely determined by the number and age of the erythrocytes. A change in erythrocyte acetylcholinesterase activity of >8% in men and 12% in women is alarming and an envioronmental cause should be investigated. Changes is plasma cholinesterase of 25-50% in a period of weeks is usual in healthy individuals and porvides little insight by itself (Sidell and Kaminskis, 1975). However, because different physiological conditions determine the levels of these two enzymes, a substantial decrease in both enzymes is a strong indication of exposure to a toxic agent.

The inhibition of BuChE does not seem to be pharmacologically important, but serves as an excellent biochemical marker for the presence of an inhibitor. Butyrylcholinesterase is sensitive to inhibitors and will be completely inactivated while acetylcholinesterase in inhibited only 50%. This is not a fixed relationship, but varies according to inhibitor and route of absorption. Plasma cholinesterase has no known

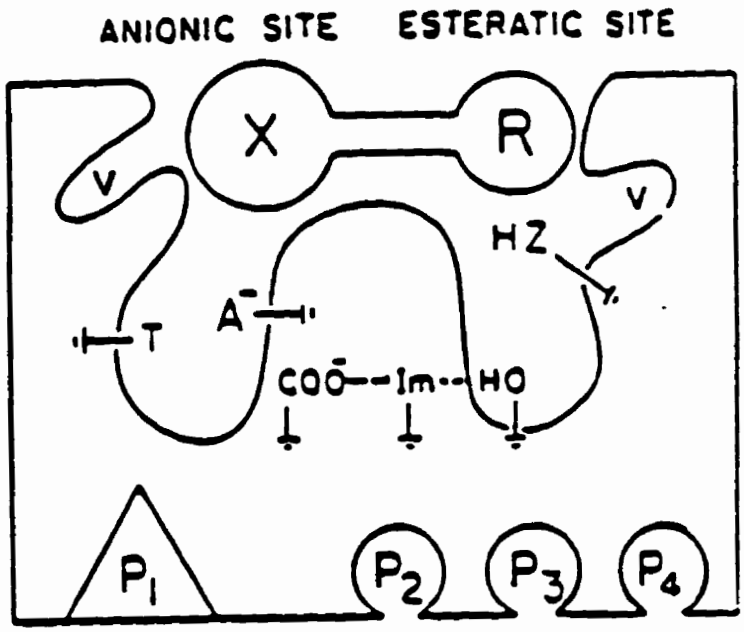
physiological function but its inhibition reflects exposure to various noxious agents.

The physiological function of erythrocyte acetylcholinesterase is not known. The enzyme is the same as that involved in synaptic transmission and its measurement is thought to mirror effects on the nervous system. The relation between inhibition in erythrocytes and nervous tissue depends on the pharmacokinetics of inhibitors but usually erythrocyte acetylcholinesterase inhibition overestimates that in nervous tissue. Erythrocyte inhibition persists longer than in nervous tissue.

The principal biologic role of AChE is believed to be the termination of impulse transmission by hydrolysis of the neurotransmitter acetycholine to acetate and choline. The hydrolysis of ACh to acetate and choline occurs on the region of the protein surfaces known as the active or catalytic site which is comprised of two subsites, the anionic and the esteratic site. (Figure 8). AChE and BuChE differ in that BuChE binds more readily to hydrophobic regions surrounding the anionic site of the catylytic site. BuChE is believed to react easily with anti-cholinesterase agents before they have a chance to inactivate AChE at brain synapses and neuromuscular junctions (Schwarz et. al., 1995). In addition to the differences between AChE and BuChE, several allelic variants of BuChE differ in their interactions with natural (glycolalkaloids of the Solanaceae) and man-made anti-cholinesterase (e.g. organo phosphates and carbamate pesticides) Soreq and Zahut (1993).

Fractionation studies conducted on different brain regions demonstrate that acetylcholinesterase enzyme activity is associated with the membrane of excitable cells. Following exposure to an AChE inhibitor, regional variations in ACh accumulation were observed and these variations corresponded to concentration of these excitable cells.

Figure 8.



RX ---- substrate

V ------ hydrophobic regions

A ------ negatively charged group at the anionic site

HZ ---- acidic group at esteratic site

P_1 - P_4 — peripheral anionic sites

(Rosenberry, T.L., 1963)

Although it has been shown that mammalian brain acetylcholinesterase is membrane bound, (Boschetti, 1996) dietary fats which affect the fatty acid composition of the brain membranes did not show an effect on the cholinergic system *in vivo* (Salvati, 1996).

The potency of an anti-cholinesterase agent *in vivo* depends on a number of factors which do not operate *in vitro*. If the agent is susceptible to detoxification in the liver or extrahepatic systems, it may prove less toxic than its *in vitro* performance would suggest. Some compounds are methylated *in vivo* making a relatively weak *in vitro* inhibitor more potent. Lipid solubility also has an important effect. Polar molecules will not readily enter the CNS, therefore noxious actions will be restricted largely to the periphery. There is evidence that the rate of penetration of a charged inhibitor through the blood-brain barrier into the CNS may vary from one region of the brain to another.

Chapter III

MATERIALS AND METHODS

3.1 Animals

The use of experimental animals conformed to the guidelines of the Canadian Council on Animal Care. All aspects of the experiment were approved by the Animal Policy and Ethics Committee of the University of Manitoba. Male weanling (21 day old) Sprague-Dawley rats (50-60g) were purchased from the University of Manitoba central breeding facility. The animals were housed separately in individual stainless steel cages and kept on a 12-12 hour light/dark cycle. The room temperature was maintained at $21 \pm 1°C$ with a relative humidity of 50%.

3.2 Experimental Protocol

The rats were fed a standard protein (modified AIN 76) semi-purified diet *ad libitum* for 5 days to allow for adaptation from the commercial lab chow diet. They were then randomly assigned to either a low protein (7.5%) or standard protein (15%) diet and fed *ad libitum* for the next 28 days.

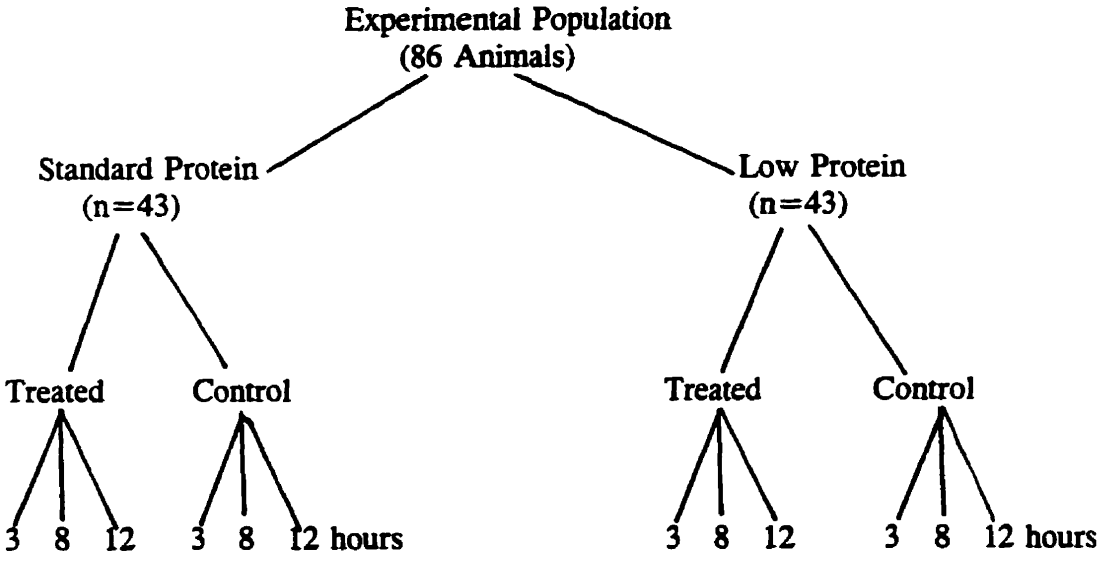
After four weeks on the dietary regimen, the animals were assigned to experimental treatment and injected intraperitoneally with α-solanine (dose level of 10 mg/KG B.W. suspended in 8% ethyl alcohol. Controls were similarly treated with only the ethyl alcohol.

At the end of the treatment period, three, eight or twelve hours post dosing, the animals were lightly anaesthetized with ether and a blood sample collected from the tail

vein. They were humanely killed by decapitation and the liver and brain excised, frozen immediately in liquid nitrogen and stored at -80°C until required for homogenate preparation and/or assay. The blood sample was centrifuged and the plasma removed to separate vials. Washed erythrocytes were diluted and stored at -80°C until assay.

3.3 Experimental Design

Table 7 summarizes the experimental design. Eighty-six animals were fed the standard protein semi-purified diet for an adaptive period fo 5 days. They were randomly assigned to standard protein (15%) or low protein (7.5%) diets for 28 days.



6-7 animals per treatment group

3.4 Diet formulation

A semi-purified diet consisting of relatively pure sources of starch, protein, lipid,

Table 8.

Diet Composition (percent weight)

Ingredient	Low protein	Standard Prot.	AIN 76
Casein[1]	8.62	17.24	20
Corn Starch[2]	35.76	31.38	15
Glucose[4]	35.76	31.38	50 (sucrose)
D-L Methionine[1]	0.15	0.30	0.3
AIN Mineral Mix[5]	3.50	3.50	3.50
AIN Vitamin Mix[5]	1.00	1.00	1.00
Choline Bitartrate[1]	0.20	0.20	0.20
Corn Oil[3]	5.00	5.00	5.00
Lard[3]	5.00	5.00	
Alphacel (fibre)[5]	5.00	5.00	5.00

1. United States Biochemical Corporation, Cleveland, Ohio.
2. St. Laurence Corn Oil, St. Laurence Starch Company, Mississauga, Ont.
3. Tenderflake Lard, Maple Leaf Foods Inc., Toronto, Ontario.
4. R-Wine Baril, Winnipeg, Manitoba.
5. ICN Pharmaceuticals Inc. Cleveland, Ohio.

crystalline vitamins and reagent grade minerals is preferred for use in studies requiring a degree of nutritional definition. Therefore, a semi-purified diet was prepared based on the National Research Council's guidelines set forth in the Nutrient Requirements of Laboratory Animals (1978). This formulation is intended for optimal growth and maintenance during the first year of life. To ensure that the diet was adequate in essential fatty acids, the percentage of corn oil was maintained at 5% in accordance with the recommendation of the American Institute of Nutrition (1977). Specifics of the diet are provided in table 8. Modifications of the AIN diet were made because the AIN 76 diet is not suitable for toxicological studies which depend on normal hepatocellular function. The toxicity of some drugs is markedly affected by the simple/complex CHO ratio of diets used in the study. In the AIN 76 formulation, sucrose is the major carbohydrate. Recommendations for change were made in the subsequent revision (AIN-76A; AIN, 1980). One of the recommendationswas to substitute glucose for sucrose, substituting cornstarch for sucrose, or varying the carbohydrate portion by using a combination of the three. None of these suggestions was recommended over the others.

3.5 Methods

Determination of AChE can be made by a variety of available methods. The hydrolysis of the ester is accomplished by the release of one equivalent amount of acid. This can be determined titrimetrically with an automated pH stat or measured by Warburg manometry as CO_2 liberated from a bicarbonate buffer by the acid. Radiochemical and fluorometric assays have been used also (Ricny et. al., 1989). The most widely used method is a spectrophotometric determination.

3.5.1 Erythrocyte Acetylcholinesterase

Erythrocyte AChE was determined by the Improved Ellman Procedure (George and Abernethy, 1983) a method used previously in this laboratory and found successful for AChE determination. The method involves the hydrolysis product, thiocholine which is detected by reaction with dithiobis nitrobenzoate (DTNB) to produce 5-thionitrobenzoate, a yellow colored anion that absorbs strongly at 412 nm.

The principle obstacle to estimating erythrocyte cholinesterase is the high background absorbance of hemoglobin pigments. The peak absorbance wavelength of the measured reaction product 5-thiobenzoate coincides with the massive Soret absorption band of hemoglobin from erythrocytes at 410 nm. George and Abernethy (1983) found that the absorbance peak of 5-thionitrobenzoate is shifted from 410 to 435 nm. in the presence of the cationic detergent benzethonium chloride (Hyamine 1622), the Soret band of hemoglobin is shifted to a shorter wavelength (405 nm) and its peak intensity decreased. Detergent micelle interactions shift the peak absorbance of 5-thionitrobenzoate from 410 to 435 nm. eliminating the spectral interference arising from the superposition of the Soret hemoglobin band.

Samples of human blood were assayed by this technique on the same day to provide an additional control.

Calculation:

Absorbance difference x dilution/ molar absorbtivity x reaction time

Dilution = 2562
Molar absorbtivity 14340 L/ mol
Reaction time = 10 mins.

IUB = micromols of propionylthiocholine hydrolyzed/ min/ per ml of packed RBC

3.5.2 Plasma Cholinesterase

Plasma cholinesterase was determined using the Improved Ellman Procedure for Erythrocyte Cholinesterase, (Geroge and Abernethy, 1983). Although, previously, this method was used successfully in this laboratory considerable difficulties were encountered. Turbidity in the assay mixture made spectrophotometric measurement impossible. Several dilutions were tested but the results were inconsistent. There was considerable intra-individual variation presumably due to the cloudiness of the solution. Centrifugation of the sample before reading did not give more reliable results.

In this method, Hyamine 1622 replaced the quinidine sulfate stopping reagent used in the method of Dietz, (1977). However, a small amount of quinidine sulphate present in the DTNB reagent selectively inhibited the plasma cholinesterase.

Consultation with one of the authors, M.Abernethy (personal communication) provided direction in selecting another more appropriate method for plasma cholinesterase. However, due to the small size of the samples collected, it was not possible to analyze them a second time.

3.5.3 Liver and Brain Acetylcholinesterase

A further review of the literature showed that the most commonly used method is that of Ellman et.al., 1961 (Hayes, 1994). Most investigators currently use this method (Rao, 1995; Saez-Valero, 1995; Roddick, 1989; Wierenga, 1992; Shuttleworth, 1990). It is extemely sensitive (ie. 10 ul sample of blood is adequate) and is applicable to either small amounts of tissue or to low concentration of enzyme. The method has been used

been used to study the enzyme in human erythrocytes and homogenates of rat brain, kidney, lungs, liver and muscle tissue. The progress of hydrolysis is followed by measurement of the rate of production of thiocholine as acetylthiocholine is hydrolyzed. (Data obtained using acetylthiocholine as substrate have shown it to be a satisfactory substitute for the natural substrate). This is accomplished by continuous reaction of the thiol with 5 5' dithiobis 2 nitrobenzoate to produce the yellow anion of 5 thio 2 nitrobenzoic acid. (Figure 9). The rate of colour production is measured at 412 nm in the spectrophotometer. The reaction with the thiol has been shown to be sufficiently rapid so as not to be rate limiting in measurement of the enzyme and in concentration that does not inhibit the enzymic hydrolysis.

The rates were calculated as follows:

$$R = \frac{\Delta A}{1{\cdot}36\,(10^4)} \times \frac{1}{(400/3120)C_o}$$

R = rate in moles of substrate hydrolyzed per min per gram of tissue

Δ A = changes in absorbance per minute

C_o = original concentration of tissue (mg/ml)

The extinction coefficient of the yellow anion is known to be 13,600 (Ellman, 1959).

3.6 Statistical Analysis

Analyses of variance (ANOVA) were carried out on the experimental data. The

Figure 9.

PRINCIPLE OF REACTIONS

$$H_2O + (CH_3)_3\bar{N}CH_2CH_2SCOCH_3 \xrightarrow{(enzyme)} (CH_3)_3\bar{N}CH_2CH_2S^- + CH_3COO^- + 2H^+$$

acetylthiocholine — thiocholine

$$(CH_3)_3\bar{N}CH_2CH_2S^- + RSSR \longrightarrow (CH_3)_3\bar{N}CH_2CH_2SSR + RS^- \quad (II)$$

5 : 5'-dithiobis-2-nitrobenzoate ion — 5-thio-2-nitro-benzoic acid (yellow)

significance between treatment groups was determined at the 5% level using Tukey's studentized Range test as described by Steel and Torrie (1960).

Data were analyzed using the Statistical Analysis (SAS), 1985 version 6.06.

CHAPTER IV

RESULTS

4.1 Animal Weight

Animals on the standard protein diet showed the greater weight gain at the end of the study period (4 weeks). At the end of week 1 and week 2 the differences were not statistically significant. However, at the end of week 3 and week 4 these differences were significant (Table 9, Figure 11).

4.2 Food Consumption

Food intake was greater on the low protein diet than on the standard protein diet (Table 10, Figure 12). These differences were statistically significant throughout the study period.

4.3 Feed Efficiency

Feed efficiency was calculated and found to different with standard protein animals having the greater efficiency and the low protein animals having the lesser efficiency (Table 11).

4.4 Enzyme Assays

The level of acetylcholinesterase and butyrylcholinesterase activity in the tissues examined was slightly reduced as a result of treatment but the difference was not statistically significant (Tables 12-23; Figures 12-23).

4.5 Protein Effect

The effect of dietary protein on the enzyme activity was observed only in the liver. In this tissue, enzyme activity was significantly lower in animals fed the low protein diet compared with animals fed the standard protein diet at each of the three time intervals -- three, eight, and twelve hours post-injection.

Table 9

Effect of Dietary Protein on Body Weight (grams)

variable	n	Low Protein	Standard Protein
week 1	43	142.0 ± 15.4[a]	146.5 ± 19.4[a]
week 2	43	196.5 ± 19.6[a]	203.0 ± 23.4[a]
week 3	43	246.7 ± 22.4[a]	259.9 ± 23.9[b]
week 4	43	291.1 ± 32.9[a]	312.0 ± 28.3[b]

Means ±S.D. denoted with different superscripts are significantly different. ($p < 0.05$)

Table 10

Effect of Dietary Protein on Food Intake (grams)

variable	n	Low Protein	Standard Protein
week 1	43	137.9 ± 22.5^a	121.0 ± 19.3^b
week 2	43	163.2 ± 29.0^a	139.9 ± 19.7^b
week 3	43	164.0 ± 16.3^a	144.3 ± 15.0^b
week 4	43	168.1 ± 21.8^a	157.7 ± 26.1^b

Means $\pm$S.D. denoted with different superscripts are significantly different. ($p<0.05$)

Table 11

Feed Efficiency

Standard Protein	Low Protein
0.38 ± 0.04^a	0.31 ± 0.05^b

RESULTS

Table 12 Acetylcholinesterase Activity in Erythrocytes 3 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	**Standard Protein**
Control	1.09 ± 0.44^a	1.24 ± 0.28^a
GA treated	1.08 ± 0.97^a	1.11 ± 0.32^a

Table 13 Acetylcholinesterase Activity in Erythrocytes 8 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	**Standard Protein**
Control	1.12 ± 1.02^a	2.28 ± 1.18^a
GA treated	1.25 ± 0.83^a	1.53 ± 0.61^a

Table 14 Acetylcholinesterase Activity in Erythrocytes 12 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	**Standard Protein**
Control	1.16 ± 0.43^a	2.09 ± 0.76^a
GA treated	0.95 ± 1.00^a	2.18 ± 1.08^a

Values are means ±SD. Means with different superscripts are significantly different ($p < 0.05$)

RESULTS

Table 15 Butyrylcholinesterase Activity in Plasma 3 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	**Standard Protein**
Control	0.44 ± 0.14^a	0.57 ± 0.20^a
GA treated	0.39 ± 0.19^a	0.48 ± 0.10^a

Table 16 Butyrylcholinesterase Activity in Plasma 8 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	**Standard Protein**
Control	0.41 ± 0.11^a	0.58 ± 0.22^a
GA treated	0.52 ± 0.30^a	0.40 ± 0.19^a

Table 17 Butyrylcholinesterase Activity in Plasma 12 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	**Standard Protein**
Control	0.42 ± 0.09^a	0.58 ± 0.29^a
GA treated	0.40 ± 0.31^a	0.60 ± 0.30^a

Values are means $\pm$ SD. Means with different superscripts are significantly different ($p < 0.05$)

Table 18 Butyrylcholinesterase Activity in Liver 3 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	Standard Protein
Control	0.89 ± 0.08^{b}	1.23 ± 0.13^{a}
GA treated	0.86 ± 0.12^{b}	1.31 ± 0.27^{a}

Table 19 Butyrylcholinesterase Activity in Liver 8 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	Standard Protein
Control	0.94 ± 0.12^{b}	1.10 ± 0.15^{a}
GA treated	0.95 ± 0.15^{b}	1.21 ± 0.28^{a}

Table 20 Butyrylcholinesterase Activity in Liver 12 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	Standard Protein
Control	0.89 ± 0.22^{c}	1.21 ± 0.27^{a}
GA treated	0.87 ± 0.12^{c}	0.99 ± 0.07^{b}

Values are means ± S.D. Means with different superscripts are significantly different ($p < 0.05$)

Table 21 Acetylcholinesterase Activity in Brain 3 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	Standard Protein
Control	9.32 ± 2.69^a	11.96 ± 4.38^a
GA treated	10.36 ± 2.30^a	10.29 ± 2.37^a

Table 22 Acetylcholinesterase Activity in Brain 8 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	Standard Protein
Control	11.44 ± 2.91^a	10.24 ± 2.10^a
GA treated	11.70 ± 4.0^a	12.64 ± 0.93^a

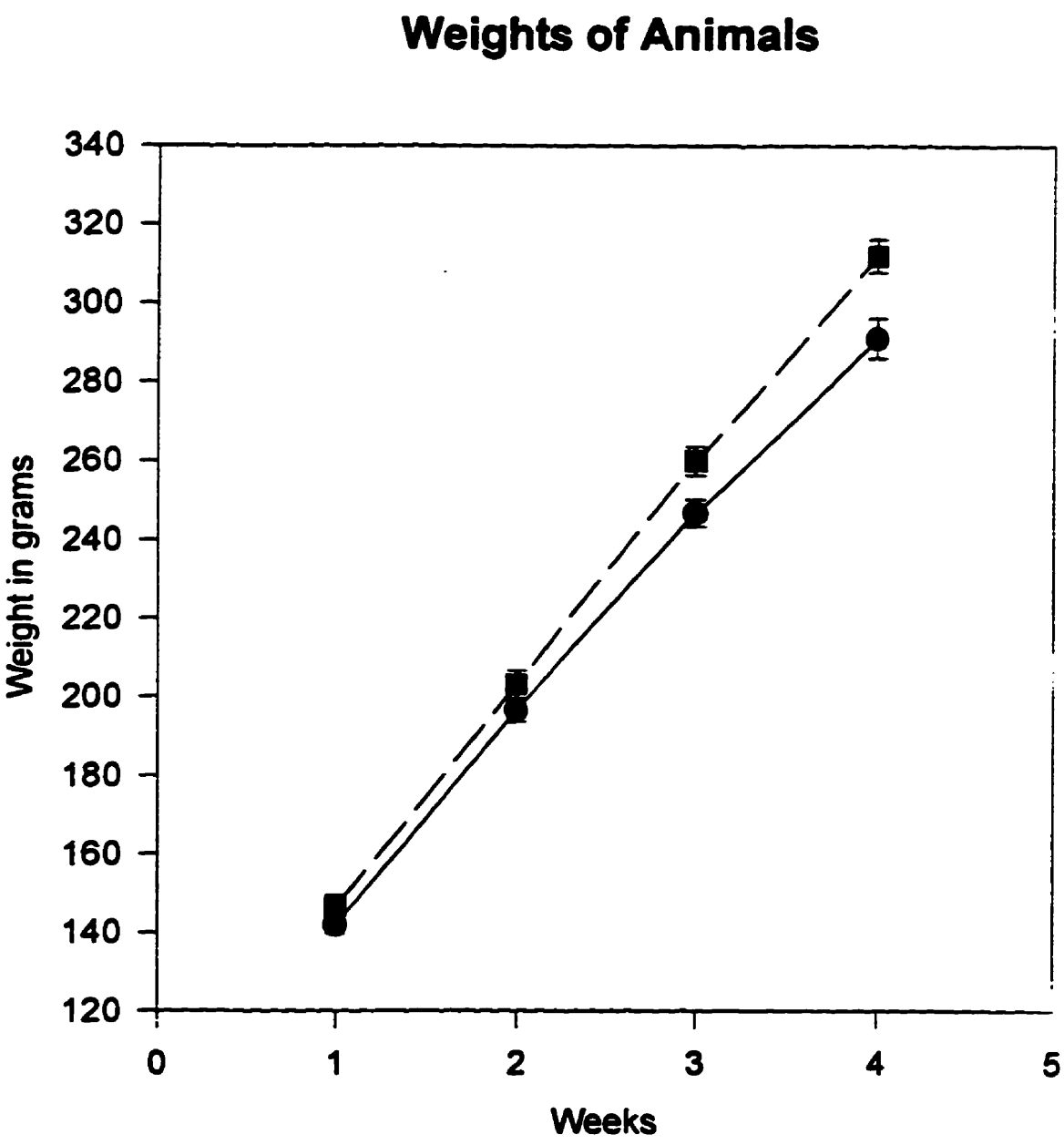
Table 23 Acetylcholinesterase Activity in Brain 12 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

	Low Protein	Standard Protein
Control	13.16 ± 2.60^a	12.46 ± 2.37^a
GA treated	10.11 ± 2.69^a	12.15 ± 3.15^a

Values are means $\pm$ SD. Means with different superscripts are significantly different ($p < 0.05$)


Low Protein
Standard Protein

Figure 10.


Weights of Animals
340
320
300
280
260
240
220
200
180
160
140
120
Weight in grams
0
1
2
3
4
5
Weeks

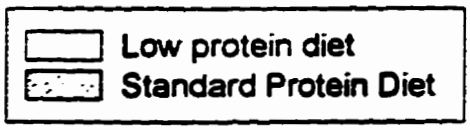
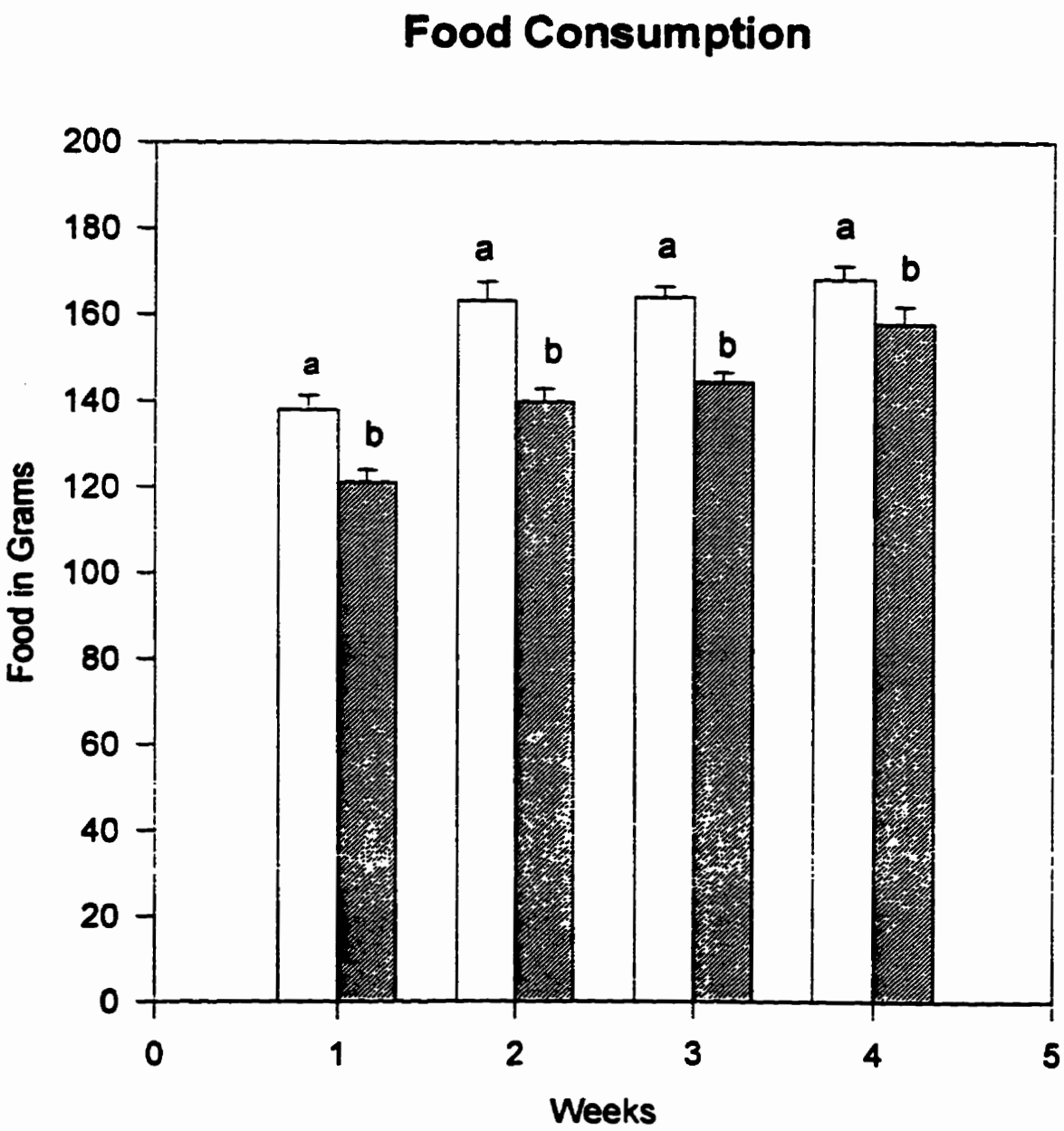


Figure 11.

Effect of Dietary Protein Level on Food Consumption



Means±SEM. Means designated with the same letter are not significantly different.($p<0.05$)

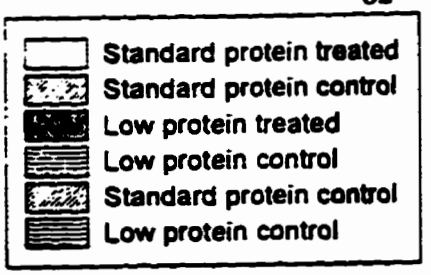
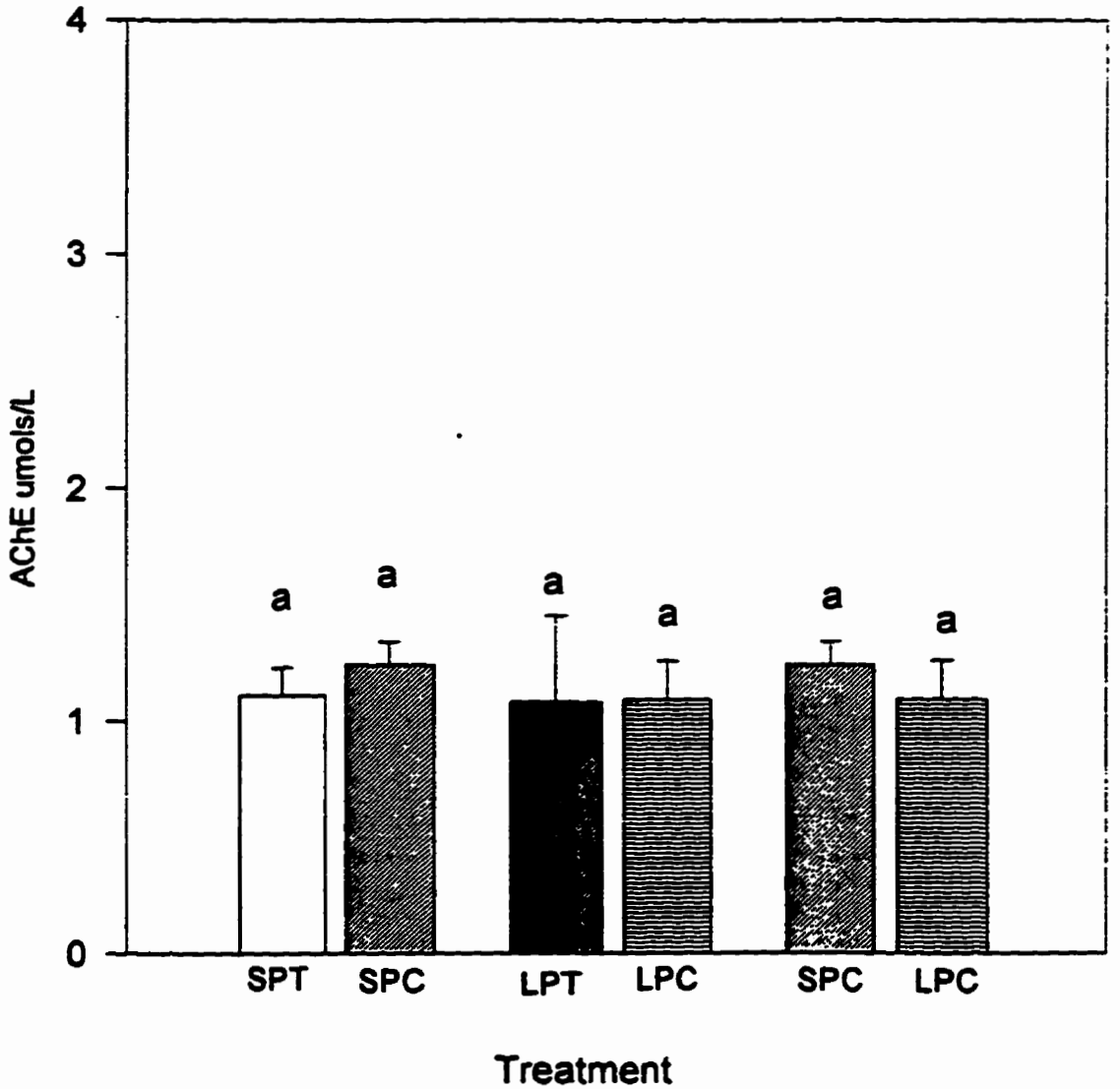


Figure 12.

Acetylcholinesterase Activity in Erythrocytes 3 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).



Means ±SEM.Means with same letter designation are not significantly different. ($p<0.05$)

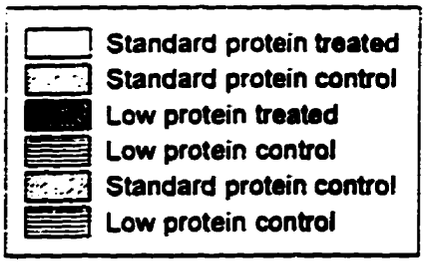
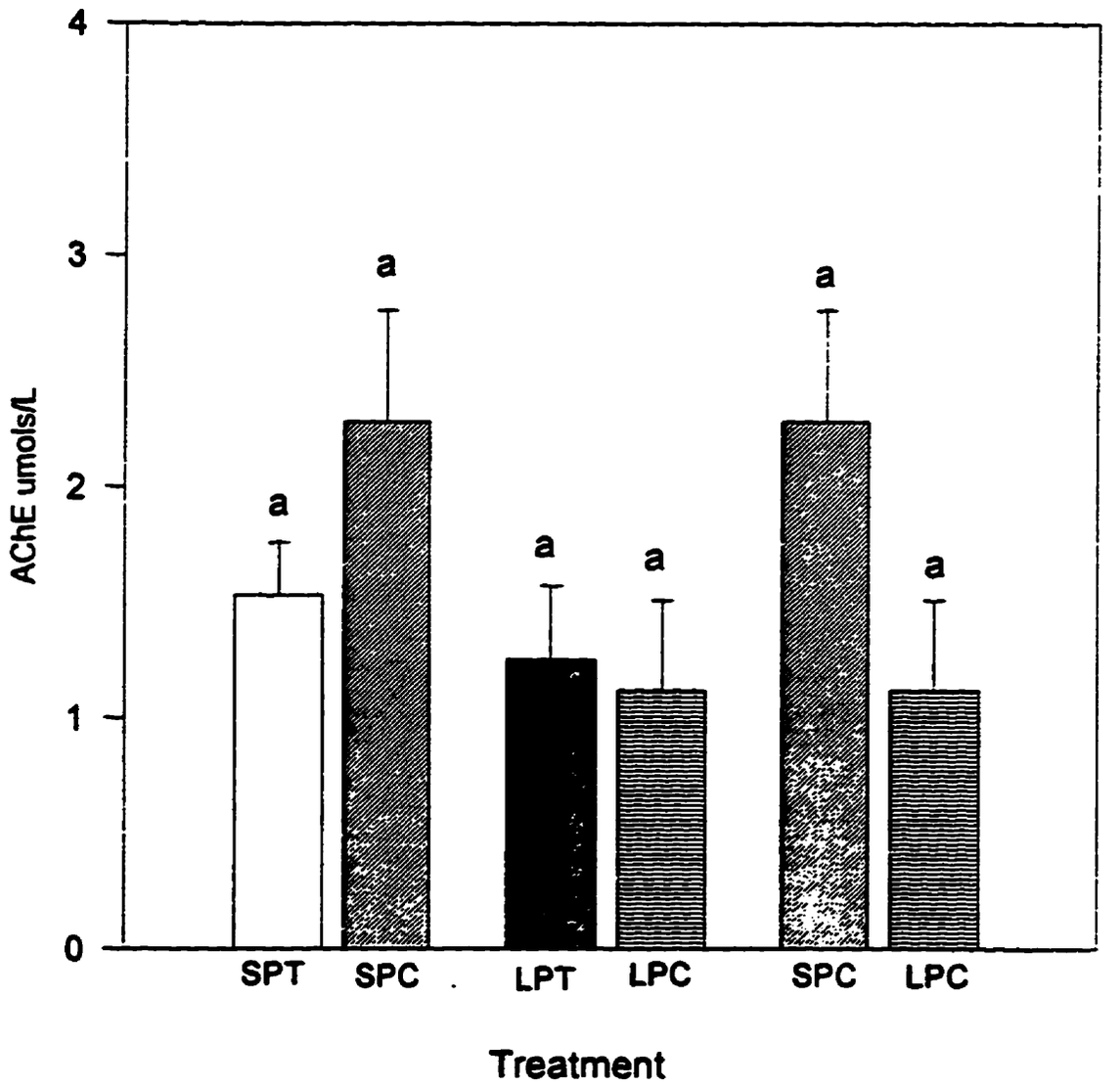


Figure 13.

Acetylcholinesterase Activity in Erythrocytes 8 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).



Means ± SEM. Means with same letter designation are not significantly different. ($p<0.05$)

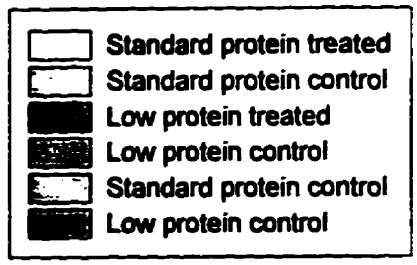
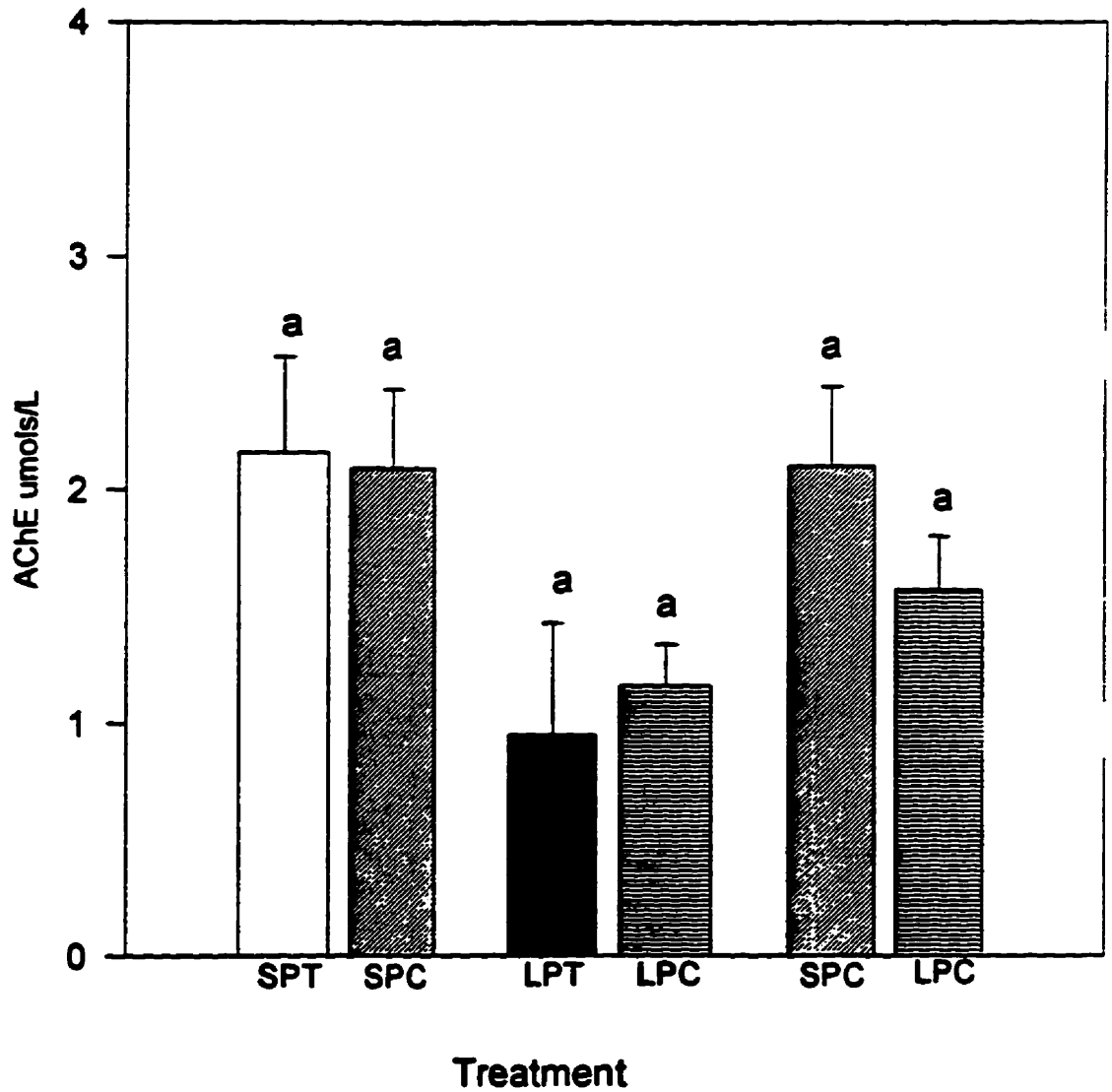


Figure 14.

Acetylcholinesterase Activity in Erythrocytes 12 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).



Means ± SEM. Means with same letter designation are not significantly different. ($p<0.05$)

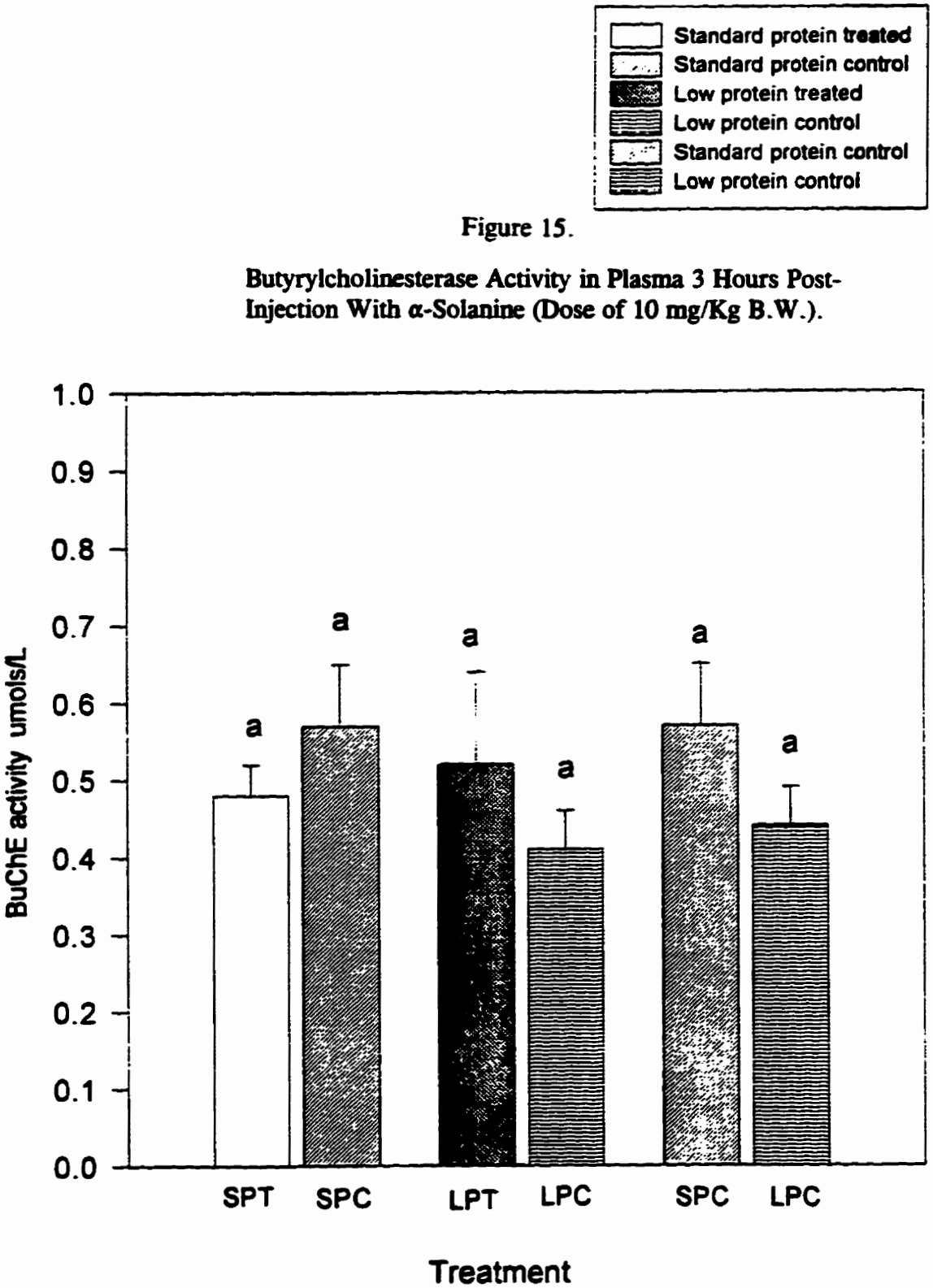
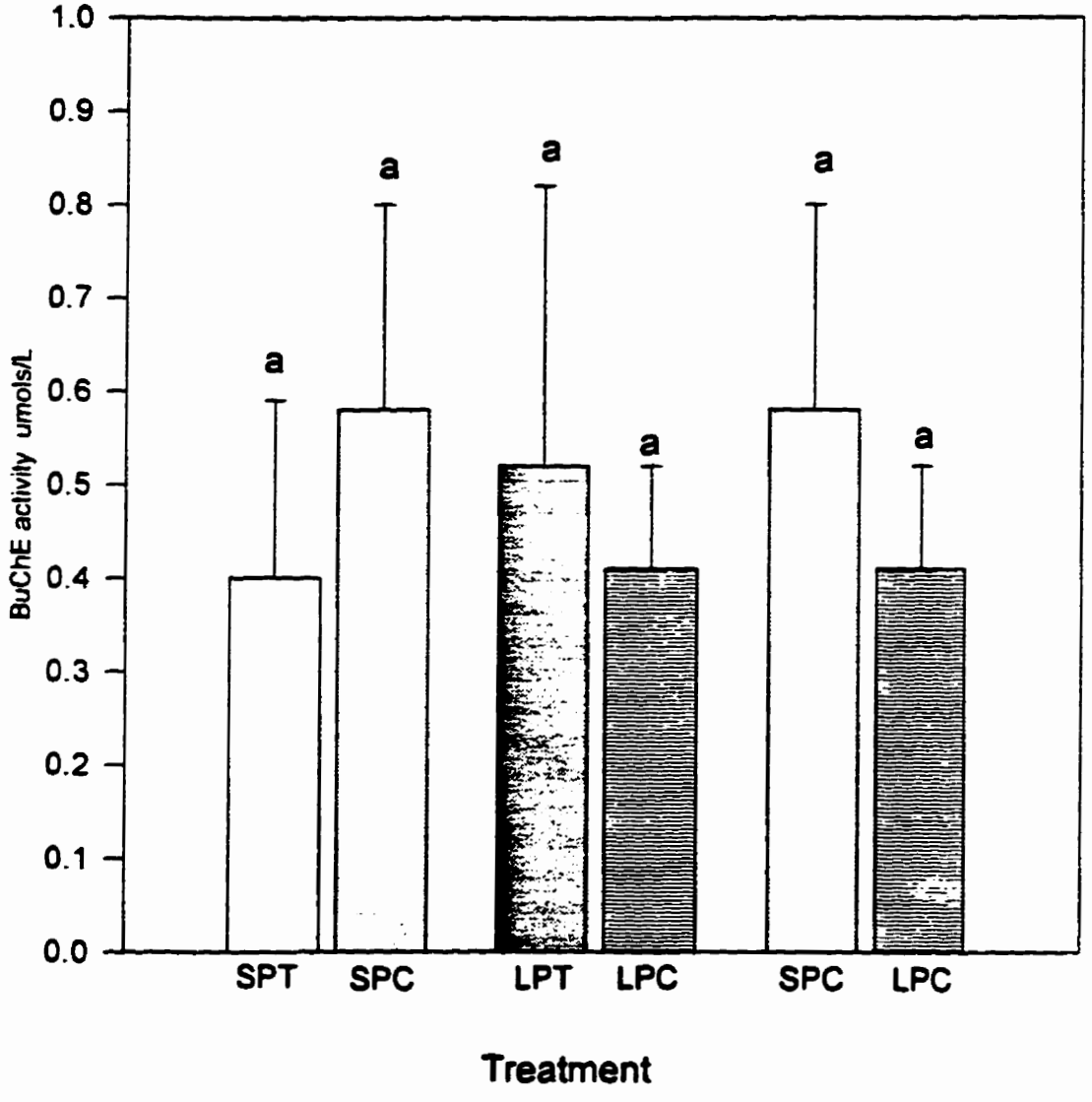


Figure 15.

Butyrylcholinesterase Activity in Plasma 3 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

Means±SEM. Means with same letter designation are not significantly different ($p<0.05$)

Figure 16. Butyrylcholinesterase Activity in Plasma 8 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).



Means ± SEM.Means with same letter designation are not significantly different ($p<0.05$)

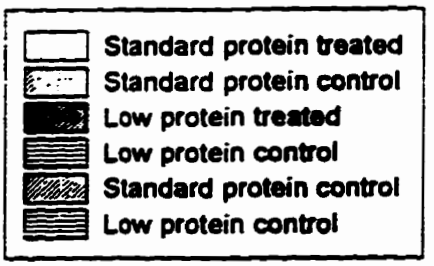
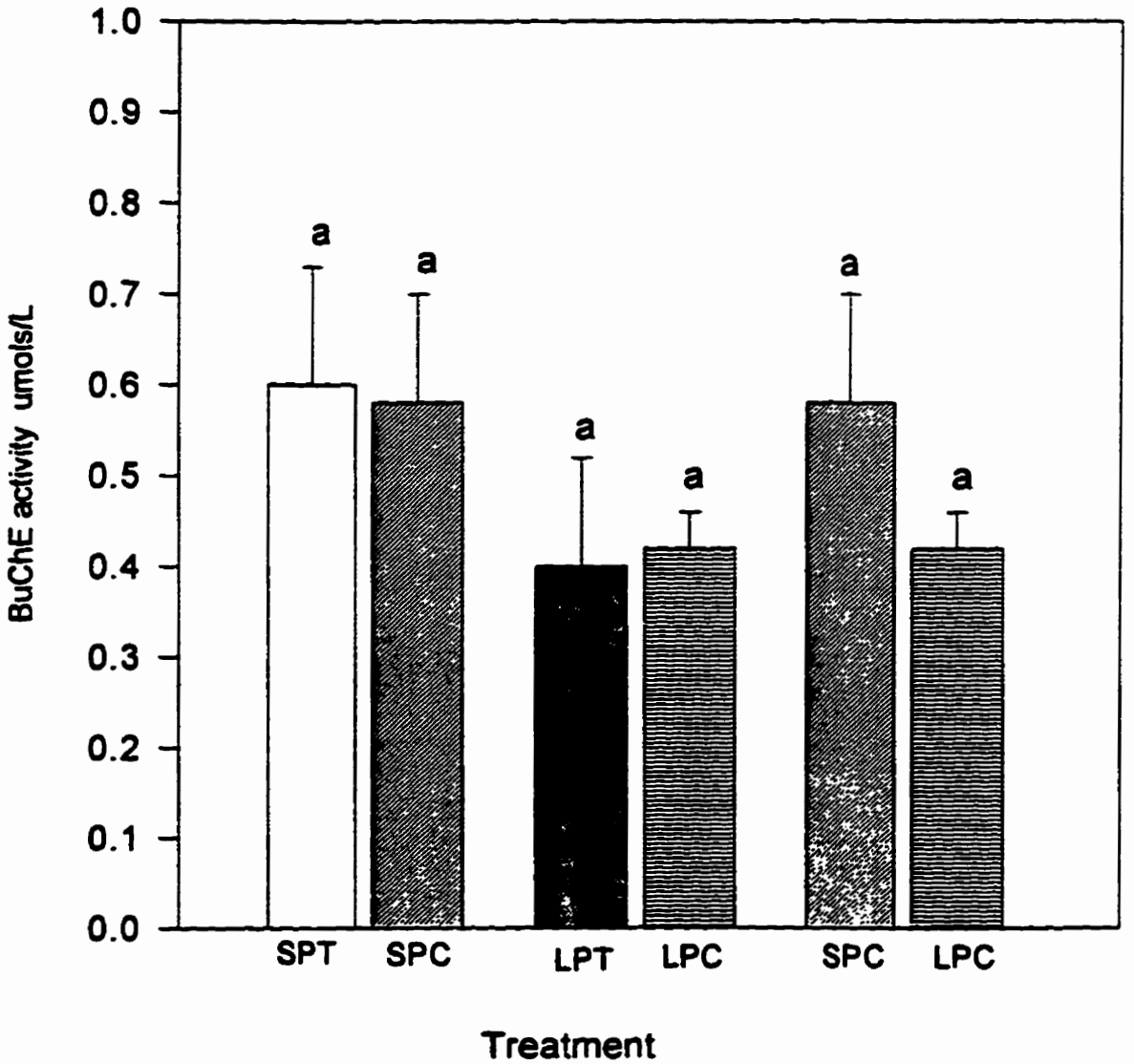


Figure 17.

Butyrylcholinesterase Activity in Plasma 12 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).



Means±SEM. Means with same letter designation are not significantly different ($p<0.05$)

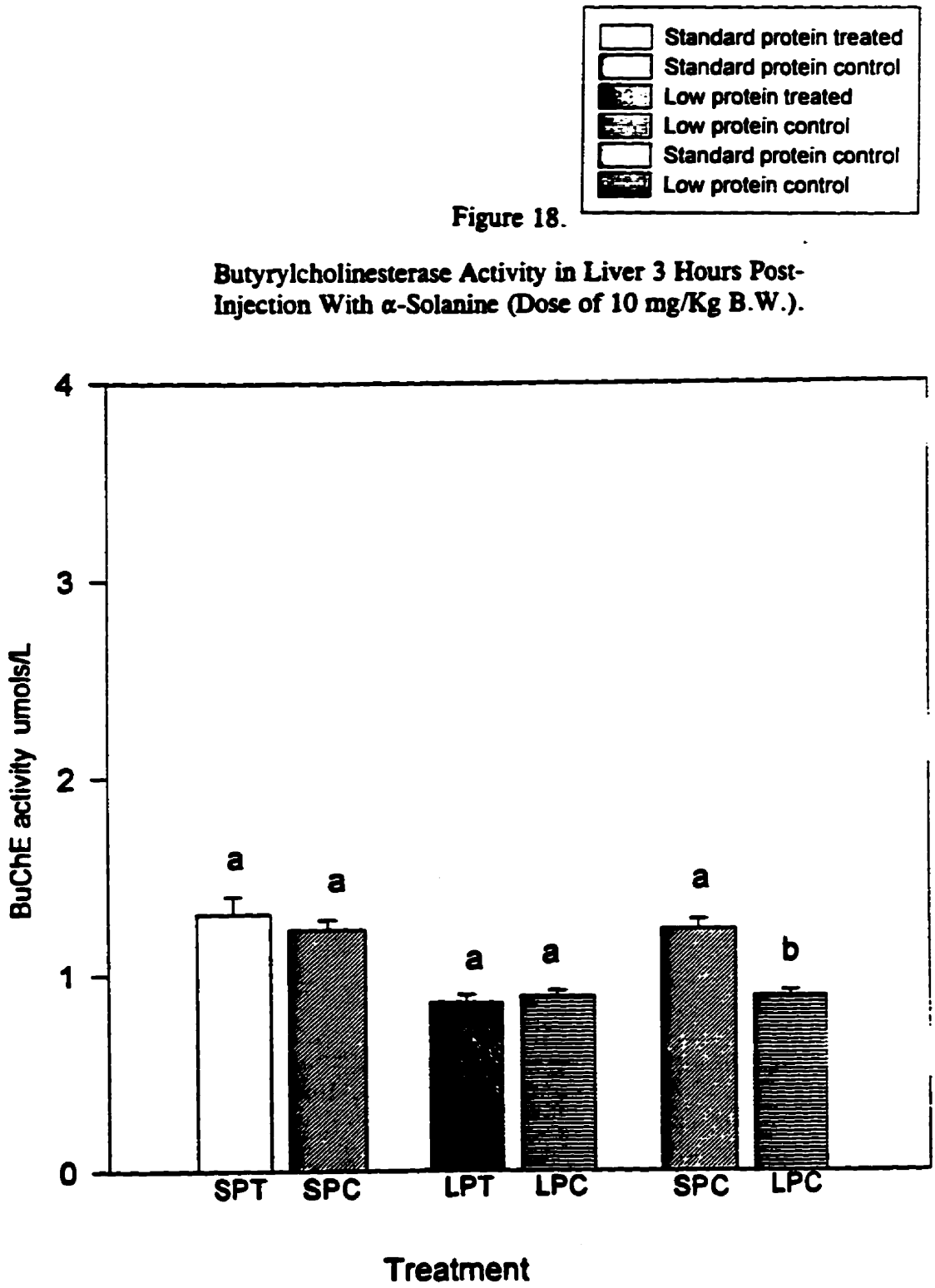


Figure 18. Butyrylcholinesterase Activity in Liver 3 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).

Means ± SEM.Means with same letter designation are not significantly different ($p<0.05$)

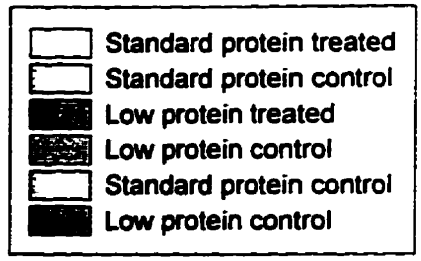
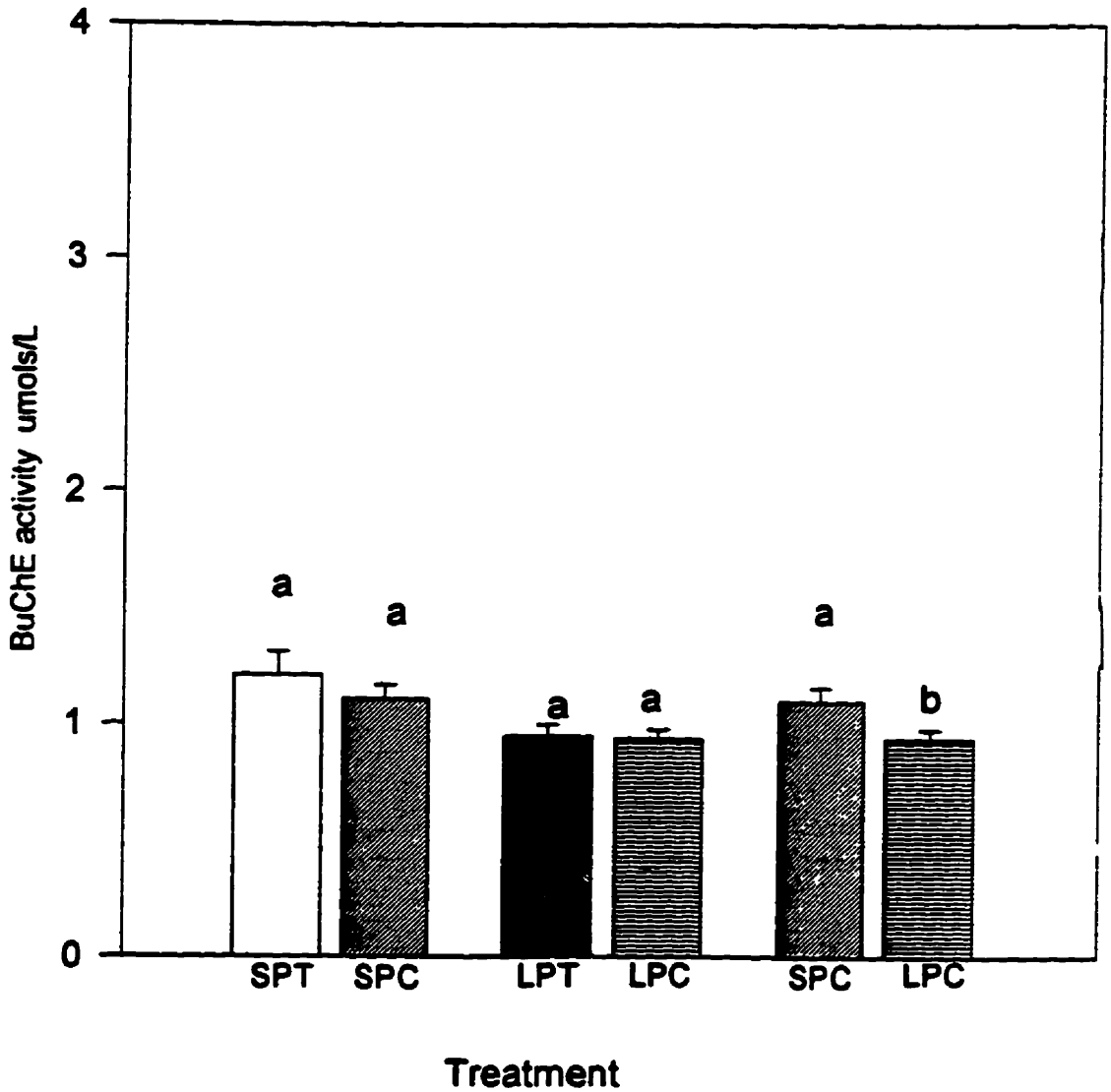


Figure 19.

Butyrylcholinesterase Activity in Liver 8 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).



Means±SEM. Means with same letter designation are not significantly different.($p<0.05$)

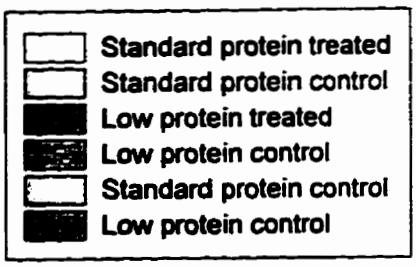
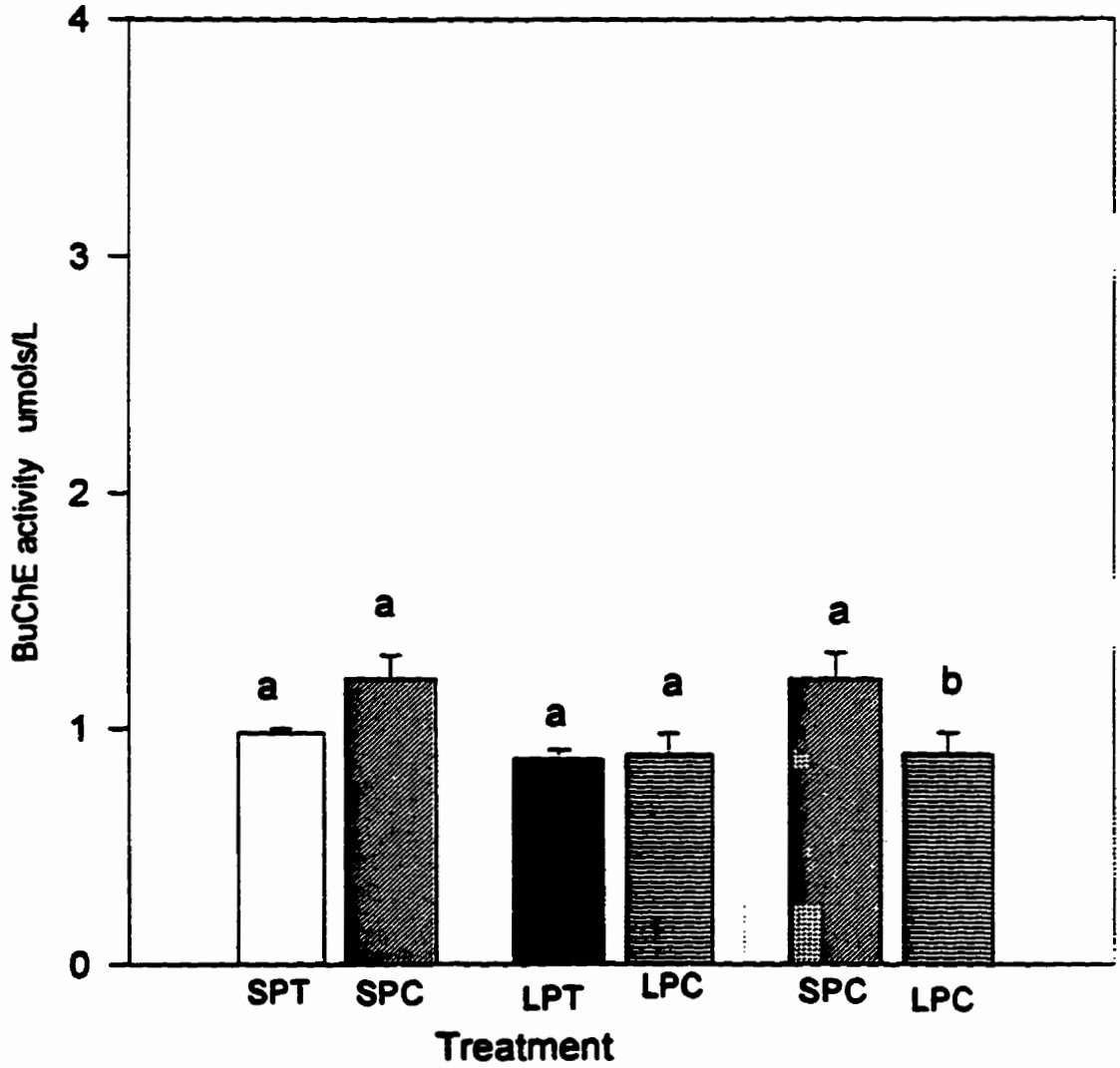


Figure 20.

Butyrylcholinesterase Activity in Liver 12 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).



Means± SEM. Means with same letter designation are not significantly different. ($p<0.05$)

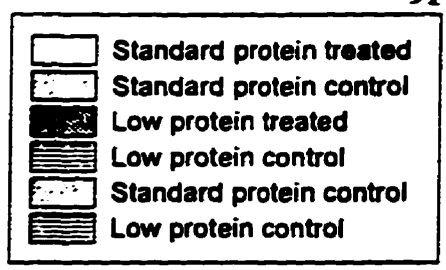
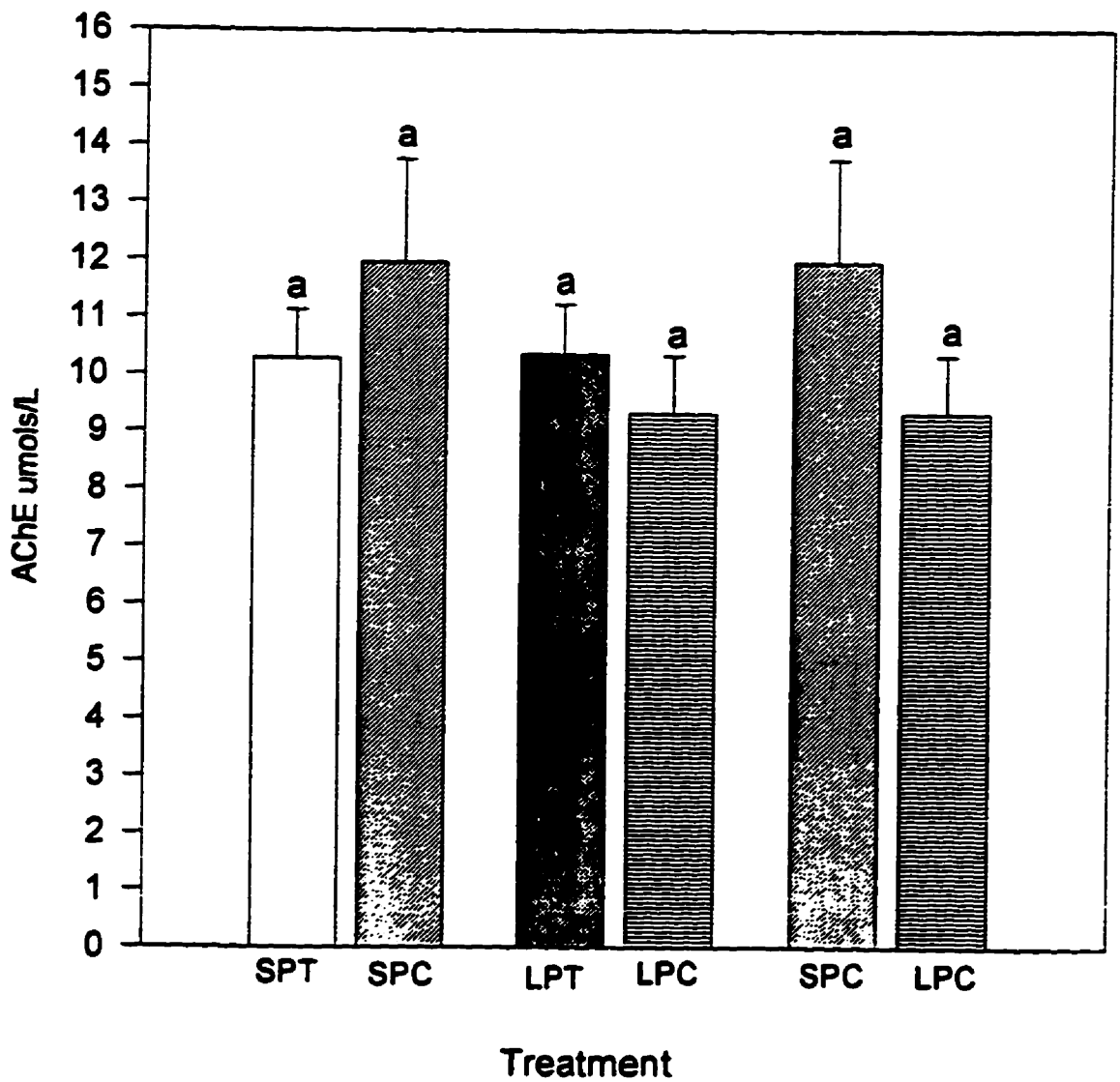


Figure 21.

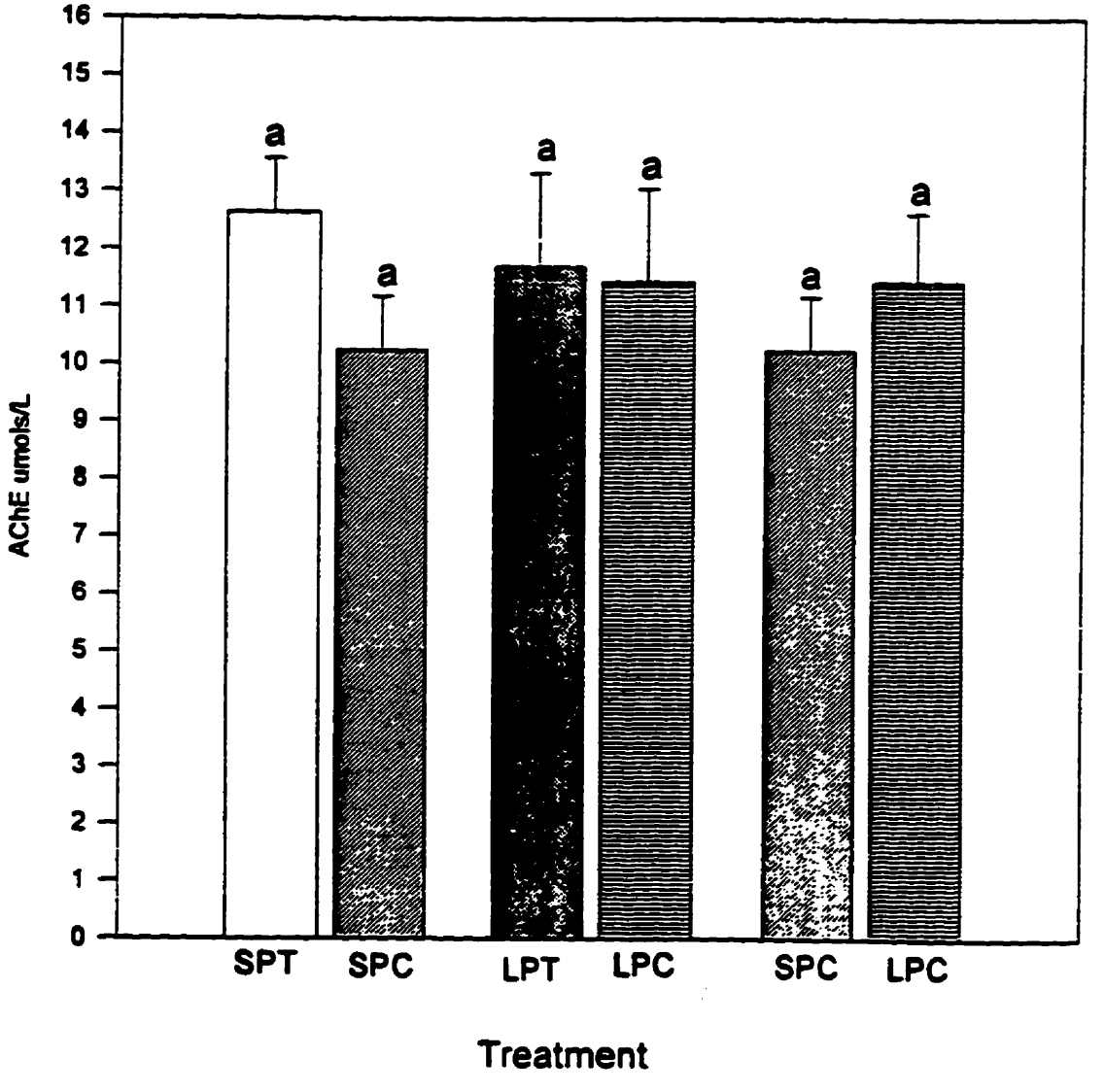
Acetylcholinesterase Activity in Brain 3 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).



Means ± SEM. Means with same letter designation are not significantly different ($p<0.05$)

Figure 22.

Acetylcholinesterase Activity in Brain 8 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).



Means ± SEM.Means with same letter designation are not significantly different ($p<0.05$)

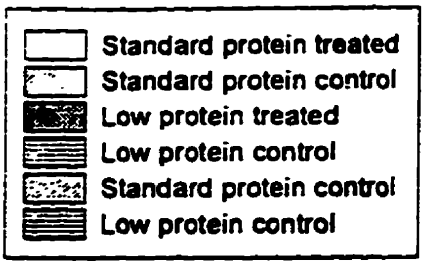
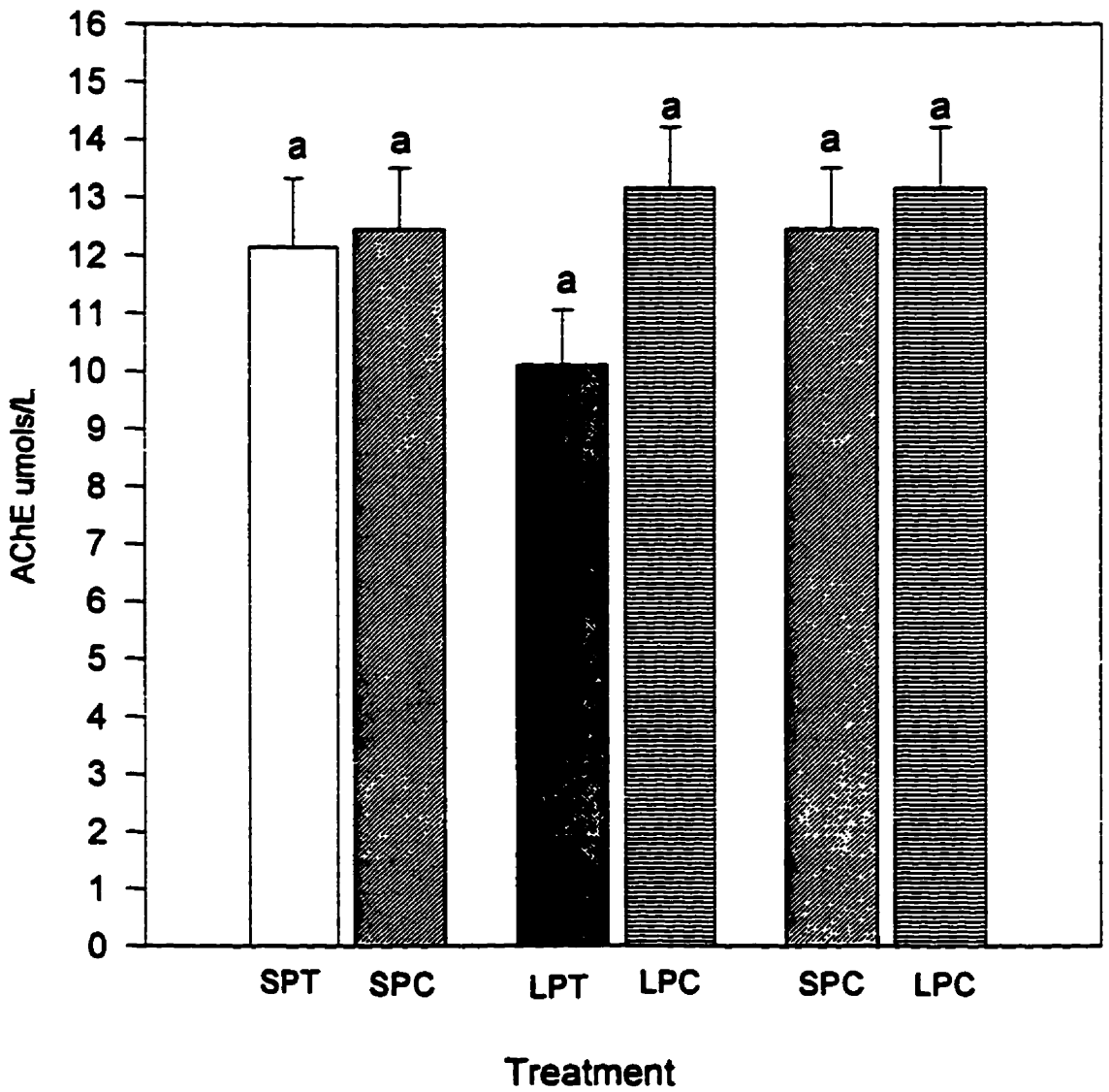


Figure 23.

Acetylcholinesterase Activity in Brain 12 Hours Post-Injection With α-Solanine (Dose of 10 mg/Kg B.W.).



Means±SEM. Means with same letter designation are not significantly different ($p<0.05$)

Chapter V

DISCUSSION

5.1 Animal Weight

Animal weight gain was greater on the standard protein (15%) diet than on the low protein (7.5%) diet which is consistent with previous work in this laboratory and that reported in the literature (Merrill and Bray, 1982; Tutelyan, 1990; Swick and Gribskov, 1983; Semon et. al., 1987; Hietnanen, 1980; Fitzpatrick et.al., 1988). The lower body weight reflects protein deficiency. The rats were in a period of maximum growth and the low protein diet did not adequately supply enough protein to meet requirements for growth and development. Young animals are most susceptible to dietary deficiencies.

5.2 Food Consumption

Greater food consumption by animals on low protein diets is consistent with much of the literature. Animals fed low protein diets may consume excess calories in an effort to meet requirements. Discrepancies in the literature may be due to the age of the animals, the nature of the low protein diet and the time of commencement or the length of the dietary exposure period.

5.3 Feed Efficiency

The measurement of feed efficiency estimates the effect of treatment on the diet utilization and its impact on growth as measured by weight gain per unit of nutrient consumed. Feed efficiency was calculated and found to be different with standard protein

animals having the greater efficiency and the low protein animals having lesser efficiency. This is also consistent with other work done in this laboratory (Fitzpatrick et.al.,1988) and much of the literature.

5.4 Enzyme Assays

Very little information is available on the effect of α-solanine on acetylcholinesterase activity *in vivo*. In a current review of literature no studies were identified which examined the effect of this toxicant on AChE activity.

This study was undertaken as a follow-up to some preliminary trials which were conducted in this laboratory (B.Paz-Aliaga, unpublished data). Previously, it was observed that α-solanine caused inhibition of the activity of AChE in several tissues in rats. By contrast, in the present study, no inhibition occurred in any of the tissues examined at either protein level with the exception of twelve hours post-injection in the liver.

The reasons for the differences in outcome remains unclear. There are two notable differences in the experimental protocol between these two studies. In the earlier study (B. Paz-Aliaga), the animals were maintained on a laboratory chow diet for the duration of the study whereas in the present study the animals were fed semi-purified diets. The composition of the diets differed considerably in the amount and type of fat. Fat in the lab chow diet consisted of beef tallow and soybean oil at a level of 5.3% (Dr. Haught, Purina Research, personal communication). The semi-purified diets contained corn oil (5%) and lard (5%). Therefore the fatty acid composition of the diet differed considerably. Most importantly, is the higher level of the 18:3 fatty acid which is 7X

greater in soybean oil than corn oil. The current recommendation of AIN is for the replacement of 5 grams of corn oil with 7 grams of soybean oil in order to increase the amount of the 18:3 fatty acid.

Diet exerts an important effect on membrane composition and physiology (Suh et.al., 1996; Tynan et.al., 1995; Pan et.al., 1994). Although the toxic effects of α-solanine are believed to be due to its inhibitory effect on AChE the neuronal membrane is also involved. There is considerable evidence that α-solanine also causes toxic effects by disruption of membranes (Roddick, 1984, 1986) leading to release of peroxidase previously enclosed in lipid vesicles. The GA interact specifically with membrane associated cholesterol resulting in complexes which cause disruption. AChE is a membrane bound enzyme and its catalytic properties depend on membrane integrity. Studies on plant cells, fungi and mammalian cells have shown that GA can induce leakage of cell contents. The leaked compounds ranged from ions to large proteins but the mechanism of the induced changes in barrier function remain unclear.

The second major difference between these studies is the method of analyses. In the earlier study all enzymes were assayed by the method of George and Abernethy, (1983).

The sample sizes were smaller (n=2 or 3) and no statistical test was applied so the differences between the treated and control group may or may not have been statistically significant. In this study, for example, if the AChE activity was calculated simply as percent of the control value the inhibition of the activity of AChE in erythrocytes at 8 hours post-injection would show a 33% inhibition in the treated group. However, these were not significantly different when analyzed statistically.

The control vehicle used in the former study (Paz, unpublished data) was distilled water whereas in the present work the vehicle in which the toxin was suspended was 8% ethyl alcohol for both the treatment group and the control group.

It is questionable whether the results of the plasma data (both studies) should be considered valid. The presence of quinidine sulphate in the DTNB reagent would effectively inhibit the cholinesterase in plasma.

In this study no inhibition of AChE occurred except in the liver at twelve hours post-injection. Because no inhibition of cholinesterase occurred in the liver at three or eight hours post-injection it is difficult to be confident of a true effect at twelve hours. The fact that this occurred may be an anomaly. Substances injected i.p. are absorbed rapidly by the hepatic portal circulation--less than 1 hour. Nishie et.al.,(1971), using tritiated α-solanine at a dose level of 5mg/Kg B.W. reported that a maximum tissue concentration following a single p.o. dose occurred at twelve hours in all tissues. Therefore, it would be expected that peak tissue concentration following i.p. injection would occur more rapidly than twelve hours following i.p. injection

The reasons for the null result in this study are not clear but several factors may be pertinent.

The dose-response curve for α-solanine rises steeply between 15-25 ug/ml (Crawford and Kocan, 1993). There is a very narrow margin between a safe dose and a toxic dose. At 10 mg/Kg B.W. the dose may have been narrowly on the side of being non-toxic in these animals.

The toxicity resulting from GA ingestion may be due to chronic or repeated exposure rather than an acute exposure. In cases where toxicity has been most severe

there is no information about previous history relating to exposure or nutritional status. In a study of human volunteer subjects (Hellenas et. al., 1992) α-solanine and α-chaconine were detected in all blood serum samples collected 1-25 hours after ingestion of a single meal of mashed potatoes which contained 1mg/Kg B.W. of GA. Detectable amounts of solanidine were found also 4-8 hours after ingestion. At these dose levels the subjects reported signs of toxicity. Because of the rapid onset and short duration it was suggested that these symptoms may have resulted from direct effects on the intestines rather than by systemic toxicity. As well, serum solanidine levels took up to 3 weeks to reach minimum which would indicate a considerable half-life for solanidine in serum. Harvey et.al., (1985) also found a strong correlation between serum solanidine concentration and normal dietary intake of potato. In addition, Claringbold (1980) provided evidence of prolonged retention of solasodine in man and hamster and concluded that once it is in the body it is eliminated over a prolonged period of time. The retention and the very low excretion of solanidine and its metabolites suggests that if it were ingested on a regular basis a sizable body burden would accumulate. At times of metabolic stress the stores might be mobilized with deleterious effects.

In this study, only the effect of pure α-solanine was examined. Considerable evidence exists that α-solanine acts in concert with α-chaconine in producing the toxicity. Keukens et. al. (1995) studied the effects of GA on model membranes and showed that GA interacted specifically with the cholesterol associated with membranes resulting in the formation of complexes which caused the membrane disruption. This was strongly dependent on the type of sterol present in the membrane and the type of sugar moiety of the GA. α-solanine had very little effect but α-chaconine which has the same aglycone

but different sugars caused significant lysis of cholesterol containing lipid vesicles. The ineffective sugar moiety of α-solanine became very effective when combined with α-chaconine. A further study (Keukens et.al., 1996) on the effects of GA on biomembranes was carried out to see if these observations were valid for biomembranes. The membrane disruptive effects were determined on intestinal epithelial cells by monitoring the leakage of lactate dehydrogenase (LDH) from cells. All the glycoalkloids induced substantial LDH leakage with potency greatest for α-tomatine and least for α-solanine.

α-tomatine > α-chaconine > α-solanine

From this study they concluded that 1) membrane permeabilization was cholesterol dependent 2) a similar order of potency for membrane disruption was seen as in the model membrane experiment and 3) α-solanine and α-chaconine showed synergism in their effect on biomembranes.

As well as the inhibition of AChE, GA induced membrane disruption may well be the cause of intoxication. Neuronal cells contain high amounts of cholesterol making them susceptible to GA which would subsequently impair conduction along the nerve. However, the relationship between membrane disruption and reported symptoms will require further research on the interaction of GA with biomembranes.

Protein Effect

When protein status was inadequate there was a reduction in enzyme activity in the positive control group. This was not observed in the brain or erythrocytes but was

evident in liver at each time period.

It has been shown that the biotransformation of lipophilic xenobiotics to hydrophilic metabolites by the phase I and phase II enzyme systems is modulated by dietary protein (Baijal and Fitzpatrick, 1996). Tutelyan et. al., (1990) provided evidence that both a decrease and an increase of protein in the diet are accompanied by significant changes in the activity of biotransformation enzymes. Low protein diets resulted in a marked increase in the activity of CDNB glutathione transferase in the intestine whereas the transferase activity was reduced in the liver. Although the liver is recognized to be the major drug metabolizing tissue, detoxification is not confined to this organ. For example, the intestine is a primary route for entry of drugs and most nutritional toxicants. Biotransformation in the intestine may serve as the first line of defence against the body's toxic burden (Hoensch et. al., 1979). Under conditions of insufficient protein supply, xenobiotic metabolizing enzymes respond differently in different tissues. Extrahepatic detoxification occurs and it is not simply an attenuated version of hepatic systems. Baijal and Fitzpatrick, (1996) showed that UDPGT isoenzyme activities in the liver and intestine were greatest on a low protein diet whereas in kidney they were greatest on a high protein diet. They also showed that isoenzymes within a tissue respond differently to protein modulation. The duration of feeding of the protein diets also affects the response by the tissue to the protein supply. Therefore, the fact that the response to protein diets is tissue and isoenzyme dependent suggests that generalizations regarding the response to protein diets based solely on hepatic studies are invalid.

CHAPTER VI

CONCLUSION

The effect of nutritional status in exacerbating the toxicity of α-solanine has not been studied previously. One objective of this study was to determine this effect as it pertained to inhibition of AChE activity. Lower AChE activity was observed in all tissues but the difference was statistically significant only in the liver. This was due undoubtedly to the wide inter-individual variation.

The inhibitory effect of α-solanine on the level of AChE activity was also evident in all tissues but statistically significant only in the liver at 12 hours post-injection. This supports the theory that toxicity from ingestion of solanine glycoalkaloids is at least in part a result of their anti-cholinesterase properties. It suggests also that other factors may play a part in producing the toxicities reported in the literature.

Because nothing is known about the relative stabilities of the hydrolysis products of glycoalkaloids in the GI tract, experiments are needed to define and identify these products of digestion. Some oral studies are required to give a realistic indication of potential health hazard.

Because bioavailability varies greatly between species, it would be advisable to use blood levels when using animal studies as a basis for risk assessment in humans.

Predicting safety limits for the consumption of potatoes and potato products based on individual GA may not be justifiable. For example, tomatoes and eggplant contain other types of GA which may lead to additional interactions when several GA-containing

foods are consumed. To accurately predict human hazard the chemical interaction between toxicant and dietary components should be considered. This study was a preliminary step in obtaining some basic toxicity tests in the laboratory.

The question of sub-chronic and chronic toxicity as a consequence of continual exposure has not been addressed. Glycoalkaloids in foods may accumulate in the body and mobilized at a time of metabolic stress. Conversely, does chronic treatment with sub-lethal doses of GA induce changes in AChE which may contribute to tolerance?

Until there is further knowledge about the toxicity of GA, a safety factor lower than 20mg/Kg B.W. should be used when establishing an acceptable daily intake.

Pre and post harvest practices as well as potato processing procedures should be monitored for possible adverse effects on the tuber GA concentration.

Appendix A

LIST OF MAJOR EQUIPMENT

1. Fisher Accumet pH meter, model 810
2. Milton Roy spectronic 3000 spectrophotometer
3. Potter-Elvejhem homogenizer
4. Sp6-300 Spectrophotometer, Pye Unicam
5. Kitchen Aid Mixer

Appendix B

NUTRIENT SUPPLIERS

Nutrient	Supplier
Corn Starch	U of M Food Services
Cellufil (non-nutritive bulk)	USB (United States Chemical) 21000 Miles Parkway, Cleveland , OH 44128
Dextrose Sugar	The R Wine Baril, Winnipeg,MB
Vitamin Free Casein	USB
D-L Methionine	USB
Mazola Corn Oil	U of M Food Services
Lard	U of M Food Services
AIN Vitamin Mixture 76	USB
AIN Mineral Mixture 76	USB
Choline Bitartrate	USB

REFERENCES

Alles GA and Hawes RC. (1940) Cholinesterases in the blood of man. *J Biological Chemistry*, **133**:375-390.

Alozie SO, Sharma RP and Salunkhe DK. (1979) Physiological disposition, subcellular distribution and tissue binding of α-chaconine(^{3}H). *Journal of Food Safety*, **1**:257-263.

Alozie SO, Sharma DK, Salunkhe DK. (1979) Excretion of α-chaconine ^{3}H a steroidal GA from Solanum Tuberosum L and its metabolites in hamsters. *Pharmacol Res Commun*, **11**:483-490.

American Institute of Nutrition (1977) Report of the AIN ad hoc committee on standards for nutritional studies. *Journal of Nutrition*, **107**:1340-1348.

Ames BN, McCann J, Yamasaki E. (1975) Methods for detecting carcinogens and mutagens with the *Salmonella* /mammalian-microsome mutagenicity test. *Mutat Res*, **31**: 347-364.

Ames BN, Profet M, Gold L. (1990a) Dietary pesticides (99.99% all natural) *Proceedings of the National Academy of Sciences of the U.S.A.*, **87**:7777-7781.

Ames BN, Profet M, Gold L. (1990b) Nature's chemicals and synthetic chemicals: Comparative toxicology, *Proceedings of the National Academy of Sciences of the U.S.A.*, **87**:7782-7786.

Baerug R. (1962) Influences of different rates and intensities of light on solanine content and cooking quality of potato tubers. *European Potato Journal*, **5**:242-251.

Baijal PK and Fitzpatrick DW. (1996) Effect of dietary protein on hepatic and extrahepatic phase I and phase II drug metabolizing enzymes. *Toxicology Letters*, **89**:99-106.

Baker LC, Lampitt LH and Meredith OB. (1955) Solanine glycoside of the potato. III. An improved method of extraction and determination. *J Sci Food Agric*, **6**:197-202

Bantle JA, Fort DJ, Rayburn Jr, DeYoung DJ and Bush SJ. (1990) Further validation of FETAX: evaluation of the developmental toxicity of five known mammalian teratogens and non-teratogens. *Drug and Chemical Toxicology*, **13**:267-277.

Bergenstrahle A, Tillberg E, Jonsson L. (1992a) Regulation of glycoalkaloid accumulation in potato tuber discs. *J Plant Physiol*, **140**: 269-275.

Blankenmeyer JT, Stringer, BK, Rayburn JR, Bantle JA, Friedman M. (1992) Effect of potato glycoalkaloids α-chaconine and α-solanine, on membrane potential of frog embryos. *J Agric Food Chem*, **40**:2022-2026.

Bomer A, and Mattis H. (1924) Der Solaninghalt der Kartoffeln. *Z Unters Nahr Genussm*, **47**:97-127.

Boschetti N, Brodbeck U. (1996) The membrane anchor of mammalian brain acetylcholinesterase consists of a single glycosylated protein of 22 kDa. *Febs Lett*, **380**(1-2):133-136.

Boschetti N, Brodbeck U, Jensen SP, Koch C, and Norgaard-Pedersen B. (1996) Monoclonal antibodies against a C-terminal peptide of human brain acetylcholinesterase distinguish between erythrocyte and brain acetylcholinesterases. *Clinical Chemistry*, **42**(1):19-23.

Boyd EM and Krupa V. (1970) Protein deficient diet and diuron toxicity. *J Agr Food Chem*, **18**:1104-1107.

Buchanan N, Davis MD, Eyberg C. (1979) Gentamicin pharmacokinetics in kwashiorkor. *Br J Clin Pharmacol*, **8**:451-353.

Buchanan N, Davis MD, Henderson DB, Muchlow JC, Rawlins MD. (1986) Acetanilide pharmacokinetics in kwashiorkor. *British Journal of Clinical Pharmacology*, **9**:525-526.

Bushway RJ, Barden ES, Wilson AM, Bushway AA. (1980) Analysis of potato glycoalkaloids by high performance liquid chromatography. *J Food Science*, **45**:1088-1089.

Bushway RJ, Ponnampalam R. (1981) α-Chaconine and α-solanine content of potato products and their stability during several modes of cooking. *J Agric Food Chem*, **29**:814-817.

Bushway RJM, Savage SA and Ferguson BS. (1987) Inhibitors of acetylcholinesterase by *solanaeous* glycoalkaloids and alkaloids. *Potato Journal*, **64**:409-413.

Bushway AA. Bushway RJ, Oh CH. (1988) Isolation, partial purification and characterization of a potato peel glycoalkaloid glycosidase. *American Potato Journal*, **65**:621-631.

Bushway AA, Bushway RJ, Oh CH. (1990) Isolation, partial purification and characterization of a potato peel α-solanine cleaving glycosidase. *American Potato Journal*, **67**:233-238.

Caldwell J. (1992) Problems and opportunities in toxicity testing arising from species differences in xenobiotic metabolism. *Toxicology Letters*, **64/5**:651-659.

Caldwell KA, Grosjean OK, Henika PR, Friedman M. (1991) Hepatic ornithine decarboxylase induction by potato glycoalkaloids in rats. *Food and Chemical Toxicology*, **29**:531-535.

Camire ME, Zhao J, Violette DA. (1993) *In vitro* binding of bile acids by extruded potato peels. *J Agric Food Chem*, **41**:2391-2394.

Campbell TC and Hayes JR. (1976) The effect of quantity and quality of dietary protein on drug metabolism. *Fed. Proc., Fed Am Soc Exp Biol*, **35**:2470-2474.

Chaube S and Swinyard CA. (1976) Teratological and toxicological studies of alkaloidal and phenolic compounds from *Solanum tuberosum*. *J Toxicology and Applied Pharmacology*, **36**:227-237.

Christiansen JA. (1977) The utilization of bitter potatoes to improve food production in the high altitude of the tropics. Ph.D. thesis, University of Cornell, Ithaca, N.Y.

Claringbold WDB, Few JD, Brace CJ, Renwick JH. (1980) The disposition and metabolism os [^{3}H]-solasodine in man and Syrian hamster. *J Steroid Biochem*. **13**:889-895.

Claringbold WDB, Few JD, Renwick JH. (1982) Kinetics and retention of solanidine in man. *Xenobiotica*, **12**:293-302.

Cole KS and Curtiss HH. (1939) Electric impedance of the Squid giant axon during activity. *J Gen Physiol*, **22**:649.

Conney AH, Pantuck EJ, Hsia KC, Garland WA, Anderson KE, Alvares AP, Kappas A. (1976) Enhanced phenacetin metabolism in human subjects fed charcoal broiled beef. *Clin Pharmacol Ther*, **20**:633-642.

Crawford L and Kocan R. (1993) Steroidal alkaloid toxicity to fish embryos. *Toxicology*

Letters, **66**:175-181.

Crawford L and Myhr B. (1995) A preliminary assessment of the toxic and mutagenic potential of steroidal alkaloids in transgenic mice. *Food and Chemical Toxicology* **33**: 191-194.

Crawford L and Wheeler EL. (1983) Microsomal P-450 induction by some secondary products from thermal oxidation of dietary lipids: epidermal hyperplasia, mutagenicity and cytochrome P-450 activities. *Cancer Letters*, **21**:211-217.

Crone C. (1986) The blood brain barrier; a modified tight epithelium. In: The Blood Brain Barrier in Health and Disease, Edited by A.J. Suckling, M.G. Reimsky and MWB. pp 17-41 Bradbury Ellis Horwood, Chichester, England.

Cronk TC Kuhn GD, McArdle FJ. (1974) The influence of stage of maturity, level of nitrogen fertilization and storage on the concentration of sooanine in tugers of three potato cultivars. *Bull Environ Contam Toxicol*, **11**:163-169.

Dale HH. (1914) The action of certain esters and ethers of choline, and their relation to muscarine. *Journal of Pharmacology and Experimental Therapeutics*, **6**:147-190.

Dale MFB, Griffiths DW, Bain H, Todd D. (1993) Glycoalkaloid increase in *Solanum tuberosum* on exposure to light. *Ann Appl Biol*. Warwick: Association of Applied Biologists, **123**(2):411-418.

Dalvi RR. (1985) Comparative assessment of the effect of solanine administered orally and intraperitoneally on hepatic dysfunction in male rats. *Japanese Journal of Veterinary Science*, **47**:657-659.

Dalvi RR, Jones RDH. (1986) Role of microsomal cytochrome p-450 containing monooxygenases in the metabolism and toxicity of certain alkaloids. *Current*

Science, **55**: 558-561.

Deahl KL and Sinden SL. (1987) A technique for the rapid detection of leptine glycoalkaloids in potato foliage. *American Potato Journal*, **64**:285-291.

Deahl KL, Cantelo WW, Sinden SL, Sanford L. (1991) The effect of light intensity on Colorado beetle resistance and foliar glycoalkaloid concentration of four *Solanum chacoense* clones. *American Potato Journal*, **68**:659-666.

Dietz AA, Rubenstein HM, Lubrano T. (1977) Colorimetric determination of serum cholinesterase and its genetic variants by the propionylthiocholine dithiobis (nitrobenzoic acid) procedure. *Selected Methods in Clinical Chemistry*, **8**:41-46.

Elliot TR. (1905) The action of adrenaline. *J Physiology*, **32**:401.

Ellman GL Courtney KD Andres VJ and Featherstone RM. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*, **7**:88-95.

FAO Yearbook. (1994) Vol.**48**, Food and Agriculture Organization of the United Nations, Rome, Italy.

Fewell AM and Roddick JG. (1993) Interactive antifungal activity of the glycoalkaloids α-solanine and α-chaconine. *Phytochemistry*, **33**(2):323-328.

Fitzpatrick DW, Hassen AM, and Arbuckle LD. (1988) Effect of dietary protein on zearalenone metabolism and toxicity in the rat. *Nutrition Research*, **8**:663-671.

Flanders KL, Hawkes JG, Radcliffe ER, Lauer FJ. (1992) Insect resistance in potatoes: sources of evolutionary relationships, morphological and chemical defenses, and ecogeographical associations. *Euphytica*, **61**:83-111.

Ford DJ, Dawson DA, Bantle JA. (1988) Development of a metabolic activation system

for the frog embryo teratogenesis assay: Xenopus (FETAX). *Teratogenesis Carcinogenesis and Mutagenesis*, **8**:251-268.

Friedman M, Rayburn JR, Bantle JA. (1991) Developmental toxicology of potato alkaloids in the frog embryo teratogenis assasy--Xenopus (FETAX). *Food Chem Toxicol*, **29**:537-547.

Friedman M and Dao L. (1992) Distribution of glycoalkaloids in potato plants and commercial potato products. *J Agric Food Chem*, **40**: 419-423.

Friedman M and Henika PR. (1992) Absence of genotoxicity of potato alkaloids α-chaconine, α-solanine and solanidine in the Ames Salmonella and adult and foetal erythrocyte micronucleus assays, *Food and Chemical Toxicology*, **30**:689-694.

Friedman M, Rayburn JR, Bantle JA. (1992) Structural and developmental toxicities of *Solanum* alkaloids in the frog embryo teratogenesis assay Xenopus (FETAX). *J Agric Food Chem*, **40**:1617-1624.

Friedman M, Levin CE, and McDonald GM. (1994) α-Tomatine determination in tomatoes by HPLC using pulsed amperometric detection. *J Agric Food Chem*, **42**:1959-1964.

Friedman M, McDonald G, Haddon WF. (1993) Kinetics of acid catalyzed hydrolysis of carbohydrate groups of potato glycoalkaloids α-chaconine and α-solanine. *J Agric Food Chem*, **41**:1397-1406.

George PM and Abernethy MH. (1983) Improved Ellman Procedure for Erythrocyte Cholinesterase, *Clin Chem*, **29**(2):365-368.

Gossen JA, de Leeuw WJF, Tan CHJ, Zwarthoff EC, Berends F, Lohman PHM, Knock DL and Vijg J. (1989) Efficient rescue of integrated shuttle vectors from

transgenic mice: a model for studying mutations *in vivo*. *Proceedings of the National Academy of Sciences of the U.S.A.*, **86**:7971-7975.

Gregory P, Sinden SL, Osman SF, Tingey WM, Chessin DA. (1981) Glycoalkaloids in wild, tuber bearing *Solanum* species. *J Agric Food Chem*, **29**:1212-1215.

Groen K, Pereboom-Deifauw DPKH, Besamusca P, Beekhof PK, Speijers GJA, Derks HJGM. (1993) Bioavailability and disposition of ^{3}H-solanine in rat and hamster. *Xenobiotica*, **23**(9):995-1005.

Gull DD. (1961) Chlorophyll and solanine changes in tubers of *Solanum tuberosum* induced by fluorescent light and a study of solanine toxicology by bioassay technique. *Dissertation Abstract*, **21**:2242-2243. PhD Thesis, Cornell University

Hall RL. (1992) Toxicological burdens and the shifting burden of toxicology. *Food Technology*, **46**(3):109-112.

Harris FW and Cockburn T. (1918) Alleged poisoning by potatoes. *Analyst*, **43**:133-137.

Harris H, And Whittaker M. (1962) Differntial inhibition of the serum cholinesterase phenotypes by solanine and solanidine. *Ann Hum Genet Lond*, **26**:73-76.

Harvey MH, McMillan M, Morgan NRA and Chan WS. (1985) Solanidine is present in sera of healthy individuals and in amounts dependent on their dietary potato consumption. *Human Toxicology*, **4**:187-194.

Harvey MH, Morris BA, McMillan M, Marks V. (1986) Potato steroidal alkaloids and neural tube defects: serum concentration fail to demonstrate a causal relation. *Human Toxicology*, **5**:249-253.

Hathcock JN. (1982) Nutritional Toxicology: Definition and Scope. In: Hathcock JN, ed. *Nutritional Toxicology*, volume I. New York: Academic Press, Publishers, 1-16.

Hathcock JN. (1987) Nutritional Toxicology: Definition and Scope. In: Hathcock JN, Ed. *Nutritional Toxicology* volume II. New York: Academic Press, Publishers, 1-58.

Hayes WH. (1994) In: Principles and Methods of Toxicology, 3rd Ed., Raven Press, New York.

Heftman E. (1983) Biogenesis of steroids in *solanaceae*. *Phytochemistry*, **22**(9):1843-1860.

Hellenas KE, Cehan E, Slanina P, Bergman K. (1992) Studies of embryotoxicity and the incidence of external malformations after continuous intravenous infusion of α-chaconine in pregnant rats. *Pharmacology and Toxicology*, **70**:381-383.

Hellenas KE, Slanina P, Loof L, Gabrielsson J. (1992) Determination of potato glycoalkaloids and their aglycone in blood serum by high-performance liquid chromatography. *Journal of Chromatography*, **573**:69-78.

Hellenas KE, Branzell C, Johnson H, Slanina P. (1995) High levels of glycoalkaloids in the established Swedish potato variety Magnum Bonum. *J Sci Food Agric*, **68**:249-255.

Hietanen E. (1980) Modification of hepatic drug metabolizing enzyme activities and their induction by dietary protein. *Gen Pharma*, **11**:443-450.

Hlywka J, Stephenson GR, Sears MK Yada RY. (1994) Effects of insect damage on glycoalkaloid content in potatoes (*Solanum tuberosum*) *J Agric Food Chem*, **42**:2545-2550.

Hoensch HP, Hutt R, Hartmann F. (1979) Biotransformation of xenobiotics in human intestinal mucosa. *Environ Health Perspec*, **33**:71-78.

Hoorn AJW, Custer L, Myrh BC, Brusick D, Grossen J, Vijg J. (1993) Detection of

chemical mutagens using Muta Mouse: a transgenic mouse model. *Mutagenesis*, **8**:7-10.

Jadhav SJ and Salunkhe DK. (1975) Formation and control of chlorophyll and glycoalkaloids in tubers of *Solanum tuberosum L* and evaluation of glycoalkaloid toxicity. *Advanced Food Research*, **21**:307-354.

Jadhav SJ, Sharma RB and Salunkhe DK. (1981) Naturally occurring toxic alkaloids in foods. *CRC Critical Review in Toxicology*, **9**:21-104

Jadhav SJ, Kumar A, Chawan JK. (1991) Glycoalkaloids. In Potato: Production, Processing, Products; Salunkhe DK, Kadam SS, Jadhav SJ Eds. CRC Press, Boca Raton Florida, pp 203-245.

Janne J, Poso H and Raina A. (1978) Polyamines in rapid growth and cancer. *Biochimica et Biophysica Acta*, **473**:241-293.

Jorgenson FS, Sundberg K, Loft GR, Arends J, Pedersen RN. (1995) Alpha-fetoprotein and acetylcholinesterase activity in first and early second-trimester amniotic fluid. *Prenatal Diagnosis*, **15**:621-625.

Kappas A, Alvares AP, Anderson KE, Pantuck EJ, Pantuck CB, Chang R, Conney AH. (1978) Effect of charcoal broiled beef on antipyrene and theophylline metabolism. *Clin Pharmacol Ther*, **23**:445-450.

Kato R, (1977) Drug metabolism under pathological and abnormal physiological states in animals and man. *Xenobiotica*, **7**:25-92

Kato R, Tani T, Yoshida A. (1980) Effect of dietary level of protein on liver microsomal drug metabolizing enzymes, urinary ascorbic acid and lipid metabolism in rats fed PCB-containing diets. *J. Nutrition*, **110**:1686-1694.

Keukens EAJ, de Vrije T, Fabrie CHJP, Demel RA, Jongen WMF, de Kruijff B. (1992) Dual specificity of sterol-mediated glycoalkaloid induced membrane disruption. *Biochimica et Biophysica Acta*, **1110**:127-136.

Keukens EAJ, de Vrije T, van den Boom C, de Waard P, Plasman HH, Thiel F, Chupin V, Jongen WMF, de Kruijff B. (1995) Molecular basis of glycolakloid induced membrane disruption. *Biochimica et Biophysica Acta*, **1240**:216-228.

Keukens EAJ, de Vrije T, Jansen LAM, de Boer H, Janssen M, de Kroon AIPM, Jongen WMF, de Kruijff B. (1996) Glycoalkaloids selectively permeabilize cholesterol containing biomembranes. *Biochimica et Biophysica Acta*, **1279**:243-250.

Klassen CD. (1972) Immaturity of the newborn rat's hepatic excretory function for oubain. *J Pharmacol Exp Ther*, **183**:520-526.

Kubo I and Fukuhara K. (1996) Steroidal Glycoalkaloids in Andean Potatoes. In: Saponins Used in Food and Agriculture, ed. Waller and Yamasaki, Plenum Press, New York, pp 405-417.

Lesinska G and Leszczynski WL. (1989) *Potato Scienc and Technology*; Elsevier, London.

Lopez de Romana, Graham GG, Mellits ED, McLean WC. (1981) Prolonged consumption of potato based diets by infants and small children. *J Nutrition*, **111**:1430-1436.

Loft AGR, Hogdall E, Larsen SO, Norgaard-Pedersen B. (1993) A comparison of amniotic fluid alpha-fetoprotein and acetylcholinesterase in the prenatal diagnosis of open neural tube defects and anterior abdominal wall defects. *Prenatal Diagnosis*, **13**:93-109.

MAFF. (1993) National Food Survey, 1992. HMSO, London.

Maga JA. (1980) Potato glycoalkaloids. *CRC Critical Reviews in Food Science and Nutrition*,**12**:371-405.

Marnay A and Nachmansohn D. (1938) Choline Esterase in Voluntary Muscle.*J Physiol*, **92**:37-47.

McQueen MJ.(1995) Chemical and analytical considerations in the utilization of cholinesterase measurements. *Clin Chim Acta*, **237**(1-2):91-105.

McMillan M and Thompson JC. (1979) An outbreak of suspected solanine poisoning in schoolboys: Examination of criteria of solanine poisoning, *Quarterly Journal of Med*, New Series **XLVIII**: 227-243.

Merrill JC, and Bray TM. (1982) The effect of dietary protein quantity on the activity of UDP-glucuronyl transferase and its physiological significance in drug metabolism. *Can J Physio Pharmacol*, **60**:1556-1561.

Millan JW amd Woollam DH. (1957) Influence of cortisone on teratogenic effects of hypervitaminosis-A. *British Medical Journal*, **5038**:196-197.

Milunsky A, Hershel J, Jick SS, Bruel CL, McLoughlin DD, Rothman KJ, Willet W. (1989) Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *Journal of the American Medical Association*, **262**:2847-2852.

Morris SC and Lee TH. (1984) The toxicity and teratogenicity of *Solanaceae* glycoalkaloids, particularly those of the potato (*Solanum tuberosum*): a review. *Food Technology in Australia*, **36**:118-124.

Myr B. (1991) Validation studies with Muta Mouse: a transgenic model for detecting

mutations *in vivo*. *Environmental and Molecular Mutagenesis*, **18**:308-315.

National Research Council, (1978) Nutrient requirements of laboratory animals. Washington: National Academy Press, Publishers.

Nishie K, Gumbman MR, Keyl AC. (1971) Pharmacology of solanine. *Toxicol Appl Pharmacol*, **19**:81.

Norred WP, Nishie K, Osman SF. (1976) Excretion, distribution and metabolic fate of ^{3}H α-chaconine. *Res Commun Chem Pathol Pharmacol*, **13**:161-171.

Oldendorf WH. (1990) The Blood Brain Barrier. In: Encyclopedia of Neuroscience. Edited by G. Adelman. pp 139-141. Birkhaeuser, Boston.

OMAF (1991) Potato production in Ontario; Publication 534. Ontario Ministry of Agriculture and Food. Toronto, Ontario.

Orgell WH, Vaidya KA, Dahm PA. (1958) Inhibition of human plasma cholinesterase *in vitro* by extracts of solanaceous plants. *Science*, **128**: 1136-1137.

Orgell WH. (1963) Inhibition of human plasma cholinesterase *in vitro* by alkaloids, glycosides and other natural substances. *Lloydia*, **26**:36-43.

Osman SF, Herb SF, Fitzpatrick TJ and Schmiedicke P. (1978) Glycoalkaloid compositions of wild and cultivated tuber bearing *Solanum* species of potential value in potato breeding programs.*J Agric Food Chem*, **26**:1246-1248.

Osman SF. (1980) Glycoalkaloids of the *Solanaceae*. *Recent Phytochemistry*, **14**:75-96.

Osman SF Sinden SL Irwin P, Deahl K, Tingey WM. (1991) Solanocardinol: a steroidal alkaloid from *Solanum neocardenasii*. *Phytochemistry*, **30**:3161-3163.

Pan DA, Hulbert AJ, Storlien LH. (1994) Dietary fats, membrane phospholipids and obesity. *J Nutrition*. **124**:1555-1565.

Parke DV and Ioannides C. (1981) The role of nutrition in toxicology. *Ann Rev Nut*, 1:207-234.

Paseshnichenko VA and Guseva AR. (1956) Quantitative determination of potato glycoalkaloids and their preparative separation. *Biochemistry* (USSR), **21**:606-611.

Patchett BJ Sunningham PS, Lill RE. (1976) Glycoalklaoid levels in New Zealand potatoes, *NZJ Exper Agric*, **5**:55-57.

Patil BC, Sharma KA, Dahm PA. (1958) Inhibition of human plasma cholinesterase *in vitro* by extracts of solanaceous plants. *Science*, **128**:1136-1137.

Patil BC, Sharma RP, Salunkhe DK, Salunkhe K. (1972) Evaluation of solanine toxicity. *Fd Cosmet Toxicol*, **10**:395-398.

Percival G and Dixon G. (1994) Glycoalkaloid concentration of potato tubers following continuous illumination. *J Sci Food Agric*, **66**:139-144.

Rao GV and Rao KSJ.(1995) Modulation in acetylcholinesterase of rat brain by pyrethroids *in vivo* and an *in vitro*kinetic study. *Journal of Neurochemistry*, **65**(5):2259-2266.

Rayburn JR, Bantle JA, and Friedman M. (1994) The role of the carbohydrate side chain of potato glycoalkaloids in developmental toxicity. *J Agric Food Chem*, **42**:1511-1515.

Rayburn JR, Friedman M, Bantle JA. (1995) Synergistic interaction of GA α-chaconine and α-solanine on developmental toxicity in Xenopus embryos. *Food Chem Toxicol*, **33**(12):1013-1019.

Renwick JH. (1972) Hypothesis: Anancephaly and spina bifida are usually preventable by avoidance of a specific but unidentifiable substance present in certain potato

tubers. *British Journal of Preventative and Social Medicine*, **26**:67-88.

Renwick JH, Claringbold WDB, Earthy ME, Few JD, McLean ACS. (1984) Neural tube defects produced in Syrian hamsters by potato glycoalkaloids. *Teratology*, **30**:371-381.

Rhoades RE and Rogers M. (1982) The Incredible Potato, National Geographic, May, 668-694.

Ricny J, Soupek J, Stanislav T. (1989) Determination of acetylcholine and choline by flow injection with immobilized enzymes and fluorometric or luminometric detection. *Analytical Biochemistry*, **176**:221-227.

Roddick JG. (1986) In Solanaceae: Biology and Systematics. ed. D'Arcy WG. Columbia University Press, New York.

Roddick JG Rijnenberg AL Osman SF. (1988) Synergistic interaction between potato glycoalkaloids α-solanine and α-chaconine in relation to destabilization of cell membranes: Ecological implications. *J Chem Ecol*, **14**:889-901.

Roddick JG. (1989) The acetylcholinesterase-inhibitory activity of steroidal glycoalkaloids and their aglycones. *Phytochemistry*, **28(10)**:2631-2634.

Rosenberry TL. (1963) Acetylcholinesterase. Handbook on Cholinesterases and Anti-cholinesterase Agents, Ed Koelle.

Ross H. (1966) The use of wild *Solanum* speciess in German potato breeding of the past and today. *American Potato Journal*, **43**:63-80.

Ross H, Pasemann P and Nitzsche W. (1978) Glycoalkaloid content of potatoes and its relationship to location, year and taste. *Z Pflanaenzuecht*, **80**:64-79.

Rothe JC. (1918) Illness following the eating of potatoes containing solanine. *Z Hyg*,

88:1-12.

Ruhl R. (1951) Report on the pathology and toxicology of solanins. *Arch Pharm*, **284**:67-74.

Russell DH. (1980) Ornithine decarboxylase as a biological and pharmacological tool. *Pharmacology*, **20**:117-129.

Saez-Valero J and Vidal CJ. (1995) Monomers and dimers of acetycholinesterase in human meningioma are anchored to the membrane by glycosyl-phosphatidylinositol. *Neuroscience Letters*, **195**:101-104.

Salunkhe DK, Wu MT Jadhav SJ. (1972) Effects of light and temperature on the formation of solanine in potato slices. *J Food Science*, **37**:969-970.

Salvati S, Attorri L, Difelice M, Campeggi LM, Pintor A, Tiburzi F, Tomassi G. (1996) Effect of detary oils on brain enzymatic activities (2'-3'-cyclic nucleotide 3'-phosphodiesterase and acetylcholinesterase) and muscarinic receptor sites in growing rats. *Journal of Nutritional Biochemistry*, **7**:113-117.

Sanford LL and Sinden SL. (1972) Inheritance of potato glycoalkaloids. *American Potato Journal*, **49**:209-217.

Sanford LL Deahl KL Sinden SL Ladd TL Jr. (1990) Foliar solanidine glycoside levels in *Solanum tuberosum* populations selected for potato leafhopper resistance. *American Potato Journal*, **67**:461-466.

SAS/STAT Guide, Version 6, (1987) SAS Institute Inc., Cary, NC.

Schreiber K. (1979) The steroidal alkaloids of *Solanum*. In *The biology and Taxonomy of the Solanaceae*, Edited by J.G.Hawkes, pp193-202. Linnean society Symposium Series no. 7. Academic Press, New York.

Semon BA, Leung PMB, Rogers QR and Gietzen DW. (1978) Effect of type of protein on food intake of rats fed high protein diets. *Physiol Behav*, **41**:451-458.

Sharma RP and Salunkhe DK. (1989) *Solanum* glycoalkaloids. In Toxicants of Plant Origin, Volume I, Alkaloids. Ed. by PR Cheeke, pp 179-236. CRC Press, Boca Raton, Florida

Shuttleworth B, de Villiers DL, Whitely C. (1990) Interaction and inhibition of acetycholinesterase from electrophorus electricus by nitrosamines. *Archives of Biochemistry and Biophysics*, **279**(2):338-344. 1990

Sidell F and Kaminskis A. (1975) Temporal intrapersonal physiologiccal variability of cholinesterase activity in human plasma and erythrocytes. *Clin Chem*, **21**(13):1961-1963.

Sinden SL and Webb RW. (1972) Effect of variety and location on the glycolakloid content of potatoes. *American Potato Journal*, **49**:334-338.

Sinden SL and Webb RW. (1974) Effect of environment on glycoalkaloid content of six potato varieties at 39 locations. Technical Bulletin no. 1472 US. Dept. of Agriculture, Agric. Res. Serv.

Sinden SL and Deahl KL. (1976) Effect of glycoalkaloids and phenolics on potato flavours.*J Food Science*, **41**:520-523.

Sinden SL, Sanford LL, Cantelo WW, Deahl KL. (1986a) Leptine glycoalkaloids and resistance to the Colorado potato beetle (Coloptera: Chrysomelidae) in *Solanum chacoense*. *Environ. Entomol.* **15**:1057-1062.

Sinden Sl, Sanford LL Deahl KL. (1986b) Segregation of leptine glycoalkaloids in *Solanum chacoense* Bitter. *J Agric Food Chem*, **34**:372-377.

Sipes G and Gandolfi AJ. (1991) Biotransformation of toxicants. In: Amdur MO, Doull J and Klassen CD, eds. *Cassarett and Doull's Toxicology. The Basic Science of Poisons*. New York: Pergamon Press, Publishers,88-126. 64-98

Slanina P. (1990) Solanine (glycoalkaloids) in potatoes: toxicological evaluation. *Food and Chemical Toxicology*, **28**:759-761.

Soreq H, and Zahut H. (1993) *Human Cholinesterases and Anticholinesterases*, Academic Press, San Diego.

Steel RGD and Torrie JH. (1960) *Principles and Procedures of Statistics*, McGraw Hill Book Co., New York.

Suh M, Wierzbicki AA, Lien E, Clandinin MT. (1996) Relationship between dietary supply of long-chain fatty acids and membrane composition of long and very long chain essential fatty acids in developing rat photoreceptors. *Lipids*, **31**(1): 61-64.

Swick RW, and Gribskov C. (1983) The effect of dietary protein levels on diet induced thermogenesis in the rat. *J Nutrition*, **113**:2289-2294.

Swinyard CA and Chaube S. (1973) Are potatoes teratogenic for experimental animals? *Teratology*, **8**:349-358.

Tingey WM. (1984) Glycoalkaloids as pest resistance factors, *American Potato Journal*, **61**:157-162.

Torun B and Chew F. (1994) Protein-energy malnutrition. In: Modern Nutrition in Health and Disease. Eds. M. Shils, J. Olson and M Shike. Lea and Febiger, USA.

Tutelyan VA, Kravchenko LV, Avrenyeva LI, Kuzmina EE.(1990) The activity of xenobiotic-metabolizing enzymes in the liver and small intestine of rats fed low

and high levels of protein. *Nutrition Research*, **10**:1119-1129.

Tynan MB, Nicholls DP, Maguire M, Steele IC, McMaster C, Moore R, Trimble ER, Pearce J. (1995) *Atherosclerosis*, **117**:245-252.

Van Gelder WMJ, Vinke JH, Scheffer JJC. (1988) Steroidal glycoalkaloids in tubers and leaves of *Solanum* species used in potato breeding. *Euphytica*, **S**:147-158.

Van Gelder WMJ, Wald N, Cuckle H, Nanchakal K. (1989) Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects second report of the collaberative acetycholinesterase study. *Prenatal Diagnosis*, **9**:813-829.

Wang XG. (1993) Teratogenic effect of potato glycoalkaloid. *Chung-Hua- Fu-Chan- Ko-Tsa-Chih*, **28**(2):73-75,121-122.

Warhany F. (1971) *Congenital Malformations*, Vol 1, Academic Press, New York.

Watanabe T, and Endo A. (1989) Species and strain differences in teratogenic effects of biotin deficiency in rodents. *Journal of Nutrition*, **119**:255-261.

Wattenberg LW. (1971) Studies of polycyclic hydrocarbon hydroxlylases of the intestine possibly related to cancer. *Cancer*, **28**: 99-102.

Wattenberg L. (1975) Effects of dietary constituents on metabolism of chemical carcinogens. *Cancer Res*, **35**:3326-3331.

Wierenga JM and Hollingworth RM. (1992) Inhibition of insect acetylcholinesterase by the potato glycoalkaloid α-chaconine. *Natural Toxins*, **1**(2):96-99.

Willimott SG. (1933) An investigation of Solanine poisoning. *The Analyst*, **58**:431-434.

Wilson GS. (1959) A small outbreak of solanine poisoning, *Monthly Bulletin of the Ministry of Public Health Laboratory Service*, **18**:207.

Woolfe JA. (1987) *The Potato in the Human Diet*, Cambridge University Press, England.

Wu MT and Salunkhe DK. (1976) Changes in glycoalkaloid content following mechanical injuries to potato tubers. *J Am Soc Hort Sci*, **101**:329-331.

Zhao J, Camire ME, Bushway AA. (1994) Glycoalkaloid content and *in vitro* glycoalkaloid solubility of extruded potato peels. *J Agric Food Chem*, **42**:2570-2573.

Zitnak A and Johnston GR. (1970) Glycoalkaloid content of B5141-6 potatoes. *Am Pot J*, **47**:256-261.