CHAPTER 2

ANTIOXIDANT STATUS DURING VARIOUS STAGES OF TAIL REGENERATION IN *HEMIDACTYLUS FLAVIVIRIDIS* SUBJECTED TO SPECIFIC COX-2 INHIBITOR

INTRODUCTION

During epimorphic caudal regeneration, the tissue undergoes a trauma of inflammation followed by wound healing with a pool of dedifferentiated cells undergoing an active cell proliferation to form regeneration blastema. The blastema further differentiates to form the lost part. Regeneration also depends upon other cellular processes involving non-muscle cells. Inflammatory cell influx also occurs sequentially; there is a predominance of polymorphonuclear leukocytes (PMNs) at the onset of the injury, which are gradually replaced by increasing numbers of phagocytosing mononuclear (MN) cells as inflammation progresses to resolution (Gilroy *et al.*, 2001). One of the earliest of these inflammatory responses, which facilitates myogenesis via phagocytosis of cellular debris are the release of chemoattractants and growth factors (Robertson *et al.*, 1993, Tidball 1995, Lescaudron *et al.*, 1999; Chazaud *et al.*, 2003). The cellular events that occur during regeneration are orchestrated by a number of growth factors and cytokines which have been shown to further generate reactive oxygen species (ROS). These ROS serve as normal signalling molecules (Suzuki and Griendling, 2003).

The ability of organisms to use molecular oxygen was a major evolutionary breakthrough that enabled the production of significantly more energy from the breakdown of foods, along with many other advantages. All living cells, from a smallest bacterium to a longest nerve cell or a biggest ovum, must carry on a variety of cellular processes to be able to stay alive. Some of these activities are proactive in nature such as gaining nourishment, and reproducing, whereas other actions are defensive in nature. Since a hoard of chemical reactions with several sets of chemicals take place in a cell constantly, a large number of by products are also produced. Some of these by products can also cause damage to the cell that produces them. Thus, although several advantages are derived from using molecular oxygen, these advantages come at a cost. One such negative effect of the use of molecular oxygen that causes damage is in form of toxic by-products known as reactive oxygen species. These ROS if left unchecked would seriously affect a cell and eventually an organism's viability

(Figure 2.1). Reactive oxygen species are natural by-products of aerobic metabolism and their production correlates with normal cell proliferation through activation of growth-related signalling pathways (Benhar *et al.*, 2002).

Reactive oxygen species can elicit a plethora of responses ranging from proliferation, to growth arrest (transient or permanent), to senescence, to cell death (through either an apoptotic or necrotic mechanism). Lower doses of oxidants are generally associated with mitogenesis, moderate doses with growth arrest, and higher doses with cell death. Other factors that determine oxidant effects include the nature of the ROS and the type of cell in which it is operating. These ROS include hydrogen peroxide, superoxide anion radicals, singlet oxygen, hydroxyl radicals and nitric oxide. It is well known that ROS generated play a critical role during regeneration of amphibian limbs (and tails). After amputation of the amphibian limb, the wound is quickly covered by a specialized epithelium, the so-called wound epithelium. It is strongly believed that this epithelium provides the necessary signals for the underlying tissues to dedifferentiate, proliferate, and form the blastema (Brockes *et al.*, 2004). All the tissues at the stump, including muscle, bone, and other mesodermal tissue, undergo dedifferentiation. The dedifferentiation process leads to the proliferation of cells to form the blastema. After a period of about 2 weeks the blastema redifferentiates to form an exact replica of the severed part (Tsonis, 1996). Due to its complexity the process of limb regeneration is regarded as a spectacular phenomenon. The expression in the wound epithelium has been studied with the hope of identifying the factors that signal the initiation of the dedifferentiation process. While several factors have been found unique to the wound epithelium, FGFs and their receptors are thought to be paramount for the signalling that leads to regeneration. In urodeles, FGF-1 and FGF-2 have been found in the wound epithelium. Interestingly, FGF-1 and FGF-2 have been implicated in the nerve dependency of limb regeneration (Mullen *et al.*, 1996; Zenjari *et al.*, 1997). Moreover, FGF-2 is known to evoke activation of antioxidant enzymes that take care of the excess of ROS production (Yong-Fang and Yongie –Jie, 2001).

However, many aspects of the regulation of regeneration remain unclear and most likely involve molecules that are yet to be defined. Prostaglandins (PGs) are one such candid group of molecules as they have been implicated in various stages of myogenesis and are synthesized by regenerating muscle (Palmer *et al.*, 1983; McLennan 1991a, 1991b; McArdle *et al.*, 1994; Trappe *et al.*, 2001). Prostaglandins are involved in myoblast proliferation

(Zalin, 1987), differentiation (Schutzle *et al.*, 1984), and fusion *in vitro* (Zalin, 1987; David and Higginbotham, 1981; Entwistle *et al.*, 1986; Rossi *et al.*, 1989; Horsley and Pavlath, 2003) and appear to play an important role in myofiber growth during development (McLennan, 1991b). In addition to their roles in myogenesis, PGs are also potent modulators of inflammation, as evidenced by the ability of inhibitors of PG synthesis known as non-steroidal anti- inflammatory drugs (NSAIDs) to ameliorate pain and inflammation following muscle injury and other types of tissue damage. These local mediators, *viz* PG, ROS and NO, modulate some of the basic cellular functions (Figure 2.1). The release of these autocoids takes place by mediators of well identified bioenzymatic complexes- cyclooxygenases (COX) for PGs and nitric-oxide synthases (NOS) for NO (Mollace *et al.*, 2005). The actions of ROS are mediated in part by cyclooxygenase by-products that are important participants in the inflammatory process (Higgs *et al.*, 1984). It is also known that COX-2 cytotoxicity mechanisms may be related to production of reactive oxygen species, production of prostanoids, or induction of apoptosis via prostaglandin E_2 (PGE_2) (Feng *et al.*, 1995; Nogawa *et al.*, 1997). Production of ROS is in narrow relationship with the metabolism of arachidonic acid (AA). Conversion of this fatty acid via the lipoxygenase pathway is associated with an increase of ROS, whereas the transformation of AA into prostaglandins via the cyclooxygenase pathway results in the opposite effect (Baud and Ardaillou, 1986). These molecules if remained unchecked can damage a wide variety of molecules within the cells, leading to oxidative stress.

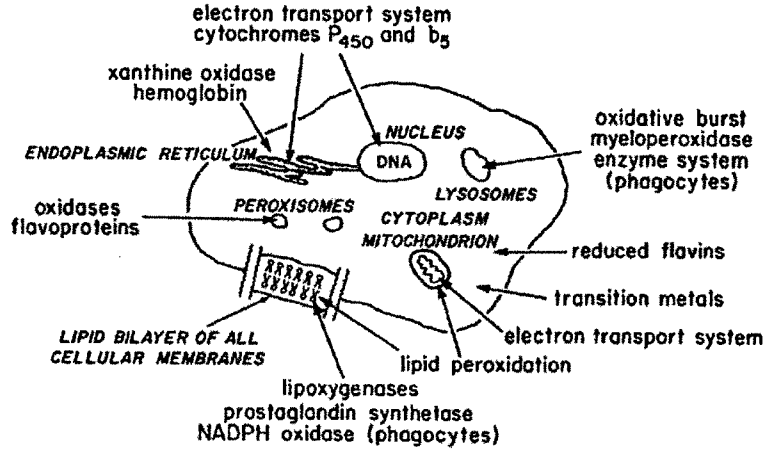

Figure 2.1. Cellular sources of free radicals. Adapted from Freeman and Crapo, 1982.

To help protect against the destructive effects of ROS, natural antioxidant enzymes are manufactured inside the body of the aerobic organisms to provide an important defence

against free radicals (Figure 2.2). Glutathione peroxidase, glutathione reductase, catalase, thioredoxin reductase, superoxide dismutase, heme oxygenase and biliverdin reductase, are some of the most important antioxidant enzymes. It was the evolution of these enzymes that made oxidative cellular metabolism possible. The enzyme superoxide dismutase converts two superoxide radicals into one hydrogen peroxide and one oxygen. To eliminate hydrogen peroxide before the Fenton Reaction can create a hydroxyl radical, organism use catalase and/or glutathione peroxidase. Glutathione peroxidase is found throughout the cell, whereas catalase is often restricted to peroxisomes. The Superoxide dismutase (SOD) molecule in the cytoplasm contains copper & zinc atoms (Cu/Zn–SOD), whereas the SOD in mitochondria contains manganese (Mn–SOD) (Blokhina *et al.*, 2003) (Figure 2.2).

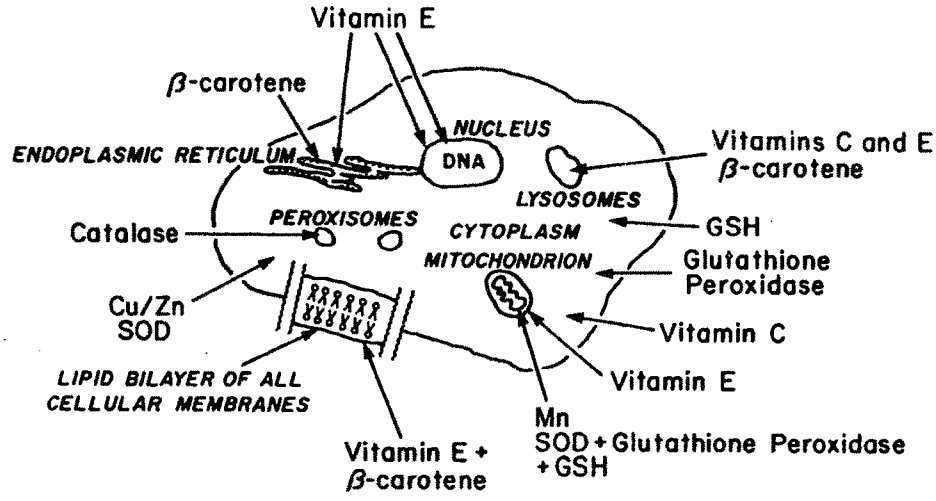

Figure 2.2 Antioxidant protection within the cell adapted from Machlin and Bendich, 1987.

The glutathione system (glutathione, glutathione peroxidase and glutathione reductase) is a key defense against hydrogen peroxide and other peroxides. There are four forms of glutathione peroxidase (GPx) enzymes: (1) cytosolic Glutathione Peroxidase (cGPx, ubiquitously distributed), (2) Phospholipid Hydroperoxidase Glutathione Peroxidase (PHGPx, in plasma membranes to reduce hydroperoxides of complex lipids), (3) plasma Glutathione Peroxidase (pGPx, in blood plasma) and (4) Gastro-Intestinal Glutathione Peroxidase (GIGPx, in the liver and GI tract only). The kidney manufactures mostly pGPX. Both pGPX and PHGPx can counteract LDL peroxidation in plasma and endothelial cells. PIGPx probably protects against dietary hydroperoxides (Brigelius-Flohe, 1999). Glutathione S-transferase (GST) is the family of enzymes comprising a long list of cytosolic, mitochondrial, and microsomal proteins which are capable of multiple reactions with a

multitude of substrates, both endogenous and xenobiotic. GST is useful in detoxifying endogenous compounds such as peroxidised lipids as well as the metabolites from xenobiotics (Storey, 1996).

Similarly, living systems contain large amounts of sulfhydryl compounds which must be maintained in the proper oxidation state to retain their biological activity. Biochemical systems contain relatively large concentrations of sulfhydryl compounds, notably glutathione and cysteine, which could provide such protection (Haugaard, 1968).

Lipid peroxidation is one of the processes for the synthesis of prostaglandin from nonenzymatic free radical-catalyzed peroxidation of arachidonic acid, the result of free radical attack of cell membrane phospholipids and low-density lipoprotein (LDL) oxidation. Therefore, the enhanced cyclooxygenase-mediated prostaglandin (PG) turnover occurring during sacrifice and biochemical processing of tissues generates malondialdehyde (MDA), a product of lipid peroxidation (LPO) (Peltola *et al.,* 1994). It is useful to estimate the MDA levels in the tissues to identify any toxic affects occurring in the regenerating system with the administration of COX-2 inhibitor.

The hypothesis that the inhibition of PGE_2 by competitive inhibition of COX-2 increases the oxidative stress of the regenerating tissue was tested by blocking the COX induced PGE_2 with the usage of NSAIDs (competitive inhibitors of COX activity). It was observed that when COX-2 was blocked by specific COXibs - etoricoxib, whereby blocking the prostaglandin pathway, caudal regeneration was hampered (Chapter 1). However, the influence of blocked PGE_2 on the production of antioxidant enzymes (to act against the ROS form in the body) of the tissues was unclear. This prompted one to study the status of antioxidant enzymes in various tissues of PGE_2 inhibited lizards.

MATERIALS AND METHODS

Animals

A total of forty Northern House Geckos of both the sexes were selected and maintained in the laboratory as described elsewhere (Materials and Methods). The animals were divided into two groups of twenty animals each and were treated as follows:

Experimental Design and Drug Dosage

Group I: This group of animals served as control to the experimental groups and injected with vehicle (Tris Buffer of pH 8.8).

Group II: The animals were injected with etoricoxib (25 mg/kg body weight).

The treatment in each group started four days prior to amputation and was continued till the animals reached differentiation stage. The drug was administered *in loco* every alternate day.

From each group, five animals which attained the wound epithelium stage, blastema and differentiation stage were selected and sacrificed. The extra animals were euthenised with excess of anaesthetic ether at the end of the experimental scheduled. The superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase(GST) activity and levels of reduced glutathione (GSH), glutathione peroxidase(Gpx), total-sulfhydryl (Total-SH) groups and malondialdehyde (MDA) were estimated in blood and tissues *viz.* liver, kidney, intestine and tail (one intact segment and the regenerate)of the animals.

STATISTICAL ANALYSIS

Statistical analysis was done using paired Student's t-test and the significance was set at $p \leq 0.05$.

RESULTS

The current experimental setup was designed to investigate the effect of PGE_2 blockage on free radical generation and the level of antioxidants during the successive stages of regeneration.

SOD: A reduction in the activity of SOD was observed in the tissues of the PGE_2 suppressed animals during the three defined stages of regeneration *viz.* wound epithelium, blastema and differentiation. However, the tail stump/regenerate registered an appreciable reduction in the activity of SOD (Table2.1-2.4, Figure 2.3).

Catalase: The activity of catalase was found reduced in the tail tissue of the animal that received etoricoxib, during all the stages of regeneration compared to that of vehicle treated lizards. However, an increase in activity of this enzyme was apparent in all the other studied tissues of the experimental animals (Table2.1-2.4, Figure 2.4).

GPx: The mean GPx activity was found decreased in the tail tissues of the treated animals except during the blastemal stage, where no significant change in activity was observed. However, the GPx activity in other tissues selected for the study showed aberrant response to COX 2 inhibition (Table2.1-2.4, Figure 2.5).

GSH: The analysis of the results revealed that the average GSH activity was high in all the tissues of the experimental animals, during wound epithelium and differentiation stages, but for the liver. However, during blastema stage, kidney as well as tail tissue from the PGE_2 suppressed lizards revealed significant reduction in GSH activity (Table2.1-2.4, Figure 2.6).

GST: The activity of GST in the caudal tissue of the treated lizards showed an obvious reduction during blastema stage. However, compared to controls during wound epithelium and differentiation stages no significant change in GST activity was observed in COX-2 suppressed animals. Nevertheless, the other tissues from the experimental animal registered by and large an increase in the GST activity compared to that of controls (Table2.1-2.4, Figure 2.7).

Total SH: Tissues of the experimental animals revealed an apparent hike in the total sulfhydrl group compared to respective controls. The notable exception however, was liver from the treated animals where a significant reduction in the activity (28%) was noticed (Table2.1-2.4, Figure 2.8).

LPO: It is vivid from the present results that the LPO level, in the etoricoxib treated lizards, got elevated in all the major tissue studies during wound epithelium and blastema stage except for regenerate. A significant reduction in the values of LPO was noticed in the caudal tissue of the experimental animal during blastema and differentiation stage (Table2.1-2.4, Figure 2.9).

DISCUSSION

A careful scan through the available literature showed scanty evidence of studies related to the role of PGE_2 in the regulation of antioxidant enzymes during reptilian tail regeneration. Therefore, the results of the current study are discussed in the light of antioxidant status during skeletal muscle healing or during inflammation of other tissues *viz.* liver, skin and muscle.

Injury to the skin initiates series of events, including inflammation, new tissue formation and matrix remodelling (Steilieng *et al.*,1999), finally leading to the partial reconstruction of the wounded tissues. Similar observation was noted during reptilian caudal regeneration. Inflammation, which occurs in response to an injurious stimulus, is a beneficial event that leads to removal of the offending factor and restoration of tissue structure and function (Gilroy *et al.*, 2001). Thus, the hallmark of a successful tissue restoration requires proper neutralization of inflammatory response. The factors which are required for the successful resolution of inflammation are those belonging to the cyclooxygenase pathways and release of ROS for destroying contaminating bacteria. There is a delicate balance in biological systems between amount of oxidants and antioxidants produced to prevent oxidative damage to the cells. The ability of cells to maintain homeostasis by preventing accumulation of excess oxidants is termed as redox homeostasis. The oxidants play an important role in wound healing by providing signalling and defence against micro organisms (Sen, 2003). However, the oxidants have to be detoxified in order to prevent damage to host cells. When, the antioxidant defence system fails to eliminate the oxidants, the alteration in homeostasis leads to oxidative stress.

In the present investigation an attempt was made to unravel the antioxidant pathways which are important for reptilian tail regeneration. In order to understand the role of prostaglandin in regeneration and also to understand how PGs regulate, if at all, the antioxidant status of the animal during such a process, it was considered worth estimating the antioxidant enzyme when the production of PGE_2 is being blocked by the *in loco* application of selective COX-2 inhibitor. In fact, the regenerative capabilities appear to depend upon the individual's potential for histogenetic and morphogenetic plasticity expressed in terms of recruitment of stem cells and/or dedifferentiated cells, cell proliferation and migration, supply of specific regulatory/trophic factors, and finally expression or reexpression of a specific developmental program (Weissman, 2000; Wadman, 2005). However, the role of individual prostanoids as well as the molecular mechanisms linking oxidative stress with caudal regeneration still remains to be clarified.

The studies showed that the administration of COX-2 inhibitor in the animal resulted in the decrease of major antioxidant enzymes in tail tissue which was in a state of inflammation after induced caudotomy. A disparate change was observed due to the differences in the amount, nature and activities of these enzymes in particular tissue. The status of the enzymes

was examined in all the stages of regeneration wherein the tissue undergoes several amendments from lost structure to a replaced organ and under these circumstance the production of ROS and its removal from the system plays an important role as stated by Steilieng *et al.,* (1999), Thannickal and Fanburg (2000), Finkel (2003), Gordillo and Sen (2003), Diegelmann and Evans (2004), Johar *et al.,* (2004).

The antioxidants are of two types: enzymatic and nonenzymatic antioxidants. Catalase, superoxide dismutase, and glutathione peroxidase are examples of enzymatic antioxidants. Superoxide dismutase and catalase are considered primary enzymes since they are involved in the direct elimination of reactive oxygen species (Halliwell and Gutteridge, 1985). Superoxide dismutase is an important defense enzyme which catalyzes the dismutation of superoxide radicals (McCord *et al.,* 1976). However, catalase is a hemoprotein which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals (Chance *et al.,* 1952). Else and Hulbert (1985) have reported that SOD in reptiles is several folds lower than that of mammals. In the present study during WE stage, reduction in SOD activity was observed in all the respective tissues resulting in the deleterious effect due to the accumulation of superoxide anion radical and hydrogen peroxide. At the WE stage, the tail of the animal, it appears is under a great oxidative stress which is evident from the increase in LPO levels and decrease in SOD and CAT activities. This increase in LPO level and decrease in SOD activity in tail is one of the biomarker for delayed healing of wound due to step up synthesis of ROS in acute and chronic wound environment (Moseley *et al.*, 2004).

Glutathione S-transferases, the most important biomolecule protecting against chemical induced toxicity, participates in the elimination of reactive intermediates by reduction of hydroperoxide in the presence of glutathione peroxidase (Meister, 1984; Nicotera and Orrenius, 1986). The decreased level of glutathione S-transferases observed in liver and tail at WE stage in the PGE_2 blocked animals which could be because of increased utilization of substrate resulting from oxidative stress (Anuradha and Selvam, 1993). The decrease in the GST activity also account for the altered arachidonic acid (AA) metabolism as it is known that GST play an important role in AA metabolism by virtue of their peroxidase activity, commonly referred to as non-selenium glutathione peroxidase activity (Halliwell, 1976). Glutathione peroxidase, a selenium containing enzyme present in significant concentrations, detoxifies H_2O_2 to H_2O through the oxidation of reduced glutathione (Bruce *et al.,* 1982).

However, reduction of glutathione peroxidase activity, observed in tail of experimental animals, has been shown as an important adaptive response to increased peroxidative stress. Therefore, the decrease of the main antioxidant enzymes could be due to the absence of PGE_2 in the tail tissue. Hence, it is logical to believe that the presence of PGE_2 might play an important role in the mode of action of these enzymes to overcome the oxidative stress. Also, the capacity of detoxification was reduced due to the administration of the drug in the animal. Therefore, it could be inferred from the present study that the requirement of PGE_2 and sufficient amount of antioxidants are essential for the formation of apical epithelial cap.

Moreover, in order to determine how the antioxidant defences of a regenerating tail responded to stress in other organs of the body we measured the activities of these enzymes in liver, kidney, intestine and blood. In the current study we found an increase in the activity of CAT, GSH and LPO level in liver, kidney intestine and blood. We found an increase in GPx level in liver and kidney at WE stage. Similarly an increase in GST was observed in kidney, intestine and blood at all the stages. However a decreased level of GST was observed in liver at WE stage. An apparent increase in total-SH was found in almost all tissues. The discrepancy of these enzymes in respective tissues might be due to several reasons. Firstly differences in the route of etoricoxib (COX-2 inhibitor) administration, time duration and dose. Secondly the state of the tissue and type of the cells present in it and the stimulus to which they have to respond. During the course of the study we also noticed that as the animal progress from an inflammatory stage to wound healed and differentiation stage, the animal no longer remains under the oxidative stress and the level of many of the antioxidant enzymes come to the basal control values.

Limb regeneration in amphibians is a representative process of epimorphosis. This type of organ regeneration, in which a mass of undifferentiated cells referred to as the "blastema" proliferate to restore the lost part of the amputated organ, is distinct from morphallaxis as observed, for instance, in Hydra, in which rearrangement of pre-existing cells and tissues mainly contribute to regeneration (Suzuki *et al.,* 2006). A significant decrease was found in SOD, CAT, GST, GSH and LPO in tail of the treated animal during blastemal stage where the cells are in intensive proliferation. Decreased SOD levels in the regenerating tail of lizard indicate decreased ability of the tissues to handle O^{-}_2 radicals. Similar findings on SOD have been reported in the tissues of mice exposed to high fluoride intake (Patel and Chinoy, 1998; Sharma and Chinoy, 1998; Vani and Reddy, 2000). Due to this the tissue undergoes an

oxidative stress leading to cellular and DNA damage and thus affecting the rate of cell proliferation. A decrease in LPO level is denoting a reduction in lipid peroxidation which one of the crucial step in prostaglandin synthesis. Therefore it can be possible that with the blockage of PGE_2 via blocking the main rate limiting enzyme COX-2 by administrating a specific COX-2 inhibitor results in to a reduction of LPO. Similar results were observed by Yadav (2005), were the decreased activity of SOD and CAT was found when the regenerating tail is treated with anti fibroblast growth factor (antiFGF-2). The decrease in GSH levels represents increased utilization due to oxidative stress (Anuradha and Selvam, 1993). The depletion of GSH content may also lower the GST activity as GSH is required as a substrate for GST activity (Rathore *et al.*, 2000). Moreover, an increase in the level of antioxidants was found in liver, kidney, intestine and blood. The increase in all the antioxidants is one of the biomarker signifying that the tissue is under oxidative stress. This increase may be due to detoxifying the toxic effect of the drug in the animal.

At differentiation stage of the regenerating tail, the proliferated cells differentiate to form the lost structure, where in the status of antioxidants were very near to the control values, thus suggesting that there is ROS burst during the inflammatory stage and proliferation stage and in later stage with the action of antioxidants the basal level of the tissue is attained.

The present study demonstrates the role of ROS in caudal regeneration of lizard tail. According to Storey (1996), adaptive changes are being evidenced from the stressed state of the animal to the antioxidant defences, both enzymatic and nonenzymatic, that are expressed by animal to cope with the oxidative stress. This difference in these reactive molecules can cause a lot of tissue damage by reacting with polyunsaturated fatty acids in cellular membranes, nucleotides in DNA, and critical sulfhydryl bonds in proteins as studied by Lawernce, 1987. It is also known that there is an increase in ROS production with the release of cytokine and also that intercellular ROS signalling may be important in physiological/pathophysiological processes characterized by regenerating epithelial cells and activated myofibroblasts (Waghray *et al.*, 2005).

Considering the experimental data discussed above, it is difficult to delineate a universal mechanism for the whole antioxidant response towards stress. It is necessary to discuss other factors in the protective mechanism of animal. ROS if remained untreated eventually leading to oxidative damage of DNA, proteins, carbohydrates and lipids (Thannickal and Fanburg,

2000). Prior, (2004) found out that higher susceptibility of a tissue towards an oxidative stress depend upon both antioxidant capacity and the level of free radicals produced by the species. The protective responses against ROS could also be under the redox control of gene expression (Zheng and Storz, 2000). Several gene products in prokaryotes are involved in the antioxidant defense including Mn-SOD (Hassan and Fridovich, 1977), catalase (Yashpe-Purer and Yashpe, 1977), hydroperoxidase I (katG), an alkylhydoperoxide reductase (ahpCF), glutathione reductase (gorA), glutaredoxin 1 (grxA), and a regulatory RNA (oxyS). The variation in gene expression could also be one of the reasons in the variation of these antioxidants. Therefore, to scrutinize the effect of increased ROS on DNA damage and proteins the rate of cell proliferation was quantified with the administration of immunofluorescent DNA marker dye (Chapter 4) and protein expression was observed with SDS-PAGE (Chapter 3).

Table 2.1: Activity of antioxidative enzymes and levels of non-enzymatic antioxidants in liver, kidney and intestine of control and COX-2 inhibited lizards, at wound epithelium (WE) Stage.

Tissues	Liver		Kidney		Intestine		Tail		Blood	
Enzymes	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
SOD	0.67±0.01	0.53±0.03*↓	0.48±0.02	0.31±0.03*↓	2.00±0.24	1.87±0.57↓	3.35±0.12	2.16±0.19**↓	4.40±0.16	3.02±0.63*↓
CAT	9.12±0.36	11.06±0.67*↑	11.04±0.30	12.87±0.74*↑	16.17±0.79	18.99±0.98*↑	22.46±0.69	19.98±0.87*↓	7.22±0.37	8.83±0.47*↑
GPx	28.78±0.69	31.78±0.82**↑	29.11±1.14	32.89±1.34*↑	28.22±2.13	24.89±3.73↓	36.44±0.87	31.78±1.59*↓	31.89±0.65	28.31±1.30*↓
GSH	9.45±0.20	11.76±0.42**↑	9.68±0.36	11.23±0.59*↑	20.12±0.61	23.28±1.20*↑	19.95±0.81	23.33±1.42**↑	7.89±0.45	9.63±0.62*↑
GST	11.80±0.40	9.33±0.75**↓	15.39±0.65	17.88±0.96*↑	8.15±0.44	9.87±0.63*↑	7.79±0.80	6.95±1.10↓	2.88±0.15	4.50±0.63*↑
Total-SH	108.84±5.4	78.76±2.92**↓	65.75±2.20	76.07±3.50*↑	2.81±0.23	4.91±0.26**↑	9.17±0.64	11.41±0.73*↑	27.12±0.88	31.66±1.16**↑
LPO	14.83±0.60	17.31±0.90*↑	13.65±0.61	15.92±0.88*↑	6.14±0.11	7.81±0.39**↑	10.15±0.37	11.83±0.32**↑	2.69±0.30	3.97±0.44*↑

@ Values are expressed as Mean ± SE, * $p \leq 0.05$, ** $p \leq 0.01$; n=5

SOD: U /mg protein, CAT: µmole of H_2O_2 consumed/minutes/mg protein, GPx: mmoles of GSH utilized/mg protein/ Min., GSH: µg / mg protein, GST: nM of CDNB-GSH conjugate formed / min/mg protein, Total-SH: µg / mg protein, LPO: nmoles of MDA formed/mg tissue

Table 2.2: Antioxidant Status of lizards at blastemal stage (BL) in control and treated animals

Tissues	Liver		Kidney		Intestine		Tail		Blood	
Enzymes	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
SOD	3.48±0.41	2.53±0.98↓	3.80±0.29	2.39±0.47*↓	4.99±0.09	3.16±0.19**↓	2.63±0.09	1.16±0.15**↓	4.58±0.45	3.87±0.69↓
CAT	18.62±0.36	21.57±0.63**↑	8.07±0.47	10.30±0.88*↑	20.83±0.87	23.77±0.83*↑	31.55±1.07	27.90±1.15*↓	13.84±0.56	15.97±0.72*↑
GPx	25.67±0.85	29.44±1.07*↑	21.89±0.82	17.12±.1.60*↓	24.89±0.91	21.67±1.12*↓	22.44±1.79	24.22±2.25↑	21.56±0.46	18.38±1.10*↓
GSH	5.62±0.41	6.11±0.94↑	9.24±0.59	6.10±0.64**↓	72.30±1.42	74.44±2.22↑	59.63±1.29	51.66±2.21**↓	12.69±0.33	14.75±0.80*↑
GST	14.69±0.56	17.41±0.98*↑	14.45±0.44	16.36±0.82*↑	10.60±0.47	13.36±0.98*↑	4.92±0.31	3.17±0.69*↓	1.53±0.14	2.79±0.35**↑
Total-SH	97.81±2.87	101.00±4.90↑	14.15±0.64	18.40±0.95**↑	83.88±2.14	74.77±3.83*↓	50.58±1.09	56.04±1.78*↑	47.83±1.07	52.24±1.47*↑
LPO	7.55±0.38	9.41±0.80*↑	12.33±0.61	13.52±0.96↑	21.45±0.46	24.42±1.25*↑	10.79±0.37	8.92±0.74*↓	1.12±0.06	2.28±0.15**↑

@ Values are expressed as Mean ± SE, * p≤0.05, ** p≤0.01; n=5

SOD: U /mg protein, CAT: µmole of H_2O_2 consumed/minutes/mg protein, GPx: mmoles of GSH utilized/mg protein/ min. ,GSH: µg / mg protein, GST: nM of CDNB-GSH conjugate formed / min/mg protein, Total-SH: µg / mg protein, LPO: nmoles of MDA formed/mg tissue

Table 2.3: Activity of antioxidative enzymes and levels of non-enzymatic antioxidants in liver, kidney and intestine of control and COX-2 inhibited lizards, at differentiation (DF) stage.

Tissues	Liver		Kidney		Intestine		Tail		Blood	
Enzymes	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
SOD	3.54±0.32	2.13±0.43*↓	2.68±0.21	1.37±0.33**↓	2.12±0.32	1.43±0.70↓	2.29±0.08	1.17±0.16**↓	4.81±0.12	3.38±0.25**↓
CAT	8.57±0.44	9.65±0.80↑	4.24±0.24	5.73±0.44**↑	4.13±0.18	5.39±0.23**↑	8.24±0.44	6.30±0.63*↓	6.16±0.27	7.81±0.56*↑
GPx	59.68±1.09	52.04±3.29*↓	53.00±1.64	49.64±3.21↓	53.00±1.41	46.56±2.57*↓	54.93±1.16	49.16±2.13*↓	28.69±0.70	24.55±1.46*↓
GSH	3.23±0.06	1.93±0.12**↓	4.92±0.45	5.80±0.68↑	3.45±0.41	4.67±0.81↑	30.10±1.49	31.52±2.09↑	17.49±0.58	20.81±0.60**↑
GST	10.61±0.80	11.67±1.21↑	13.64±0.53	17.59±1.73*↑	4.67±0.65	5.41±0.98↑	2.80±1.08	3.21±0.23↑	2.22±0.39	1.75±0.49↓
Total-SH	24.35±1.46	28.22±1.62↑	7.67±0.24	6.68±0.67↓	3.99±0.21	4.33±0.49↑	12.87±0.49	11.19±1.54↓	38.56±1.24	43.87±1.72*↑
LPO	10.40±0.19	14.69±1.13**↑	4.15±0.33	5.66±0.48*↑	6.82±0.38	5.21±0.58*↓	3.51±0.18	2.75±0.21*↓	0.22±0.015	0.05±0.006*↓

[@] Values are expressed as Mean ± SE, * $p \leq 0.05$, ** $p \leq 0.01$; n=5

SOD: U /mg protein, CAT: µmole of H_2O_2 consumed/minutes/mg protein, GPx: mmoles of GSH utilized/mg protein/ min. ,GSH: µg / mg protein,

GST: nM of CDNB-GSH conjugate formed / min/mg protein, Total-SH: µg / mg protein, LPO: nmoles of MDA formed/mg tissue

Table 2. 4: Percentage increase / decrease in antioxidant status of various tissues

Parameters	Wound epithelial stage					Blastema stage					Differentiation stage				
	Liver	Kidney	Intestine	Tail	Blood	Liver	Kidney	Intestine	Tail	Blood	Liver	Kidney	Intestine	Tail	Blood
SOD	21↓	35↓	06↓	36↓	31↓	27↓	37↓	37↓	56↓	16↓	40↓	49↓	33↓	49↓	30↓
CAT	21↑	17↑	17↑	11↓	22↑	16↑	28↑	14↑	12↓	15↑	13↑	35↑	31↑	24↓	27↑
GPx	10↑	13↑	12↓	13↓	11↓	15↑	22↓	13↓	08↑	15↓	13↓	06↓	12↓	11↓	14↓
GSH	24↑	16↑	16↑	17↑	22↑	09↑	34↓	03↑	13↓	16↑	40↓	18↑	35↑	05↑	19↑
GST	21↓	16↑	21↑	11↓	56↑	19↑	13↑	26↑	36↓	82↑	10↑	29↑	16↑	15↑	21↓
TOTAL-SH	28↓	16↑	75↑	24↑	17↑	03↑	30↑	11↓	11↑	09↑	16↑	13↓	09↑	13↓	14↑
LPO	17↑	17↑	27↑	17↑	48↑	25↑	10↑	14↑	17↓	104↑	41↑	36↑	24↓	22↓	77↓

Figure 2.3: Superoxide Dismutase (SOD) activity in the tissues of control and treated at various stages of regeneration

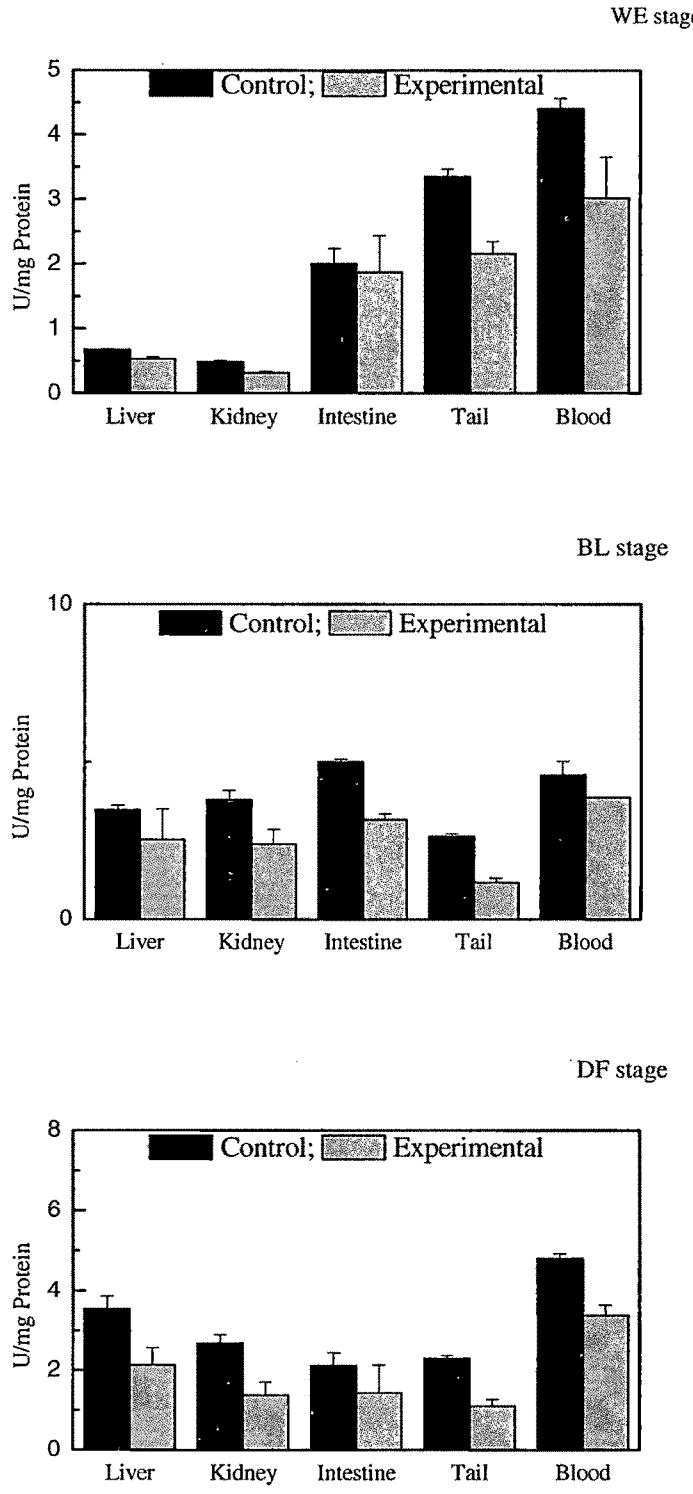

Figure 2.4: Catalase (CAT) activity in the tissues of control and treated at various stages of regeneration

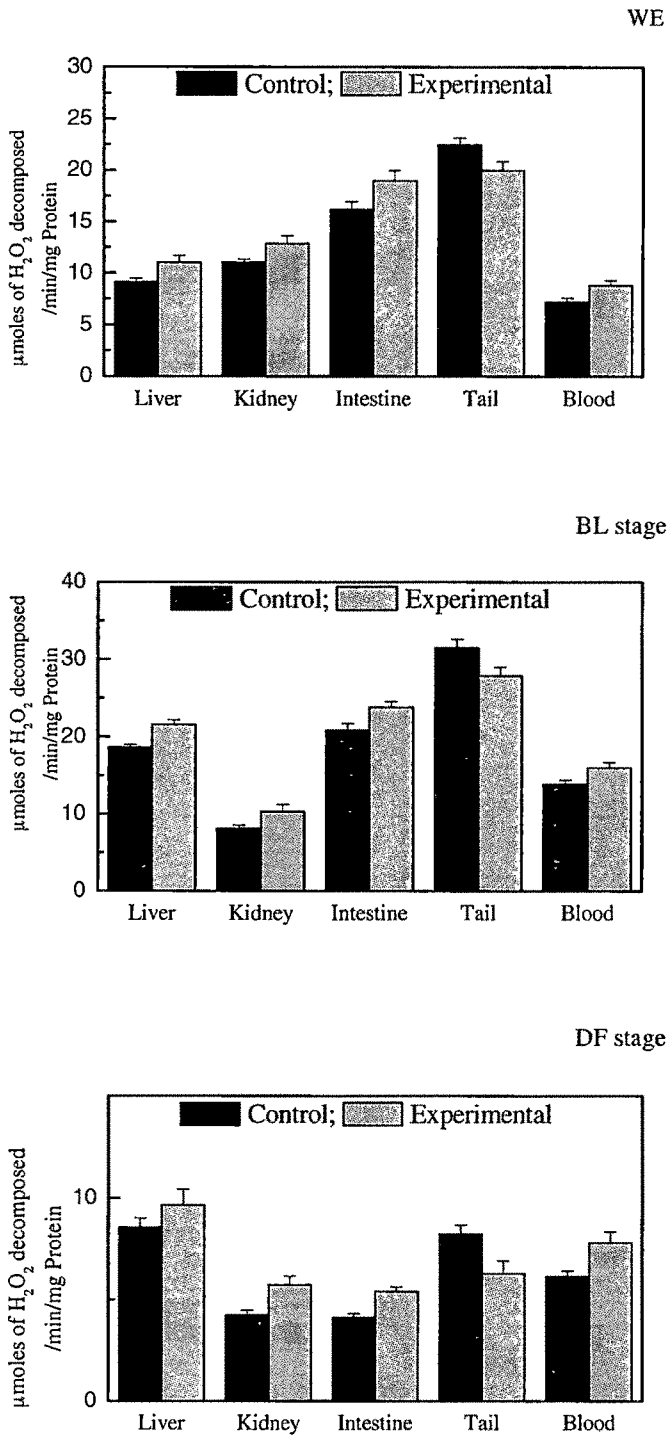

Figure 2.5: Glutathione Peroxidase (GPx) activity in the tissues of control and treated at various stages of regeneration

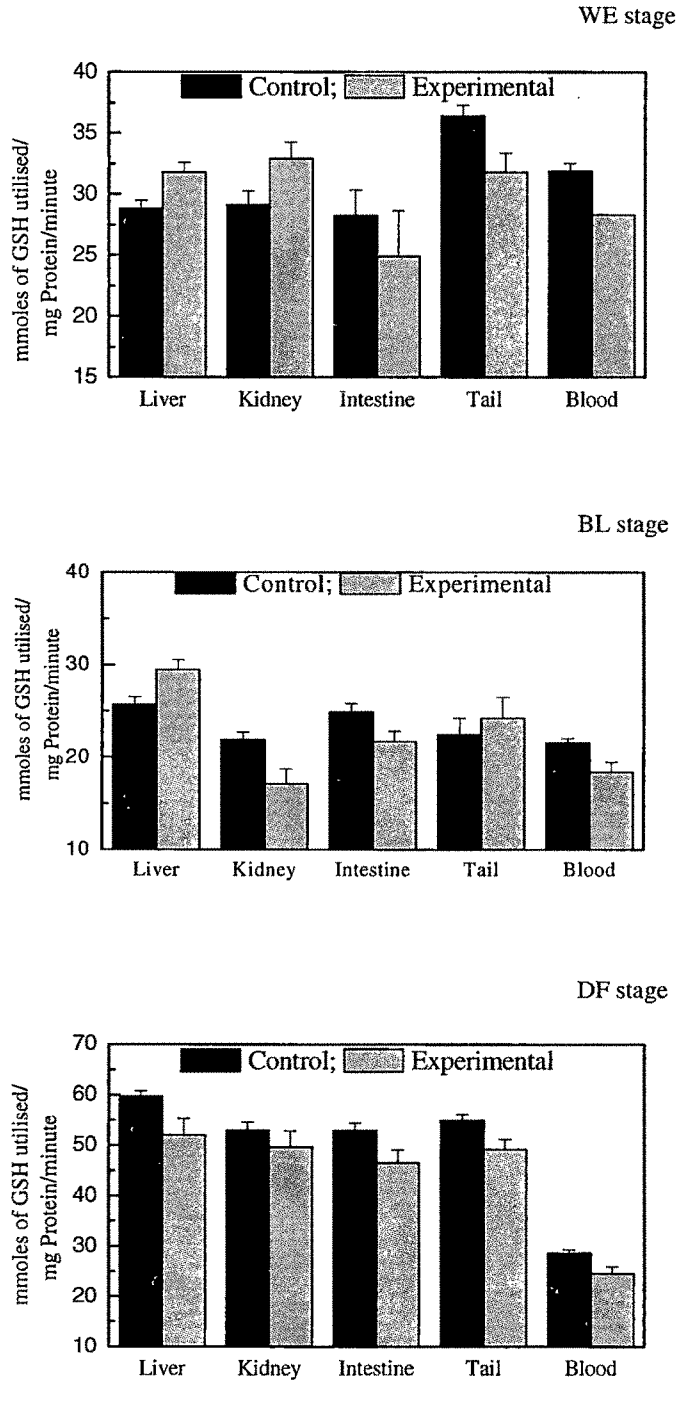

Figure 2.6: Reduced Glutathione (GSH) levels in the tissues of control and treated at various stages of regeneration

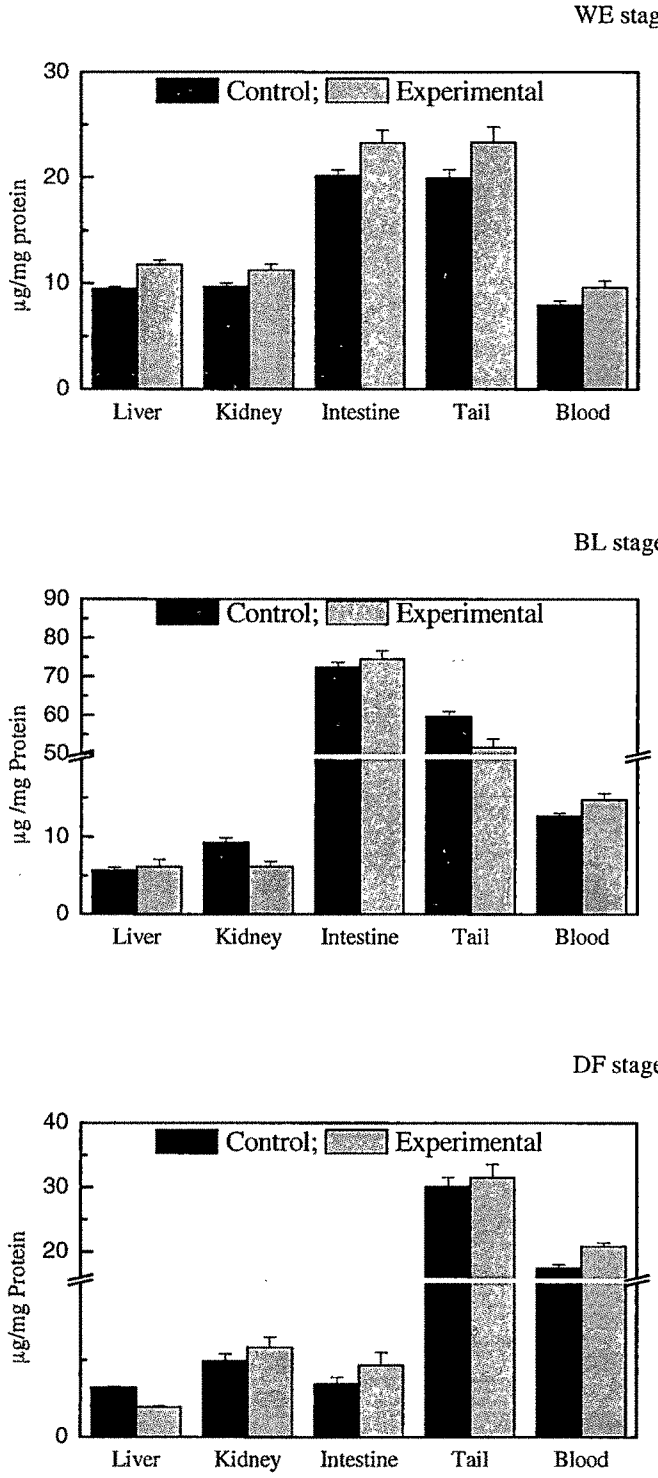

Figure 2.7: Glutathione-S-Transferase (GST) activity in the tissues of control and treated at various stages of regeneration

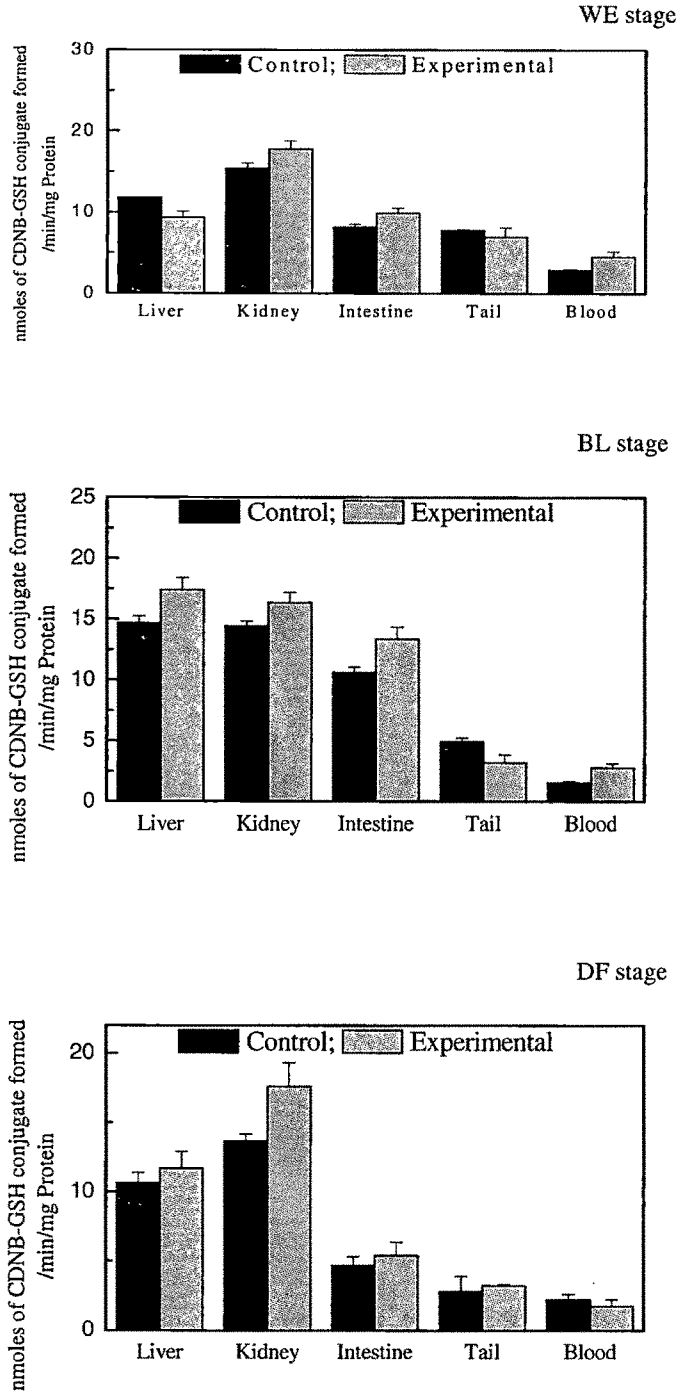

Figure 2.8: Total-SH levels in the tissues of control and treated at various stages of regeneration

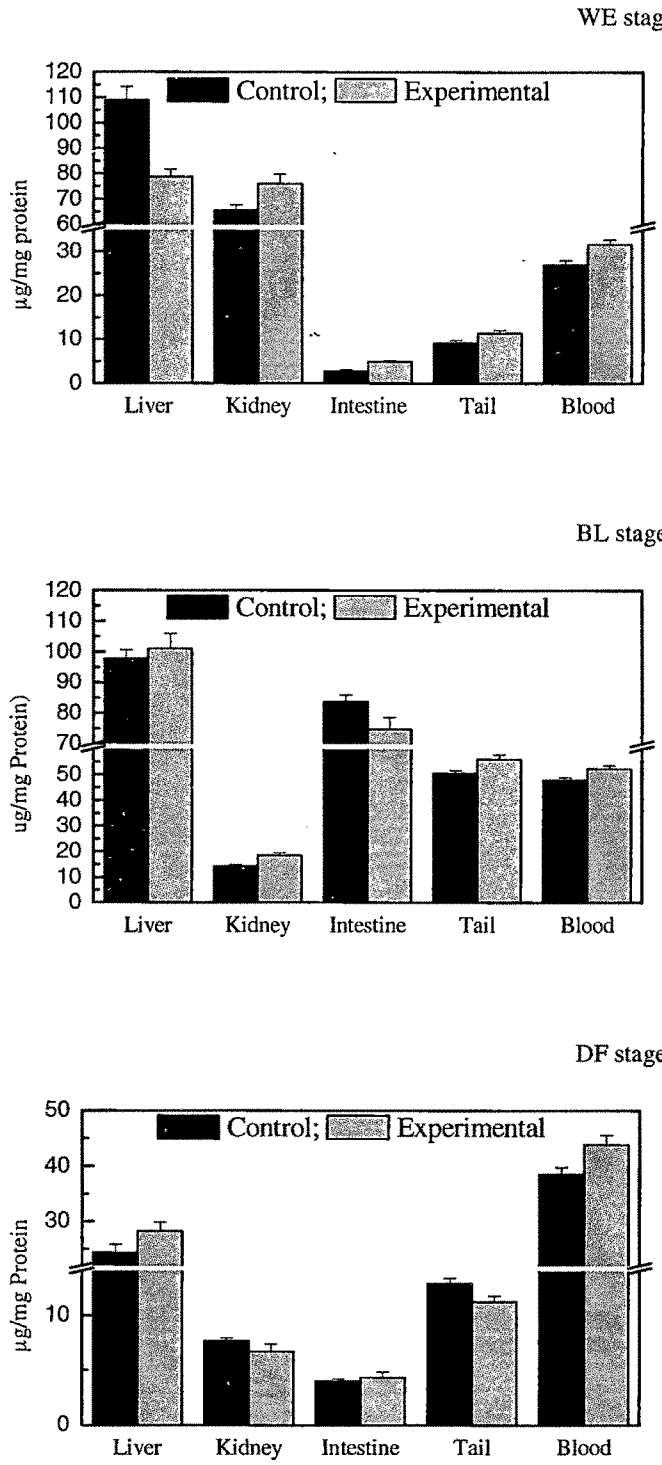

Figure 2.9: Lipid Peroxidation levels in the tissues of control and treated at various stages of regeneration