EFFECT OF T-2 TOXIN, A TRICHOTHECENE MYCOTOXIN, ON THE CENTRAL NERVOUS SYSTEM IN RATS

BY

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ABSTRACT

T-2 toxin has been shown to affect the nervous system, however, neurochemical mechanism of T-2 toxin action has not been documented well. To investigate the effect of T-2 toxin on the central nervous system (CNS), three sets of experiments were conducted. In the first experiment, rats were dosed orally with 0, 0.1, 1.0 or 2.5 mg T-2 kg^{-1} BW; killed 2, 6 and 10 hrs post dosing; and brain nuclei were analyzed for monoamines and their metabolites. T-2 treatment increased serotonin (5-HT) and 5-hydroxy-3-indoleacetic acid (5-HIAA) throughout the brain, produced a transient increase in nucleus raphe magnus (NRM) and locus coeruleus (LC) norepinephrine (NE) and a decrease in substantia nigra (SN) NE. T-2 altered dihydroxyphenylacetic acid (DOPAC), but did not effect epinephrine (EP) and dopamine (DA). Few treatment differences were observed, with 0.1 mg T-2 kg^{-1} BW (2% of the LD_{50}), affecting brain monoamines in the similar manner to higher dosages, which suggests that T-2 may affect the CNS directly. In a second study rats were fed 0, 2.5 or 10 ppm T-2 toxin daily in a semi-synthetic diet, and killed after 7 or 14 days. NRM's 5-HT, 5-HIAA and NE increased in a dose dependent manner and a transient DA increase was observed. Animals fed 10 ppm T-2 had increased SN EP after 7

days, and decreased SN NE after 14 days. In the paraventricular nucleus and medial forebrain bundle, DOPAC concentration was lower in T-2 treated animals at all levels of toxin. In the third study, rats were dosed intraperitoneally (ip) with 0, 0.2 or 1 mg T-2 kg-1 BW. Two hrs post treatment blood-brain barrier (BBB) permeability, protein synthesis, and MAO enzyme activity were determined. BBB permeability increased for mannitol, a small molecular weight saccharide, throughout the brain, but not for dextran. Acute T-2 treatment decreased brain protein synthesis, however, no effect on MAO enzyme activity was observed. Rats were fed diets containing 0, 2.5 and 10 ppm T-2 for 7 days and blood-brain barrier (BBB) permeability, protein synthesis, and MAO enzyme activity were determined. Dietary T-2 toxin increased BBB permeability to mannitol in cerebellum and pons+medulla, while permeability to dextran was not affected in any brain region examined. In T-2 toxin fed rats brain MAO enzyme activity decreased, however, no effect on protein synthesis was observed. The results suggest that T-2 toxin directly affects the CNS. That T-2 affects BBB transport, brain protein synthesis and enzyme activity which may provide an explanation for the observed neurochemical perturbations and physiological manifestations of trichothecene intoxication.

TABLE OF CONTENTS

PAGE

ACKNOWLEDGEMENT. iv

ABSTRACT v

TABLE OF CONTENTS. vii

LIST OF TABLES x

LIST OF FIGURES. xii

LIST OF ABBREVIATIONS. xiii

CHAPTER

1. INTRODUCTION 1

2. REVIEW OF LITERATURE 6

CHEMICAL AND TOXICOLOGICAL ASPECTS OF T-2 TOXIN

Chemistry And Metabolism of T-2 Toxin and Related Trichothecene Mycotoxins 6

Lethal Toxicity of T-2 Toxin 10

Toxic Expressions Of T-2 Toxin in Non-Neural Systems 12

Central Nervous System Effects Of T-2 Toxin. . . 18

Biochemical Mechanism of T-2 Toxicity. 23

THE CENTRAL NERVOUS SYSTEM 28

Anatomy of Catecholamine and Serotonin Neurons in the Central Nervous System 28

CHAPTER PAGE

Dopamine Neurons 28

Norepinephrine Neurons 29

Epinephrine Neurons. 29

Serotonin Neurons. 32

Biosynthesis and Metabolism of Catecholamines and Serotonin. 34

Physiological Functions of Catecholamines and Serotonin 42

Blood-Brain Barrier: Anatomy and Physiology. . . 45

3. EFFECT OF A SINGLE ORAL T-2 TOXIN EXPOSURE ON REGIONAL MONOAMINE CONCENTRATIONS IN THE RAT?. . . 49

Abstract 49

Introduction 50

Materials and Methods. 53

Results. 57

Discussion 63

4. EFFECT OF DIETARY T-2 TOXIN ON BIOGENIC MONOAMINES IN DISCRETE AREAS OF RAT BRAIN INTUBATED WITH SINGLE LOW DOSES OF T-2 TOXIN 70

Abstract 70

Introduction 71

Materials and Methods. 74

Results. 76

Discussion 79

CHAPTER PAGE

5. EFFECT OF T-2 TOXIN ON BLOOD-BRAIN BARRIER PERMEABILITY, MONOAMINE OXIDASE ACTIVITY AND PROTEIN SYNTHESIS IN RATS. 89

Abstract 89

Introduction 90

Materials and Methods. 93

Results. 99

Discussion 103

6. GENERAL DISCUSSION AND CONCLUSION. 110

REFERENCES 117

LIST OF TABLES

Table		Page
2-1	LD_{50} values of T-2 toxin and related trichothecene	11
3-1	5-HT concentrations in discrete brain regions of T-2 toxin intubated rats	58
3-2	5-HIAA concentrations in discrete brain regions of T-2 toxin intubated rats	60
3-3	DA concentrations in discrete brain regions of T-2 toxin intubated rats	61
3-4	DOPAC concentrations in discrete brain regions of T-2 toxin intubated rats	62
3-5	NE concentrations in discrete brain regions in T-2 toxin intubated rats	64
3-6	EP concentrations in discrete brain regions of T-2 toxin intubated rats	65
4-1	Composition of semi-synthetic diet.	75
4-2	Food consumption and body weight of rats. . . .	77
4-3	5-HT levels in brain nuclei in rat fed T-2 toxin containing diet	80
4-4	5-HIAA levels in brain nuclei in rat fed T-2 toxin containing diet	81
4-5	DA levels in brain nuclei in rat fed T-2 toxin containing diet	82
4-6	DOPAC levels in brain nuclei in rat fed T-2 toxin containing diet	83
4-7	NE levels in brain nuclei in rat fed T-2 toxin containing diet.	84

Table		Page
4-8	EP levels in brain nuclei in rat fed T-2 toxin containing diet	85
5-1	Brain uptake index: Regional brain uptake of mannitol in rats treated with a single intraperitoneal dose of T-2 toxin	100
5-2	Brain uptake index: Regional brain uptake of dextran in rats treated with a single intraperitoneal dose of T-2 toxin	101
5-3	Brain metabolism: Protein synthesis and monoamine oxidase enzyme activity in brain tissue of rats given an intraperitoneal injection of T-2 toxin.	102
5-4	Brain uptake index: Brain region uptake of mannitol in rats fed diets containing 10 ppm T-2 toxin.	104
5-5	Brain uptake index: Brain region uptake of dextran in rats fed a diets containing 10 ppm T-2 toxin.	105
5-6	T-2 toxin feeding trial - protein synthesis and monoamine oxidase enzyme activity in brain tissue of rats fed a semi-synthetic diet containing 2.5 or 10 ppm T-2 toxin for 7 days.	106

LIST OF FIGURES

Figure		Page
2-1	Chemical structures of T-2 toxin and related trichothecene mycotoxins	7
2-2	Schematic diagram illustrating the distribution of the main central neuronal pathways containing dopamine.	30
2-3	Diagram of the projections of the locus coeruleus norepinephrine neurons viewed in the sagittal plane	31
2-4	Schematic diagram illustrating the distribution of the main serotonin-containing pathways in the rat central nervous system.	33
2-5	The primary pathway in the formation of catecholamines.	36
2-6	The metabolic pathways available for the synthesis and metabolism of serotonin	40
4-1	Daily food intake of rats fed T-2 toxin containing diet for fourteen days	78

LIST OF ABBREVIATIONS

Blood-brain barrier	BBB
Body weight	BW
Catechol-O-methyltransferase	COMT
Central nervous system	CNS
Deoxynivalenol	DON
Dihydroxy-phenylalanine	DOPA
3,4-dihydroxyphenylacetic acid	DOPAC
3,4-Dihydroxyphenyl (ethylene)-glycol	DOPEG
Dopamine	DA
4-Dydroxy-3-methoxyphenyl (ethylene)-glycol	MHPG
Epinephrine	EP
5-Hydroxy-3-indole acetic acid	5-HIAA
Large neutral amino acid	LNAA
Locus coeruleus	LC
Medial forebrain bundle	MFB
3-Methoxy-4-hydroxyphenylacetic acid or Homovanillic acid	HVA
Monoamine oxidase	MAO
Norepinephrine	NE
Nucleus accumbens	NA
Nucleus raphe magnus	NRM
Olfactory tubercle	OT

Paraventricular nucleus of the hypothalamus	PVN
Serotonin or 5-hydroxytryptamine	5-HT
Substantia nigra	SN

CHAPTER 1

INTRODUCTION

Mold spores are ubiquitously distributed in nature and under suitable environmental conditions they germinate to produce fungal growth. The secondary metabolites produced by fungi, called mycotoxins, can spoil foods, animals feeds, or the raw materials used in their manufacture, making them potentially toxic to animal and humans (Fitzpatrick, 1989).

Trichothecene mycotoxins are a group of chemically related metabolites produced mainly by *Fusarium* molds, and are also synthesized by *Myrothecium, Trichoderma, Cephalosporium,* and *Stachybotrys* mold (Ueno, 1980). Trichothecenes are potent mycotoxins, with DON considered one of the least toxic and T-2 toxin one of the most toxic of this group, based on the LD_{50} values (Ueno, 1984).

T-2 toxin contamination of agriculture products has been reported worldwide (Pathre and Mirocha, 1979). In Canada, trichothecenes received little attention until there was a outbreak of mycotoxicosis (Puls and Greenway, 1976). In the Peace River region of British Columbia wet, cool harvest conditions resulted in mold growth and trichothecene production, with T-2 toxin was identified as a major

contaminant the grain and the cause of the mycotoxicosis (Greenway and Puls, 1976).

T-2 toxin intoxication is characterized by multisystem disorders including alimentary tract, cardiovascular and respiratory malfunctioning. T-2 toxin affects reproductive function, disrupts hemopoiesis and energy metabolism, and depresses immune function (Ueno, 1980). T-2 toxin affects the central nervous system (CNS) with T-2 intoxication characterized by impaired function. Clinical signs of intoxication include motor, sensory and autonomic nerve system malfunctioning (Feuerstein *et al.* 1989) as well as changed behaviors (Forsyth *et al.* 1977). Collectively, these observations suggests T-2 toxin is neurotoxic.

The biogenic monoamines serotonin (5-HT), dopamine (DA), norepinephrine (NE) and epinephrine (EP) are neurotransmitters in the CNS, and their regional concentration controls diverse physiological functions (Cooper *et al.* 1991). They are involved in regulation of autonomic nerve function, feeding behavior, motor activity, body temperature as well as having a role in the control of immune function. The physiological manifestations of T-2 intoxication, especially the neural disturbances may be due to the effect of the toxin on these transmitters. Only recently have attempts been made to investigate the central neurochemical mechanism underlying these pathophysiological

manifestations. Investigators report that acute T-2 toxin treatment affects the concentrations of brain biogenic monoamines and their metabolites (Boyd *et al.* 1988; Cavan *et al.* 1988; Chi *et al.* 1980; Weekley *et al.* 1989). However, these neurochemical perturbations have not been well documented and there is no consensus on the physiological effects of T-2 toxin.

Previous investigators examined the effect of T-2 toxin on biogenic monoamine concentrations in whole brain or in dissected main brain regions. However, the distribution of biogenic monoamines in the CNS is not uniform, monoamine neurons are present in clusters and axons of the neurons travel along specific areas. Therefore, it is of more physiological and clinical significance to examine biogenic monoamine levels in specific brain regions or nuclei which contain either the major cell groups or the important projections of catecholamines and serotonin systems.

Furthermore, previous experiments on the neurochemical effects of T-2 toxin used dosages approaching the LD_{50} value. Such high doses made it impossible to ascertain whether T-2's CNS effect is a direct action, or is secondary to T-2's peripheral effect, such as cardiovascular failure. Therefore, dose-response acute experiments and chronic feeding trials, designed to determine the effect of low

levels of T-2 toxin exposure on brain biogenic monoamine levels are needed to clarify this issue..

The mechanism of T-2 toxin action on the brain biogenic monoamines has yet to be determined. Major factors affecting brain biogenic monoamine levels include the availability of precursors for monoamine synthesis, which is partly controlled by the specific amino acid transport systems of the blood-brain barrier, and activity of the enzymes which are responsible for synthesis and degradation of monoamines (Cooper *et al.* 1991). The effect of T-2 on these factors may account for the central effect of the trichothecenes. We hypothesized that T-2 toxin affects brain biogenic monoamine concentrations and, that alterations of brain biogenic monoamine concentrations are associated with effects of T-2 toxin on factors which are related to the synthesis or degradation of biogenic monoamines in the CNS.

Therefore, the general objectives of this thesis research are to characterize, in more neurochemical detail the effect of trichothecene T-2 toxin, especially at low doses on central biogenic monoamine concentrations and to probe the mechanism which is related T-2 toxin induced central neurochemical perturbations. The specific purposes included:

1) To examine the dose and time related acute effects of T-2 toxin on regional concentrations of monoamines and

their metabolites using a micropunch technique for sampling selected brain areas which are either the main cell groups or the important projections of monoamine systems in the brain.

2) To examine the effect of dietary T-2 toxin on regional concentrations of monoamines and their metabolites in the brain areas which have been indicated to be sensitive to T-2 toxin treatment in the acute experiment.

3) To examine the effect of both acute T-2 toxin treatment by ip and chronic exposure to T-2 toxin by feed on the blood-brain barrier permeability.

4) To examine the effect of both ip T-2 toxin treatment and dietary T-2 toxin on activity of brain monoamine oxidase, a major enzyme involved in metabolic breakdown of monoamines.

5) To observe *in vitro* the effect of both acute T-2 toxin treatment and dietary T-2 toxin on protein synthesis of the brain.

CHAPTER 2

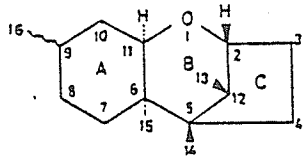
REVIEW OF LITERATURE

CHEMICAL AND TOXICOLOGICAL ASPECTS OF T-2 TOXIN

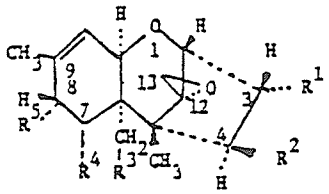
Chemistry And Metabolism of T-2 Toxin and Related Trichothecene mycotoxins.

CHEMISTRY: Trichothecene mycotoxins are a group of chemical derivatives of a "trichothecene" ring system. The structure and numbering system for this ring are shown in Figure 2-1a. All naturally occurring trichothecene contain an olefinic bond at C-9, 10, an epoxy ring at 12, 13 and they are classified as 12, 13-epoxytrichothecenes. In general, the carbon atoms at 3, 4, 7, 8, 14, and 15 are occupied by hydrogen, hydroxyl, acyl, additional epoxide, or macrocyclic ring functions (Figure 2-1b). In some groups, the carbon-8 is substituted by a ketone base (C=O). With the development of sophisticated analytical methods, more than a hundred of trichothecenes have been identified, and most of these mycotoxins have derivatives. Trichothecenes have been classified into four groups A, B, C, and D according to structural and fungal characteristics. Substitutions of R^{1-5} by different chemical group result in

A. Structure and numbering system of trichothecene



B. T-2 toxin and related trichothecenes



Trichothecene	R^1	R^2	R^3	R^4	R^5
T-2 TOXIN	OH	$COCH_3$	$COCH_3$	H	$OCOCH_2(CH_3)_2$
HT-2 TOXIN	OH	OH	$COCH_3$	H	$OCOCH_2(CH_3)_2$
T-2 TRIOL	H	H	H	H	$OCOCH_2(CH_3)_2$
T-2 TETRIOL	H	H	H	H	H

FIGURE 2-1 Chemical structure of T-2 toxin and related trichothecenes

the derivatives of the parent trichothecene. T-2 toxin belongs to group A of this classification and represents the so-called 'simple' trichothecenes, which carry only simple substituents in the 3-ring structure. The substituents in R positions of T-2 toxin are $R^1 = R^4 = H$; $R^2 = R^3 = -COCH_3$; $R^5 = -COCH_2CH(CH_3)_2$) (Figure 2-1b).

METABOLISM: T-2 toxin distribution and excretion was investigated in broiler chicks given a single dose of [^{3}H]-labelled T-2 toxin (Chi *et al*, 1978). Radioactivity reached a maximum concentrations 4 hrs after dosing in most tissue. The gastrointestinal tract and bile, including the gall bladder, contained the highest specific radioactivity among organs and tissues examined during the 48 hr test period, indicating the highest metabolic transformation and excretion rate of T-2 toxin in this organs. The distribution of T-2 toxin in the brain was not examined in this study.

Most trichothecenes, including T-2 toxin and DON, are lipophilic and should readily penetrate the blood-brain barrier (BBB). When the distribution [^{14}C]-DON was examined, the brain, like other organs examined, had its highest distributions of radioactivity three hours after systemic administration (Prelusky *et al*. 1986 and 1990). Thus rapid uptake into the brain make it possible for trichothecenes to directly affect the brain.

Following systemic application of T-2 toxin, there are two main metabolic pathways, a stepwise hydrolysis of the three ester groups in T-2 toxin and oxidation at 3'-carbon of the iso-valeroxy residue at position 8. Rat liver homogenate hydrolyses T-2 toxin rapidly to HT-2 toxin and finally to T-2 tetraol and hydroxy derivatives including metabolites with open 12,13-epoxide ring, a process which leads to much less toxic products. Brain metabolism of T-2 toxin, however, does not lead to measurable oxidized metabolites because of low levels of cytochrome P-450 oxidase, but leads to de-esterified products, i.e. HT-2 toxin, T-2 triol and T-2 tetraol in sequence (Bergmann *et al.* 1985 and 1988). The cerebral hydrolase, a enzyme responsible for the conversion of T-2 toxin to HT-2 toxin in the brain, exhibits pronounced specificity. When T-2 toxin was incubated with rat brain homogenate without blood content, HT-2 toxin was the sole metabolite (Yagen *et al.* 1991). HT-2 toxin is six times more toxic than T-2 toxin, when applied directly to the rat brain (Bergmann *et al.* 1985). That is metabolism of T-2 toxin in the brain is toxic activation processes and results in production of more potent neurotoxic metabolites. Formation and accumulation of HT-2 toxin in the brain, upon direct T-2 administration, would cause more neurological damage than systemic exposure. Thus, the neurological toxic effects of T-2 toxin on the CNS

may not be specific, however, the selective metabolic fate of the toxin in the brain makes the toxic expression there different from that observed in other systems (Bergmann *et al*. 1985).

Lethal Toxicity of T-2 Toxin.

The toxicity was compared between T-2 toxin and several other trichothecene mycotoxins in mice and rats (Ueno, 1984) (Table 2-1). The LD_{50} values of T-2 toxin in 6-week-old mice and rats were 10.5 and 5.2 mg kg^{-1} BW respectively when administrated by mouth (po). Intraperitoneal (ip) injection and intravenous (iv) injection of T-2 toxin resulted in LD_{50} values 5.2 and 4.2 mg kg^{-1} BW respectively in mice. The LD_{50} values of DON and 3-acetyldeoxynivalenol ranged from 34 to 70 mg kg^{-1} BW in mice, about 10 times higher than those of T-2 toxin. Newborn and immature mice were much more susceptible than adults to T-2 toxin exposure. In rat the LD_{50} values for intramuscular (im) injection of T-2 toxin was 0.85 mg kg^{-1} BW (Chan and Gentry, 1984).

The acute toxicity of T-2 toxin was compared in several species when administrated by iv, ip, sc, po and intratracheal (Fairhurst *et al* 1987). The LD_{50} values observed ranged from 1.0 to 14 mg T-2 kg^{-1} BW in different species. The mouse was shown to be less sensitive to T-2 toxin exposure than the rat, which agrees with the

Table 2-1. LD_{50} values (mg kg^{-1} body weight) of T-2 toxin and related trichothecenes

Animals	Routes	T-2 toxin	Fusarenon-X	Nivalenol	DON	3-Acetyldeoxy-nivalenol
Mice (male)						
6-week-old	po	10.5	4.5		46	34
	ip	5.2	3.4	4.1	70	49
	sc	2.1	4.2			
	iv	4.2	3.4	6.3		
4-week-old	sc	1.6				
New born	sc	0.15	0.23			
Rats (male)						
6-week-old	po	5.2				

po - per oral; ip - intraperitoneal injection; sc - subcutaneous injection; iv - venus injection.

observations of Ueno (1984). In chicken, the LD_{50} value of T-2 toxin were 4.0 mg kg^{-1} BW when the animal received a single oral dose of the toxin (Huff *et al.* 1980). Kubena and colleagues (1989) compared combined toxicity of T-2 toxin and DON with their individual toxicity in broiler chickens, and observed that their combination produced a synergistic toxic effect. Only an additive effect, however, was observed when T-2 toxin and diacetoxyscirpenol (DAS), another trichothecene mycotoxin were administrated together (Hoerr and Carlton, 1981).

In summary, the LD_{50} values of T-2 toxin is ten times less than that of DON. The toxicity of trichothecenes varies with the animal species, with rat being more sensitive to trichothecenes than mice and, young mice are more sensitive than matured mice. There is potential for a synergistic toxic effect between the various trichothecenes.

Toxic Expressions Of T-2 Toxin in The Non-Neural Systems

Effect of T-2 toxin on the cardiovascular system has been observed in both man and experimental animals. The patients with alimentary toxic aleukia, a human health problem in Russia, associated with the ingestion of moldy cereals infected with T-2-producing strains of fusarium, had a disease syndrome characterized by tachycardia, cyanosis, headache and cold in the extremities. Blood pressure of seriously affected people fell to 80/50 mm Hg, significantly

lower than normal values of 120/80 mm Hg and the heart rate was 180 beats min^{-1}, significantly greater than the normal value, less than 100 beats min^{-1} (Bubien *et al.* 1989).

Cardiovascular effects of T-2 toxin were observed in experimental animals. T-2 toxin treated rats had reduced contractility of myocardium and blood pressure (Magnuson *et al.* 1987). In swine, acute T-2 toxin exposure decreased blood pressure to shock level (diastolic pressure less than 40 mm Hg) and reduced blood flow to the brain, heart, kidney and gastrointestinal tract significantly (Beasley *et al.* 1987; Lundeen *et al.* 1986). These effects may be due to a direct action of T-2 toxin on cardiovascular system. Pathological changes were observed in the heart of rats treated with high repeated dosage of T-2 toxin (Yarom *et al.* 1983a). Heart lesions included interstitial edema, damaged myocytes and disrupted small blood vessels accompanied by myofibrillar disorganization, dilation of sarcoplasmic reticulum and formation of hypercontraction bands (Pang *et al.* 1986). In cultured myocardial cells, T-2 toxin decreased the beat rate and inotropic response of the myocyte (Yarom *et al.* 1986). However, the calculated dose needed to elicit pathological changes in the heart was relatively high. That is T-2 toxin is capable of damaging the heart directly, however, the lethal dose was too high to make this the cause of cardiovascular failure *in vivo*. An

indirect mechanism for the development of T-2 toxin induced cardiovascular failure has been suggested (Yarom *et al.* 1983b and 1986). It has been reported that T-2 toxin treated rats demonstrated a high peripheral resistance and a simultaneous increase in catecholamine release from adrenal medulla, suggesting an involvement of sympathetic nerve system in changed hemodynamics (Siren and Feuerstein, 1986). Furthermore, altered blood pressure and elevations in arterial plasma catecholamines, observed in conscious rats and guinea pigs, were not obtained in pithed rats, following iv administration of T-2 toxin (Feuerstein *et al.* 1985). Taking these results as a whole, it would suggest that the central sympathetic nervous system play an important role in T-2 toxin induced cardiovascular failure. Decreased myocardium contractility and altered sinus node cell function after T-2 toxin treatment could be a result of suppressed sympathetic activity.

The hematological system is an important site of trichothecenes' action. Subacute systemic T-2 toxin administration decreased hematocrit, white blood cell count and serum alkaline phosphatase activity in rabbits (Gentry and Cooper, 1981). T-2 toxin is hemolytic, causing complete erythrocyte hemolysis after a lag period, the length of which depended upon toxin concentration (Segal *et al.* 1983). The hemolytic action of T-2 toxin depended upon the species.

Man, pig, rabbit, guinea pig, horse, dog, rat, and mouse erythrocytes were all lysed by T-2 toxin, but cow, sheep, goat, buffalo, and deer erythrocytes were resistant to hemolysis by T-2 toxin. The different erythrocyte susceptibility was thought to be related to their membrane composition. That is ruminant animal erythrocytes contain little or no phosphatidylcholine and it has been suggested that phosphatidylcholine is required for T-2 toxin to exert its hemolytic action.

T-2 toxin affect platelet behavior. When human platelet was intubated with T-2 toxin a dose related inhibition of platelet aggregation and release of dense bodies was observed (Yarom *et al*. 1984). T-2 toxin affects blood coagulating function. A single iv dose of 0.25 mg kg^{-1} BW T-2 toxin decreased the plasma activity of several blood coagulating factors (Gentry and Cooper, 1983). The deleterious effect of trichothecene mycotoxins to both erythrocytes and platelet observed *in vivo* or *in vitro* could be the result of their general cytotoxic effects.

T-2 toxin has been shown to affect the immune function (Corrier, 1991). Hematological changes observed in the animals acutely exposed to T-2 toxin include lymphopenia and leucopenia, suggesting that lymphocytopoiesis was affected by trichothecenes (Gentry *et al*. 1984). Histological observation indicated extensive necrosis occurred in

lymphoid organs, such as thymus and lymph nodes in acutely trichothecene-treated animals (Fekete *et al*. 1989, Forsell *et al*. 1987). Chronic exposure to T-2 toxin resulted in lower plasma immunoglobulin IgG, IgM and complement protein B concentrations in cattle (Mann *et al*. 1983). In sheep, an oral treatment with T-2 toxin 0.3 and 0.6 mg kg^{-1} BW caused leucopenia and lymphopenia (Friend *et al*. 1983). At necropsy, lymphoid atrophy of mesenteric lymph nodes and spleens was most marked. Depressed immune function was also demonstrated when animals fed low dose T-2 toxin containing diet (Friend *et al*. 1983; Fekete *et al*. 1989). These observations clearly indicated that lymphoid system is sensitive to T-2 toxin exposure and that immune toxicity is an important feature of T-2 mycotoxicosis.

Emesis and reduced food intake are common symptoms in acute, subacute and chronic trichothecene-treated animals (Forsyth *et al*. 1977; Ueno, 1984). Damage of digestive function by T-2 toxin exposure may contribute to these pathological manifestations but can not account for all changes in feeding behavior, especially at low dosages, at which T-2 toxin exposure did not cause discernable digestive tract damage, but still resulted in suppressed food intake and weight gain (Fekete *et al*. 1989). DON, a trichothecene mycotoxin with much lower toxicity compared with T-2 toxin, demonstrated even more potent action in inducing emesis in

experimental animals. An oral dose of 0.2 mg DON kg^{-1} BW, a dosage of 0.4% of LD_{50} value, would cause vomiting in swine (Forsyth *et al.* 1977). At this level, DON would not cause distress of alimentary tract.

Feeding behavior is centrally controlled through certain neural structures mainly located in the hypothalamus (Martin *et al.* 1991). CNS neurotransmitters, especially catecholamines and serotonin have been shown to play an important role in feeding behavior regulation (Leibowitz, 1982). Therefore, suppressed food intake and body weight gain after trichothecene exposure could be due to their effects on feeding controlling system or on related neurotransmitters in the brain.

In summary, T-2 toxin intoxication is characterized by a multisystem disorder. However, based on the information reviewed in this chapter, it is impossible to attribute all of the T-2 toxin-induced pathophysiological manifestations in the peripheral systems to local toxicity. Altered sympathetic nerve activity and neurobehaviors observed in T-2 toxin treated animals suggest a role of the CNS in T-2 toxicity. It is necessary to realize that malfunction of the CNS could result in pathophysiological changes in both central and peripheral systems. For example, T-2 toxin could caused the cardiovascular malfunction by affecting the central autonomic nerve system. It is possible that T-2

affects both cardiovascular system and the CNS directly. These effects may be independent of each other but interact synergically resulting in cardiovascular failure.

Central Nervous System Effects Of T-2 Toxin.

The effect of T-2 toxin on the CNS has received limited attention, but evidence of T-2 toxin neurotoxicity exists. Rats acutely exposed to T-2 toxin had neurobehavioral changes with a decrease in the motor activity and an impairment in the passive avoidance test being most prominent behavioral manifestation of toxicity (Sirkka *et al.* 1992). In chickens, it has been observed that dietary T-2 toxin caused abnormal positioning of wing, hysteroid seizures, and impaired righting reflex. The incidence of behavioral symptoms depended on the length of exposure and T-2's concentration in the diet (Wyatt *et al.* (1973). Chicken acutely dosed with T-2 toxin exhibited inactivity, loss of appetite and restlessness, and developed diarrhoea, panting and coma (Chi *et al.* 1977). These observations suggest that T-2 toxin affect nervous system.

A series experiments by Bergmann and colleagues (1985 and 1988) demonstrated that T-2 toxin has greater lethal toxicity when applied directly to the brain than systemic administration. Direct application of T-2 toxin to rat brain causes death with doses that are only 1-2% of those required by systemic administration (Bergmann *et al.* 1985).

It has been suggested that higher toxicity of T-2 toxin upon direct cerebral administration is due to its limited metabolism in the CNS (Bergmann *et al.* 1988).

Only recently have attempts been made to characterize the central neurochemical processes associated with the neural symptoms caused by T-2 toxin exposure. The effects of acute T-2 toxin treatment on neuronal DNA, RNA and protein in selected brain areas of rats were examined (Ballough *et al.* 1989; Martin *et al.* 1986a). Rats were dosed intraperitoneally with 0.75, 1.0, 1.5 and 6.0 mg T-2 toxin kg^{-1} BW and decapitated 8 hrs post exposure. Microscopic observations revealed no gross evidence of T-2 toxin induced brain cytopathology (Martin *et al.* 1986a). While cortical and striatal neurons exhibited a dose-dependent decrease in DNA hydrolysability (i.e. impaired chromatin activity) and protein level. Moderate RNA depletion was evidenced in the cerebral cortex with 1.5 mg T-2 toxin kg^{-1} BW in the striatum with a 6 mg T-2 toxin kg^{-1} BW dose (Martin *et al.* 1986a; Ballough *et al.* 1989). These observation suggested T-2 toxin inhibits protein synthesis in central neuron cells.

Acute exposure to trichothecenes perturbs biogenic monoamine metabolism in several animal models including chicks, rats and swine, however, the mechanism of trichothecene action is unknown (Prelusky *et al.* 1992). In

chickens, a single intubation of 2.5 mg T-2 toxin kg^{-1} BW affected whole brain catecholamines, with DA concentration significantly increased, while brain NE was reduced (Chi *et al*. 1981). In subsequent studies, intubation of T-2 toxin or DON did not affect whole brain concentrations of monoamines in rat. However, when brains were dissected into five brain regions, increased regional 5-HT and 5-hydroxy-3-indole acetic acid (5-HIAA), and decreased regional NE and DA concentrations were observed (Boyd *et al*. 1988; Fitzpatrick *et al*. 1988b). In rats, MacDonald *et al*. (1988) observed that intubation of T-2 toxin caused an initial increases in whole brain tryptophan and 5-HT, followed by an increase in DA. Weekley and coworkers (1989) reported that in rats intraperitoneal T-2 toxin administration increased cerebral and brain stem tryptophan, but reduced 5-HT in the same areas. However, in our laboratory it was observed that T-2 toxin or DON intubation elevated regional concentrations of the indoleamines, 5-HT and 5-HIAA in all brain regions examined, whereas NE and DA levels were not significantly altered (Boyd *et al*. 1988; Fitzpatrick *et al*. 1988b). In swine, acute intravenous administration of DON elevated NE and depressed DA concentrations in the hypothalamus, cerebellum and frontal cortex, but had few effects on the indoleamines (Prelusky *et al*. 1992). Prelusky suggested that changes in brain monoamine concentrations in

trichothecene treated animals, depend upon the toxin and species involved. However, given the limited number of experiments conducted, the plethora of experimental designs and analytical techniques employed, the lack of consensus on the central effects of trichothecenes is understandable.

Based on the information reviewed in this chapter, several questions related to the effect of T-2 toxin on brain monoamine metabolism need to be addressed. The dosages used in previous experiments were from 2 to 2.5 mg kg^{-1} BW which close to a half of LD_{50} value. Considering the natural contamination levels of T-2 toxin in agriculture commodities and the potential effects on human and agricultural animals, it is of the importance to examine the effect of T-2 toxin on the CNS at lower doses.

To date, numerous experiments have been conducted to examine the acute effect of T-2 toxin on biogenic monoamine (Cavan *et al.* 1988; Chi *et al.* 1981; MacDonald *et al.* 1988; Boyd *et al.* 1988; Fitzpatrick *et al.* 1988b). However, these effects have not been demonstrated in feeding trial. When administered by feed, T-2 toxin could have a different toxic expression compared with acute exposure. A chronic exposure to low dose of T-2 toxin by feed resulted in reduced food intake and feed efficiency (Fekete *et al.* 1989) as well as suppressed immune function (Jagadeesan *et al.* 1982). Since central monoamines have been shown to be involved in energy

metabolism, feeding behavior regulation and immune modulation, the pathophysiological manifestations occurred in T-2 fed animals could be related to the chronic effects of this mycotoxin on central monoamine metabolism.

So far, no effort has been made to determine the mechanism of T-2 toxin induced central neurochemical alterations. The factors which may influence brain biogenic monoamine levels include the precursor availability for monoamine synthesis and the activities of the enzymes which are involved in the synthesis and degradation of monoamines in the brain. T-2 toxin could alter brain biogenic monoamine levels by affecting these factors. It has been observed T-2 toxin treatment alter large neutral amino acid concentrations in both blood (Cavan *et al.* 1988) and the brain (Weekley *et al.* 1989). Additionally, T-2 toxin are potent inhibitor of protein and nucleic acid synthesis (Martin *et al.* 1986a,b; Ueno, 1980), therefore, T-2 toxin may influence brain biogenic amine concentrations by inhibiting synthesis of the enzymes which are responsible for degradation of monoamines. Since the substance exchange between the brain and blood is largely mediated by the BBB, a speculation could be made that the altered brain amino acid and biogenic monoamine concentrations after T-2 treatment may be, in part, caused by an increase in BBB permeability. T-2 toxin has been shown to cause lesions in

the blood vascular system. In rat T-2 toxin-induced pathology in the hear was characterized by dilation and swelling of the microvessels, damage to the endothelial cell membrane, and tearing of the vessel wall (Yarom *et al.* 1983a). In isolated rat heart similar damages occurred in the microvessels when T-2 toxin was directly infused into the heart (Yarom *et al.* 1983b). Since the BBB is formed with endothelial cells through the tight junction, T-2 toxin may also interrupt brain endothelial cell membrane which constitutes the BBB. Changes of BBB integrity and specific transport functioning could alter influx of large neutral amino acid from plasma to the brain, thereby affect the biogenic monoamine synthesis.

Biochemical Mechanism of T-2 Toxicity.

The toxic effects of trichothecenes in mammals, birds, farm animals and yeast have been attributed to their cytotoxicity. Several mechanisms for trichothecene cytotoxicity have been suggested, including inhibition of protein synthesis (McLaughlin *et al.* 1977; Rosenstein and Lafarge-Frayssinet, 1983; Ueno, 1980), damage of cell membrane function (Gyongyossy-Issa *et al.* 1986), impaired mitochondrial respiration function and energy metabolism (Koshinsky *et al.* 1988; Pace, 1983). The inhibition of protein synthesis is considered the critical feature for cell toxicity (Feinberg *et al.* 1989).

Inhibition of DNA and protein synthesis by T-2 toxin has been demonstrated. DNA and protein synthesis was inhibited in spleen, thymus and bone marrow in mice given a single dose of T-2 toxin (Rosenstein and Lafarge-Frayssinet, 1983). This effect was time specific with the inhibitory effect observed being reversed in bone marrow and liver 20 hrs after a single exposure to the toxin, however, T-2 effect on thymus and spleen was longer lasting. T-2 toxin inhibited DNA and protein synthesis both in hepatoma cells and in phytohemagglutinin (PHA)-stimulated lymphocytes (Rosenstein and Lafarge-Frayssinet, 1983). Replication and translation rates of DNA were comparably diminished in hepatoma cells by T-2 toxin, where in PHA-stimulated lymphoid cells, DNA synthesis was inhibited to a greater extent than was protein synthesis. Lymphocytes were shown to be more sensitive to T-2 toxin action than were tumor cells (Rosenstein and Lafarge-Frayssinet, 1983). This observation may provide an explanation for the extensive damages observed in lymphoid tissues in acute and subacute trichothecene intoxication (Fekete *et al.* 1989).

Inhibition of protein synthesis by trichothecenes was also demonstrated in eukaryotic and yeast cells. In mammalian cells, Wei and colleagues (1974) observed that protein synthesis was almost completely inhibited when the cell intubation medium contained trichodermin concentration

of 2.5 or 25 μM. In yeast cells, 30 μM trichodermin completely inhibited protein synthesis (McLaughlin *et al.* 1977). It has been suggested that trichothecenes suppress protein synthesis by inhibiting peptidyl transferase (Feinberg and Mclaughlin, 1989). This enzyme is part of the 60S ribosomal subunit and is involved in elongation and termination during peptide synthesis. Trichothecenes have been demonstrated to be able to bind 60S ribosomal subunit and inhibit activity of peptidyl transferase *in vitro* (Cundliffe *et al.* 1974; McLaughlin *et al.* 1977).

Inhibition of DNA and RNA by trichothecenes was reported in yeast and eukaryotic cells (Cundliffe *et al.* 1974; Feinberg and Mclaughlin, 1989; McLaughlin *et al.* 1977). However, this effect appears to be a secondary effect in that alteration in cellular DNA or RNA synthesis can be explained by the primary effect of trichothecenes on protein synthesis (Feinberg and Mclaughlin, 1989). However, protein synthesis inhibition can not explain all the cytotoxic actions of trichothecenes. For example, T-2 toxin can alters the morphology of erythrocytes and result in hemolysis at very low concentration (Segal *et al.* 1983). Since erythrocytes lack nuclei and protein synthetic capacity, the hemolytic action of T-2 toxin must be due to other mechanisms, not the inhibition of protein.

It has been suggested that trichothecene mycotoxins may exert their cytotoxicity by acting directly on the cell plasma membrane. To gain access to the cytoplasm and various cell organelles, trichothecenes must pass through the plasma membrane. T-2 toxin is a lipophilic and easily enters the plasma membrane. T-2 toxin is also one of the amphipathic substances which have both hydrophilic and hydrophobic ends. Thus, T-2 toxin could interact with the membrane and cause membrane perturbations, similar to those caused by other amphipaths (Khachatourians, 1990). Pace and Watts (1989) examined subcellular distribution of [^{3}H]-T-2 toxin in a perfused rat liver noting that after 5 min, the plasma membrane fraction contained 38% of labelled T-2, a concentration greater than that observed for the smooth endoplasmic reticulum, mitochondria and nuclear fractions, which were 27, 10 and 7%, respectively. Although T-2 toxin was concentrated in the membrane for a limited period, this distribution provides T-2 the opportunity to exert its noxious effect there.

Bunner and Morris (1988) examined perturbations in cell membrane function induced by T-2 toxin in L-6 myoblast. T-2 toxin was shown to affect numerous cell membrane functions, with many of these effects starting at a concentration of less than 4 pg ml^{-1}, which is in the molar range of steroid hormone which also produce significant cellular effects.

Observed perturbations of membrane functions were noted within 10 min of exposure to T-2 toxin, a time interval is too short to attribute these effects directly to protein synthesis inhibition since even short-lived membrane proteins have half-life measured in hours (Bunner and Morris, 1988). Therefore, T-2 toxin directly influences membrane function and perturbation in membrane functions are an important feature of trichothecene cytotoxicity.

Impaired mitochondrial function is a prominent feature of trichothecene cytotoxicity. Ultrastructural studies indicated that the mitochondrion membrane and the rough endoplasmic reticulum were most susceptible to T-2 toxin (Trusal and O'Brien, 1986). Pace (1983) observed that T-2 toxin inhibited oxygen utilization of rat liver mitochondria and site I along the electron transport chain was identified as the principal locale of T-2 toxin action. A similar inhibition of electron transport function was observed in yeast, with 0.1 and 0.2 mM T-2 toxin causing a 24 and 68% decrease in oxygen consumption, respectively (Koshinsky *et al.* 1988). This mitochondrial effect is another example of T-2 induced breakdown of membrane function.

THE CENTRAL CATECHOLAMINE AND SEROTONIN NERVOUS SYSTEM

Anatomy Of Catecholamine And Serotonin Neurons In The CNS.

Only with the development of histofluorescence and immunohistochemistry techniques in the last two decades has it been possible to map the monoamine neuronal system in the CNS (Falck *et al.* 1962). The cellular organization of catecholamines and serotonin systems in the brain and spinal cord has been extensively studied. A briefly description of catecholaminergic and serotoninergic cell groups and their major axonal projections in the CNS follows.

Dopamine Neurons: Dopamine-containing neurons can be divided into three main groups: nigrostriatal, mesocortical, and tuberohypophysial (Bjorklund *et al.* 1984; Cooper *et al.* 1991). The major dopaminergic tract in brain originates in the zona compacta of the substantia nigra and sends axons that provide a dense innervation to the caudate nucleus and putamen of the corpus striatum with nearly 80% of all brain DA is found in the corpus striatum. Dopamine-containing cell bodies that lie medial to substantia nigra provide a diffuse, but modest, innervation to the forebrain, including the frontal and cingulate cortex, septum, nucleus accumbens and olfactory tubercle. Dopamine-containing cell bodies in the arcuate and peraventricular nuclei of the hypothalamus send axons that innervate the intermediate lobe of the pituitary and the median eminence. These neurons play an

important role in regulating the release of pituitary hormone, especially prolactin. In addition to these major pathways, dopamine-containing neurons have been found in the olfactory bulb and in the neural retina. Figure 2-2 shows the main central neuronal pathways containing DA.

Norepinephrine Neurons: The NE cell groups divide into two major groups, the locus coeruleus (LC) group and the lateral tegmental group (Cooper *et al.* 1991). NE cell groups are confined to the lower brainstem, with the most rostral located in the pons in the LC, and the lateral pontine reticular formation. More caudally located cell groups are found in the ventrolateral part of the medulla oblongata (Bjorklund *et al.* 1984; Weiner and Molinoff, 1989). The LC cell groups have three projections descending to end in the spinal cord, projecting to cerebellum, and ascending anteriorly through the medial forebrain bundle (MFB) to innervate the entire cerebral cortex and hippocampus (Figure 2-3) (Cooper *et al.* 1991). The NE neurons of the lateral tegmentum system send fibers that innervate the brain stem and hypothalamus (Bjorklund *et al.* 1984; Weiner and Molinoff, 1989).

Epinephrine Neurons: Epinephrine neurons have a much more restricted distribution compared to DA and NE systems,

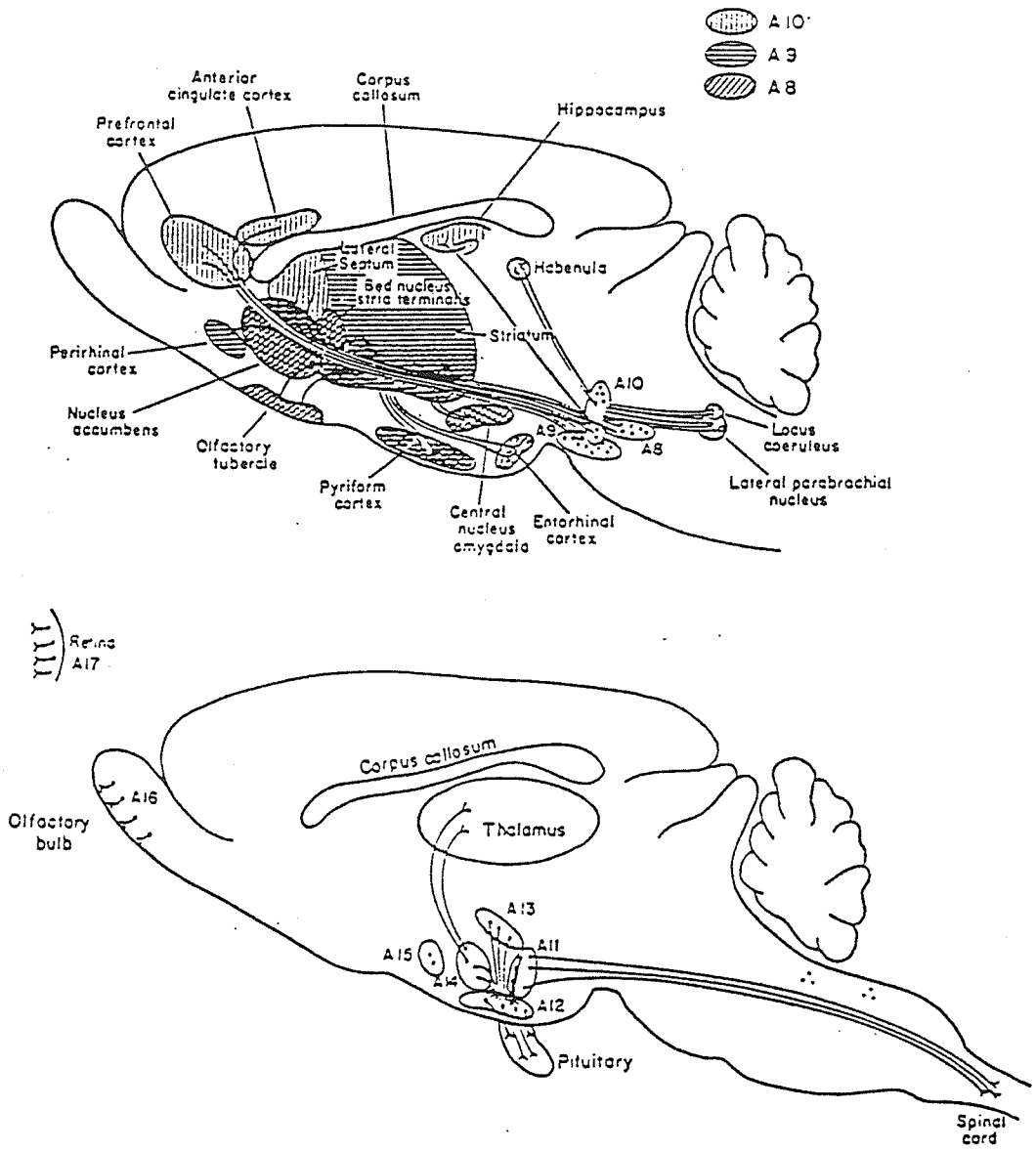


Figure 2-2. Schematic diagram illustrating the distribution of the main central neuronal pathways containing dopamine. The stippled regions indicate the major nerve terminal areas and their cell groups of origin. The cell groups in this figure are named according to the nomenclature of Dahlstrom and Fuxe (1965).

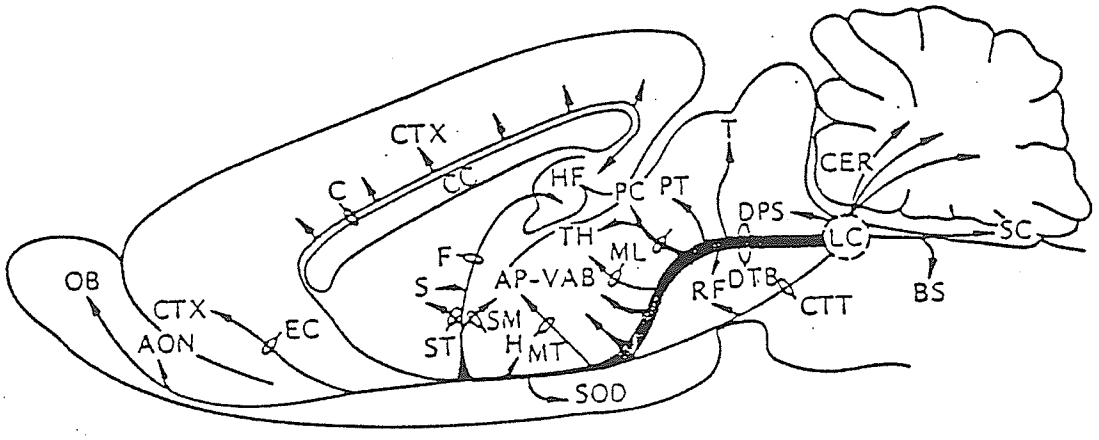


Figure 2-3. Diagram of the projections of the locus coeruleus viewed in the sagittal plane. Abbreviations: AON, anterior olfactory nucleus; C, cingulum; CC, corpus callosum; CER, cerebellum; CTT, central tegmental tract; CTX, cerebral neocortex; DPS, dorsal peraventricular system; DTB, dorsal catecholamine bundle; EC, external capsule; F, fornix; H, hypothalamus; HF, hippocampal formation; LC, locus coeruleus; ML, medial lemniscus; MT, mammilothalamic tract; OB, olfactory bulb; PC, posterior commissure; PT pretectal area; RF, reticular formation; S, septal area; SC, spinal cord; SM, stria medullaris; SOD, supraoptic decussations; ST, stria terminalus; T, tectum; TH, thalamus.

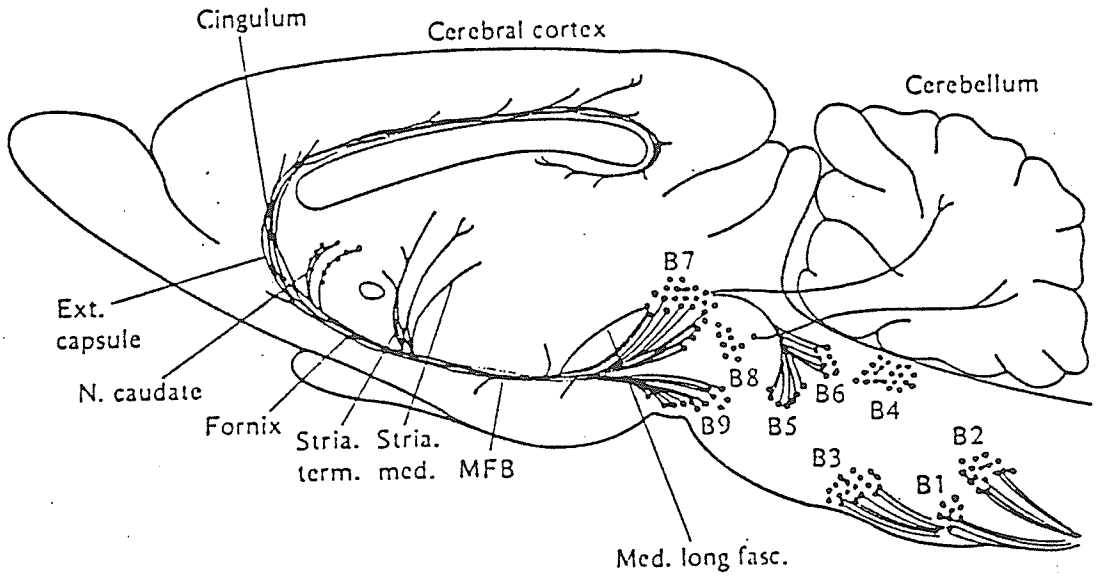


Figure 2-4. Schematic diagram illustrating the distribution of the main serotonin-containing pathways in the rat central nervous system. (Modified after G. Breese, Biosynthesis and Metabolism of Catecholamines and Serotonin, *Handbook of Psychopharmacology*, Vol. I, 1975).

constitute a major source of the ascending pathways and account for approximate 80% of the forebrain 5-HT terminals. In the rat, the principal ascending 5-HT pathways consist of medial and lateral bundles along the ventral aspect of the MFB. The medial pathway innervates limbic structures, the hypothalamus and preoptic area. The lateral pathway innervates the cingulate cortex. A third minor pathway originates from dorsal nuclei and innervates the caudate nucleus.

In addition to the nine 5-HT nuclei described classically, recent immunocytochemical localization of 5-HT has also detected reactive cells in the area postrema and in the caudal locus coeruleus, as well as in and around the interpeduncular nucleus (Cooper *et al.* 1991).

Biosynthesis And Metabolism Of Catecholamines And Serotonin

Catecholamines.

CATECHOLAMINES: The catecholamines DA, NE and EP share a common amino acid precursor, tyrosine, for their synthesis (Cooper *et al.* 1991). Tyrosine is a nonessential amino acid. Phenylalanine must be converted to tyrosine before it can be used to synthesize catecholamines. This conversion is catalyzed by phenylalanine hydroxylase in the liver. Tyrosine is converted to dihydroxy-phenylalanine (DOPA) in a reaction catalyzed by tyrosine hydroxylase. This conversion is considered the initial step in catecholamine synthesis

(Cooper *et al.* 1991). DOPA is decarboxylated to DA by aromatic-L-amino acid decarboxylase. Dopamine as an end-product of this synthetic pathway is utilized by specific neurons as a neurotransmitter. In other neurons, it may be converted to NE by the action of dopamine-ß-hydroxylase, while in still other neurons NE can be N-methylated to EP by phenylethanolamine-N-methyltransferase. The primary pathway of catecholamine synthesis is shown in Figure 2-5. All three catecholamine neurotransmitters may not function in the same neuron, the enzyme complement of the neuron dictates which catecholamine(s) it will produce (Cooper *et al.* 1991; Fernstrom, 1990).

Tyrosine hydroxylation, the initial reaction in the metabolic pathway, is rate-limiting and thought to be the control point for catecholamine synthesis (Fernstrom, 1990). Tyrosine hydroxylase is a mixed-function oxidase that uses molecular oxygen and tyrosine as substrates, and biopterin as a cofactor for the synthesis of DOPA. Tyrosine hydroxylase has multiple controls on its activity, including direct end-product inhibition and indirect phosphorylation-mediated effects (Fernstrom, 1990). It has a low K_m value for tyrosine and under normal condition is saturated by the tissue concentrations of endogenous tyrosine. Therefore, the influence of tyrosine levels on hydroxylase enzyme activity has not been thought physiologically important with

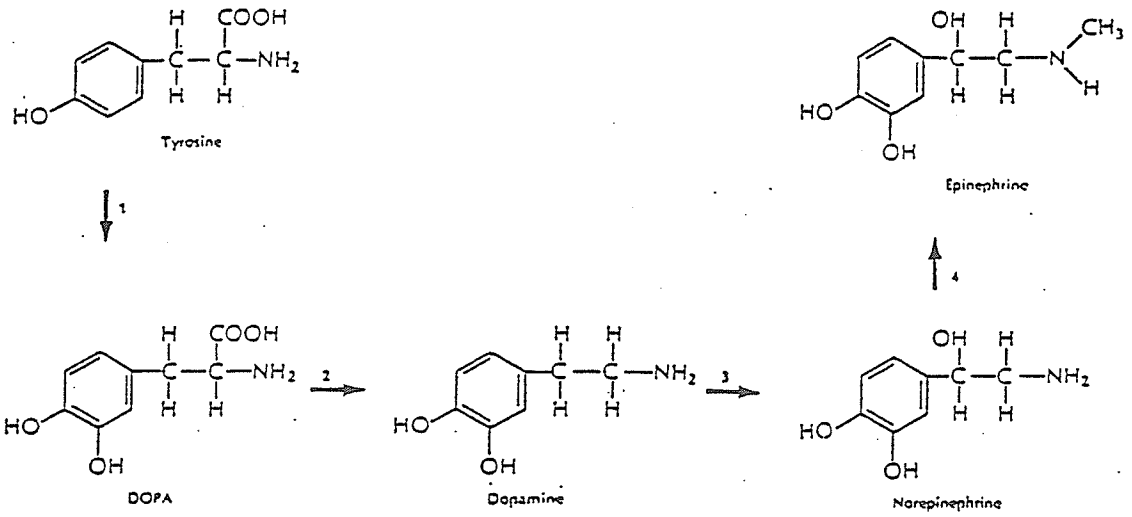


Figure 2-5. The primary pathway in the formation of catecholamine: (1) tyrosine hydroxylase; (2) aromatic amino-acid decarboxylase; (3) dopamine-ß-hydroxylase; (4) phenylethanolamine-N-methyl-transferase.

some exceptions. That is tyrosine levels can affect catecholamine synthesis in the brain region containing high firing-rate neurons (ie mesocortical area) or when catecholamine neurons are first activated with a drug or a physiological challenge such as cold stress (Fernstrom, 1990). In other words, under specialized conditions, availability of tyrosine in the brain can be an important factor to influence synthesis and the functions of catecholamine systems.

In the brain, approximately 80% of DA is deaminated and oxidized forming 3,4-dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase (MAO) and aldehyde dehydrogenase, and 20% of DA O-methylated to 3-methoxytyramine by catechol-o-methyltransferase (COMT), then deaminated into 3-methoxy-4-hydroxyphenyl-acetaldehyde (Kopin, 1985). In most species DOPAC is O-methylated primarily to 3-methoxy-4-hydroxy-phenylacetic acid (homovanillic acid, HVA), with DOPAC conjugation with sulphate being only a minor metabolic pathway. Around 40% of the HVA is conjugated and the remainder is eliminated as free acid. The relative concentrations of DOPAC and HVA and their conjugates differ among the various brain regions and with species. In rat brain DOPAC and HVA levels are similar and present mainly in conjugated form. Whereas in other species, free HVA predominates (Kopin, 1985). These metabolites can be

released from brain into the circulation possibly through active acid transport processes. DOPAC or HVA concentrations in the brain or plasma have been used to evaluate DA metabolism and functional activity of dopaminergic neurons in the brain (Cooper *et al.* 1991; Kopin, 1985).

The initial pathway for metabolism of NE in the brain and cerebral-spinal fluid involves MAO catalyzed oxidative deamination followed by reduction to 3,4-dihydroxyphenyl (ethylene)-glycol (DOPEG) and subsequent methylation to 4-hydroxy-3-methoxyphenyl (ethylene)-glycol (MHPG) (Cooper *et al.* 1991; Kopin, 1985). There are, however, species differences in the metabolism of DHPG; in mice the glycol metabolites are almost all unconjugated, whereas in rats, most of the metabolites are present in the conjugated form. In primates, as in mice, free MHPG appears to be the major NE metabolite released from the brain into the blood (Kopin, 1985).

Concentration of EP in the brain is quite low. Metabolism of EP in the brain may be similar in the adrenal medulla. It is possible that brain EP can be metabolized to MHPG and vanillyl mandelic acid. The role of conjugation in EP metabolism has not been adequately defined (Kopin, 1985).

Serotonin: Tryptophan is the precursor for 5-HT synthesis. 5-HT synthesis from tryptophan occurs in a two-step reaction

sequence with tryptophan first hydroxylated to 5-hydroxy-tryptophan, in a reaction catalyzed by tryptophan hydroxylase and then decarboxylated to 5-HT (Figure 2-6), in a reaction mediated by aromatic-L-amino acid decarboxylase, the enzyme which also catalyzes the decarboxylation of DOPA to DA (Green, 1989). Tryptophan hydroxylation is considered as the rate-limiting step in 5-HT synthesis. According to enzymatic kinetics study, brain tryptophan hydroxylase is not saturated at physiological concentration and fluctuation of brain tryptophan concentration affects 5-HT synthesis (Carlsson and Lindqvist, 1972; Wurtman *et al*. 1980). Therefore, any factor which alters tryptophan level in plasma or brain can affect 5-HT synthesis in the brain.

The primary metabolite of 5-HT is 5-hydroxyindolein in the same neuron, the enzyme complement of the neuronacetaldehyde. This reaction is catalyzed by MAO enzyme, preferentially by the MAO-A isoenzyme. 5-Hydroxy-indoleacet-aldehyde is oxidized to 5-hydroxyindoleacetic acid by NAD^+-dependent aldehyde dehydrogenase (Figure 2-6). The regional distribution of 5-HIAA in the brain is similar to that of 5-HT, a result of the precursor product relationship (Green, 1989). Measurement of 5-HIAA in brain tissue and calculation of the ratio of 5-HIAA to 5-HT can provide useful information about functional and metabolic activities of central serotonergic system.

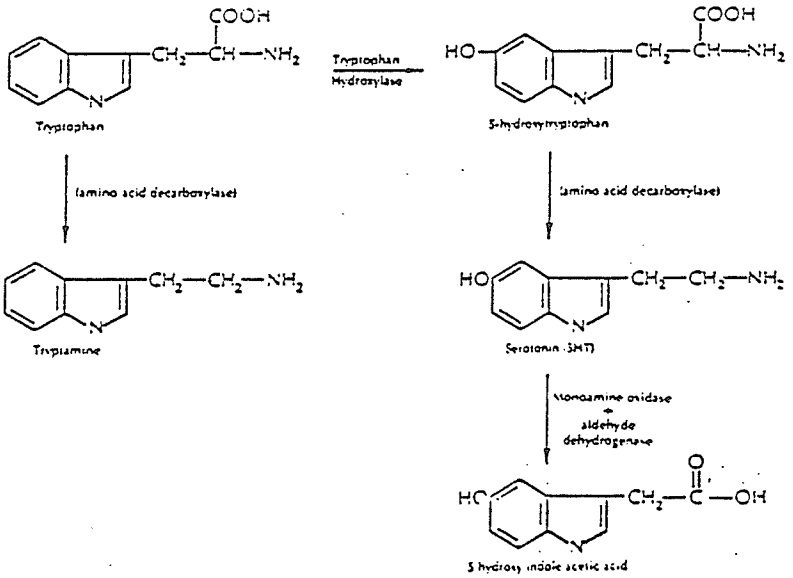


Figure 2-6. The metabolic pathways available for the synthesis and metabolism of serotonin.

Transport of amino acids across the BBB takes place via specific transport mechanisms (Gjedde, 1988; Skeie *et al.* 1990). All of the large neutral amino acid (LNAA) which include aromatic amino acids tyrosine and tryptophan, and branch-chain amino acids alanine, leucine and isoleucine share the common transport system (Skeie *et al.* 1990). This transport system is normally saturated and LNAA compete for transport into the brain with each others. The absolute amount of any individual LNAA entering the brain depends upon the ratio of this amino acid to the other LNAA rather than its absolute concentration in the circulation (Gjedde, 1988). An increased ratio of tryptophan to the other LNAA through diet manipulation or administration of tryptophan resulted in enhanced 5-HT synthesis (Wurtman *et al.* 1980). Insulin affects the ratio of tryptophan to the other LNAA in the plasma by affecting the uptake of amino acids by cells. Tryptophan displays a different behavior than the other LNAA. Insulin promotes the transport of branch-chain amino acids into cells, but dose not affect cellular tryptophan uptake (Fernstrom *et al.* 1972). Thus, elevated plasma insulin increases the ratio of tryptophan to the other LNAA, increasing the transport of tryptophan into the brain (Jamnicky *et al.* 1991). Furthermore, tryptophan is bound to plasma albumin at a specific site. The stoichiometry of this binding was shown to be one molecule of tryptophan to

one of albumin. This property is unique to tryptophan; no other amino acid binds to albumin. Under normal physiologic conditions, about 90% of the total tryptophan in plasma is bound to albumin (Skeie *et al.* 1990). It has been suggested that only free tryptophan is able to compete with other LNAA for transport into the brain. The factors such as free fatty acids which promote dissociation of tryptophan from albumin can result in a increase of tryptophan to enter the brain (Green, 1989; Skeie *et al.* 1990).

Physiological Functions of Catecholamines and Serotonin.

The catecholamines DA, NE, and EP are neurotransmitters or hormones in biological system. Norepinephrine is the principal postganglionic, sympathetic neurotransmitter in both peripheral and CNS. Dopamine has biological activity in peripheral tissues, particularly in the kidney, and serves as a neurotransmitter in several important pathways in the CNS. Epinephrine, a hormone released from the adrenal gland stimulates catecholamine receptors in a variety of organs. Small amounts of EP are also found in the CNS, particularly in the brain stem. However, information concerning the physiological function of EP neurons in the CNS is still very limited, but based on its distribution in specific brain regions attention has been directed to their possible role in neuroendocrine mechanisms

and blood pressure control (Cooper *et al*. 1991; Weiner and Molinoff, 1989).

Briefly, functions in which catecholamines may be involved in the CNS include modulation of autonomic activity, cardiovascular function and blood pressure, eating and sexual behaviors, endocrine secretion, body temperature, locomotor activity, emesis as well as pain. Peripherally, they play important role in regulation of gastrointestinal tract movement, secretion of digestive glands, nutrient metabolism and energy utilization (Green, 1989; Weiner and Molinoff, 1989).

5-HT has an ubiquitous distribution in both central and peripheral nervous systems. Research has indicated that 5-HT, as a neurotransmitter in the CNS, is associated with many physiological functions (Green, 1989). Based on the pharmacological, biochemical and behavior observations, brain 5-HT involved in feeding behavior regulation and body weight control (Leibowitz, 1982). 5-HT was shown to have an inhibitory influence on feeding (Beczkowska and Bodnar, 1991). Administration of 5-HT and its agonist norfenfluramine into the medial hypothalamus, or in hypothalamic paraventricular nucleus resulted in a dose-dependent suppression of feeding (Leibowitz and Brown, 1980), and a decrease in meal size, duration of meals and rate of eating was observed. While the latency to meal

onset and the frequency of meals taken was not effected, suggesting that endogenous 5-HT may primarily be responsible for the termination of feeding, rather than the initiation of feeding (Leibowitz and Shor-Posner, 1986). Changed preference for specific macronutrients after central 5-HT administration has also been described.

Experimental evidence supports the role of 5-HT in thermoregulation (Hillegaart, 1991). Increase of 5-HT concentration in the brain, especially in the anterior hypothalamus by peripherally administration of 5-hydroxytryptophan, or centrally injection of 5-HT to cerebral fluid or directly to anterior hypothalamus resulted in hyperthermia. Cooling induced 5-HT release from the anterior hypothalamus (Myers, 1973).

5-HT has also been associated with modulation of immune function. Administration of 5-HT precursor 5-hydroxy-tryptophan resulted in a depressed state of immunity (Devoino and Ilyutchenok, 1968). In contrast, the tryptophan hydroxylase inhibitor, p-chlorophenylalanine, has been found to have a potentiating effect upon T-cell dependent response (Hall and Goldstein, 1985). Destruction of the midbrain raphe nucleus, a 5-HT cell group, was correlated with a increase in immune responsiveness (Eremina and Devoino, 1973). Collectively, these studies suggest that brain 5-HT plays an inhibitory role in immune response.

Blood-Brain Barrier: Anatomy And Physiology.

The brain endothelial cells are structurally and functionally different from endothelial cells in capillaries of other organs. Structurally, the BBB is a modified tight epithelium (Crone, 1986). The nonneural capillary wall is made up of flat endothelial cells, joined by loose junctions, with gaps between cells providing a major route for exchange of most molecules across the wall. However, in the brain continuous tight junctions are present between the endothelial cells. These junctions prevent transcapillary movement of polar molecules varying in size from protein to ions, and there are no transendothelial pathway. Thus, substance exchanges between blood plasma and brain must pass through these membranes and the cytoplasm of the endothelial cell. These two membranes and the interposed cytoplasm constitute the BBB (Oldendorf, 1990). The term BBB implies a general impermeability, which is not true. Within healthy adult brain, there is a continuous BBB, except for small regions in the floor of the third ventricle and the area postrema. These regions have capillaries which structurally resembling nonneural capillaries and may provide sites for brain directly to receive information from circulation (Green, 1989; Oldendorf, 1990). Furthermore, there is active substance exchange cross the BBB (Oldendorf, 1990). Therefore, the BBB is selectively permeable, and it is

selective permeability that functions to control substance exchanges between the brain and circulation and to keep the brain in a special environment required for normal CNS function.

The permeability of the BBB to substances depends upon lipid solubility, polarity, molecular size and existence of specific transport system on the endothelial cells (Crone, 1986; Oldendorf, 1990). Lipid soluble compounds such as ethanol, freely cross the BBB. The BBB is almost impermeable for hydrophilic and polar molecules, such as acetylcholine and catecholamines, thereby isolating the brain from plasma neurotransmitters.

Water, because of its small molecular size, readily enters the brain by diffusion. The half-time of the exchange of brain water varies between 12 and 25 seconds. Glucose, monocarboxylic acids (L-lactate, pyruvate and ketone bodies), and amino acids have low lipid solubility but easily enter the brain. The permeability of BBB to these compounds is mediated by specific transport proteins in the plasma membranes of the endothelial cells comprising the BBB.

Generally, the BBB functioning to maintain a unique and homeostatic environment for brain. Oldendorf (1990) defined the functions of the BBB in following aspects:

1) Exclusion of blood-borne toxic substances. Many foreign substances and endogenous metabolites might find their way into brain extra cellular fluid in much greater concentrations than allowed by the BBB. Most of these will be polar and not a substrate for any of the BBB carrier systems. Just as the renal tubule will not resorb most foreign substances not needed by the body, the BBB carries out a similar role in brain.
2) Protection from systemic neurotransmitters and hormones. The BBB is quite impermeable to neurotransmitters such as NE and 5-HT. Sudden bursts of these substances in plasma will not result in similar bursts in brain extra cellular fluid. Similarly, circulating peptide which could cause perturbations in brain growth and function are excluded.
3) Maintenance of brain electrolyte levels. It is possible that the BBB control of ions to move in or out of the brain. These processes are essential for maintaining substantial ionic gradients exist across the BBB. Some organic anions, such as organic acidic metabolic end products and iodide, are actively pumped out of brain.
4) Modulation of substrate entry by saturable transport system. The BBB has several saturable transport systems which could present the brain with nutrients at a

relatively constant rate in the presence of shifting blood plasma levels.

5) Chemical barrier of endothelial cell enzymes. Endothelial cells contain various active enzyme system. Passing from blood to brain expose substances to endothelial cell cytoplasmic enzymes. Chemical modification of the substances can prevent them from penetrating the outer membrane to affect the brain.

Although the BBB has both a physical (impermeability to hydrophilic substances) and a metabolic (metabolic enzymes in the endothelial cytosol) barriers, which provide an efficient protective system controlling the passage of chemicals from the blood into the cerebral tissue (Minn *et al.* 1991), this barrier can be disturbed by either physical or chemical factors. Perturbation of the BBB due to physical stimulation or chemical exposure has been reported (d'Avella *et al.* 1992; Stewart, *et al.* 1988).

CHAPTER 3

BIOGENIC MONOAMINE CONCENTRATIONS IN DISCRETE BRAIN AREAS OF RATS INTUBATED WITH SINGLE LOW DOSES OF T-2 TOXIN

ABSTRACT

T-2 toxin is a trichothecene mycotoxin which has been shown to affect the CNS. Only recently have attempts been made to characterize the neurochemical perturbations associated with T-2 intoxication. To examine the dose-dependent effect of T-2 toxin on regional brain biogenic monoamines and selected metabolites, male rats were orally dosed with T-2 toxin in corn oil at 0.1, 1.0 or 2.5 mg kg^{-1} BW. At 2, 6 and 10 hrs post dosing, rats were killed, brains were collected and stored at -80° until analyzed. Seven brain nuclei, including nucleus raphe magnus, paraventricular nucleus, locus coeruleus, substantia nigra, medial forebrain bundle, nucleus accumbens and olfactory tubercle, were extracted for neurochemical analysis. T-2 toxin treatment increased 5-hydroxy-3-indoleacetic acid and serotonin throughout the rat brain at 2 hrs post-dosing, and produced a transient increases in norepinephrine in the nucleus raphe magnus and a temporary decrease in the substantia nigra. No regional changes in epinephrine,

dopamine or dihydroxyphenylacetic acid concentration were observed. Few differences were observed between treatment, with the 0.1 mg kg^{-1} T-2 toxin treatment (2% of the LD_{50}), significantly affecting brain monoamines. It had been suggested that neurological manifestations of T-2 toxin are the result of brain hypoxia, however, the altered profile of brain monoamines observed at dosages which do not alter heart function, suggests that T-2 toxin may affect the CNS directly.

INTRODUCTION

Filamentous mold spores are ubiquitous in nature and under suitable environmental conditions in the field or in storage they germinate and fungal growth occurs. During this growth process, toxic secondary metabolites, known collectively as mycotoxins, are synthesized and these natural fungal products have the potential to disrupt protein and nucleic acid metabolism in man and animals (Mills, 1990).

The trichothecenes are a group of chemically related mycotoxins produced by Fusarium mold species endemic to North American agricultural products (Abramson *et al.* 1987). Trichothecenes can be potent, with T-2 toxin (3α-hydroxy-4ß,15-diacetoxy-8α-(3-methylbutyrloxy)-12,13-epoxy trichothec-9-ene) being one of the most toxic (Ueno, 1984). Ingestion of T-2 reduces food intake, irritates cutaneous

and mucous membranes, and is pathogenic in numerous tissues (Kravchenko *et al.* 1986; Ueno, 1984; Yarom and Yagen, 1986), disrupts hemopoiesis and the functional integrity of the cardiovascular, digestive and immunological systems (Fairhurst *et al. 1987;* Friend *et al.* 1983; Yarom and Yagen, 1986). Acute T-2 toxicosis is characterized by hemorrhages, sepsis and cardiopulmonary failure (Kravchenko *et al.* 1986; Ueno, 1984; Yarom *et al.* 1983). The effects of T-2 toxin on the CNS have received limited attention (Carson and Smith, 1983; Chi *et al.* 1977; Martin *et al.* 1986; Wyatt *et al.* 1973). Dietary T-2 produces abnormal positioning of the wing, hysteroid seizures, and impaired righting reflex in young chickens, with the incidence of neural symptoms dependent on the length of exposure to T-2 toxin and its dietary concentration (Wyatt *et al.* 1973). Acutely dosed birds became inactive, inappetent and developed diarrhea, panting and coma (Chi *et al.* 1977). In the rat, dietary T-2 depresses feed consumption and body weight (Carson and Smith, 1983). Acutely dosed rats became subdued with a hunched posture, exhibiting sluggishness with skeletal-motor weakness and ataxia of the hind limbs (Fairhurst *et al.* 1987). All discernable signs of nervous system were depressant in nature (Fairhurst *et al.* 1987). Signs of neural intoxication and pathology are observed when

trichothecenes are centrally administered as brain implants (Bergmann *et al.* 1988) and when rats were exposed at intraperitoneal dosages approaching the LD_{50} for T-2 toxin (Ballough *et al.* 1989).

Only recently have attempts been made to characterize the neurochemical mechanisms or sites underlying these neurobehavioral, and pathophysiological symptoms (Boyd *et al.* 1988; Cavan *et al.* 1988; Chi *et al.* 1980; Fitzpatrick *et al.* 1988b; MacDonald *et al.* 1988; Weekley *et al.* 1989). For example, in chickens T-2 toxin increases whole brain DA, while reducing NE (Chi *et al.* 1980). In rats, MacDonald and coworkers (1988) observed that acute ingestion of T-2 toxin produced an initial elevation in whole brain levels of the amino acid tryptophan and 5-HT, followed by elevations in whole brain DA. Weekly *et al* (1989) reported that intraperitoneal T-2 toxin administration elevated cerebral and brainstem tryptophan concentrations, but reduced 5-HT in rats. However, Fitzpatrick and collaborators (1988b) observed that the oral administration of T-2 toxin or the trichothecene DON, increased the concentrations of 5-HT and its metabolite 5-HIAA, but had minimal effect on NE and DA concentrations in the five brain regions analyzed in rats. In young chickens, however, T-2 toxin and DON increased regional 5-HIAA without altering 5-HT, and decreased regional NE and DA concentrations. These observations

suggest that the trichothecenes' ability to influence biogenic monoamine metabolism may not be species specific.

Therefore, this study was designed to characterize, in more neurochemical detail the dose related effects of T-2 toxicity on regional metabolism of selected brain monoamines and their metabolites using a micropunch technique for sampling discrete brain areas.

MATERIALS AND METHODS

Animals: Sprague-Dawley male rats weighing 140-160 g were obtained from University of Manitoba breeding facility in two groups of 60 animals. All animals were housed separately in galvanized steel cages and kept on a 14-10 hours light-dark cycle. The room temperature was maintained at 21 ± 1°C with a relative humidity of 50%. Animals were given a 5 day acclimatization period during which they were fed a semi-purified diet and water ad libitum. Use of experimental animals conformed to the guidelines of the Canadian Council on Animal Care.

Treatment: Following a 5 day adaption period each rat was assigned, in a counter balanced order, to receive one of the four doses of T-2 toxin (corn oil vehicle, 0.1, 1.0 or 2.5 mg T-2 toxin kg^{-1} BW) and, for each dose , assigned to one of three sampling intervals (2, 6 or 10 hrs). On the day of the experiment diet was removed at 0600 hr for all

rats. Later, at 1000, 1400 or 1800 hr the single dose of T-2 toxin was administered through esophageal intubation. T-2 toxin (Myco-Lab Co., Chesterfield, Missouri) was dissolved in 0.5 ml corn oil and intubated into the stomach using a plastic infant feeding tube attached to a syringe. Following intubation, at 2000 hr, the rats were sacrificed by decapitation, producing the three (2, 6 and 10 hr) sampling intervals. Accordingly, the depravation time interpolated between the removal of the diet and sacrifice was constant (14 hrs) for rats assigned to every dose x sampling interval condition.

Tissue Dissection and Preparation: Rats were killed by decapitation at 2000 h, which resulted in sample intervals of 2, 6 or 10 hrs. The brains were then immediately extracted and frozen in liquid nitrogen and stored at -80°C until dissection. All tissue samples were analyzed within 60 days. The seven brain regions selected for neurochemical analysis were the nucleus raphe magnus (NRM), paraventricular nucleus of the hypothalamus (PVN), locus coeruleus (LC), substantia nigra (SN), medial forebrain bundle (MFB) at the level of the hypothalamus, nucleus accumbens (NA) and olfactory tubercle (OT). These regions constitute main cell groups or fibre projection systems for NE, DA or 5-HT (Biorklund *et al.* 1984).

Brains were transferred a -20°C freezer 20 min prior to sectioning. A temperature controlled microtome (Mino, IEC Equipment Ltd., Needham Heights, Massachusetts) set at -8°C, was used to slice the tissue. Brain were sliced into 0.5 mm sections and placed on glass slides. When the sectioning approached regions containing the specific nuclei, 20 μm sections were cut and brain slices were temporarily stored at -20°C prior to dissecting the nuclei. The procedure for tissue microdissection, extraction and preparation for analysis follows that described by Palkovits and Brownstein (1988). Visual identification of the relevant nuclei and pathways was done under a dissecting microscope. Dissection was performed with stainless steel trochars, 0.5-1.5 mm diameters or a scalpel depending on the region being dissected. Brain slices were held on a cold plate (Flexi-cool, FTS Systems Inc., Stone Ridge, New York) precooled to -10°C during dissection. A trocar with a diameter smaller than nucleus, was used to isolate the nucleus. The pellets punched from the brain sections were placed into 1.5 ml centrifuge tubes that contained 0.5 ml of 0.1 N perchloric acid buffer solution with 50 μM EDTA (Mallinckrodt Inc., Paris, Kentucky). Sample preparations were performed at 1-4°C. The tissue pellets were homogenized with a micropestle. Homogenate was centrifuged at 16,000 x g for 30 min (Eppendorf Microcentrifuge 5414, Brinkmann, Westbury,

New York) and the supernatant filtered through 0.45 μm nylon filter (MSI, Westboro, Massachusetts). The supernatant was analyzed for NE, EP, DA, DOPAC, 5-HT and 5-HIAA. The supernatant of OT and AN were diluted 5 times using 0.1 N perchloric acid buffer prior to analysis. Protein concentrations of tissue homogenate were determined according to Lowry *et al.* (1951) and the concentrations of monoamines were expressed as ng mg^{-1} protein.

HPLC Analysis: Catechol standards and 3,4-dihydroxybenzylamine hydrobromide, the internal standard, were obtained from Sigma Chemical Co. (St. Louis, Missouri). Solvents were HPLC grade; other chemicals were reagent grade and were obtained from Fisher Scientific (Ottawa, Ontario). High-performance liquid chromatography was performed using a Beckman Model 116M Solvent Delivery Module liquid chromatograph (Boyd *et al.* 1988). The analytical column was an Ultrasphere IP, C_{18} column (250 x 4.6 mm ID, 5 μm particle size) (Beckman Toronto, Ontario). A precolumn was used to protect the column. Electrochemical detection was accomplished using an EAS Coulochem detector, model 5100A (Bedford, Massachusetts). The catechols were oxidized and reduced at the applied potentials of +0.25, +0.1 and −0.45 v (60 x 10) using a porous graphite electrode.

The mobile phase was a modification of the buffer used by Martin *et al* (1983) consisting of 75 mM sodium phosphate

and 1.064 mM of octane sulphonate (Eastman Kodak Co., Rochester, New York) as an ion-pair reagent, 50 μM EDTA and 11.5% acetonitrile (Mallinckrodt Inc., Paris, Kentucky). The mobile solution was adjusted to a final pH of 3.25 using phosphoric acid. The flow rate was maintained at 1.0 ml min^{-1}.

Statistical Analysis: For each of the sampled brain area, monoamine and metabolite concentrations were analyzed with a 4 x 3 (Dose x Sampling Interval) factorial analyses of variance (ANOVAs). Linear contrasts (SAS Institute INC.) yielded comparisons on main effects, interactions, or a priori hypotheses. An overall alpha level of $p \leq 0.05$ was set for statistical comparisons.

RESULTS

T-2 toxin affected 5-HT concentrations in six of seven brain regions examined (Table 3-1). Toxin intubated rats exhibited greater overall mean 5-HT concentrations in the NRM, LC, MFB and NA regions than did vehicle treated controls. This effect was transient. That is, 5-HT concentrations were greater in T-2 treated animals than controls 2 and 6 hrs post dosing, with significant differences observed for NRM, LC, SN and NA 2 hrs post dosing, MFB and PVN 6 hrs post dosing, however, no differences observed at the 10 hr sampling interval.

Table 3-1. 5-HT concentrations in discrete brain regions of T-2 toxin intubated rats

Region of Brain	Sampling Interval (hr)	Vehicle Control	T-2 toxin treatment (mg kg^{-1} bw) 0.1	1.0	2.5	Treatment Means#
NRM	2	5.9 ± 0.6^{a}	6.5 ± 0.4^{ab}	8.3 ± 0.4^{c}	7.8 ± 0.9^{bc}	7.5*
	6	5.7 ± 0.5^{a}	7.3 ± 0.5^{b}	6.6 ± 0.4^{ab}	6.6 ± 0.7^{ab}	6.8
	10	5.9 ± 0.4	7.2 ± 0.6	6.4 ± 0.5	6.4 ± 0.4	6.7
	Means	5.8^{a}	7.0^{b}	7.1^{b}	6.9^{b}	
LC	2	8.2 ± 0.4^{a}	8.7 ± 0.6^{ab}	10.5 ± 0.4^{b}	10.2 ± 1.3^{b}	9.8*
	6	7.9 ± 0.3	8.6 ± 0.7	7.8 ± 0.6	9.2 ± 0.6	8.5
	10	7.2 ± 0.5	8.7 ± 0.8	8.0 ± 0.8	8.1 ± 0.5	8.3
	Means	7.8^{a}	8.7^{ab}	8.8^{ab}	9.2^{b}	
SN	2	$12.8 \pm 0.6^{a}_{I\dagger}$	14.3 ± 0.6^{ab}	15.9 ± 0.8^{b}	14.0 ± 0.4^{ab}	14.7*
	6	$13.7 \pm 0.7_{I,II}$	14.5 ± 0.6	13.5 ± 0.9	13.9 ± 0.5	14.0
	10	$15.1 \pm 1.0^{a}_{II}$	15.2 ± 1.0^{ab}	13.0 ± 0.6^{abc}	12.7 ± 0.7^{c}	13.6
	Means	13.9	14.7	14.1	13.5	
MFB	2	8.6 ± 0.6^{a}	8.8 ± 0.7^{a}	10.6 ± 0.6^{b}	9.6 ± 0.6^{ab}	9.7
	6	7.6 ± 0.6^{a}	9.6 ± 0.5^{b}	8.8 ± 0.5^{ab}	9.3 ± 0.7^{b}	9.2*
	10	8.5 ± 0.4	9.7 ± 0.6	9.1 ± 0.4	8.4 ± 0.3	9.1
	Means	8.2^{a}	9.4^{b}	9.5^{b}	9.1^{ab}	
PVN	2	$9.2 \pm 0.8^{a}_{I}$	9.5 ± 0.8^{a}	12.4 ± 0.9^{b}	10.6 ± 0.8^{ab}	10.8
	6	$9.0 \pm 0.9^{a}_{I,II}$	11.6 ± 0.7^{b}	9.7 ± 0.6^{ab}	12.3 ± 1.1^{b}	11.2*
	10	$11.2 \pm 0.4_{II}$	10.4 ± 0.8	9.8 ± 0.5	9.4 ± 0.7	9.9
	Means	9.8	10.5	10.6	10.8	
NA	2	3.4 ± 0.2^{a}	4.0 ± 0.4^{a}	5.0 ± 0.4^{b}	4.3 ± 0.5^{ab}	4.4*
	6	4.3 ± 0.4	4.8 ± 0.5	5.4 ± 0.6	4.1 ± 0.2	4.8
	10	4.0 ± 0.3	4.2 ± 0.3	3.8 ± 0.3	4.8 ± 0.3	4.2
	Means	4.0^{a}	4.3^{ab}	4.8^{b}	4.4^{ab}	
OT	2	9.3 ± 0.4	8.8 ± 0.7	9.6 ± 0.6	9.5 ± 0.7	9.3
	6	8.2 ± 0.5	8.1 ± 0.3	9.1 ± 0.7	9.2 ± 0.4	8.8
	10	9.0 ± 0.6	9.2 ± 0.6	8.0 ± 0.5	8.8 ± 0.4	8.7
	Means	8.8	8.7	8.9	9.2	

Values are expressed in ng mg^{-1} protein and represents the mean ± MSE of eight animals.

Means within row designated with different letter superscript are significantly different, $p < 0.05$.

\# Represents the mean of 24 animals.

* Mean treatment value significantly different from vehicle control value, $p < 0.05$.

† Means control values with different numerical subscript are significantly different, $P < 0.05$.

Differences in 5-HT concentrations were observed between T-2 toxin treatment groups. In general, 5-HT concentrations were greater at the 2 hr sample interval in animals treated with 1.0 or 2.5 mg T-2 toxin kg^{-1} bw, than rats treated with the lower dosage.

Relative to the vehicle treated controls, T-2 toxin treated animals displayed several instances where greater mean concentrations of 5-HIAA in the NRM, MFB and PVN occurred (Table 3-2). 5-HIAA concentrations in T-2 treated rats were significantly greater in the NRM and MFB 2 hrs post dosing, while SN 5-HIAA levels appeared to decrease 6 and 10 when compared to control values. Few differences were observed between treatment groups, and no changes 5-HIAA concentrations were observed in the AN or OT.

With the exception of the LC regional DA concentrations for the 6 hr sampling interval, T-2 toxin treatment did not affect DA (Table 3-3). Regional DOPAC concentrations were affected by T-2 toxin treatment, with increased DOPAC concentrations observed in the LC, MFB and PVN, and decreased DOPAC in the OT 6 and 10 hrs post dosing (Table 3-4).

Norepinephrine concentrations in the NRM, LC and MFB were affected by T-2 toxin, with NE concentrations greater in toxin treated animals in the NRM and LC at 2 and 6 hrs post dosing, respectively, while for the MFB, mean treatment

Table 3-2. 5-HIAA concentrations in discrete brain regions of T-2 toxin intubated rat

Region of Brain	Sampling Interval (hr)	Vehicle Control	T-2 toxin treatment (mg kg^{-1} bw) 0.1	1.0	2.5	Treatment Means#
NRM	2	9.1 ± 0.5[a]	10.0 ± 0.7[ab]	12.1 ± 0.9[b]	10.0 ± 0.5[ab]	10.7*
	6	9.5 ± 0.5	11.5 ± 0.6	9.5 ± 0.5	10.1 ± 0.5	10.4
	10	9.2 ± 0.5	10.0 ± 0.7	10.2 ± 0.7	11.5 ± 1.1	10.6
	Means	9.4[a]	10.5[ab]	11.2[b]	11.2[b]	
LC	2	8.2 ± 0.7	9.7 ± 0.4	9.0 ± 0.6	8.1 ± 0.9	8.9
	6	8.7 ± 0.6	8.1 ± 0.5	7.5 ± 0.6	7.5 ± 0.3	7.7
	10	7.0 ± 0.6[a]	7.5 ± 0.7[ab]	8.3 ± 0.8[ab]	9.2 ± 0.8[b]	8.3
	Means	8.0	8.4	7.8	8.3	
SN	2	7.4 ± 0.4	8.0 ± 0.4	8.5 ± 0.3	7.2 ± 0.5	7.9
	6	8.4 ± 0.6[a]	7.9 ± 0.3[ab]	7.0 ± 0.5[b]	8.1 ± 0.2[ab]	7.7
	10	8.3 ± 0.4[a]	8.6 ± 0.3[a]	8.1 ± 0.6[ab]	7.1 ± 0.2[b]	7.9
	Means	8.0	8.2	7.9	7.5	
MFB	2	5.8 ± 0.8[a]	5.7 ± 0.7[a]	6.3 ± 0.7[ab]	7.0 ± 1.2[b]	6.3
	6	6.2 ± 1.1	6.1 ± 0.7	6.8 ± 0.8	6.4 ± 0.5	6.4
	10	5.8 ± 0.9	6.3 ± 0.7	6.5 ± 1.1	6.1 ± 1.3	6.3
	Means	5.9[a]	6.0[ab]	6.5[b]	6.5[b]	
PVN	2	6.4 ± 0.5	8.5 ± 1.4	6.9 ± 0.5	8.0 ± 0.7	7.8
	6	6.2 ± 0.5	6.4 ± 0.5	7.3 ± 1.1	7.4 ± 0.8	7.0
	10	6.8 ± 0.5	6.3 ± 0.6	7.6 ± 0.5	8.1 ± 1.0	7.3
	Means	6.5[a]	7.1[ab]	7.3[ab]	7.8[b]	
AN	2	4.2 ± 0.5	4.6 ± 0.3	5.0 ± 0.6	4.5 ± 0.2	4.7
	6	5.0 ± 0.4	4.2 ± 0.2	4.8 ± 0.6	5.0 ± 0.4	4.7
	10	4.9 ± 0.4	4.8 ± 0.4	5.0 ± 0.3	6.0 ± 0.7	5.3
	Means	4.7	4.5	4.9	5.2	
OT	2	3.1 ± 0.3	2.9 ± 0.3	2.8 ± 0.1	2.7 ± 0.1	2.8
	6	2.4 ± 0.3	2.5 ± 0.3	2.4 ± 0.3	2.9 ± 0.2	2.6
	10	3.6 ± 0.2	2.4 ± 0.3	2.5 ± 0.4	2.8 ± 0.3	2.6
	Means	3.0	2.6	2.6	2.8	

Values are expressed in ng mg^{-1} protein and represents the mean ± MSE of eight animals.

Means within row designated with different letter superscript are significantly different, $p < 0.05$.

\# Represents the mean of 24 animals.

* Mean treatment value significantly different from vehicle control value, $p < 0.05$.

Table 3-3. DA concentrations in discrete brain regions of T-2 toxin intubated rats

Region of Brain	Sampling Interval (hr)	Vehicle Control	T-2 toxin treatment (mg kg^{-1} bw) 0.1	1.0	2.5	Treatment Means#
NRM	2	0.40 ± 0.04	0.52 ± 0.04	0.56 ± 0.04	0.63 ± 0.08	0.57
	6	0.55 ± 0.08	0.64 ± 0.07	0.61 ± 0.07	0.72 ± 0.11	0.66
	10	0.65 ± 0.27	0.71 ± 0.10	0.47 ± 0.03	0.45 ± 0.04	0.54
	Means	0.55	0.62	0.56	0.60	
LC	2	1.3 ± 0.2	1.7 ± 0.2	1.8 ± 0.3	1.8 ± 0.4	1.8
	6	1.2 ± 0.2[a]	2.0 ± 0.4[ab]	2.1 ± 0.5[b]	1.7 ± 0.2[ab]	1.9*
	10	1.5 ± 0.2	1.4 ± 0.3	1.4 ± 0.2	1.5 ± 0.3	1.4
	Means	1.4	1.7	1.8	1.7	
SN	2	11.4 ± 1.2	11.1 ± 1.2	11.1 ± 0.7	12.2 ± 0.9	11.5
	6	12.8 ± 1.7	10.5 ± 1.0	11.4 ± 0.9	12.4 ± 1.2	11.4
	10	11.3 ± 0.5	13.0 ± 0.9	10.5 ± 1.0	9.8 ± 0.8	11.1
	Means	11.8	11.5	11.0	11.5	
MFB	2	2.1 ± 0.3	1.8 ± 0.1	2.0 ± 0.2	2.3 ± 0.2	2.1
	6	1.9 ± 0.2	2.4 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.3
	10	1.9 ± 0.2	2.1 ± 0.2	1.9 ± 0.1	2.2 ± 0.3	2.1
	Means	2.0	2.1	2.1	2.2	
PVN	2	7.8 ± 0.7	8.8 ± 0.9	9.3 ± 0.9	8.6 ± 1.0	8.9
	6	7.4 ± 0.9	9.0 ± 0.9	9.8 ± 1.6	9.6 ± 1.3	9.5
	10	8.1 ± 0.7	9.1 ± 0.9	6.8 ± 0.7	8.7 ± 1.4	8.2
	Means	7.8	9.0	8.8	9.0	
NA	2	64.7 ± 2.3	64.0 ± 4.7	76.3 ± 4.2	67.5 ± 6.6	69.3
	6	73.4 ± 6.4	70.8 ± 5.6	68.2 ± 5.7	73.7 ± 6.1	70.9
	10	66.7 ± 3.4	71.9 ± 6.2	62.8 ± 3.6	67.9 ± 3.0	67.5
	Means	68.3	68.9	69.1	69.7	
OT	2	74.4 ± 3.0	72.2 ± 4.4	77.4 ± 5.4	77.1 ± 4.7	75.6
	6	80.8 ± 2.4	78.2 ± 3.5	73.1 ± 4.7	77.6 ± 6.2	76.3
	10	75.6 ± 3.5	79.5 ± 4.5	70.3 ± 4.5	70.2 ± 4.3	73.3
	Means	76.9	76.6	73.6	75.0	

Values are expressed in ng mg^{-1} protein and represents the mean ± MSE of eight animals.

Means within row designated with different letter superscript are significantly different, $p < 0.05$.

\# Represents the mean of 24 animals.

* Mean treatment value significantly different from vehicle control value, $p < 0.05$.

Table 3-4. DOPAC concentrations in discrete brain regions of T-2 toxin intubated rats

Region of Brain	Sampling Interval (hr)	Vehicle Control	T-2 toxin treatment (mg kg^{-1} bw) 0.1	1.0	2.5	Treatment Means#
LC	2	2.7 ± 0.6	2.6 ± 0.5	3.4 ± 0.4	2.8 ± 0.7	2.9
	6	1.7 ± 0.2^{a}	2.9 ± 0.6^{ab}	2.6 ± 0.2^{ab}	3.2 ± 0.5^{b}	2.9*
	10	1.9 ± 0.4	2.3 ± 0.4	2.4 ± 0.6	2.4 ± 0.5	2.4
	Means	2.1	2.6	2.8	2.8	
SN	2	2.9 ± 0.3	3.0 ± 0.4	3.0 ± 0.2	3.0 ± 0.1	3.0
	6	2.8 ± 0.3	2.9 ± 0.2	2.9 ± 0.2	3.0 ± 0.3	2.9
	10	2.9 ± 0.2	3.2 ± 0.3	2.6 ± 0.3	2.3 ± 0.2	2.7
	Means	2.9	3.0	2.8	2.8	
MFB	2	0.8 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6
	6	0.6 ± 0.1^{a}	1.4 ± 0.4^{b}	0.8 ± 0.1^{a}	0.7 ± 0.1^{a}	1.0*
	10	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6
	Means	0.6^{a}	0.9^{b}	0.7^{ab}	0.6^{a}	
PVN	2	2.0 ± 0.7	2.0 ± 0.6	2.3 ± 0.6	1.6 ± 0.3	2.0
	6	2.0 ± 0.3^{a}	1.8 ± 0.2^{a}	2.0 ± 0.5^{a}	3.9 ± 1.0^{b}	2.6
	10	1.8 ± 0.3^{a}	3.0 ± 1.0^{b}	3.1 ± 0.8^{b}	3.2 ± 1.0^{b}	3.1*
	Means	2.0	2.2	2.5	2.9	
NA	2	19.2 ± 1.6	16.7 ± 1.3	20.4 ± 1.4	17.7 ± 1.6	18.3
	6	18.9 ± 1.5	18.9 ± 1.3	17.7 ± 1.2	19.4 ± 1.5	18.7
	10	19.0 ± 1.6	19.1 ± 1.6	17.6 ± 1.3	17.1 ± 1.2	17.9
	Means	19.0	18.2	18.6	18.1	
OT	2	10.7 ± 0.4 $_{I}$†	9.9 ± 0.9	11.3 ± 0.9	10.9 ± 1.0	10.7
	6	13.3 ± 1.6^{a} $_{I}$	11.2 ± 0.8^{ab}	10.5 ± 1.0^{b}	11.3 ± 0.6^{ab}	11.0*
	10	12.3 ± 1.0 $_{I,II}$	10.9 ± 1.0	9.9 ± 0.6	10.5 ± 0.5	10.4
	Means	12.1^{a}	10.7^{ab}	10.6^{b}	10.9^{ab}	

Values are expressed in ng mg^{-1} protein and represents the mean ± MSE of eight animals.

Means within row designated with different letter superscript are significantly different, $p < 0.05$.

Represents the mean of 24 animals.

* Mean treatment value significantly different from vehicle control value, $p < 0.05$.

† Means control values with different numerical subscript are significantly different, $P < 0.05$.

NE concentrations were greater that control values (Table 3-5). SN NE concentrations were lower in T-2 toxin treated rats than vehicle treated controls, however, the decrease in NE observed at the 2 hr sampling interval was not detected at the 6 and 10 hrs post dosing. None of the T-2 toxin doses significantly influenced NE concentrations in the PVN, NA and OT nuclei. No differences in EP level in the PVN or MFB (Table 3-6), and the EP concentration was insufficient for quantitative analysis in the other nuclei examined.

DISCUSSION

These results indicate that a single intubation of T-2 toxin disrupts the metabolism of the indoleamines, increasing 5-HIAA and 5-HT concentrations increased throughout the rat brain. A transient increase in NE in the NRM and SN, but no alteration in DA or DOPAC concentrations was observed, suggesting that in rats the dopaminergic and noradrenergic systems appear less sensitive to the effects of T-2 toxin than the indoleamine system. These data are consistent with previous reports (Boyd *et al.* 1988; Fitzpatrick *et al.* 1988b), that single oral doses of 2.5 mg kg^{-1} BW of T-2 toxin or DON resulted in increased 5-HIAA and 5-HT in the pons and medulla, cerebellum, hypothalamus, hippocampus and cerebral cortex, but produces few effects on the catecholamine systems. Our observation that T-2 treatments of 0.1 mg kg^{-1} BW affect brain monoamine

Table 3-6. EP concentrations in discrete brain regions of T-2 toxin intubated rats

Region of Brain	Sampling Interval (hr)	Vehicle Control	T-2 toxin treatment (mg kg^{-1} bw) 0.1	1.0	2.5	Treatment Means
PVN	2	1.86 ± 0.48	1.78 ± 0.54	1.82 ± 0.64	1.95 ± 0.61	1.85
	6	1.72 ± 0.70	1.57 ± 0.84	1.26 ± 0.39	1.32 ± 0.81	1.38
	10	1.50 ± 0.50	1.30 ± 0.27	1.24 ± 0.52	0.09 ± 0.22	0.88
	Means	1.69	1.55	1.44	1.12	
MFB	2	0.49 ± 0.09	0.42 ± 0.07	0.43 ± 0.11	0.42 ± 0.08	0.42
	6	0.36 ± 0.10	0.40 ± 0.09	0.28 ± 0.07	0.36 ± 0.09	0.35
	10	0.34 ± 0.06	0.36 ± 0.08	0.27 ± 0.08	0.15 ± 0.05	0.26
	Means	0.39	0.39	0.33	0.31	

Means within row designated with different letter superscript are significantly different, $p < 0.05$.

\# Represents the mean of 24 animals.

demonstrates that these effects can be obtained at considerably lower doses.

The effect of T-2 toxin on brain monoamines and the resulting neurochemical imbalance, may contribute to the physiological and behavioral manifestation of trichothecene intoxication. For example, the regulation of ingestive behavior depends, in part, upon the reciprocal relationship between PVN 5-HT and NE, an increase in NE stimulating feeding behavior, while increases in 5-HT suppressing feeding (Leibowitz, 1980).

Satiation is modulated by both central and peripheral mechanisms (Carruba *et al*. 1986). NRM, a large caudal serotonergic cell group, projects down the spinal column influencing the pregangleonic activation of the sympathetic nervous system (Baum and Shrophire, 1975). Accordingly, disrupted NRM 5-HT metabolism may influence food intake by altering hormone secretion, peristaltic contractions or thermal energetics. Therefore, increased 5-HT in the PVN and the NRM observed in T-2 toxin treated rats provides a neurochemical explanation for decreased feed consumption associated with trichothecene intoxication (Ueno, 1984).

The MFB provides a pathway for the fibers from dorsal and medial raphes, two serotonin-containing nuclei located in the midbrain and projecting to most areas of the forebrain (Steinbusch, 1984). Therefore, a 5-HT increase in

MFB may be amplified through their effect on other brain regions to alter peripheral organ function and account for the behavioral disturbances observed in T-2 toxin treated animals.

In rats the neurochemical effects of trichothecenes, including T-2, have been related to cardiac insufficiency induced systemic hypoxia (Ballough *et al.* 1989; Martin *et al.* 1986a,b). For example, Ballough *et al* (1989) observed a dose-dependent decrease in systolic blood pressure. However the lower dosages of T-2 toxin (0.45 and 0.68 mg kg^{-1} BW i.p.) which produced only moderate hypotension, was as effective or more effective in inducing a supraoptic-magnocellular cytopathogenesis than the highest dosages (1.35 mg kg^{-1} BW by i.p.). The effects observed in the present study were obtained at dosages as low as 0.1 mg kg^{-1} BW (2% of the LD_{50} value) (Ueno, 1984), a dosage far too modest to alter myocardial function (Yarom *et al.* 1983; Yarom and Yagen 1986). Additionally, the observation that DON, a trichothecene with negligible cardiovascular effects, but alters brain monoamine metabolism, suggests a primary central site of trichothecene action (Fitzpatrick *et al.* 1988b). Collectively, these observations strongly suggest that the trichothecenes effect on the CNS is not hemodynamically mediated.

The fact that trichothecene mycotoxins are potent inhibitors of protein and nucleic acid synthesis (Ueno, 1984), suggests that DON and T-2 may influence brain biogenic monoamine concentration by directly inhibiting protein synthesis (Chi *et al.* 1980). Therefore, increased concentration of 5-HT in animals dosed with DON or T-2 toxin may reflect an effect of these mycotoxins on the synthesis of MAO, the enzyme required for the metabolic breakdown of 5-HT. However, the observed increase in 5-HIAA and the lack of effect on the catecholamine transmitters which are also inactivated by MAO (Fernstrom, 1990), does not support the suggestion that trichothecene toxicity is due to a direct inhibition of protein synthesis (Chi *et al.* 1980).

T-2 toxin is pathogenic to the vascular system, causing dilation and swelling of microvessels, damage to the plasma membrane, and tearing of the blood vessel wall (Yarom and Yagen, 1986). Therefore, T-2 toxin could compromise the endothelial cells in the capillaries of the blood brain barrier and alter amino acid transport into the brain. Increased concentrations of tryptophan (Cavan *et al.* 1988; Weekley *et al.* 1989) and tyrosine (Weekley *et al.* 1989) have been observed in the brain of T-2 toxin treated rats.

Dopamine and NE share precursors and biosynthetic pathway (Nagatsu, 1973). Tyrosine hydroxylase, the rate-limiting enzyme for catecholamine biosynthesis, has multiple

controls on its activity, including end-product inhibition and is generally insensitive to the influence of tyrosine concentration (Gessa, 1974; Fernstrom, 1990). However, kinetic studies indicate that tryptophan hydroxylase, the rate limiting enzyme in 5-HT synthesis, is not saturated at a physiological concentration of tryptophan (Fernstrom, 1990). Accordingly, 5-HT synthesis depends on the brain precursor concentration, while DA and NE biosynthesis are not responsive to precursor concentrations. Therefore, increased brain 5-HT and 5-HIAA may be a reflection of increased concentration of precursors, and explain the observation that trichothecenes affect the indoleamines, while the dopaminergic and catecholamine systems are less sensitive to the effects of T-2 toxin (Boyd *et al.* 1988; Fitzpatrick *et al.* 1988b).

CHAPTER 4

EFFECT OF DIETARY T-2 TOXIN ON BIOGENIC MONOAMINES IN DISCRETE AREAS OF RAT BRAIN

ABSTRACT

Physiological manifestations of T-2 toxicosis may be explained, in part, by the effect of trichothecenes on the CNS. Acute T-2 toxin treatments alter brain biogenic monoamine concentrations, however, these perturbations have not been well documented or demonstrated in feeding trials. To examine the effect of dietary T-2 toxin on regional brain biogenic monoamines and their metabolites, male rats (175 g) were fed a semi-synthetic diet containing 0, 2.5 or 10 ppm T-2 toxin for either 7 or 14 days. Whole brains were collected, stored at -80°C until sectioning and micropunching, and four brain nuclei were analyzed by high performance liquid chromatography, using electrochemical detection. A reduction in feed consumption, body weight gain and feed efficiency was observed. These effects were transient and tissue necrosis was not observed, therefore, the notion that trichothecene induced feed refusal was due to irritation of the oral cavity was not supported. T-2 toxin affected brain biogenic monoamine concentrations. Nucleus raphe magnus' 5-HT, 5-HIAA and NE increased in a

dose dependent manner, and a transient increase in DA was observed. In the SN, animals fed 10 ppm T-2 had increased EP after 7 days, and decreased NE after 14 days, when compared to controls. PVN and MFB bundle dihydroxy-phenylacetic acid concentrations were lower in T-2 toxin treated rats than control animals. The observed effects of T-2 toxin on brain monoamines and the resulting neurochemical imbalance may account for the physiological manifestation of trichothecene intoxication.

INTRODUCTION

T-2 toxin is an naturally occurring trichothecene mycotoxin synthesized by various *Fusarium* molds (Ueno, 1986). T-2 toxin is endemic to western Canada (Abramson *et al.* 1987; Mills, 1990; Puls and Greenway, 1976). It is one of the most potent trichothecenes. The ingestion of T-2 toxin contaminated grain and grain products results in serious mycotoxicosis in man and animals (Bhat *et al.* 1989; Puls and Greenway, 1976).

T-2 toxicosis is a multisystem disorder (Khachatourians, 1990; Ueno, 1986). Ingestion of T-2 reduces food intake, irritates cutaneous and mucous membranes, and is pathogenic in numerous tissues (Kravchenko *et al.* 1986). T-2 toxin interfere with hemopoiesis and disrupts the functional integrity of the cardiovascular, digestive and immunological systems (Fairhurst *et al.* 1987;

Friend *et al.* 1983). Acute T-2 toxicosis is characterized by hemorrhages, sepsis and cardiopulmonary failure (Kravchenko *et al.* 1986; Yarom *et al.* 1983; Ueno, 1986). Neurological dysfunction, including impaired motor, sensory, and autonomic nervous system functioning (Bergmann *et al.* 1988; Lorenzana *et al.* 1985; Wyatt *et al.* 1973) as well as changed feeding behavior (Wellman *et al.* 1989) are manifestations of trichothecene intoxication.

The effects of trichothecenes on the CNS have received limited attention and while T-2 toxin alters neurotransmitter concentrations in chicks, rats and swine, the mechanism of trichothecene action is still unknown (Prelusky *et al.* 1992; also see chapter 3). In poultry, Chi and coworkers (1981) reported that with T-2 toxin intubation whole brain catecholamines were affected, with DA concentration significantly increased, whereas brain NE was reduced. In subsequent studies, intubation of T-2 toxin or the DON, a trichothecene which affects the CNS (Huff *et al.* 1981), did not affect whole brain concentrations of monoamines, however, when brains were dissected into five brain regions, increased 5-HT, 5-HIAA and decreased regional NE and DA concentrations were observed (Boyd *et al.* 1988; Fitzpatrick *et al.* 1988b). In rats, MacDonald *et al.* (1988) observed that intubation of T-2 toxin caused an initial increases in whole brain tryptophan and 5-HT, followed by an

increase in DA. Weekley and coworkers (1989) reported that intraperitoneal T-2 toxin administration increased cerebral and brain stem tryptophan, but reduced 5-HT in rats. However, we observed that T-2 toxin or DON intubation elevated regional concentrations of the indoleamines, 5-HT and 5-HIAA in all brain regions examined, whereas NE and DA levels were not significantly altered (Boyd *et al.* 1988; Fitzpatrick *et al.* 1988b). Similar results were observed when a micropunch technique was used to isolate discrete brain areas of rats intubated with T-2 toxin (chapter 3). In swine, acute intravenous administration of DON elevated NE and depressed DA concentrations in the hypothalamus, cerebellum and frontal cortex, but had few effects on the indoleamines (Prelusky *et al.* 1992). Prelusky suggested that changes in brain monoamine concentration in trichothecene treated animals, depends upon the toxin and species involved. However, given the limited number of experiments conducted, the plethora of experimental designs and analytical techniques employed, the lack of consensus on the central effects of trichothecenes is understandable.

Therefore, this study was designed to characterize, in more neurochemical detail the effects of dietary T-2 toxin on regional metabolism of selected brain monoamines and their metabolites using a micropunch technique for sampling discrete brain areas.

MATERIALS AND METHODS

Animals and maintenance: Sixty Sprague-Dawley male rats weighing 160-185 g were purchased from the University of Manitoba central breeding facility. All animals were hosed separately in galvanized steel cages and kept on a 14-10 hours light-dark cycle. The room temperature was maintained at 21 ± 1°C with a relative humidity of 50%. Animals were given a 2-day acclimatization during which they were fed the standard semi-synthetic diet (Table 4-1), formulated according to National Academy of Sciences-National Research Council guidelines (1978). Animals were randomly assigned to treatment groups, with 10 animals per group and fed the standard diet containing 0, 2.5 or 10 ppm T-2 toxin for 7 or 14 days. Use of experimental animals confirmed to the guidelines of the Canadian Council on animal care.

Tissue dissection and preparation: Upon decapitation, brains were immediately extracted and frozen in liquid nitrogen and stored at -80°C until sectioned and micropunched. All tissue samples were analyzed within 30 days. The four brain regions selected for neurochemical analysis were the NRM, PVN of the hypothalamus, SN and MFB at the level of the hypothalamus. These regions constitute main cell groups or fibre projection systems for NE, DA or 5-HT (Bjorklund *et al.* 1984) and were previously shown to

Table 4-1 Composition of experimental diet

Ingredients	Content (%)
Casein[1]	17.24
DL-methionine[1]	0.30
Choline bitartrate[1]	0.20
Corn oil[2]	5.00
Lard[3]	5.00
Corn starch[4]	31.38
Glucose[5]	31.38
Vitamin pre-mixture[1]	1.00
Mineral pre-mixture[1]	3.50
Cellulose[1]	5.00

[1]United States Biochemical Corporation, Cleveland, OH.

[2]St. Lawrence corn oil, St. Lawrence Starch Company, Mississauga, Ontario.

[3]Tenderflake lard, Maple Leaf Foods Inc., Toronto, Ontario.

[4]St. Lawrence corn starch, St. Lawrence Starch Company, Mississauga, Ontario.

[5]R-Wine Barrel, Winnipeg, Manitoba.

sensitive to T-2 toxin (Chapter 3). The methods and procedures for brain slicing, nucleus isolation, sample preparation, and analysis are that same as those previously described.

Statistical analysis: Data were analyzed using the Statistical Analysis System, Inc. (SAS, 1985). Treatments were analyzed by analysis of variance and means, within the same feeding period, compared using Duncan's t-test, $P<0.05$.

RESULTS

Dietary T-2 toxin altered feed consumption, weight gain and feed efficiency (Table 4-2). T-2 toxin animals consumed less diet than control animals; 24.8, 21.7 and 11.1 g per day in week one, and 24.5, 22.7 and 16.7 g per day during week two for the control, 2.5 ppm T-2 and 10 ppm T-2 animals respectively. The effects of T-2 toxin on food intake was transient with animals fed the 10 ppm T-2 toxin diet increasing their daily food consumption (11.1 vs 16.7 g, $P < 0.05$) (Figure 4-1), average daily weight gain (2 vs 5.7 g, $P < 0.05$) and feed efficiency (0.15 vs 0.34, $P < 0.05$) during week two. Dietary trichothecene exposure, calculated from the food intakes, during weeks one and two was 54 and 56 μg per day for animals on the 2.5 ppm T-2 toxin diet and 110 and 167 μg per day for animals on the 10 ppm T-2 toxin diet.

Table 4-2 Feed consumption, feed efficiency and weight gain of rats fed semi-purified diets containing T-2 toxin.

Sampling interval	T-2 (PPM)	Weight (g) Initial	Final	Gain	Feed intake (g)	Feed efficiency
7 days	0.0	176 ± 3.7	248 ± 4.1[a]	72 ± 2.1[a]	174 ± 5[a]	0.42 ± 0.01[a]
	2.5	176 ± 2.7	236 ± 4.6[a]	60 ± 2.8[b]	152 ± 4[b]	0.40 ± 0.01[a]
	10.0	170 ± 3.9	184 ± 7.0[b]	14 ± 4.6[c]	78 ± 4[c]	0.15 ± 0.01[b]
14 days	0.0	169 ± 2.2	298 ± 5.5[a]	129 ± 4[a]	346 ± 8[a]	0.37 ± 0.01[a]
	2.5	173 ± 3.2	286 ± 5.5[a]	113 ± 3[b]	311 ± 9[b]	0.36 ± 0.01[a]
	10.0	169 ± 2.2	221 ± 5.4[b]	54 ± 3[c]	195 ± 6[c]	0.27 ± 0.02[b]

Mean ± MSE of 10 animals.

Means within sampling intervals not designated with the same letter superscript are significantly different, $P<0.05$, Duncan's test.

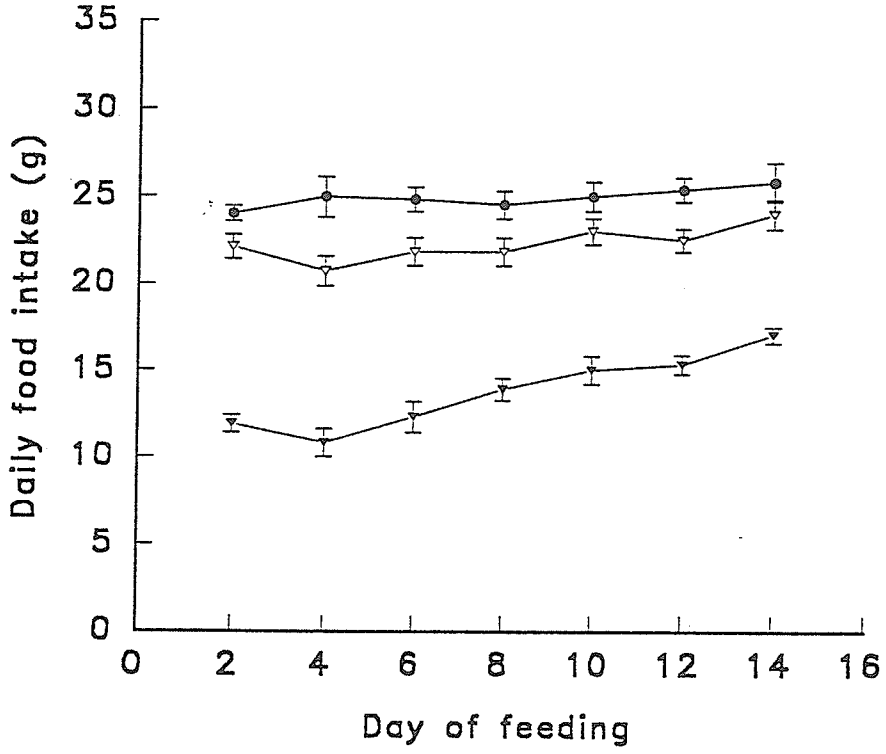


Figure 4-1. Daily food intake of the rats fed T-2 toxin-containing diet. Mean (S.E.).

● - control; ▽ - 2.5 ppm; ▼ - 10 ppm

T-2 toxin affected biogenic monoamine and metabolite concentrations in the MFB, SN and NRM. T-2 toxin treated animals had greater 5-HT and 5-HIAA concentrations in the NRM than control animals after 7 and 14 day (Tables 4-3 and 4-4). Regional DA concentrations were greater in SN and NRM in 10 ppm T-2 toxin treated animals after 7 days (Table 4-5), but returned to control values after 14 days. In general, regional DOPAC levels were lower in T-2 treated animals than controls, with PVN and MFB concentrations being significantly lower than control values at 7 and 14 days, respectively (Table 4-6). T-2 toxin feeding altered NE concentrations in the NRM and SN (Table 4-7). NE concentration in the NRM increased in a dose dependent manner and significant difference was observed in the 10 ppm groups compared to the control. Substantia nigra NE reduced after 14 day feeding of 10 PPM T-2 toxin. A transient increase in SN EP was observed, with animals fed 10 ppm T-2 having greater EP concentrations than controls after 7 days feeding (Table 4-8).

DISCUSSION

The depressions in growth rate, feed consumption and feed efficiency observed in animals fed diets containing 2.5 or 10 ppm T-2 toxin were consistent with previous reports (Carson and Smith, 1983; Rukmini *et al*. 1980, Marasas *et al*. 1969). Ueno (1977) suggested that trichothecene induced

Table 4-3 5-HT concentration in discrete brain regions of rats fed semi-purified diets containing T-2 toxin.

Sampling interval	T-2 (PPM)	Nuclei			
		PVN	MFB	SN	NRM
7 days	0.0	11.0 ± 0.6	12.9 ± 0.4	20.1 ± 0.6	8.4 ± 0.6
	2.5	12.1 ± 0.7	13.1 ± 0.6	19.8 ± 0.7	9.7 ± 0.6
	10.0	12.5 ± 0.6	13.9 ± 0.7	18.7 ± 0.8	9.5 ± 0.3
14 days	0.0	12.1 ± 0.6	13.8 ± 0.4	20.7 ± 1.1	8.4 ± 0.4[a]
	2.5	13.4 ± 1.1	13.2 ± 0.6	22.1 ± 0.6	9.5 ± 0.3[a]
	10.0	13.7 ± 0.4	13.3 ± 0.4	22.0 ± 1.2	11.5 ± 0.3[b]

Mean ± MSE of 10 animals.

Means within sampling intervals not designated with the same letter superscript are significantly different, $P<0.05$, Duncan's test.

Table 4-4 HIAA concentration in discrete brain regions of rats fed semi-purified diets containing T-2 toxin.

Sampling interval	T-2 (PPM)	Nuclei			
		PVN	MFB	SN	NRM
7 days	0.0	10.9 ± 0.5	11.3 ± 0.6	14.1 ± 0.6	16.1 ± 0.4[a]
	2.5	11.5 ± 0.7	11.9 ± 0.8	14.0 ± 0.6	19.7 ± 1.0[b]
	10.0	10.4 ± 0.7	11.8 ± 0.8	13.3 ± 0.6	18.0 ± 0.7[ab]
14 days	0.0	10.7 ± 0.4	11.3 ± 0.4	13.6 ± 0.5	16.3 ± 0.6[a]
	2.5	12.1 ± 0.9	12.0 ± 0.8	14.5 ± 0.6	18.0 ± 0.4[a]
	10.0	10.5 ± 0.5	10.6 ± 0.60	13.8 ± 0.6	20.3 ± 0.9[b]

Mean ± MSE of 10 animals.

Means within sampling intervals not designated with the same letter superscript are significantly different, $P<0.05$, Duncan's test.

Table 4-5 DA concentration in discrete brain regions of rats fed semi-purified diets containing T-2 toxin.

Sampling interval	T-2 (PPM)	Nuclei			
		PVN	MFB	SN	NRM
7 days	0.0	6.23 ± 0.37	2.79 ± 0.24	12.8 ± 0.7[a]	0.84 ± 0.03[a]
	2.5	6.62 ± 0.36	3.10 ± 0.27	12.1 ± 1.0[a]	0.98 ± 0.05[b]
	10.0	6.16 ± 0.42	3.32 ± 0.37	15.8 ± 1.2[b]	1.03 ± 0.05[b]
14 days	0.0	7.43 ± 0.42	3.12 ± 0.14	14.2 ± 0.7	0.99 ± 0.05
	2.5	7.79 ± 0.46	2.96 ± 0.22	14.8 ± 1.1	0.97 ± 0.06
	10.0	6.88 ± 0.36	2.69 ± 0.21	13.4 ± 0.8	0.99 ± 0.07

Mean ± MSE of 10 animals.

Means within sampling intervals not designated with the same letter superscript are significantly different, $P<0.05$, Duncan's test.

Table 4-6 DOPAC concentration in discrete brain regions of rats fed semi-purified diets containing T-2 toxin.

Sampling interval	T-2 (PPM)	Nuclei			
		PVN	MFB	SN	NRM
7 days	0.0	1.94 ± 0.09[a]	1.05 ± 0.11	4.08 ± 0.19	ND
	2.5	2.01 ± 0.17[a]	1.03 ± 0.07	4.04 ± 0.36	ND
	10.0	1.44 ± 0.17[b]	0.94 ± 0.15	4.44 ± 0.32	ND
14 days	0.0	1.90 ± 0.11	1.02 ± 0.05[a]	4.32 ± 0.13	ND
	2.5	1.79 ± 0.11	0.90 ± 0.09[ab]	4.41 ± 0.20	ND
	10.0	1.81 ± 0.09	0.78 ± 0.06[b]	3.92 ± 0.14	ND

ND: not detectable.

Mean ± MSE of 10 animals.

Means within sampling intervals not designated with the same letter superscript are significantly different, $P<0.05$, Duncan's test.

Table 4-7 NE concentration in discrete brain regions of rats fed semi-purified diets containing T-2 toxin.

Sampling interval	T-2 (PPM)	Nuclei			
		PVN	MFB	SN	NRM
7 days	0.0	56.1 ± 4.3	18.8 ± 0.9	2.6 ± 0.2	6.0 ± 0.5[a]
	2.5	59.7 ± 4.6	20.5 ± 0.6	3.0 ± 0.3	7.0 ± 0.4[ab]
	10.0	55.4 ± 4.0	21.4 ± 1.0	3.3 ± 0.3	7.2 ± 0.3[b]
14 days	0.0	58.2 ± 5.3	18.4 ± 1.1	3.2 ± 0.2[a]	6.0 ± 0.3[a]
	2.5	67.8 ± 4.8	19.8 ± 1.0	3.3 ± 0.2[a]	6.4 ± 0.3[a]
	10.0	61.8 ± 4.3	17.9 ± 1.2	2.6 ± 0.2[b]	7.7 ± 0.4[b]

Mean ± MSE of 10 animals.

Means within sampling intervals not designated with the same letter superscript are significantly different, $P<0.05$, Duncan's test.

Table 4-8 EP concentration in discrete brain regions of rats fed semi-purified diets containing T-2 toxin.

Sampling interval	T-2 (PPM)	Nuclei			
		PVN	MFB	SN	NRM
7 days	0.0	5.20 ± 0.46	2.10 ± 0.23	0.97 ± 0.08[a]	1.30 ± 0.16
	2.5	5.83 ± 0.49	2.12 ± 0.18	0.86 ± 0.09[a]	1.15 ± 0.16
	10.0	6.10 ± 0.47	2.70 ± 0.29	1.43 ± 0.11[b]	1.54 ± 0.20
14 days	0.0	4.92 ± 0.46	1.61 ± 0.12	1.00 ± 0.09	1.21 ± 0.09
	2.5	5.19 ± 0.41	1.56 ± 0.15	0.98 ± 0.12	1.16 ± 0.12
	10.0	4.66 ± 0.14	1.55 ± 0.08	1.04 ± 0.11	1.23 ± 0.16

Mean ± MSE of 10 animals.

Means within sampling intervals not designated with the same letter superscript are significantly different, $P<0.05$, Duncan's test.

feed refusal was due to irritation and/or inflammation of the oral cavity and gastrointestinal tract. Our observations do not support this notion. At the levels of T-2 exposure in the current experiment, no acute effects of T-2 toxin were observed (Ueno, 1986), no signs of tissue necrosis or haemorrhage were observed (Marasas *et al.* 1969) and the effects of T-2 on feed intake, growth and feed efficiency were transient. If tissue necrosis were principally responsible for feed refusal, prolonged exposure to T-2 toxin would have exacerbated this condition and feed refusal would have continued for the 2 week duration of the experiment.

The NRM was the most sensitive region among the seven nuclei examined to neurochemical changes induced by T-2 toxin (Chapter 3). The dose-dependent increases in 5-HT, 5-HIAA and NE concentrations observed in the NRM are consistent with previous observations (Boyd *et al.* 1988; Fitzpatrick *et al.* 1988b; Chapter 3).

Neurochemical imbalance may account for the physiological manifestations of trichothecene intoxication. Feeding is modulated by both central and peripheral mechanisms (Carruba *et al.* 1986). NRM, a large caudal serotoninergic cell groups, projects down the spinal column influencing the pregangleonic activation of the sympathetic nervous system (Baum and Shrophire, 1975). Disrupted NRM

monoamine metabolism may influence food intake by altering hormone secretion, peristaltic contractions or thermal energetics. The regulation of ingestive behavior depends, in part, on the reciprocal relationship between PVN 5-HT and NE, an increase in NE stimulating feeding behavior, while increases in 5-HT suppressing feeding (Leibowitz, 1980). Therefore, increased 5-HT in the PVN and NRM observed in T-2 treated rats may provide a biochemical explanation for decreased feed consumption associated with trichothecene intoxication (Ueno, 1977).

Intubation with T-2 toxin elevates brain 5-HT and 5-HIAA and produces modest changes in the catecholamine transmitters in rats (Boyd *et al.* 1988; Macdonald *et al.* 1988). These perturbations occurred after animals received a single, large dosage of T-2 toxin, 2 - 2.5 mg kg^{-1} BW, approximately 450 μg of the trichothecene. Their physiological significance has been questioned by Prelusky *et al.* (1992), who suggested that these neurochemical effects were probably the nonspecific consequence of the lethal doses of toxin. The trichothecene doses in question were 5 to 10 fold greater than the daily animal exposure of this current experiment. Therefore, our observation of altered monoamine and metabolite concentrations confirms the significance of the previous reports, further supporting the

belief that non lethal doses of T-2 toxin influence brain biogenic monoamine metabolism (Chapter 3).

The central influence of the trichothecenes has been considered to be secondary to toxin induced hypoxia (Martin *et al*. 1986; Ballough *et al*. 1989). However, this notion seems difficult to reconcile with observations that (a) lower dosages of T-2 toxin, which produces only moderate systemic effects, are as effective or more effective in inducing a supraoptic-magnocellular cytopathogenesis than the highest dosages (Ballough *et al*. 1989), (b) significant changes in brain monoamines were observed in rats intubated with T-2 toxin at dosages of 0.1 mg kg^{-1} BW, 2% of the LD_{50} value (Chapter 3), or fed diets containing 2.5 ppm T-2 toxin, dosages too modest to alter heart function (Ballough *et al*. 1989; Yarom *et al*. 1983), and (c) DON alters brain monoamine metabolism without inducing hypoxia (Boyd *et al*. 1988; Ueno, 1986). Furthermore, the trichothecene fusarenon-X (FX) induced emesis in dogs was suppressed by preliminary administration of chlorpromazine and metoclopramide (Matsuoka *et al*. 1979). These results suggest a direct action of FX in the stimulation of the chemoreceptive trigger zone in the medulla oblongata. These observations, taken collectively, strongly suggest that the trichothecenes directly affect the CNS.

CHAPTER 5

EFFECT OF T-2 TOXIN ON BLOOD-BRAIN BARRIER PERMEABILITY, MONOAMINE OXIDASE ACTIVITY AND PROTEIN SYNTHESIS IN RATS

ABSTRACT:

Exposure to or consumption of the mycotoxin T-2 toxin results in a disruption of brain biogenic monoamine metabolism. We have suggested T-2's neurochemical perturbations are a reflection of increased blood-brain barrier (BBB) permeability or altered brain enzyme activities, however, the mechanism of T-2 action has not been determined. To examine T-2's effect on the blood-brain barrier rats were dosed with 0, 0.2 and 1 mg T-2 kg^{-1} BW ip, and 2 hrs post dosing, brain permeability was determined using [^{14}C]-mannitol and [^{14}C]-dextran with [^{3}H]-water as the diffusible reference. Permeability increases were observed in all brain regions examined for mannitol, but not for dextran. To observe T-2's effect on brain protein synthesis and enzyme activity, animals were dosed with 0 or 1 mg T-2 kg^{-1} BW ip. Two hrs post treatment, T-2 reduced *in vitro* protein synthesis, as determined by C^{14}-leucine incorporation, but did not affect *in vitro* MAO enzyme activity, as determined by H_2O_2 production. When rats were fed a semipurified diet containing 10 ppm T-2 for 7 days,

BBB permeability increases were observed for mannitol but not for dextran. Dietary T-2 treatment reduced protein synthesis and MAO enzyme activity was significantly lower than control values. These observations support the notion that T-2 affects amino acid transport into the brain, altering precursor availability and neurotransmitter synthesis. That T-2 inhibition of protein synthesis may reduce MAO enzyme activity, contributing to the perturbations in brain neurotransmitters previously observed. Collectively, T-2 toxin's affect on the BBB, protein synthesis and MAO enzyme activity may account for the neurochemical imbalance observed in T-2 intoxication.

INTRODUCTION:

T-2 toxin (3α-hydroxy-4ß,15-diacetoxy-8α-(3-methylbutyrloxy)-12,13-epoxy trichothec-9-ene) is a trichothecene mycotoxin endemic to western Canada (Mills, 1990). Trichothecene intoxication is characterized by an injury of hematopoietic and immune-competence systems, disturbed functions of the cardiovascular system and gastrointestinal tract, hemorrhage syndrome, leucopenia and thrombocytopenia (Khachatourians, 1990; Ueno, 1986). Neurological dysfunctions involving motor, sensory and autonomic nervous systems (Bergmann *et al.* 1988) and feeding behavior (Wellman *et al.* 1989) have been observed. T-2 is one of the more potent trichothecenes (Ueno, 1986).

Exposure to the trichothecene mycotoxins, T-2 toxin or deoxynivalenol (DON), alters brain neurotransmitter concentrations, however, the mechanism of toxic action is still unknown (Prelusky *et al.* 1992). In poultry, Chi and coworkers (1981) reported that T-2 toxin intubation elevated whole brain dopamine (DA) concentration, while reducing whole brain norepinephrine (NE). They suggested this profile was consistent with the notion that T-2's capacity to inhibit protein synthesis interferes with the production of the enzyme dopamine-ß-hydroxylase, reducing the rate of DA hydroxylation to NE. In rats, MacDonald *et al.* (1988) observed that T-2 toxin intubation produced short term increases in tryptophan and 5-hydroxytryptamine (5-HT), followed by an increase in DA. They concluded that these changes in biogenic amines reflected the dual contribution of alterations precursor availability and selective disruptions of membrane amino acid transport (Cavan *et al.* 1988; MacDonald *et al.* 1988). T-2 is lipophilic and an amphipathic molecule and would be expected to cause membrane perturbations similar to those caused by other amphipathic (Gyongyossy-Issa *et al.* 1988). However, the observation that T-2 treatment increased both tryptophan and tyrosine, precursors to both the catecholamines and indoleamines, suggested to Weekley and coworkers (1989) that changes in the transport system were unlikely. In swine, Prelusky *et*

al. (1992) observed that acute intravenous administration of the DON elevated NE and depressed DA concentrations in the hypothalamus, cerebellum and frontal cortex, but had few effects on the indoleamines. The absence of any apparent link between these alterations and any neurochemical model of anorexia suggested to the investigators a peripheral, rather than central, primary site of trichothecene action.

It has been suggested that neurochemical perturbations are due to the systemic effects of trichothecenes, specifically that intoxication leads to cardiac insufficiency and hypoxemia with changes in brain monoamines considered a secondary effect (Martin *et al.* 1986; Ballough *et al.* 1989). This would seem somewhat inconsistent with the observation that DON, a trichothecene with negligible cardiovascular effects, alters brain monoamine metabolism (Fitzpatrick *et al.* 1988b) or our observation that 0.1 mg T-2 toxin kg^{-1} BW treatment, 2% of the LD_{50}, a dosage too modest to affect heart function, still altered brain monoamine concentrations (chapter 3).

The blood brain barrier (BBB), a continuous layer of capillary endothelial cells, contributes to the maintenance of normal brain metabolism and function by controlling the distribution of substances between blood plasma and the brain (Betz *et al.* 1989). Capillary endothelial cells are highly susceptible to T-2 toxin treatment (Yarom *et al.*

1986). The cytotoxic properties of T-2 toxin can inhibit nucleic acid and protein synthesis, and disrupt cytoplasmic membrane function (Trusal *et al.* 1986). Therefore, T-2 toxin may disrupt monoamine metabolism directly by inhibiting the synthesis of the enzymes responsible for the metabolic breakdown of neurotransmitters or by indirectly by compromising the BBB permeability. Accordingly, this study was undertaken to characterized the effect of T-2 toxin on brain protein synthesis, monoamine oxidase (MAO) activity and BBB permeability.

MATERIALS AND METHODS:

Animals and maintenance. Use of experimental animals conformed to the guidelines of the Canadian Council on Animal Care. Sprague-Dawley male rats were purchased from University of Manitoba breeding facility. All animals were received when required for the following two experimental studies. All animals were housed separately in galvanized steel cages and kept on a 14/10 hours light/-dark cycle. The room temperature was maintained at 21 ± 1 °C with a relative humidity of 50%. Animals were introduced into the animal holding facility, allowed 5 days to adjust to the new feeding regimen. Animals to be fed T-2 toxin were given a 2-day acclimatization period during which they were fed a semi-purified diet (Kiritsy *et al.* 1987), formulated

according to National Academy of Sciences-National Research Council guidelines (1978) and water ad libitum.

Acute T-2 toxin treatment. Rats weighing 180-200 g were randomly assigned into five groups, with 10 animals per group. To examine BBB permeability, animals were given single dose of T-2 toxin (Myco-Lab Co., Chesterfield, Missouri) dissolved in corn oil, at dosage of 0, 0.2 or 1.0 mg T-2 kg^{-1} body weight, intraperitoneally. To examine the effect of T-2 on MAO enzyme activity and protein synthesis, rats were given a single dose of 0 or 1.0 mg T-2 kg^{-1} body weight intraperitoneally. Two hours post injection, either BBB permeability was examined or the brains were collected for the determination of MAO enzyme activity and *in vitro* protein synthesis.

Chronic T-2 toxin treatment. Animals having initial body weight 130 g were randomly assigned to treatment groups, with 10 animals per group and fed, *ad libitum,* for 7 days. For the BBB permeability study, animals were fed a standard diet containing 0 or 10 ppm T-2 toxin, while, for the MAO enzyme activity and protein synthesis assay rats were fed a standard diet containing 0, 2.5 and 10 ppm T-2 toxin (chapter 4).

Brain uptake studies (brain uptake index, BUI). D-[^{14}C]-mannitol (50 mCi $mmol^{-1}$) with a molecular weight of

182.2, [^{14}C]-dextran (50 mCi mmol^{-1}) with a molecular weight of 70,000 and [^{3}H]-water (25 mCi g^{-1}) were purchased from New England Nuclear Co. (Boston, Massachusetts). Animals were anesthetized with intraperitoneal injection of sodium pentobarbital (60 mg kg^{-1} BW). Brain uptake of D-[^{14}C]-mannitol or [^{14}C]-dextran was determined by a modification of the single-injection technique of Oldendorf (1981). Approximately, 15 min after the administration of the anesthetic, the right carotid artery was exposed and cannulated with a 27-gauge needle. The needle was left intact throughout the procedure. An injection mixture was prepared that contained approximately 0.75 μCi of a [^{14}C]-labelled substance, 1.0 μCi [^{3}H]-water and sufficient Ringer's solution buffered to pH 7.55 to bring the injection volume to 200 μl. The test solution was rapidly injected to prevent the mixing of the 200-μl bolus with the blood. After 5 s, the time according to inulin clearance studies necessary for the bolus to complete one microcirculatory passage of the brain (Oldendorf 1981), the rat was decapitated. Immediately following decapitation the brain were removed from the cranium and frozen in powdered dry ice. Six main brain regions including lateral cortex, hypothalamus, hippocampus, midbrain, cerebellum, and pons and medulla ipsilateral to the injection were dissected according to Glowinski and Iversen (1966) and put into 20 ml

glass vials. The brain tissue was digested with 1 ml aliquots of NCS tissue solubilizer (Amersham Co., Illinois, USA) in a shaking water bath set at 45 °C, and then mixed into 10 ml aliquots of ready safe liquid scintillation cocktail (Fullerton, California). To increase the counting efficiency 50 μl of glacial acetic acid was added. Radioactivity was measured with a Beckman LS-6000 liquid scintillation counter, equipped with an external standard and automatic quench control for counting dual isotope samples. The counting efficiency was greater than 47% for ^{3}H and 71% for ^{14}C.

The brain uptake index (BUI) was calculated as follows: BUI = [^{14}C dpm (brain)/$^{3}H_2O$ dpm brain]/[^{14}C dpm (mixture)/$^{3}H_2O$ dpm (mixture)] X 100. Because the movement into the brain of ^{14}C substrates was expressed relative to the [^{3}H]-water reference, measurement of brain weight was not necessary (Oldendorf, 1981).

Monoamine oxidase enzyme assay. The mitochondria-enriched brain tissue fraction was prepared according to Kalaria *et al.* (1987). Upon decapitation, brains were immediately removed from the cranium, the right cerebral hemisphere was dissected and homogenized in 10 ml of chilled 0.3 M sucrose 0.01 M sodium phosphate buffer, pH 7.4. Sample preparations were performed at 1-4 °C. Homogenate was centrifuged at 1000 X g for 10 min (Beckman L5-50B

ultracentrifuge, rotor type 50 Ti). The supernatant was recentrifuged at 50,000 X g for 20 min and the pellets was washed in 50 mM Tris/HCl pH 7.4 buffer and dissolved in 1.5 ml of the Tris/HCl buffer. Samples were stored at -80 °C. Protein concentration were determined according to Lowry *et al.* (1951).

MAO activity was determined by a modification of the method described by Szutowicz *et al.* (1984). The assay medium contained 100 mM sodium phosphate buffer pH 7.4, 1.0 mM substrate and 3.1 mM sodium azide in a final volume of 0.75 ml. Octopamine, benzylamine and tyramine specific for type A, type B and for both MAO enzyme forms were used as substrates. The addition of 0.75 ml of the sample, containing 0.4 mg protein initiated the reaction and the assay was performed in a shaking water bath at 37 °C. After 20 min, the addition of 0.75 ml H_2O_2 measuring solution, containing 0.5 M phosphate-citrate buffer pH 4.0, 1.8 mM ABTS, and 6 U of horseradish peroxidase, stopped the reaction. Fifteen seconds later, 0.3 ml of 0.75 M HCl containing 5% sodium dodecyl sulfate was added, thoroughly mixed and the coloured product was measure at 414 nm. Blank assays in which the reaction medium did not contain substrate were run. Readings were matched with a H_2O_2 standard curve. The production of H_2O_2 was demonstrated to be linear with intubation time and protein concentration

(unpublished data). Oxidase activity was expressed as nmol of H_2O_2 produced mg^{-1} protein min^{-1}.

***In vitro* protein synthesis.** Protein synthesis was determined by a modification of the method of Schliebs *et al.* (1985). Upon decapitation, brains were immediately removed from the cranium, the left cerebral hemisphere was dissected and homogenized 10 ml of chilled minimum essential medium (MEM) cell culture medium pH 7.4 (Sigma Chemical Co., St. Louis, Missouri). Homogenate protein concentrations were determined according to Lowry *et al.* (1951). Duplicated 1.2 ml homogenate aliquots were put into 25 ml pyramid flasks containing 4 ml of MEM solution and the flask placed into a shaking water bath at 37 °C. After 10 min preincubation period, 75 μCi of ^{14}C labelled L-leucine (314.8 mCi $mmol^{-1}$, New England Nuclear Co., Boston, Massachusetts) was added. After 60 min, 5 ml 10% trichloric acid (TCA) solution was added to stop the reaction and precipitate protein. Flask contents were centrifuged at 2800 X g (International Equipment Co., Model CS, Boston Massachusetts) for 15 min. The pellets were washed twice with 5% TCA, then dissolved into 1.5 ml NCS tissue solubilizer and then mixed into 10 ml aliquots of ready safe liquid scintillation cocktail (Fullerton, California). Radioactivity was measured with a Beckman LS-6000 liquid scintillation counter, equipped with an external standard

and automatic quench control for counting dual isotope samples. Preliminary experiments demonstrated that L-leucine incorporation rate was linear with intubation time for 90 min and with protein within the range of 2.5 to 16 mg (unpublished data). Leucine incorporation rate was calculated in DPM mg^{-1} protein hr^{-1}.

Statistical analysis. Data were analyzed using the Statistical Analysis System, Inc. (SAS, 1985).

RESULTS:

T-2 toxin increased the regional brain uptake of mannitol in a dose dependent manner (Table 5-1). Intraperitoneal treatment increased BUI for all brain regions examined for both dosages, with animals receiving 1 mg T-2 kg^{-1} BW having significantly greater mannitol uptake than controls. Intraperitoneal T-2 toxin treatment did not affect regional brain uptake of dextran (Table 5-2). Two hours post treatment, protein synthesis as determined by leucine incorporation rate was significantly lower in T-2 treated animals as compared to control (Table 5-3). While MAO activity was lower for all three measures of enzyme activity in T-2 animals, these differences were not significant.

Table 5-1 Brain uptake index: Regional brain uptake of mannitol in rats treated with a single intraperitoneal dose of T-2 toxin.

Brain Region	Vehicle Control	T-2 Toxin (mg kg^{-1} BW) 0.2	1.0
Lateral Cortex	1.5 ± 0.2[a]	1.9 ± 0.2[a]	3.3 ± 0.5[b]
Hypothalamus	3.6 ± 0.6[a]	5.0 ± 0.6[ab]	6.4 ± 1.1[b]
Hippocampus	3.3 ± 0.5[a]	4.0 ± 0.8[a]	6.8 ± 1.0[b]
Midbrain	1.7 ± 0.4[a]	2.4 ± 0.4[ab]	4.6 ± 1.2[b]
Cerebellum	14.8 ± 1.3[a]	18.6 ± 3.0[ab]	24.4 ± 2.9[b]
Pons + Medulla	19.1 ± 4.0[a]	26.5 ± 3.9[ab]	36.6 ± 6.1[b]

N = 10, mean ± SEM.

Each value is expressed as the ratio of ^{14}C-mannitol to ^{3}H-water.

Means designated with different superscripts are significantly different, $P < 0.05$, Duncan's test.

Table 5-2 Brain uptake index: Regional brain uptake of dextran in rats treated with a single intraperitoneal dose of T-2 toxin.

Brain Region	Vehicle Control	T-2 Toxin (mg kg^{-1} BW) 0.2	1.0
Lateral Cortex	2.4 ± 0.3	3.0 ± 0.3	2.6 ± 0.2
Hypothalamus	6.2 ± 1.1	8.0 ± 1.0	8.2 ± 1.0
Hippocampus	3.6 ± 0.5	4.5 ± 0.7	5.0 ± 0.7
Midbrain	3.4 ± 0.3	4.5 ± 0.4	3.9 ± 0.6
Cerebellum	17.0 ± 2.2	18.0 ± 3.8	18.9 ± 3.6
Pons + Medulla	42.7 ± 3.0	41.7 ± 8.0	50.5 ± 10.1

N = 10, mean ± SEM.

Each value is expressed as the ratio of ^{14}C-dextran to ^{3}H-water.

Means designated with different superscripts are significantly different, $P < 0.05$, Duncan's test.

Table 5-3 Brain metabolism: Protein synthesis and monoamine oxidase enzyme activity in brain tissue of rats given an intraperitoneal injection of T-2 toxin

Group	Protein Synthesis@	MAO Activity#		
		Tyramine	Octopamine	Benzylamine
control	28.0 ± 0.5	4.9 ± 0.2	2.0 ± 0.1	1.5 ± 0.1
T-2 toxin	26.3 ± 0.4*	4.5 ± 0.2	1.8 ± 0.1	1.4 ± 0.2

N = 10, mean ± SEM.

@ ^{14}C-L-Leucine incorporated, DPM mg^{-1} protein hr^{-1}.

nmol H_2O_2 mg^{-1} protein min^{-1}.

* Significantly different from control, $p < 0.05$, t-test.

Dietary T-2 altered feed consumption, weight gain and feed efficiency (data not presented) in a manner consistent with previous observations (chapter 3). Dietary T-2 increased brain uptake of mannitol, with significant differences in BUI observed in the cerebellum and the pons and medulla region (Table 5-4). Dietary T-2 did not affect dextran uptake in any of the brain regions examined (Table 5-5). The rate of brain protein synthesis tended to be lower in animals fed T-2 toxin, however, these differences were not significant (Table 5-6). Brain MAO activities toward octopamine, benzylamine and tyramine were significantly lower in animals fed either 2.5 or 10 ppm T-2 toxin compared to control values (Table 5-6).

DISCUSSION:

To gain access to the cytoplasm and various cell organelles, trichothecenes must pass through the plasma membrane. T-2 toxin is a lipophilic, an amphipathic molecule that could enter the membrane and cause membrane perturbations, similar to those caused by other amphipaths (Khachatourians, 1990). Pace and Watts (1989) examined subcellular distribution of [^{3}H]-T-2 toxin in a perfused rat liver noting that after 5 min, the plasma membrane fraction contained 38% of the labelled T-2 toxin, a concentration greater than that observed for the smooth endoplasmic reticulum, mitochondria and nuclear fractions, 27, 10 and

Table 5-4 Brain uptake index: brain region uptake of mannitol in rats fed diets containing 10 ppm t-2 toxin.

Brain Region	Control	10 ppm T-2 toxin
Lateral Cortex	2.2 ± 0.1	2.3 ± 0.2
Hippocampus	3.9 ± 0.2	4.5 ± 0.3
Hypothalamus	4.2 ± 0.4	4.1 ± 0.3
Midbrain	2.2 ± 0.1	2.4 ± 0.2
Cerebellum	5.8 ± 0.4	7.5 ± 0.7*
Pons + Medulla	12.1 ± 1.1	17.0 ± 1.4*

N = 10, mean ± SEM.

Each value is expressed as the ratio of ^{14}C-mannitol to ^{3}H-water.

* Significantly different from control values $P < 0.05$, t-test.

Table 5-5 Brain uptake index: brain region uptake of dextran in rats fed diets containing 10 ppm t-2 toxin.

Brain Region	Control	10 ppm T-2 toxin
Lateral Cortex	2.2 ± 0.1	2.6 ± 0.2
Hippocampus	4.8 ± 0.5	5.2 ± 0.6
Hypothalamus	5.1 ± 0.2	5.3 ± 0.3
Midbrain	2.8 ± 0.2	2.9 ± 0.3
Cerebellum	9.9 ± 1.2	10.5 ± 1.2
Pons + Medulla	19.2 ± 2.4	17.8 ± 0.9

N = 10, mean ± SEM.

Each value is expressed as the ratio of ^{14}C-dextran to ^{3}H-water.

* Significantly different from control values $P < 0.05$, t-test.

Table 5-6 T-2 toxin feeding trial - protein synthesis and monoamine oxidase enzyme activity in brain tissue of rats fed a semipurified diet containing 2.5 Or 10 ppm t-2 toxin for 7 days.

T-2 toxin	Protein Synthesis@	MAO Activity#		
		Tyramine	Octopamine	Benzylamine
0	28.4 ± 0.6	5.3 ± 0.1[a]	2.0 ± 0.1[a]	1.5 ± 0.1[a]
2.5	28.1 ± 0.6	4.7 ± 0.2[b]	1.6 ± 0.1[b]	1.2 ± 0.1[b]
10	27.9 ± 0.5	4.6 ± 0.1[b]	1.7 ± 0.1[b]	1.2 ± 0.1[b]

N = 10, mean ± SEM.

@ - ^{14}C-L-Leucine incorporated, DPM mg^{-1} protein hr^{-1}.

- nmol H_2O_2 mg^{-1} protein min^{-1}.

Means designated with different superscripts are significantly different, $P < 0.05$, Duncan's test.

7%, respectively. Although T-2 toxin was concentrated in the membrane for a limited period, this distribution provides T-2 the opportunity to exert its noxious effect there. Bunner and Morris (1988) reported that T-2 toxin affected multiple cell membrane functions, with many of these effects starting at a concentration of less than 4 pg ml^{-1}, which is in the molar range of steroid hormone. Membrane function perturbations were noted within 10 min of exposure, a time interval is too short to attribute T-2's effects to protein synthesis inhibition (Bunner and Morris, 1988). Therefore, T-2 toxin directly influences membrane function and perturbation in membrane functions is an important feature of trichothecene cytotoxicity.

The BBB comprises a continuous layer of endothelial cells that are joined by seamless junctions and contributes to the maintenance of normal brain metabolism and physiology (Betz *et al.* 1989). The transfer of essential nutrients such as the essential amino acids, from blood to brain is carried out through the specific facilitated transport systems (Pardridge, 1983). The increased BUI for mannitol observed after T-2 treatment suggests that this toxin may disrupt the functional integrity of this barrier. The resulting impairment in substance exchanges could alter brain biogenic monoamine homeostasis and contribute to the elevated regional brain catecholamine and 5-HT levels that

we have obtained in T-2 toxin treated animals (chapters 3 and 4). T-2 exerts cytotoxic effects on myocardial (Yarom *et al.* 1986) and skin (Ueno, 1984) endothelial cells. T-2 toxin inhibits protein and nucleic acid synthesis (Ueno, 1980) and breaks down cell membrane (Bunner *et al.* 1988). Therefore, the effect of T-2 on the BBB may reflect its disruption of endothelial cells.

The decrease in MAO enzyme activity observed may be due to T-2 toxin induced inhibition in protein synthesis (Ueno, 1986). T-2 inhibits protein synthesis by suppressing peptidyl transferase activity (Cundliffe *et al.* 1974). Trusal and coworkers (1986) demonstrated *in vitro* that inhibition was dependent upon T-2 dosage and duration of exposure in hepatocytes. At a low dosages, protein synthesis was initially inhibited initially, but activity recovered to near control levels, even with continued toxin exposure. At higher dosages of the toxin, protein synthetic rates were able to recover from a short period of exposure, but not when the exposure was prolonged (Trusal *et al.* 1986). This may explain our observations of a decrease in protein synthesis in brain tissue of rats given an ip injection of T-2 and the lack of effect of dietary trichothecene on protein synthesis. Therefore, the observed effect of T-2 on MAO enzyme activity may be due to T-2's initial effect on protein synthesis.

Inhibition of brain MAO activities after T-2 toxin administration may contribute to increases of brain monoamine concentrations. However, this notion is difficult to reconcile with the increased concentrations of 5-HIAA, a major 5-HT metabolite, observed in T-2 treated rats (chapters 3 and 4). One possibility is that the increased 5-HIAA concentrations is secondary to T-2-induced disruption of the BBB endothelial cell acidic transport system that is responsible for moving acidic monoamine metabolites out of the brain. Inhibition of this system with probenecid promotes an accumulation of brain 5-HIAA (Cooper *et al.* 1991). Therefore, a thorough investigation on the dynamics of brain monoamine metabolism is needed to clarify the relationship between T-2 toxicity, brain biogenic monoamine concentrations and enzyme activity, precursor availability and metabolite clearance.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

Previously, observations on the effect of T-2 toxin on central monoamine concentrations were made by using whole brain analysis (Cavan *et al.* 1988; Chi *et al.* 1980; MacDonald *et al.* 1988). The brain is a heterogeneous organ having different functions dependent upon its anatomical regions, in which an uneven distribution of monoamine transmitters has been confirmed (Ungerstedt, 1971). Therefore, it is anticipated that regional brain analysis would be more informative than whole brain analysis. This lab previously examined the acute effect of T-2 toxin and DON on whole brain and regional concentrations of monoamines and their metabolites in rats (Boyd *et al.* 1988; Fitzpatrick *et al.* 1988b). No alterations of whole brain monoamines or their metabolites were observed, however, when five selected brain areas were dissected and analyzed, significant changes of 5-HT and 5-HIAA concentrations were demonstrated in all the area examined in T-2 treated rats. T-2 toxin induced alterations of regional brain monoamines were also reported by other investigators (Weekly *et al.* 1989).

Compared with whole brain analysis, regional brain analysis could provide more specific information and a better understanding of the central effect of T-2 toxin. However, the limitations of regional analysis should not be overlooked. Most brain neurons are distributed in clusters called neural cell bodies or nuclei. Thus, analysis of monoamines and their metabolites in specific cell bodies would be even more informative than analysis of main brain regions. Also, examination of the effect of T-2 toxin administration on brain monoamine concentrations in selected monoamine cell bodies or important projections made it possible to associate neurochemical changes due to T-2 exposure with alteration of physiological function based upon known neuroanatomical and neurophysiological information.

In the first experiment, Chapter 3, acute experiments were conducted in rats using micropunch technique to sample the selected brain areas to examine the effect of T-2 toxin on central monoamines and their metabolites. These regions represented major cell groups or important projections of dopaminergic, norepinephrinergic or serotoninergic systems in the brain (Biorklund *et al*. 1984). The results indicate that T-2 toxin affects biogenic monoamine metabolism and that the effect was primarily in the serotoninergic system, with few effects observed for dopaminergic system. That is,

a single oral dose of T-2 toxin increased 5-HIAA and 5-HT throughout the brain. Increased NE in the NRM and LC, whereas a transient decrease in the SN NE were also observed in T-2 intubated animals. The indoleamines, 5-HT and 5-HIAA were more sensitive to T-2 treatment, an observation which is consistent with our previous observation made in dissected main brain regions (Fitzpatrick *et al.* 1988b, Boyd *et al.* 1988). Previous reports were based upon dosages of 2-2.5 mg T-2 kg^{-1} BW (Cavan *et al.* 1988; Boyd *et al.* 1988; Chi *et al.* 1980; Fitzpatrick *et al.* 1988b; MacDonald *et al.* 1988). Therefore, the neurochemical effects of the toxin, observed at dosage of 0.1 mg T-2 kg^{-1} BW, 2% of the LD_{50} (Ueno, 1984), suggest that T-2 is a more potent CNS toxin than previous thought. The neurological manifestations of T-2 intoxication have been attributed to the systemic effects of this toxin, that is the central effect is secondary to T-2 induced cardiovascular failure and hypoxia. However, 0.1 mg T-2 kg^{-1} BW, a dosage insufficient to produce cardiovascular effect (Yarom *et al.* 1983; Yarom and Yagen 1986), produced CNS effects suggesting that T-2 toxin may affect the CNS directly.

In the second experiment, Chapter 4, the effect of dietary T-2 toxin at levels of 2.5 and 10 ppm on monoamines in brain regions shown to be sensitive to T-2 toxin were examined. A reduction in feed intake, body weight gain and

feed efficiency were observed in T-2 toxin feeding animals. However, these effects were transient and no overt tissue necrosis or bleeding were observed, therefore, the notion that trichothecene induced feed refusal was due to irritation of the oral cavity was not supported (Ueno, 1977). Dietary T-2 toxin affected brain monoamine concentrations, however, the pattern of altered monoamines and metabolites observed in the acute trial was not observed. In the NRM, 5-HT, 5-HIAA and NE increased in a dose dependent manner, and a transient increase in DA was observed. In the SN, animals fed 10 ppm T-2 toxin had increased EP after 7 days, and decreased NE after 14 days. Decreased DOPAC in the PVN and MFB was also observed. Because of the physiological role of monoamines in feeding behavior regulation and body weight control (Leibowitz, 1980), the perturbations of monoamine metabolism in the CNS may account for the physiological manifestations of trichothecene intoxication such as feed refusal and low body weight gain. The neurochemical alterations caused by T-2 toxin exposure were observed at 2.5 and 10 ppm exposures, which do not produce systematic effects (Marasas *et al.* 1969; Ueno, 1986; Yarom *et al.* 1983), strengthens the notion that trichothecene mycotoxins directly affect the CNS.

Altered regional concentrations of biogenic monoamines and their metabolites in selected brain nuclei of T-2

treated rats indicate the perturbations of monoamine transmitter metabolism. However, the functional state of neurotransmitter system is more dependent upon the turnover rate of the transmitters. For a better understanding of T-2 toxin effect on the CNS, It is necessary to examine the turnover rate of serotonin, DA and NE in the brain.

The first two studies were toxicity trials, therefore, was necessary probe the mechanism through which T-2 toxin affect brain biogenic monoamines. BBB permeability to different size of molecules, brain protein synthesis and MAO enzyme activity in T-2 treated rats was examined in the third experiment (Chapter 5). The administration of 0.2 or 1.0 mg T-2 kg^{-1} BW of by i.p. resulted in an increase in BBB permeability to small molecular substance mannitol (MW 182.4) in dose dependent manner in all six major regions of the brain. BBB permeability to dextran, MW 70,000, was not changed by T-2 treatment. Rats fed diets containing 10 ppm T-2 toxin had increased BBB permeability to mannitol in two of the regions examined. Again, T-2 treatment did not affect BBB permeability to dextran. These results suggested that the BBB, the system essential to control the brain environment, was compromised by T-2 toxin. It is expected that the detrimental effect of T-2 toxin on BBB integrity and physiological function could result in more adverse effect on the brain function.

Acute T-2 toxin treatment inhibited the protein synthesis as indicated by decreased incorporation rate of ^{14}C-L-leucine into protein, however, this inhibition was not observed in the feeding trial. These observations are in agreement with Rosenstein and Lafarge-Frayssinet (1983) that T-2 toxin treatment at a low dose caused an initial decrease in protein synthesis and 20 hrs post dosing protein synthesis returned to the normal level.

Decreased MAO enzyme activity in T-2 toxin fed rats was demonstrated. This may contribute to the increase in brain 5-HT, NE and DA concentrations. However, elevated 5-HIAA and DOPAC, the major metabolites of 5-HT and DA after MAO action respectively were also observed in some brain regions of T-2 treated animals. The significance of MAO inhibition in T-2 toxin induced perturbations of brain biogenic monoamine transmitters needs further investigation.

In conclusions: 1) Acute intubation of T-2 toxin affects the concentrations of monoamines and their metabolites in selected brain regions. The neurochemical changes were observed at dosage which producing negligible effect on hemodynamics, suggesting the T-2 toxin may directly affects the CNS. 2) Dietary T-2 toxin at levels of 2.5 and 10 ppm alters monoamine metabolism in selected brain regions. The nucleus raphe magnus was more sensitive to T-2 toxin treatment, which is in agreement with the observations

REFERENCES

Abramson, D., Clear, R.M. and Nowichi, T.W. (1987). Fusaria and trichothecene mycotoxins in 1985 Manitoba wheat. *Can. J. Pl. Sci.* **67**, 611-617.

Ballough, G.P., Pritchard, G.A., Miller-Patrick, K., Kan, R.K. and Anthony, A. (1989). Cytophotometric analysis of T-2 toxin induced alterations in chromatin condensation and neuronal nuclear volume of rat supraoptic-magnocellular neurons. *Life Sci.* **45**, 189-196.

Baum, T. and Shrophire, A., (1975). Inhibition of efferent sympathetic nerve activity by 5-hydroxytryptophan and centrally administered 5-hydroxytryptamine. *Neuropharmacol.* **14**, 227-233.

Beasley, V.R., Lundeen, G.R., Poppenga, R.H. and Buck, W.B. (1987). Distribution of blood flow to the gastrointestinal tract of swine during T-2 toxin-induced shock. *Fundam. Appl. Toxicol.* **9**, 588-594.

Beczkowska, I. and Bodnar, R.J. (1991). Naloxone and serotonin receptor subtype antagonists: interactive effects upon deprivation-induced intake. *Pharmacol. Biochem. Behav.* **38**, 605-610.

Bergmann, F., Soffer, D. and Yagen, B. (1988). Cerebral toxicity of the trichothecene toxin T-2, of the products of its hydrolysis and of some related toxins. *Toxicon,* **26**, 923-930.

Bergmann, F., Yagen, B. and Soffer, D. (1985). Toxic and lethal effects of T-2 toxin upon intracerebral administration to rats. *Arch. Toxicol.* **58**, 40-45.

Bhat, R.V., Beedu, S.R., Ramakrishna, Y. and Munshi, K.L. (1989). Outbreak of trichothecene mycotoxicosis associated with the consumption of mold-damaged wheat products in Kashmir valley. *Lancet*, **1**, 35-37.

Bjorklund, A., Hokfelt, T. and Kuhar M.J. (1984). Classical transmitters in the central nervous system. *Handbook of Chemical Neuroanatomy*, Volume 3, Elsevier, Amsterdam.

Boyd, K.E., Fitzpatrick, D.W., Wilson, J.R. and Wilson, L.M. (1988). Effect of T-2 toxin on brain biogenic monoamines in rats and chickens. *Can. J. Vet. Res.* **52**, 181-185.

Bubien, J.K. and Woods, W.T. (1987). Direct and reflex cardiovascular effects of trichothecene mycotoxins. *Toxicon,* **25**, 325-331.

Bunner, D.L. and Morris, E.R. (1988). Alteration of multiple cell membrane functions in L-6 myoblasts by T-2 toxin: an important mechanism of action. *Toxicol. Appl. Pharmacol.* **92**, 113-121.

Carlsson, A. and Lindqvist, M. (1972). The effect of L-tryptophan and some psychotropic drugs on the formation of 5-hydroxytryptophan in the mouse brain *in vivo*. *J. Neural Transmission*, **33**, 23-43.

Carruba, M.O., Mantegazza, P., Memo, M., Missale, C., Pizzi, M. and Spano, P.F. (1986). Peripheral and central mechanisms of action of serotonergic anorectic drugs. In: *Serotonergic System, Feeding and Body Weight Regulation*, Edited by S. Nicolaidis, pp 105-113. Academic Press, New York.

Carson, M.S. and Smith, T.K. (1983). Effect of feeding alfalfa and refined plant fibers on the toxicity and metabolism of T-2 toxin in rats. *J. Nutr.* **113,** 304-313.

Cavan, K.R., MacDonald, E.J. and Smith, T.K. (1988). Potential for dietary amino acid precursors of neurotransmitters to overcome neurochemical changes in acute T-2 toxicosis in rats. *J. Nutr.* **118**, 901-907.

Chan, P.K-C. and Gentry, P.A. (1984). LD_{50} values and serum biochemical changes induced by T-2 toxin in rats and rabbits. *Toxicol. Appl. Pharmacol.* **73**, 402-410.

Chan, P.K-C. and Gentry, P.A. (1984). Inhibition of bovine platelet function by T-2 toxin, HT-2 toxin, diacetoxyscirpenol and deoxynivalenol. *Fd. Chem. Toxic.* **22**, 643-648.

Chi, M.S., El-Halawani, M.E., Waibel, P.E. and Mirocha, C.J. (1981). Effects of T-2 toxin on brain catecholamines and selected blood components in growing chickens. *Poult. Sci.* **60**, 137-141.

Chi, M.S., Mirocha, C.J., Kurtz, H.J., Weaver, G., Bates, F., Shimoda, W. and Burmeister, H.R. (1977). Acute toxicity of T-2 toxin in broiler chicks and lying hens. *Poult. Sci.* **56**, 103-116.

Chi, M.S., Robison, T.S., Swanson, C.J. and Shimoda, W. (1978). Excretion and tissue distribution of radioactivity from tritium-labelled T-2 toxin in chicks. *Toxicol. Appl. Pharmacol.* **45**, 391-402.

Cooper, J.R., Bloom, F.E. and Roth, R.H. (1991). *The Biochemical Basis of Neuropharmacology*. Oxford University Press, New York.

Corrier, D.E. (1991). Mycotoxicosis: mechanisms of immunosuppression. *Vet. Immun. and Immunopathol.* **30**, 73-87.

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. Rumsby and M.W.B., pp 17-41. Bradbury Ellis Horwood, Chichester England .

Cundliffe, E., Cannon, M., and Davis, J. (1974). Mechanism of inhibition of eukaryotic protein synthesis by trichothecene fungal toxins. *Proc. Natl. Acad. Sci. (USA)* **71**, 30-42.

Dahlstrom, A. and Fuxe, K. (1964). Evidence for the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. *Acta Physiol. Scand.* **62**, 1-55.

d'Avella, D., Cicciarello, R., Albiero, F., Mesiti, M., Gagliardi, M.E., Russi, E., d'Aquino, A., Tomasello, F. and d'Aquino, S. (1992). Quantitative study of blood-brain barrier permeability changes after experimental whole-brain radiation. *Neurosurgery*, **30**, 30-34.

Deloach, J.R., Gyongyossy-Issa, M.I.C. and Khachatourians, G.G. (1989). Species-specific hemolysis of erythrocytes by T-2 toxin. *Toxicol. Appl. Pharmacol.* **97**, 107-112.

Devoino, L.V. and Ilyutchenok, R.Y. (1968). Influence of some drugs on the immune response. II. Effects of serotonin, 5-hydroxytryptophan, reserpine and iproniazid on delayed hypersensitivity. *Eur. J. Pharmacol.* **4**, 449-456.

Eremina, O.F. and Devoino, L.V. (1973). Production of humoral antibodies in rabbits with destruction of the nucleus of the midbrain raphe, *Bull. Exp. Biol. Med.* **75**, 149-151.

Fairhurst, S., Marrs, T.C., Parker, H.C., Scawin, J.W. and Swanston, D.W. (1987). Acute toxicity of T-2 toxin in rats, mice, guinea pigs, and pigeons. *Toxicol.* **43**, 31-49.

Falck, B., Hillarp, N., Thieme, G. and Thorp, A. (1962). Fluorescence of catecholamines and related compounds with formaldehyde. *J. Histochem. Cytochem.* **10**, 348-354.

Feinberg, B. and Mclaughlin, S. (1989). Biochemical mechanism of action of trichothecene mycotoxins. In: *Trichothecene Mycotoxins: Pathophysiologic Effects*, Volume I. Edited by V.R. Beasley, pp 28-33. CRC Press, Inc., Florida.

Fekete, S., Tamas, J., Vanyi, A., Glavits, R. and Bata, A. (1989). Effect of T-2 toxin on feed intake, digestion and pathology of rabbits. *Lab. Anim. Sci.* **39**, 603-606.

Fernstrom, J.D. (1990). Aromatic amino acids and monoamine synthesis in the central nervous system: influence of the diet. *J. Nutr. Biochem.* **1**, 508-517.

Fernstrom, J.D., Wurtman, R.J. (1972). Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science*, **178**, 414-416.

Feuerstein, G., Goldstein, D.S., Ramwell, P.W., Zerbe, R.L., Lux Jr, W.E., Faden, A.I. and Bayorh, M.A. (1985). Cardio-respiratory, sympathetic and biochemical response to T-2 toxin in the guinea pig and rat. *J. Pharmacol. Exp. Therap.* **232**, 786-794.

Feuerstein, G., Lorenzana, R.M. and Beasley, V.R. (1989). Effects of trichothecene mycotoxins on the nervous system. In: *Trichothecene Mycotoxicosis: Pathophysiologic Effects, II*. Edited by V.R. Beasley, pp 112-122. CRC Press, Inc., Florida.

Fitzpatrick, D.W. (1989). Mycotoxins in the food chain: nutritional and toxicological considerations. *Can. J. Physiol. Pharmacol.* **68**, 979-981.

Fitzpatrick, D.W., Boyd, K.E. and Watts, B.M. (1988a). Comparison of the trichothecenes deoxynivalenol and T-2 toxin for their effects on brain biogenic monoamines in the rat. *Toxicol. Letters*, **40**, 241-245.

Fitzpatrick, D.W., Boyd, K.E., Wilson L.M. and Wilson, J.R. (1988b). Effect of trichothecene deoxynivalenol on brain biogenic monoamines concentrations in rats and chickens. *J. Environ. Sci. Health*, **B23**, 159-170.

Forsell, J.H., Jensen, R., Tai, J.H., Witt, M., Lin, W.S. and Pestka, J.J. (1987). Comparison of acute toxicities of deoxynivalenol (vomitoxin) and 15-acetyldeoxynivalenol in the B6C3F_1 mouse. *Fd. Chem. Toxic.* **25**, 155-162.

Forsyth, D.M., Yoshizawn, T., Mnooka, N. and Tuite, S. (1977). Emetic and refusal activity of deoxynivalenol to swine. *Appl. Environ. Microbiol.* **34**, 547-552.

Fowler, J.S. (1990). Enzyme activity: monoamine oxidase. In: *Neuroreceptors, Neurotransmitters and Enzymes.* Edited by J.J. Frost and H.N. Wagner, pp 179-192. Raven Press, Ltd., New York.

Friend, S.C.E., Babiuk, L.A. and Schiefer, H.B. (1983). The effects of dietary T-2 toxin on the immunological function and herpes reactivation in Swiss mice. *Toxicol. Appl. Pharmacol.* **69**, 234-244.

Friend, S.C.E., Hancock, D.S., Schiefer, H.B. and Babiuk, L.A. (1983). Experimental T-2 toxicosis in sheep. *Can. J. Comp. Med.* **47**, 291-297.

Gentry, P.A. and Cooper, M.L. (1981). Effect of fusarium T-2 toxin on hematological and biochemical parameters in the rabbit. *Can. J. Comp. Med.* **45**, 400-405.

Gentry, P.A. and Cooper, M.L. (1983). Effect of intravenous administration of T-2 toxin on blood coagulation in calves. *Am. J. Vet. Res.* **44**, 741-746.

Gentry, P.A., Ross, M.L. and Chan, P.K-C. (1984). Effect of T-2 toxin on bovine hematological and serum enzyme parameters. *Human Toxicol.* **26**, 24-28.

Gessa, G.L. (1974). Serum free tryptophan: control of brain concentrations of tryptophan and of synthesis of 5-hydroxytryptamine. In: *Aromatic Amino Acids In The Brain*. CIBA Foundation Symposium 22, pp 207-216. Elsevier, Amsterdam.

Gjedde, A. (1988). Exchange diffusion of large neutral amino acids between blood and brain. In: *Peptide and Amino Acid Transport Mechanism in The Central Nervous System*. Edited by L. Rakic, D.J. Begley, H. Davson and B.V. Zlokovic, pp 209-217. Macmillan Press Ltd., London.

Green, J.P. (1989). Histamine and serotonin. In: *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects*, 4th Edition. Edited by G.J. Siegel, pp 233-251. Raven Press, Ltd., New York.

Greenway, J.A. and Puls, R. (1976). Fusariotoxicosis from barley in British Columbia. I. Natural occurrence and diagnosis. *Can. J. Comp. Med.* **40**, 12-15.

Gyongyossy-Issa, M.I.C. and Khachatourians, G.G. (1984). Interaction of T-2 toxin with murine lymphocytes. *Biochim. Biophys. Acta*, **803**, 197-202.

Gyongyossy-Issa, M.I.C., Khanna, V. and Khachatourians, G.G. (1986). Changes induced by T-2 toxin in the erythrocyte membrane. *Fd. Chem. Toxic.* **24**, 311-317.

Hall, N.R. and Goldstein, A.L. (1985). *Neurotransmitters and host defense. In: Neural Modulation of Immunity.* Edited by R. Guillemin, M. Cohn and T. Melnechuk, pp 143-154. Raven Press, New York.

Hillegaart, V. (1991a). Effects of local application of 5-HT and 8-OH-DPAT into the dorsal and median raphe nuclei on core temperature in the rat. *Psycopharmacol.* **103**, 291-296.

Hoerr, F.J. and Carlton, W.W. (1981). The toxicity of T-2 toxin and diacetoxyscirpenol in combination for broiler chickens. *Fd. Cosmet. Toxicol.* **19**, 185-188.

Huff, W.E., Doerr, J.A., Hamilton, P.B. and Vesonder, R.F. (1981). Acute toxicity of vomitoxin (deoxynivalenol) in broiler chickens. *Poult. Sci.* **60**, 1412-1414.

Jagadeesan, V., Rukmini, C., Vijayaraghavan, M. and Tulpule, P.G. (1982). Immune studies with T-2 toxin: effect of feeding and withdrawal in monkeys. *Fd. Chem. Toxic.* **20**, 83-87.

Jamnicky, B., Slijepcevic, M., Hadzija, M., Juretic, D. and Borcic, O. (1991). Tryptophan content in serum and brain of long-term insulin-treated diabetic rats. *Acta Diabetol. Lat.* **28**, 11-18.

Khachatourians, G.G. (1990). Metabolic effects of trichothecene T-2 toxin. *Can. J. Physio. Pharmacol.* **68**, 1004-1008.

Kopin, I.J. (1985). Catecholamine metabolism: basic aspects and clinical significance. *Pharmacol. Reviews*, **37**, 334-358.

Koshinsky, H., Honour, S. and Khachatourians, G. (1988). T-2 toxin inhibits mitochondrial function in yeast. *Biochem. Biophysic. Res. Communic.* **151**, 809-814.

Kravchenko, L.V., Tutelyan, V.A., Vasilyev, A.V., Kranauskas, A.E. and Avrenyeva, L.I. (1986). Biochemical changes in subacute mycotoxicosis induced by T-2 toxin in rats. *Toxicol.* **42**, 77-83.

Kubena, L.F., Huff, W.E. and Harvey, R.B. (1989). Individual and combined toxicity of deoxynivalenol and T-2 toxin in broiler chicks. *Poult. Sci.* **68**, 622-626.

Leibowitz, S.F. (1980). Neurochemical systems of the hypothalamus-control of feeding and drinking behavior and water-electrolyte excretion. In *Handbook of the Hypothalamus (Vol. VI, Part A), Behavioral Studies of the Hypothalamus*. Edited by P.J. Morgane and J. Panksepp, pp 299-437. Marcel Decker, New York.

Leibowitz, S.F. (1982). Hypothalamus catecholamine system in regulation to control of eating behavior and mechanism of reward. In: *The Neural Basis of Feeding and Reward.* Edited by B.G. Hoebel and D. Novin, pp 241-258. Haer Institute, Brunswick, Maine.

Leibowitz, S.F. and Brown, L.L. (1980). Histochemical and pharmacological analysis of catecholaminergic projections to the perifornical hypothalamus in relation to feeding inhibition. *Brain Res.* **201**, 315-345.

Leibowitz, S.F. and Shor-Posner, G. (1986). Brain serotonin and eating. In: *Serotoninergic System, Feeding and Body Weight Regulation.* Edited by S. Nicolaidis, pp 1-14. Academic press Inc., London.

Lorenzana, R.M., Beasley, V.R., Buck, W.B., Ghent, A.W., Lundeen, G.R. and Poppenga, R.H. (1985). Experimental T-2 toxicosis in swine. I. Change in cardiac output, aortic mean pressure, catecholamines, 6-keto-PGF_{12}, thromboxane B_2 and acid-base parameters. *Fundam. Appl. Toxicol.* **5**, 879-885.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.

Lundeen, G.R., Poppenga, R.H., Beasley, V.R., Buck, W.B., Tranquilli, W.J. and Lambert, R.J. (1986). Systemic distribution of blood flow during T-2 toxin induced shock in swine. *Fundam. Appl. Toxicol.* **7**, 309-323.

MacDonald, E.J., Cavan, K.R. and Smith, T.K. (1988). Effect of acute oral doses of T-2 toxin on tissue concentrations of biogenic amines in the rat. *J. Anim. Sci.* **66**, 434-441.

Magnuson, B.A., Schiefer, H.B. and Hancock, D.S. (1987). Cardiovascular effects of mycotoxin T-2 after topical application in rats. *Can. J. Physiol. Pharmacol.* **65**, 799-802.

Mann, D.D., Beuning, G.M., Hook, B. and Osweiler, G.D. (1983). Effects of T-2 toxin on bovine serum proteins. *Am. J. Vet. Res.* **44**, 1757-1759.

Marasas, W.F.O., Bamburg, J.R., Smalley, E.B., Strong, F.M., Ragland, W.L. and Degurse, P.E. (1969). Toxic effects on trout, rats and mice of T-2 toxin produced by the fungus Fusarium tricinctum (Cd.) Snyd. et Hans. *Toxicol. Appl. Pharmacol.* **15**, 471-482.

Martin, L.J., Doebler, J.A. and Anthony, A. (1986b). Scanning cytophotometric analysis of brain neuronal nuclear chromatin changes in acute T-2 toxin-treated rats. *Toxicol Appl. Pharmacol.* **85**, 207-214.

Martin, L.J., Morse, J.D. and Anthony, A. (1986a). Quantitative cytophotometric analysis of brain neuronal RNA and protein changes in acute T-2 mycotoxin poisoned rats. *Toxicon*, **24**, 933-941.

Martin, R.J., Bailey, B.A. and Downer, R.G.H. (1983). Rapid estimation of catecholamines, octopamine and 5-hydroxy tyramine in biological tissues using high performance liquid chromatography with colorimetric detection. *J. Chromatog.* **278**, 265-274.

Martin, R.J., White, B.D. and Hulsey, M.G. (1991). The regulation of body weight. *American Scientist*, **79**, 528-541.

McLaughlin, C.S., Vaughn, M.H., Campbell, I.M., Wei, C-M., Stafford, M.E. and Hansen, B.S. (1977). Inhibition of protein synthesis by trichothecenes. In: *Mycotoxin in Human and Animal Health*. Edited by J.V. Rodricks, C.W. Hesseltine and M.A. Mehlman, pp 263-270. Pathotox Publishers, Illinois.

Mills, J. T. (1990). Mycotoxins and toxigenic fungi on cereal grains in western Canada. *Can. J. Physiol. Pharmacol.* **68**, 982-986.

Minn, A., Ghersi-Egea, J-F., Perrin, R., Leininger, B. and Siest, G. (1991). Drug metabolizing enzymes in the brain and cerebral microvessels. *Brain Res. Reviews*, **16**, 65-82.

Mutsuoka, Y., Kubota, K. and Ueno, Y. (1979). General pharmacological studies of fusarenon-x, a trichothecene mycotoxin from Fusarium species. *Toxic. Appl. Pharmacol.* **50**, 87-94.

Myers, R.D. (1973). The role of hypothalamic serotonin in thermoregulation. In: *Serotonin and Behavior*. Edited by J. Barchas and E. Usdin, pp 293-302. Academic Press, New York.

Nagatsu, T. (1973). Biosynthesis of catecholamines. In: *Biochemistry of Catecholamines. The Biochemical Method.* Edited by T. Nagatsu, pp 9-87. University Park Press.

National Research Council. (1978). Nutrient requirement of laboratory animals. Washington: National Academy Press.

Oldendorf, W.H. (1970) Measurement of brain uptake of radiolabelled substances using a tritiated water internal standard. *Brain Res.* **24**, 372-376.

Oldendorf, W. (1990). The blood-brain barrier. In: *Encyclopedia of Neuroscience.* Edited by G. Adelman, pp 139-141. Birkhaeuser, Boston.

Pace, J.G. (1983). Effect of T-2 mycotoxin on rat liver mitochondrial electron transport system. *Toxicon*, **21**, 675-680.

Pace, J. and Watts, M. (1989). Hepatic subcellular distribution of [^{3}H]T-2 toxin. *Toxicon*, **27**, 1307-1311.

Palkovits, M. and Brownstein, M.J. (eds) (1988). *Maps and Guide to Microdissection of the Rat Brain.* Elsevier, Amsterdam.

Pang, V.F., Adams, J.H., Beasley, V.R. Buck, W.B. and Haschek, W.M. (1986) Myocardial and pancreatic lesions induced by T-2 toxin, a trichothecene mycotoxin, in swine. *Vet. Pathol.* **23**, 310-319.

Pathre, S.V.and Mirocha, C.J. (1979). Trichothecenes: natural occurrence and potential hazard. *J. Am. Oil Chem Society*, **56**, 820-823.

Prelusky, D.B., Hamilton, R.M.G., Trenholm, H.L. and Miller, J.D. (1986). Tissue distribution and excretion of radioactivity following administration of ^{14}C-labelled deoxynivalenol to white leghorn hens. *Fundam. Appl. Toxicol.* **7**, 635-645.

Prelusky, D.B., Young, J.M., Thompson, B.K. and Trenholm, H.L. (1992). Effect of deoxynivalenol on neurotransmitters in discrete regions of swine brain. *Arch. Environ. Contamin. Toxicol.* **22**, 36-40.

Puls, R. and Greenway, J.A. (1976). Fusariotoxicosis from barley in British Columbia. II. Analysis and toxicity of suspected barley. *Can. J. Comp. Med.* **40**, 16-20.

Rosenstein, Y. and Lafarge-Frayssinet, C. (1983). Inhibitory effect of fusarium T-2 toxin on lymphoid DNA and protein synthesis. *Toxicol. Appl. Pharmacol.* **70**, 283-288.

Rukmini, C., Prased, J.S., and Rao, K. (1980). Effects of feeding T-2 toxin to rats and monkeys. *Food Cosmet. Toxicol.* **18**, 267-272.

SAS Institute INC. (1985). *SAS Introductory Guide*. Cary, North Carolina.

Segal, R., Milo-Goldzweig, I., Joffe, A.Z. and Yagen, B. (1983). Trichothecene-induced hemolysis. I. The hemolytic activity of T-2 toxin. *Toxicol. Appl. Pharmacol.* **70**, 343-349.

Sirkka, U., Nieminen, S.A. and Ylitalo, P. (1992) Acute neurobehavioral toxicity of trichothecene T-2 toxin in the rat. *Phyarmacol. Toxicol.* **70**, 111-114

Siren, A.L. and Feuerstein, G. (1986). Effect of T-2 toxin on regional blood flow and vascular resistance in the conscious rat. *Toxicol. Appl. Pharmacol.* **83**, 438-444.

Skeie, B., Kvetan, V., Gil, K.M., Rothkopf, M.M., Newsholme, E.A. and Askanazi, J. (1990). Branch-chain amino acids: Their metabolism and clinical utility. *Critical Care Med.* **18**, 549-571.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76-85.

Steinbusch, H.W.M. (1984). Serotonin-immunoreactive neurons and their projections in the CNS. In: *Handbook of Chemical Neuroanatomy*, Volume 3, Part II: Classical Transmitters and Transmitter Receptors in the CNS. Edited by A. Bjorklund, T. Hokfelt and M.J. Kuhar pp 68-125. Elsevier, Amsterdam.

Stewart, P.A., Hayakawa, E.M. and Carlen, P.L. (1988). Ethanol and pentobarbital in combination increase blood brain barrier permeability to horseradish peroxidase. *Brain Res.* **443**, 12-20.

Tomar, R.S., Blakley, B.R. and DeCoteau, W.E. (1988). *In vitro* effects of T-2 toxin on the mitogen responsiveness and antibody-producing ability of human lymphocytes. *Toxicol. Letters*, **40**, 109-117.

Trusal, L.R. and O'Brien, G.C. (1986). Ultrastructural effects of T-2 mycotoxin on rat hepatocytes in vitro. *Toxicon*, **24**, 481-488.

Ueno, Y. (1977). Trichothecenes: overview address. In *Mycotoxins in Human and Animal Health*. Edited by J.V. Rodericks, C.W. Hesseltine and M.A. Mehlman. pp. 189-207. Pathotox Publishers, Inc., Park Forest South, IL.

Ueno, Y. (1980). Trichothecene mycotoxins mycology, Chemistry, and Toxicology. In: *Advances in Nutritional Research*, Volume 3. Edited by H. Draper, pp 301-353. Plenum Press, New York.

Ueno, Y. (1984). Toxicological features of T-2 toxin and related trichothecenes. *Fundam. Appl. Toxicol.* **4**, S124-132.

Ueno, Y. (1986). Trichothecenes as environmental toxicants. In: *Reviews in Environmental Toxicology 2*. Edited by E. Hodgson, pp 303-341. Elsevier, Amsterdam.

Weekley, L.B., O'Rear, C.E., Kimbrough, T.D. and Llewellyn, G.C. (1989). Acute and chronic effects of the trichothecene mycotoxin T-2 on rat brain regional concentrations of serotonin, tryptophan, and tyrosine. *Vet. Hum. Toxicol.* **31**, 221-225.

Wei, C-M., Campbell, I.M., McLaughlin, C.S. and Vaughn, M.H. (1974). Binding of trichodermin to mammalian ribosomes and its inhibition by other 12,13-epoxytrichothecenes. *Mol. Cell. Biochem.* **31**, 215-224.

Weiner, N. and Molinoff, P.B. (1989). Catecholamines. In: Basic Neurochemistry: *Molecular, Cellular and Medical Aspects*. 4th edition. Edited by G.J. Siegel, pp 233-251. Raven Press, Ltd., New York.

Wellman, P.J., Rowe, L.D., Clark, D.E. and Cockroft, R.D. (1989). Effects of T-2 toxin on saccharin aversion and food consumption in adult rats. *Physiology and Behavior.* **45**, 501-506.

Wurtman, R.J., Hefti, F. and Melamed, E. (1980). Precursor control of neurotransmitter synthesis. *Pharmacol. Rev.* **32**, 315-335.

Wyatt, R.D., Colwell, W.M., Hamilton, P.B. and Burmeister, H.R. (1973). Neural disturbances in chickens caused by dietary T-2 toxin. *Appl. Microbiol.* **26**, 757-761.

Yagen, B., Bergmann, F., Barel, S. and Sintov, A. (1991) Metabolism of T-2 toxin by rat brain homogenate. *Biochem. Phyarmacol.* **42**, 949-951.

Yarom, R., Hasin, Y., Raz, S., Shimoni, Y., Fixler, R. and Yagen, B. (1986). T-2 toxin effect on cultured myocardial cells. *Toxicol. Letters*, **31**, 1-8.

Yarom, R., More, R., Eldor, A. and Yagen, B. (1984). The effect of T-2 toxin on human platelet. *Toxicol. Appl. Pharmacol.* **73**, 210-217.

Yarom, R., More, R., Sherman, Y. and Yagen, B. (1983a). T-2 toxin-induced pathology in the heart of rats. *Br. J. exp. Path.* **64**, 570-577.

Yarom, R., More, R., Raz, S., Shimoni, Y., Sorel, O. and Yagen, B. (1983b). T-2 toxin effect on isolated rat heart. *Basic. Res. Cardiol.* **78**, 623-630.

Yarom, R. and Yagen, B. (1986). T-2 toxin effect on the ultrastructure of myocardial microvasculature. *Br. J. Exp. Path.* **67**, 55-63.