HORMONAL AND PHYSICAL INTERACTIONS WITH ACETYLCHOLINESTERASE AND CATIONS IN THE PORCINE BRAIN AND HYPOPHYSES IN A HOT HUMID CLIMATE

BY

DAVID OLUSOJI ADEJUMO
B.Sc. ANIMAL SCIENCE (IBADAN)
M.Sc. ANIMAL SCIENCE (IBADAN)

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ABSTRACT

Prenatal and Postnatal changes in the physiological development of the porcine brain were determined. The results indicated a decline in amniotic fluid volume with increased gestation length. Marked fetal brain and body development was observed between four and six weeks of gestation where mean embryo weight increased by 48,878% from four weeks to six weeks with a concomitant increase of 1,300% in mean fetal brain weight.

Acetylcholinesterase (AChE) activity in the amniotic fluid declined progressively with age ($r=-0.56$) while AChE activity in the fetal brain increased with gestation length ($r=0.88$). Total protein in both the amniotic fluid and in fetal brain did not correlate significantly with gestation length ($P>0.05$). Specific acetylcholinesterase (SAChE) activities in the amniotic fluid declined significantly between four and six weeks of gestation while activity in the fetal brain increased significantly from 5.12 ± 0.39 at 6 weeks to 21.54 ± 2.6 at 12 weeks.

Postnatally, AChE activity declined significantly with age in the pons, hypothalamus, midbrain, medulla and hypophyses while no significant changes were observed in the cerebellum, cerebral cortex and hippocampus. A significant rise was observed in the amygdala. Total protein increased significantly with age in all brain regions and hypophyses while SAChE activities declined steadily with age. Significant and Positive correlations were observed in the calcium and sodium content of the embryonic

brain while negative correlations were observed in the copper and zinc content. Postnatally, Positive correlations were observed in the calcium, magnesium, potassium, sodium, copper and zinc contents of the pons, cerebellum, medulla and midbrain.

The effects of castration at different ages and hormonal therapy on brain and hypophyseal physiology of pigs were also evaluated. Castration significantly depressed AChE activity in the cerebellum, amygdala, hippocampus, hypothalamus, midbrain and medulla in all age groups except at 7-8 months of age while testosterone maintained AChE activity at levels similar to controls. The cortex was not significantly affected except in the Pre-weaners where a depression was recorded. AChE activity in the adenohypophyses of testosterone-treated castrates and controls were similar and inferior to the untreated castrates. Protein levels of all the brain regions and hypophyses of boars were depressed by castration. In addition treated castrates were still inferior to the controls. A decline was also observed in the concentrations of calcium, sodium, potassium, copper and zinc in castrated boars. Exogenous Progesterone or estradiol administration also significantly depressed AChE activity in the cerebellum, amygdala, hippocampus, midbrain and the medulla with the Progesterone - induced depression being significanlty lower than that caused by estradiol.

Total protein in the brain regions of ovariectomized gilts was depressed by estrogen and progesterone but to varying extents. Progesterone tended to elevate magnesium and zinc in the amygdala,

hippocampus and cerebellum while estradiol facilitated retention of copper and potassium in several brain regions and the neurohypophyses.

Testosterone injection also significantly depressed AChE activity in all brain regions. However no significant differences were observed in the hypophyses. Testosterone further depressed total protein levels in the cerebellum, hypothalamus, cortex, medulla and elevated it in the pons. Testosterone injection in gilts also depressed calcium levels in all brain regions and hypophyses while causing a rise in the magnesium, zinc, and potassium levels in several regions.

Lastly, heat stress caused significant increases in respiratory rates and rectal temperatures of heat-stressed boars. Heat stress also elevated AChE and SAChE activities in the Pons, cerebellum, amygdala, hippocampus and medulla. No significant effect was found in the cortex. Total protein levels in the heat-stressed pigs were generally inferior to the controls but the amygdala and cortex were unaffected. Heat stress significantly increased calcium, potassium levels and depressed magnesium, zinc and copper levels in several regions.

Water deprivation also depressed AChE activity in the amygdala, medulla and hippocampus but no effect was observed in the cortex. Total protein levels were also depressed by water deprivation in several regions

whereas SAChE activities were elevated in water deprived animals above the controls. Water deprivation resulted in a decline in Calcium and Sodium levels of several regions while increases were recorded in magnesium, potassium and zinc concentrations in some brain regions and the hypophyses.

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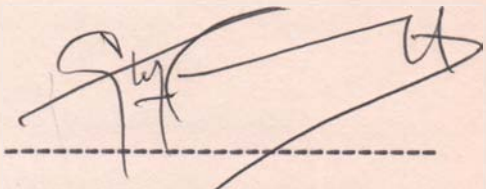
Upon all, I wish to thank God Almighty for giving me the good health and courage to carry the work through.

ADEJUMO D.O.

AUGUST 1983.

CERTIFICATION

I certify that this work was carried out by Mr. David Olusoji Adejumo in the Department of Animal Science, University of Ibadan.



Professor G.N. EGBUNIKE

B.Sc., Ph.D. (Ibadan)

Professor in the Department of

Animal Science,

University of Ibadan.

September 1983.

DEDICATION

This piece of work is dedicated to My mother, Mrs. C. Wuraola Adejumo, my Father, Rev. Cannon M. Oluyemi Adejumo and my brothers Babatunde (Sir T.) and Gboyega (Gbogus) Adejumo.

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CHAPTER ONE
INTRODUCTION

The demand for pork as a source of protein and energy for the growing world population requires that production be increased in regions which at first glance do not seem suitable for pig keeping and economic pork production. However, the limited ability of the pig to withstand stress presents an obstacle to the attainment of these goals.

About one third of the world's pig population is kept in the tropics at temperatures well above the comfort zone for sexually mature pigs. i.e. around 15°C (Steinbach, 1971). Also pigs have a higher body temperature and are less efficient than most other domestic animals in their rate of body heat loss. They are thus particularly susceptible to heat (Ingram, 1965).

Ibadan, located in the rain forest zone has a climate characterized by annual temperature of 26.6°C and Relative humidity of 71 percent.

Therefore, in order to make positive, practical suggestions to improve pig rearing in the tropics, a knowledge of the physiological reaction of pigs to changed environmental conditions is essential.

To survive, an animal must positively react to changes in its environment. This necessitates mechanisms for detecting such changes and putting the appropriate responses into operation.

The environment of an animal may be the chemical constituents or the external environment characterized by factors such as climate, disease, feed and management system.

While it is well established that the normal growth of an animal hinges on provision of well prepared feeds that are balanced in terms of energy, protein, vitamins, minerals and other additives, it is also well known that the proper functioning of an animal absolutely depends on a sometimes precarious balance between the circulating hormones inside it and the external environmental factors.

Thus it is not surprising that an animal that suffers from hormonal dysfunction may consume a lot of balanced feed and still be in a state of negative nitrogen balance. It is also clear that both the animal's internal environment as well as the external environment exert profound effects on the productivity of the animal.

Physiological studies have revealed that cells function very well within narrow limits. Quite small fluctuations in osmotic pressure, temperature or the amounts of chemical substances can disrupt biochemical activities and in extreme cases may kill the cells altogether.

It is now known that hormones and enzymes are of vital importance to the physiological integrity of the animal and do contribute to its productivity. It is also becoming increasingly apparent that nervous and endocrine systems both function to integrate the organism and are not so divergent and sharply delimited as was formerly assumed.

In addition, the products of the coordinatory systems take part in every bodily function and exert gross effects on the mental states and behavioural patterns of the individual, and that important actions may be exerted during developmental stages as well as in mature organisms.

Many endocrine glands, through their secretions affect the nervous system, on the other hand endocrine organs are frequently stimulated or inhibited by products of the nervous system.

A common physiologic attribute of these two systems is their ability to synthesize and release chemical agents or neurotransmitter that are capable of taking part in the chemical integration of the animals. thus specialized nerve cells produce transmitters that act as chemical messengers either locally e.g. Acetylcholine (ACh) at synaptic junctions or at a distance e.g. oxytocin from the neurohypophysis.

The brain, by virture of its position as being the most complex organ in the body enjoys considerable protection from abuse through the highly developed blood-cerebrospinal fluid barrier and the blood- brain barrier.

However it is also known that the brain is one of the least resistant organs to stress and the final collapse of the brain function due to stress also marks the death of the animal. It is also established that the brain plays a modulating role on all body functions and through its control on the hypophyseal hormonal pathways keeps the level of hormonal production and metabolism of the other glands in check.

The hypophyses, apart from being partly evolved from the

hypothalamus is wholy subservient to the brain. It secretes at least nine hormones, six of which directly monitor the release of other endocrine glands concerned with the production of sex-hormones, growth and body metabolism. It is able to perform such a delicate and complex duty by means of its classic short-loop and long- loop feed back systems.

There can no longer be any doubt that the relationship between the nervous and endocrine systems is one of reciprocity. The modern view is that the brain is not only an endocrine organ, but also a hormone target.

These studies were therefore aimed at

(1) Investigating the development of the pig fetal brain during gestation and after birth to four months of age.

(2) Evaluating the effects of castration and ovariectomy on certain brain and pituitary gland functions. The study was further extended to evaluate the effect of hormonal replacement therapy on such functions.

(3) Evaluating the effects of testosterone on the brain and pituitary gland function in intact gilts.

(4) Investigating the effects of environmental stress such as heat exposure or water deprivation on the brain and pituitary gland functions.

The parameters studied were:

(i) Acetylcholinesterase (AChE) activity

(ii) Protein concentration (iii) Cations concentration (Calcium, Magnesium, potassium, Sodium, Copper and Zinc).

The tropical climate is essentially stressful and has direct effect on the productivity of the animal.

In addition, the levels of circulating hormones, particularly the gonadal steroids have been known to adjust in response to stimuli arising from the exterior as well as from within the organism. It is thus essential that effects of these hormones and of their absence on AChE activities of the brain regions and hypophyses be monitored.

The brain has been divided into certain regions because the organ itself is anatomically and functionally divided into many parts and although the functions of some brain regions overlap each other there is considerable evidence in support of the fact that there is marked differential distribution of enzymes, proteins, minerals and other chemical constituents in these regions.

The protein and mineral(cations) concentrations of these brain regions and hypophyses were also monitored because environmental stimuli and hormones have been known to exert some effects on protein and mineral metabolism in the rat brain. Furthermore, the brain contains complex enzyme systems and these metals are either contained in the enzyme or are activated by them and in some cases the requirement is specific for a particular metal such that the removal of the metal completely inactivates the enzyme.

The metals are also essential in the transmission of nervous impulse and their absence abolishes nervous transmission.

Where possible, the study has been sex-specific because studies on animal behaviour have indicated sex differences within animals.

Arnold, (1980) defined this "organizational hypothesis" as a quantitative or qualitative difference in the synaptic contacts made among neuronal populations involved in a behaviour.

For purposes of clarity the studies have been reported in five chapters viz:

Chapter three in the first part deals with the Ontogenic changes in AChE activity and mineral concentrations of the embryonic brain from four to twelve weeks of gestation.

The second part deals with the development of the same parameters in the pig brain from birth to four months of age.

Chapter four investigates the effects of prepubertal and post pubertal castration with or without testosterone therapy on the brain and hypophyseal AChE activity and cations.

Chapter five considers the effects of ovariectomy with or without estradiol or progesterone therapy or the brain and hypophyseal AChE activity and cations in the gilt.

Chapter six reports the effect of testosterone on the brain and hypophyseal AChE activity and cations in the gilt.

Chapter seven deals with the effects of environmental stress on brain and hypophyseal AChE activity and cations. This is reported in three sections.

The first two sections deal with short-term heat exposure of boars to sunlight while the third investigates the effects of acute water deprivation on the brain and hypophyseal parameters.

CHAPTER TWO

INTRODUCTORY LITERATURE REVIEW

2.1.1 THE BRAIN: ANATOMICAL DIFFERENTIATION AND FUNCTIONS

For the purpose of this study, certain brain regions would be considered.

The brain is an enlongate, oval body, widest in the caudal third (Fig. 2.1)

It is anatomically and functionally divided into many parts but for the purpose of this study the regions investigated were:

1. Pons
2. Cerebellum
3. Amygdala
4. Hippocampus
5. Hypothalamus
6. Cerebral cortex
7. Mid Brain (mesencephalon)
8. Medulla oblongata.

The heterogeneity of cell types and regional differences in the brain have a major influence on the movement of materials in and out of brain tissues and the functions of the various regions overlap each other.

THE DIENCEPHALON: It includes the epithalamus, subthalamus and hypothalamus.

The hypothalamus lies below or ventral to the thalamus and forms the floor and part of the inferior lateral walls of the third ventricle.

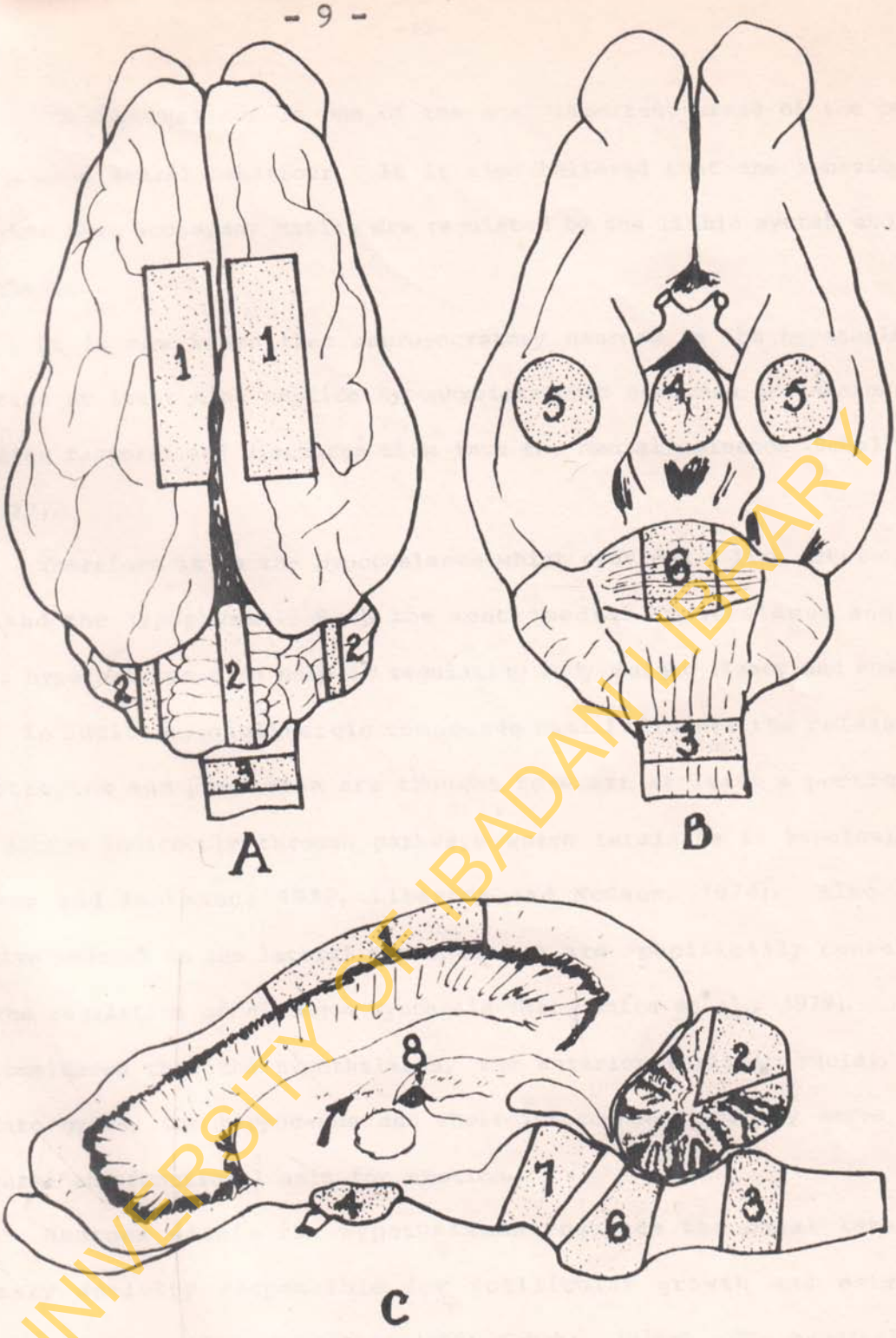


Fig. 2.1. Diagramatic representation of the porcine brain. A: Dorsal view. B: Ventral view. C: Midsagittal view. 1: Cerebral cortex; 2: Cerebellum ; 3: Medulla oblongata; 4: Hypothalamus; 5: Amygdala; 6: Pons Varoli; 7: Mesencephalon (Midbrain); 8: Hippocampus with the arrow showing the point and direction of tracing from the septum pellucidum (From Egbunike 1981)

The hypothalamus is one of the most important areas of the brain and regulates sexual behaviour. It is also believed that the behavioural components that accompany mating are regulated by the limbic system and the hypothalamus.

It is now known that neurosecretory neurons in the hypothalamus synthetize at least nine peptide hypophysiotrophic hormones (releasing and inhibiting factors) and discharge them into the medial eminence (Schally et al., 1977).

Therefore it is the hypothalamus which provides a link between the brain and the hypophyses. Both the ventromedial hypothalamus and the lateral hypothalamus also help in regulating body weight (Keesy and Powley, 1975). In addition, cholinergic compounds that influence the release of gonadotropins and prolactin are thought to exert at least a portion of their action indirectly through pathways which terminate in hypothalamus (Hahlweg and Jonkmann, 1932, Liberton and McCann, 1974). Also ACh-sensitive neurons in the lateral hypothalamus are specifically concerned with the regulation of glucagon synthesis (Matsushita et al., 1979). It is also considered that the hypothalamus, the anterior thalamic nuclei, the cingulate gyrus, the hippocamus and their interconnections may serve as a structural and functional unit for emotion.

Neurons within the hypothalamus regulate the basal level of pituitary activity responsible for folllicular growth and estrogen secretion (Elerko, 1966; Everetts, 1969; Gorski, 1971a). The hypothalamus is also involved in the control of pain.

2.1.2 THE BRAIN STEM

The brain stem is defined anatomically to include the medulla oblongata, pons and midbrain (Mesencephalon)

2.1.2.1 THE PONS

The pons lies ventral to the cerebellum and anterior to the medulla from which it is seperated by a groove through which the abducen, facial and acoustic nerves emerge. The AChE-containing fibres of the fore brain are assumed to be derived in large part from the brain stem. These form the ascending cholinergic reticular system. Lewis and Shutte (1967) demonstrated the presence of considerable AChE-containing fibres in the rat hippocampus, brain stem, medial septa, mid brain to the thalamus, hind brain (cerebellum) cortex and amygdaloid bodies through various pathways (Gerebtzoff, 1959, Koelle, 1950).

The pons integrates basic reflex behaviour and cruder intellectual functions. Hence damage to the pons usually results in facial paralysis.

2.1.2.2 MEDULLA OBLONGATA

It is the pyramid-shaped portion of the brain stem between the spinal cord and the pons. The lower half contains a central canal.

The medulla oblongata together with the pons are divided into the motor and sensory pathways. The medulla oblongata maintains nervous co-ordination and muscle tone. It also co-ordinates reflexes concerned with respiration, swallowing, vomitting and cardiovascular controls. Other functions include regulation of blood pressure, salivation and response due to stimuli.

2.1.3 THE LIMBIC SYSTEM

The limbic system is also referred to as rhinencephalon and it includes the amygdala, hippocampus, cingulate gyrus and septum.

The amygdala is a small spherical gray mass located in the roof of the terminal part of the inferior horn of the lateral ventricle.

The hippocampus extends along the interiomedian aspect of the temporal lobe from the area of the splenium of the corpus callosum to the Uncus (see Fig. 2.1).

The limbic system has been demonstrated to influence gonadotropin secretion, the stimuli arriving from the amygdala being facilitatory and those of the hippocampus inhibitory (Koikegami et al., 1954, Bunn and Everett, 1957; Velasko and Taleisnik, 1969a, b, Kawakami, et al., 1971).

Injection of cholinergic agents into either the hippocampus or the amygdala can induce seizures not produced by injections of control drugs (Grossman, 1963; McLean, 1955).

Hippocampus pyramidal cells receive a cholinergic input from the septum. They also project back to the septum where they contribute to the feed back regulation of the septal cholinergic neurons (Lewis et al.,1967).

Glick et al (1973) also postulated that newly synthetized ACh released at the hippocampal synapse is essential for mice to learn a passive avoidance response. The hippocampus forms one of the richest cholinergic structures in the brain (Lewis and Shutte, 1967). The amygdala exerts a modulating-influence on the hypothalamic-hypophyseal system for the secretion of certain trophic hormones (Koikegami et al., 1954).

2.1.4 **THE BRAIN**

It is also known as mesencephalon. It is a short portion of the brain between the pons and cerebral hemispheres. The mid brain controls the eye muscles, involuntary movements and posture.

2.1.5 **CEREBRAL CORTEX**

The cerebral cortex controls all sensory, (olfactory, sight, smell etc.) and memory functions. It also controls all co-ordinated movements which include control of voluntary movements of the skeletal muscle. AChE is particularly active in the cingulate cortex and part of paleocortex - which are regions which regulate behaviour (Gerebtzoff, 1959).

It is of interest to note that depriving the eyes of light reduces the amount of AChE in the retina (Glow and Rose, 1964) while a visually enriched environment combined with training in visual problems results in an increase in the cortical content of AChE (Holoway, 1966). Behavioural stimulation also depletes AChE activity in the visual cortex (Krech et at., 1966).

2.1.6 **CEREBELLUM**

The cerebellum is a great oval folded dorsal expansion of the hind brain. It is located in the posterior fossa of the skull and seperated from the overlying cerebrum by an extension of dura matter, the tentorium cerebili.

The cerebellum keeps the individual oriented in space by controlling balance and maintenance of posture, equilibirum and fine adjustments of movements. It also controls the anti-gravity muscles of the body (Chusid, 1970).

Lewis and Shutte, (1967) demonstrated presence of considerable AChE activity in the rat hind brain and cerebellum among other brain parts.

2.2 THE HYPOPHYSES

The pituitary glands or hypophyses are joined anatomically to the hypothalamus by a slender stalk and are believed to be subservient to and to have partly evolved from the hypothalamus.

The hypophyses consists of an adenohypophysis and a neurohypophysis and these two subdivisions are distinctly different in embryonic origin and in histological composition.

The hypophyses secrete at least nine hormones; six of these hormones are from the adenohypophysis. They all exert their effects indirectly by stimulating the functional activities of other endocrine glands.

The hormones of the neurohypophysis (Vasopressin and oxytocin) are the products of hypothalamic secretory cells and are stored and released from the pars nervosa.

All neurons within the hypothalamus regulate the basal level of pituitary activity responsible for gonadotropin secretion (Elerko, 1966; Everett, 1969).

Apart from the gonadotropins, the adenohypophysis secretes two hormones; Adrenocorticotrophin (ACTH) and Thyrotrophin (TSH) which are useful parameters of emotional disturbances such as fear, anxiety (Dupont et al., 1973), or response to cold stress or non-specific stress (Fortier, 1973). For instance, Dupont et al (1971) a, b) found a positive correlation between passive avoidance learning and the plasma corticosterone concentration, used as an index of ACTH release in rats.

Contrariwise, active avoidance learning proved inversely related to plasma corticosterone level and positively related to the plasma TSH concentration.

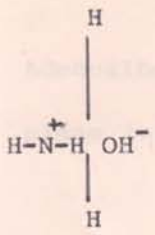
It is also well known that the maintenance of a basic level of gonadotropin secretion depends on the adenohypophysis which in turn depends on its connection with the hypothalamus.

The developmental changes of several enzyme and hormone systems in the rat brain from fetal life to birth and onwards with particular emphasis on their contributions to sexual differentiation have been investigated by a number of workers.

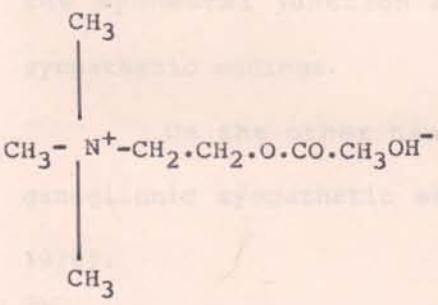
Before the roles of these enzymes and hormones are reviewed it is necessary to review the role of the enzyme investigated in this study.

2.3 THE TRANSMITTER SUBSTANCE

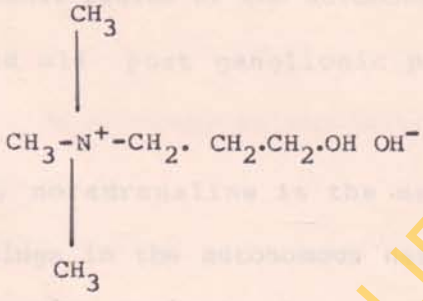
The transmitter substance at the majority of synapses is acetylcholine (ACh) which is the acetyl derivative of chlorine which may be considered to be a substituted ammonium hydroxide compound thus:



Ammonium hydroxide



Acetyl choline



choline

Acetycholine has been found to be present in three distinct subcellular compartments namely in nerve endings, axons and bodies of nerve cells (de Robertis, 1964).

A further candidate for consideration as a transmitter in the central nervous system on the grounds of its presence in brain extracts is noradrenaline.

Nerve fibres which form and release acetylcholine as transmitters are called cholinergic while those which form and release an adrenaline-like substance (now known to be non-adrenaline) are known as adrenergic.

2.3.1 BIOSYNTHESIS OF ACETYLCHOLINE

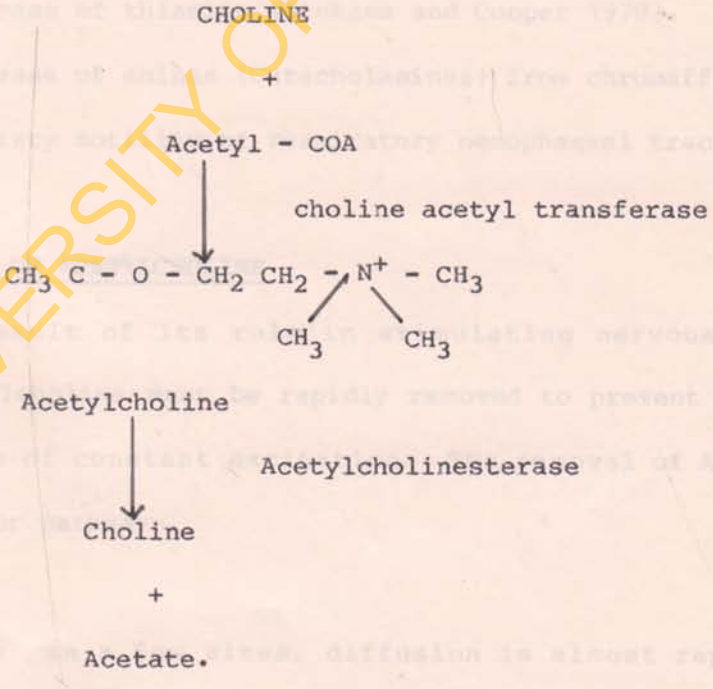
Acetylcholine is synthesized by a specific enzyme choline acetylase or choline acetyltransferase which occurs in all cholinergic neurons.

In addition to choline acetylase, free choline, acetylco-enzyme A, Adenosine triphosphate (ATP) and glucose are required. The synthesis takes place in the mitochondria (Ganong, 1979).

Acetylcholine is therefore the mediator at all synapses between preganglionic and postganglionic fibres of the autonomic nervous system, at the myoneural junction and all post ganglionic para-sympathetic and sympathetic endings.

On the other hand, noradrenaline is the mediator at most post ganaglionic sympathetic endings in the autonomous nervous system (Ganong, 1979).

The mode of action of ACh can either be muscarinic or nicotinic depending on the ganglionic location of the cholinergic neurons. The biosynthesis and catabolism of ACh is as shown below:



2.3.2 GROSS FUNCTION OF ACETYLCHOLINE

Acetylcholine has been known to be responsible for aggressive behaviour in cats and local infusion of ACh produces renal vasolidation in rat kidney (Daugherty et al., 1968).

ACh also causes excitation of the heat production pathway with inhibition of the heat loss pathway (Findlay and Thompson, 1968). This action is also considered to be temperature regulatory and is discussed in more detail in subsequent chapters.

Newly synthesized ACh appears to be more readily released on nerve stimulation than depot or stored ACh. About half of the choline produced by AChE activity is re-utilized to synthesize new ACh (Collier and Ilson, 1977).

Other extra-neurotransmitter activity of ACh are:

1) Stimulation of inorganic phosphate into phospholipids.
2) Release of thiamine (Itokama and Cooper 1970).
3) Release of amines (Catecholamines) from chromaffin cells.
4) Ciliary motility of respiratory oesophageal tracts.

2.3.3 REMOVAL OF ACETYLCHOLINE

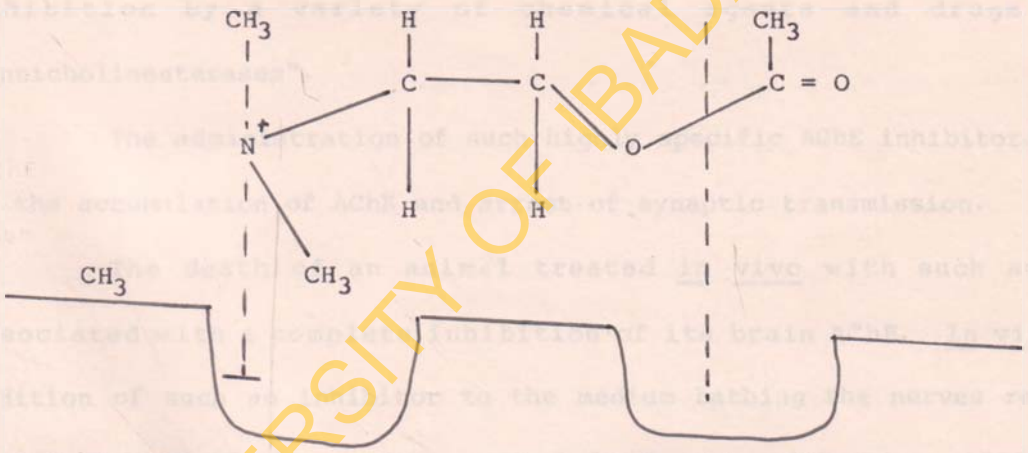
As a result of its role in stimulating nervous impulses in organisms, acetylcholine must be rapidly removed to prevent the body from being in a state of constant excitation. The removal of ACh is brought about by two major pathways.

1) **DIFFUSION:** In a few sites, diffusion is almost rapid enough to account for the rate of decay in action of ACh. However, in most sites, there is a barrier to free diffusion of ACh.

2) **ACETYLCHOLINESTERASE (AChE) HYDROLYSIS OF ACh:** AChE is a glycoprotein enzyme (Leuzinger and Baker, 1967, Ciliv and Ozano, 1972) and is partly associated with the smooth endoplasmic reticulum of cholinergic axons (Kasa, 1970; Tennyson et al., 1968, Somogyi et al., 1975). The mode of action of AChE involves the hydrolysis of ACh into its physiologically inactive products: acetate and choline.

2.3.4 STRUCTURE OF ACETYLCHOLINE

Two separate areas can be distinguished on the active surface of AChE. These consist of an anionc (N^+ ATTRACTIVE) site and an esteratic (ester-binding) site. (Augustinsson, 1963). The structure is given below:



The anionic site is capable of coulombic interaction with the trimethyl ammonium group of acetylcholine and this interaction facilitates a favourable orientation on the enzyme surface so that the hydrolysis of ACh is carried out by the esteratic site.

The hydroxyl of serine is the main functional group of the esteratic site (Kabachuik et al. 1970).

Since it is also known that the functional groups of the esteratic and anionic sites that react directly with the substrate are probably very similar, if not identical in the two main types of cholinesterases: Specific ChE (E.C. 3.1.1.7) and Pseudo ChE (E.C. 3.1.1.8), it is therefore logical to suppose that the type of cholinsterases is determined not by differences in the structure of the active centres but by some differences in the structure of various areas around the active centres.

2.3.5 MAJOR FACTORS AFFECTING AChE ACTIVITY

The following factors have been known to influence AChE activity:

INHIBITORS

One of the most fruitful studies of AChE has centered on its inhibition by a variety of chemical agents and drugs called "anticholinesterases".

The administration of such highly specific AChE inhibitors results in the accumulation of AChE and arrest of synaptic transmission.

The death of an animal treated *in vivo* with such agents is associated with a complete inhibition of its brain AChE. *In vitro*, the addition of such an inhibitor to the medium bathing the nerves results in an alteration of the action potential (Mazur and Harrow, 1966). Such inhibitors include physostigmine (eserine) and neostigmine which are reversible inhibitors.

Diisopropyl fluorophosphate (DFP) is an irreversible inhibitor and is the most powerful inhibitor even at concentrations as low as 1×10^{-10}M.

It is also a powerful nerve poison but the kidney possesses a mechanism for detoxifying it (Mazur and Harrow, 1966).

2.3.6 GROSS EFFECTS OF AChE INHIBITION

Since the destruction of AChE prolongs the effects of ACh, persons suffering from AChE inhibition may exhibit convulsive contractions of all muscles upon the slightest stimulation.

AChE inhibition in rats reduced food intake, and increased the level of water intake (Adams, 1983). It also reduced the level of spontaneous activity.

Cholinesterase inhibition also produced increased sexual behaviour with induced aggressive behaviour in conscious rats (Hoyland et al., 1970; Ferguson et al., 1970) and also increased locomotor activity (Fibiger and Campbell, 1971).

Inhibition by eserine has been known to cause gross emotional, autonomic and motor phenomena such as agitation, fear-like responses, anger, rage, itching, respiratory embarrasment, salivation, ataxia, tremor, circling and clonic-tonic convulsions in rabbits (Beleslin et al., 1973).

Studies by Kozar et al (1976) indicate that recovery of AChE activity in some brain areas after chemical depression preceeds the return of the relevant behaviour to base-line level.

CHAPTER THREE

PRENATAL AND POSTNATAL CHANGES IN THE DEVELOPMENT OF THE PORCINE BRAIN

LITERATURE REVIEW

3.1. DEVELOPMENT OF FETAL BRAIN DURING GESTATION

The somatic stability or vigour of newborn animals is associated with their physiological development during pregnancy.

Several workers have emphasized the need for a study of the changes taking place in the body composition of pig fetuses. Such knowledge enhances correct feeding of the pregnant sow as well as that of the piglets after birth.

Warwick (1928), Mitchell et al (1931), Urbany (1952) and Pomery (1960) have made detailed studies of fetal weight gains during pregnancy.

Their results indicate that very little fetal weight gains occur up to the 50th day of pregnancy. More recent result from Padalikova etal (1972) and Newland and Davis (1974) indicate that by the 80th day of pregnancy, the fetus weighs up to 30 - 40% of its final weight. They further linked this with a very rapid development during the last 30 days of intra uterine life. On the other hand, Day (1972) reported that nearly complete morphologic development takes place around mid-gestation.

3.2. NEONATAL DEVELOPMENT AND SEXUAL DIFFERENTIATION OF THE BRAIN

Information is still very scanty on the metabolic activities occuring in the brain of the fetal pig. However, some considerable

amount of work has been done on other species particularly the rat. This may not however be consolatory because the work of Reis et al (1977) indicates that some of these events during fetal growth are species-specific and are not paralleled by observations made in other species.

For instance, McEwen (1978,a,b) observed that during the late fetal life in the rat, the brain undergoes a series of changes which appear to be related to its susceptibility to hormones in undergoing sexual differentiation. These include:

(1) Final cell divisions of neurons of hypothalamus and the preoptic area.
(2) Onset of testosterone secretion
(3) Appearance of the enzymes involved in testosterone metabolism and
(4) Perinatal appearance and increase in the concentration of steroid receptors.

The research also revealed that although testosterone is the major secretory product of the testes involved in brain sexual differentation, It does not seem to be testosterone which actually brings about sexual differentation but rather a metabolite produced in some cases within the brain itself.

The brain also contains enzymes for producing the two major metabolites of testosterone which are 5 dihydrotestosterone (DHT) and estradiol.

In the guinea pig, in the early fetal life, the cerebral cortex contains low but constant concentrations of respiratory enzymes e.g. Cytochrome C.

The concentration of these enzymes increase sharply at the time of morphologic differentiation and the onset of electrical activity in the nerve cells. The adult level is reached or approximated at birth. The same pattern between functional development and brain enzyme concentrations was observed with cholinesterase and carbonic anhydrase.

Colenbrander et al(1978) during their study with the pig observed that fetal serum testosterone concentrations are elevated between 40 to 60 days post coitum and decreased between 60 to 100 days.

Moon and Hardy (1973) however reported that in the fetal pig, the leydig cells are highly differentiated between 35 to 40 days post coitus.

However, it has not actually been established that the same hormone is responsible for sex differentiation of the brain in all animals, e.g in song birds, androgens(and presumably androgen receptors) are involved in the differentiation of the brain's capacity to produce song (Nottebohm, 1980).

Yet in the quail, estrogens may be involved in the differentiation of reproductive behaviours (Adkins, 1975, Hutchson, 1978). In certain mammals, (the guinea pig and rhesus monkey) the androgen pathway appears to be essential for sexual differentiation of masculine sexual behaviour (Gold foot etal 1975) where as the estrogen pathways appears to be involved in masculinization of hamsters (Paup etal 1974) and in rats(Booth, 1978).

Thus with respect to the suppression of feminine characteristics

(defeminization), the estrogen pathway appears to be of primary importance in a number of mammalian species (guinea pig and rat), (Gold foot etal 1975, McEwen etal 1977).

The involvement of one of these two pathways is based upon a variety of evidence. First the efficacy of DHT in producing brain sexual differentiation is an index of androgen pathway involvement. Since DHT is not aromatizable, the efficacy of estrogens in producing sexual differentiation in brain is an indication of estrogen pathway involvement.

Second, the action of various inhibitors provides complementary data to the actions of the various antagonists although there are complications in the use of inhibitory drugs. For the androgen pathway, the anti-androgen flutamide (Neri, 1977) and Cyprotene acetate (CA)(Neumann and Steinbeck, 1974) are effective antagonists. Progesterone, a preferential substrate for 5- reductase (Massa and Martini, 1971) may also have anti-androgenic activity.

The responsiveness of developing neural tissue to androgens and estrogens is determined by the appearance of receptor systems during the perinatal period. The estrogen receptor system of the rat brain becomes detectable about fetal day 17(Vito etal, 1979) and increases rapidly during 1 or 2 days before birth and in the 5 or 6 days right after birth (Maclusky etal 1979 a,b). This increase coincides with the onset of the critical period for the defeminizing aspect of rat brain sexual differentation and it may represent a critical and even rate-limiting step in this process (McEwen, 1980 b).

The progestin receptor system is below the limits of detection in the rat brain at birth but increases rapidly during the first 10 days of life in parallel with the estrogen receptor system (Maclusky and

McEwen, 1980). Thus these receptors are present during the time that progestins have their blocking action over Exogenous estrogen(E2) and Testosterone(T)-induced sexual differentiation (Kincl and Maqueo, 1965, McEwen etal, 1979). By the end of the first 10 days of life, estrogen inducibility of the progestin receptor system is begining to emerge (Maclosky and McEwen, 1980).

Evidence from research on mammlian brains show that the peak of cell divisions of hypothalamic and preoptic area neurons occur prior to fetal day 17, which is also before the critical period (Ifft, 1972). This implies that changes involved in sexual differentiation are located in these two brain areas. However, it is inadisable to rule out changes in other brain regions in which some cell division may be occuring during the critical period.

The pioneering work of Dorner and Stavdt (1968, 1969) and more recent work (Staudt and Dorner, 1976) have indicated the presence of anatomical sex differences in rats with reports that there is a sex difference in the size of nuclei in neurons in the preoptic area, the anterior and ventromedial hypothalamus and amygdala. Dyer and his co-workers (1976) using electrophysiological techniques demonstrated that in intact male rats, neurons of the preoptic area which project to the medial basal hypothalamus receive more synaptic connections from the amygdala than do similar neurons in intact females. Hence neonatally- castrated males are similar to intact females in this regard and neonatally androgenized females are intermediate between males and females.

Raisman and Field (1971, 1973) provided the first siginificant anatomical evidence for an organizational sex-difference in the brain. They found a sexual dimorphism in the number of synapses on dendritic spines stemming from non-amygdaloid afferents in the preoptic area of

the rat. This difference was reversed by neonatal androgenization of females or neonatal castration of males.

A second dimorphism in the dorso medial preoptic area has also been reported by Greenough etal (1977) who detected a sex difference in the topographical distribution of golgi-stained dendrites in the hamster which can also be manipulated appropriately by neonatal steroid treatments.

Ryan and Arnold (1979) found that there are sexual differences in topographical distributions of AChE and catecholamines in the brain. Such findings were the first step toward using histochemical and biochemical measures of cholinergic and catecholaminergic function to study the normal development of this area and how to explore how certain experimental interventions disturb this normal development. It is however the view of Gorski (1973) that the production by the neonatal testes of a substance, presumably androgen is the factor which determines the course of development of the brain.

3.3. MINERAL COMPOSITION OF THE FETUS.

Few studies have been made of the mineral content of pig fetuses. Mitchell etal (1931), Urbany (1952), Salmon-legagneur (1968) all found an increase in the crude ash content of the pig fetus from the early fetal life to 112th day of pregnancy. Mitchell etal (1931) also reported an increase in calcium content with intrauterine age. During the period, calcium content increased steadily from 0.26g/kg wet matter at 31 days to 2.5-2.7g on 56th day reaching 3.6g on 70th day and suddenly doubling to 8.2g on the 98th dday. Shortly before parturition, the level rose to 10.9g/kg wet matter.(wm)

The same trend was recorded for phosphorus. Sodium concentration

was found to decrease with gestation length while potassium content rose (Pomeroy, 1960). Magnesium in the 43-day old fetus was 0.11g/kg wm (Urbany 1952) and tripled by 113th day to 0.31g/kg wm (Pomeroy 1960). Nitrogen (as on index of protein content) increased rapidly between 30 days to 45 days of pregnancy stabilizing thereafter till the 90th day after which it increased till parturition time.

Hurley (1981) found that fetuses with zinc deficiency during prenatal development results in fetal abnormalities.

3.4. POSTNATAU DEVELOPMENT OF THE PIG BRAIN AND HYPOPHYSES

In the adult guinea pig brain, metabolic activity is highest in the cerebral cortex and cerebellum.

The high energy requirement of most pontions of the brain is related to the transport of ions, the synthesis of ACh and the metabolism of glutamic acid.

Report by Reis etal(1977) indicate that age-dependent changes occur in the concentrations of neurotransmitters in the brain and the adrenals of the rat. Further study also indicated that some of these changes during aging may be a species-specific event. Cotman etal(1978), found that AChE activity and AChE levels in the hippocampus of both young and adult rats are similar.

A contradictory report by Schreff etal(1980) states that a progessive decline of AChE activity occurs in the rat brain regions with age.

According to Curtis etal (1967), developmental age may not necessarily coincide with chronological age.

There is also a conflict as to the relationship between birth weight and chemical maturity. Curtis etal (1967) found negative correlation between the two while Widdowson (1950) found a positive correlation and Pomeroy (1960) could not detect any significant correlation.

Halasz etal (1968), 1971, a,b) showed that the development of the rat brain takes place to a great extent after birth and also that a large number of cells in the cerebellum and cerebrum are formed after birth.

Age-related studies on the endocrine system suggest that the endocrine system is involved in the ageing process(Ascheim, 1976, Dilman, 1970, Mills and Mahesh, 1978, Finkelstein etal 1972).

However, Morrison etal(1981) disagreed with these views when he reported an increase in growth hormone release in rams with increasing age. An interesting comparison between chronological development in humans with that of sheep was carried out by Broody (1945) who calculated that 28 months of age in sheep is chronologically equivalent to 24 years of age in humans.

The pineal gland and the amygdala have been implicated in playing some role in puberty (Relkin, 1971).

Colenbrander etal (1978) observed a rise in serum testosterone level in the pig from birth till the 3rd week of age declining thereafter until the 18th week when it started rising again. This rise continued until the second month followed by another decline probably accompanied by other biochemical and morphologic changes.

The dependence of AChE system on age has been discovered for some

time (Moudgil and kanungo, 1973) and Davies(1979) observed a decline in AChE activity in human brain with age.

AChE activity of cerebral hemisphere of normal rat is highest at 9 weeks and decreases thereafter. No change was reported in the cerebellum (Moudgil and kanungo, 1973). They further postulated that since the 9-week old rat is still in the learning phase, a higher activity of AChE at this stage may facilitate the learning process. The decrease in the enzyme activity in the adult and old rats may also be due to a loss of nerve cells. Also, the rate of synthesis of the enzyme may decrease after the growth period.

The constant different activity level recorded in the cerebellum suggests a differential metabolic activity of different parts of the brain as a function of age. In pigs, the establishment of synaptic junctions and glial cell multiplication followed by myelination takes place during the period of "brain growth spurt" which in pigs occur during the first five post-natal weeks (Davison and Dobbing, 1968). Kova'cs(1971) also noted that the rate of protein synthesis in rat brain varies with age. In the cerebrum and cerebellum, the highest rate was followed by a decrease. Moudgil etal(1973) observed peak levels of AChE in the cerebral cortex of rats at 9 weeks of age followed by a 50% decrease at 29 weeks and 65% decrease thereafter.

3.5. BODY MINERAL COMPOSITION OF THE PIG FETUS

Brooks etal (1962) found positive correlations between potassium concentration, ether extract, ash and protein content with developmental maturity. potassium content (Curtis etal 1967) has been suggested as an indication of the fat-lean composition of the whole body because they observed a positive correlation between potassium and moisture, lean content and protein concentration and a negative correlation with fat content.

Calcium fluctuates although it tends to decrease with increasing body weight (Freese 1958). Other minerals were found to be fluctuating (Manners and McCrea, 1963, Kirchgessner and Kellner, 1972 and Spray and Widdowson, 1950).

MATERIALS AND METHODS.

Two experiments were conducted. The first was concerned with the development of the brain and hypophyses functions during gestation while the second looked into the postnatal changes in the same parameters.

3.1.1. ANIMALS AND MANAGEMENT

The Large White pigs used in these experiments were already adapted to the climatic conditions of Ibadan, housed in dwarf-walled, concrete- floored, corrugated iron-roofed pens and generally managed as already described (Egbunike and Steinbach, 1972).

In the first experiment, 15 sows bred and ascertained to be pregnant were slaughtered 4, 6, 8, 10, and 12 weeks post-coitum. Each slaughter group comprised three sows. After slaughter, the reproductive tracts were removed in toto and taken to the laboratory for processing.

In the second experiment, 40 Large White piglets were selected at birth with both sexes equally represented. They were allowed to remain with their dams with no creep feeding until they were weaned at five weeks (except those that were sacrificed at day old).

Thereafter, they were fed with grower's ration ad libitum (Egbunike, 1973). Four piglets per sex were slaughtered at day-old, one month, two months, three months and four months of age.

3.1.2. TISSUE PROCESSING.

After the animals in the first experiment had been slaughtered, the whole reproductive tract was weighed, opened longitudinally and amniotic fluid collected into sample tubes. Thereafter, the embryos were removed and separately homogenized whole (4-week-old embryos). With older embryos, the brain was carefully dissected from the Skull, freed from adhering meninges and blood clot and homogenized whole (1% w/v) in 0.1m ice-cold phosphate buffer (PH 7.4) using a potter-Elvehjem glass-glass homogenizer.

With the animals slaughtered postnatally, the heads were quickly sawn open and samples were taken from the following regions of the brain as described by Egbunike(1981). Hypothalamus, cerebellum, Cerebral cortex, amygdala, midbrain (Mesencephalon), pons, hippocampus and medulla oblongata. The adenohypophyses and neurohypophyses were also removed for processing. All brain and hypophyseal samples were then homogenized (1% w/v) as stated earlier. To ensure minimal decline of enzyme activity, all samples were analysed within 5 hours after homogenization. for the determination of the minerals, aliquots of samples were stored frozen at -20°C for analyses.

3.3.1. ACETYLCHOLINESTERASE ASSAY

The method used was a modification of the colorimetric method of Ellman etal (1961) which measured the rate of hydrolysis of acetylthiocholine iodide substrate to thiocholine and acetate using 5:5-dithiobis-2-nitrobenzoate (DTNB: Aldrich chemical company) as the colour reagent.

TEST PRINCIPLE

(1) $H_2O + (CH_3)_3NCH_2CH_2SCOCH_3 \xrightarrow{AChE} (CH_3)NH_2CH_2SH + CH_3COO^-$
Acetylthiocholine → Thiocholine acetate

(11) $(CH_3)_3 NCH_2 CH_2S^- +$ DTNB 2-nitro-5-mercapbenzoate
thiocholine.

3.3.2. PROCEDURE

The reaction mixture in a glass cuvette contained 2.6 ml of 0.1m phosphate buffer, 0.4ml of the tissue homogenate and 0.1ml of DTNB. The cuvette was then inverted with the cap on to mix the contents and inserted in an Eppendorf Photometer (1101M) as a blank.

The cuvette was then taken out and the reaction started by adding 0.02ml of 0.075M substrate(sigma) to the mixture.

The contents were then quickly mixed and the increase in absorbance over 4 minutes was measured at 405nm.

The rate of hydrolysis was calculated as follows:

$$R = \frac{A}{1.36(10^{-4})} \times \frac{1}{(400/3120)} = 5.74(10^{-4})\, A / CO$$

Where R = Rate of hydrolysis per minute.
A = change in absorbance per minute.
CO = Original concentration of tissue (10mg in 1ml)

With the dilution rate used, the change in optical density was multiplied by a constant 14.35 to give the AChE activity as Umole/g wet weight/min.

3.4.1 DETERMINATION OF TOTAL PROTEIN

The biuret method of Weichselbaum (1946) was used. Test Principle: Protein forms a coloured complex with cupric ions in an alkaline medium.

Procedure:

5.0 ml of the biuret solution was added to a clean class test-tube followed by 0.1ml of the tissue homogenate. The contents were then mixed and incubated for 30 minutes with occasional shaking at a room temperature of about 25°c.

A glass cuvette containing about 3ml of the biuret solution with 0.1ml of distilled water was used as a blank. About 3ml of the test sample was poured into a clean glass cuvette and the absorbance measured against the reagent blank at Hg 546hm.

The protein concentration (C) of the sample was determined by multiplying the absorbance by a constant 19 obtained from a regression curve and was expressed in g/100 ml.

3.5.1 CALCULATION FOR SPECIFIC AChE ACTIVITY

The AChE activity of the sample was divided by its

total protein concentration to give the specific AChE activity in Umole/g protein/min.

3.6. MINERAL ANALYSES

The mineral contents of the samples were determined by the standard procedures of Willis (1961, 1962), David(1958,1960) and Gatehouse and Willis (1961). Calcium, Magnessium, Copper, Iron and Zinc were determined by flaming in a Perkin-Elmer atomic absorption spectrophotometer 703 using different lamps. Sodium and Potassium were determined by a Corning 400 flame photometer.

All assays were expressed in parts per million (ppm or mg/litre).

3.7. STATISTICAL ANALYSIS

All results were subjected to a multi-factor analysis of variance by a digital stored computer. Treatment means ± standard error of means (S.E.M) were compared using the studentized least significant difference method (Steel and Torrie, 1960).

Where necessary, correlation and regression analyses were also carried out relating AChE activities, Protein concentrations and cation levels with gestational age.

3.8. RESULTS

A decline in amniotic fluid volume with gestation was observed from about ten weeks onwards. At four weeks of gestation, the brains of the fetuses could not be dissected out, hence, the whole fetus was

used for the biochemical assay. It was also observed that fetuses at the upper end of the uterine horns (near the uterotubal junction) were slightly heavier than those at the cervical end but these differences were not significant (P>0.05)

By the sixth week of gestation, the fetuses had developed enough for the brains to be taken out and by 8 weeks, they could be clearly differentiated into females and males.

3.8.1. INTRA UTERINE DEVELOPMENT

The reproductive tract weights, embryo weights and brain weights from four to 12 weeks of gestation are summarized in table 3.1. The reproductive tract maintained a steady increase in weight with advancing gestation. Thus by six weeks, the reproductive tract had increased by 7.80% while mean embryo weight increased by 48,878% from a mere 0.06 ± 0.03g at four weeks to 27.43 ± 1.47g at 6 weeks and mean brain weight increased by 300.0%.

At eight weeks of gestation mean reproductive tract weight increased by 114,7% while mean embryo weight increased by 132.00% and mean brain weight by 1,082.00%. By ten weeks, of mean reproductive tract weight was 8.28 ± 0.18g showing an increase of 343% while mean embryo weight increased by 326.40% and mean brain weight by 159.1%. At twelve weeks, mean reproductive tract weight showed only a slight increase of 4.4% but mean embryo weight still recorded a substantial 102% increase with brain weight declining by 2.2%. From the foregoing, it is evident that rapid development of the embryo occuring between the 4th and 6th weeks of gestation. It also marks a period of spectacular brain spurt which continues progressively until 10 weeks

TABLE 3.1

CHANGES IN THE WEIGHTS OF THE REPRODUCTIVE TRACT, EMBRYO AND THE EMBRYONIC BRAIN DURING GESTATION. (means + S.E.M).

LENGHT OF GESTATION	REPRODUCTIVE TRCT WEIGHT (kg)	EMBRYO WEIGHT (g)	EMBRYONIC BRAIN WEIGHT (g)
4 Weeks	0.81 (0.05)	0.06 (0.03)	0.06 (0.03)
6 Weeks	0.87 (0.02)	27.43 (1.47)	0.78 (0.002)
8 Weeks	1.868 (0.01)	63.78 (2.53)	9.27 (0.13)
10 Weeks	8.28 (0.18)	271.94 (11.48)	24.02 (0.74)
12 Weeks	8.64 (1.51)	548.69 (2.53)	23.49 (0.89)

TABLE 3.2

CHANGES IN AChE ACTIVITY, PROTEIN CONCENTRATION AND SAChE ACTIVITY IN THE CONCEPTUS DURING GESTATION

LENGHT OF GESTATION	AChE ACTIVITY Amniotic Fluid	AChE ACTIVITY Embryonic Brain	PROTEIN CONCENTRATN Amniotic Fluid	PROTEIN CONCENTRATN Embryonic Brain	SAChE ACTIVITY Amniotic Fluid	SAChE ACTIVITY Embryonic Brain
4 Weeks	0.492 (0.03)	0.31 (0.09)	0.11 (0.04)	0.23 (0.02)	4.69 (1.68)	1.35 (0.34)
6 Weeks	0.19 (0.01)	1.16 (0.07)	0.13 (0.01)	0.23 (0.03)	1.53 (0.21)	5.13 (0.39)
8 Weeks	0.21 (0.004)	1.34 (0.09)	0.12 (0.01)	0.12 (0.02)	1.86 (0.19)	11.42 (1.50)
10 Weeks	0.22 (0.007)	1.713 (0.11)	0.14 (0.002)	0.21 (0.03)	1.57 (0.04)	8.27 (0.59)
12 Weeks	0.26 (0.06)	3.26 (0.96)	0.13 (0.02)	0.15 (0.04)	2.06 (0.73)	21.54 (2.60)

* Values are means and the standard errors are in parentheses.

of age. It is also worth mentioning that while mean embryo weight and mean reproductive tract weights increase very linearly up to 12 weeks of gestation, brain growth rate had reduced between the tenth and twelfth week of gestation.

3.8.2 AChE ACTIVITIES, PROTEIN CONCENTRATION AND SAChE ACTIVITIES IN THE CONCEPTUS FROM FOUR WEEKS TO TWELVE WEEKS OF GESTATION

Table 3.2 Shows mean AChE activities, protein concentrations and SAChE activity in the conceptus.

AChE activity in the amniotic fluid decreased very sharply from 0.49 $\pm$ 0.03 at four weeks to 0.19 $\pm$ 0.01 at six weeks and thereafter maintained a fairly steady profile to twelve weeks of age. This is further shown by the highly significant but negative correlation coefficient of -0.56 ($P<0.05$) between amniotic AChE and gestation length (Table 3.3) and the regression curve in fig. 3.1.

AChE activity in the embryo brain increased linearly from 1.16 $\pm$ 0.06 at 6 weeks to 3.26 $\pm$ 0.96 at 12 weeks with a highly significant and positive correlation coefficient of 0.88 ($P<0.001$) (table 3.3). regression between embryobrain AChE and gestation (fig. 3.1).

TABLE 3.3

REGRESSION TABLE OF AChE ACTIVITY, PROTEIN CONCENTRATION AND SAChE ACTIVITY IN THE AMNIOTIC FLUID AND EMBRYONIC BRAIN ON AGE

Y	X	PREDICTION EQUATION	r	SIG.
AChE ACTIVITY	AGE	y=a+bx	-0.56	
Amniotic fluid		y=-0.453-0.022x	-0.5	*
Embryonic Brain		y=-0.38+0.64x	0.88	***
PROTEIN CONCENTRATION				
Amniotic fluid		y=0.11=0.004x	0.31	n.s
Embryonic Brain		y=0.21-0.004x	-0.11	n.s
SAChE ACTIVITY				
Amniotic fluid		y=-3.90-0.52x	-0.54	*
Embryonic Brain		y=-0.88 + 3.03x	0.86	***

* = P<0.05, ** = P<0.01, *** = P<0.001, n.s=P>0.05.

TABLE 3.4

CHANGES IN THE MINERAL PROFILE OF THE EMBRYONIC BRAIN DURING GESTATION *

LENGHT OF GESTATION	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM	COPPER	ZINC
4 Weeks	1.61 (0.01)	1.34 (0.03)	15.61 (0.50)	531.75 (1.18)	0.51 (0.004)	0.72 (0.04)
6 Weeks	1.502 (0.02)	2.438 (0.02)	26.09 (0.58)	530.00 (1.08)	0.46 (0.01)	0.46 (0.02)
8 Weeks	1.67 (0.03)	2.02 (0.08)	16.64 (0.54)	526.75 (1.18)	0.23 (0.005)	0.44 (0.01)
10 Weeks	1.67 (0.03)	1.55 (0.05)	18.19 (0.25)	545.50 (2.10)	0.11 (0.003)	0.35 (0.04)
12 Weeks	1.99 (0.05)	1.41 (0.01)	20.90 (0.58)	554.75 (1.89)	0.12 (0.002)	0.40 (0.01)

* Values are means and the standard errors are in parentheses.

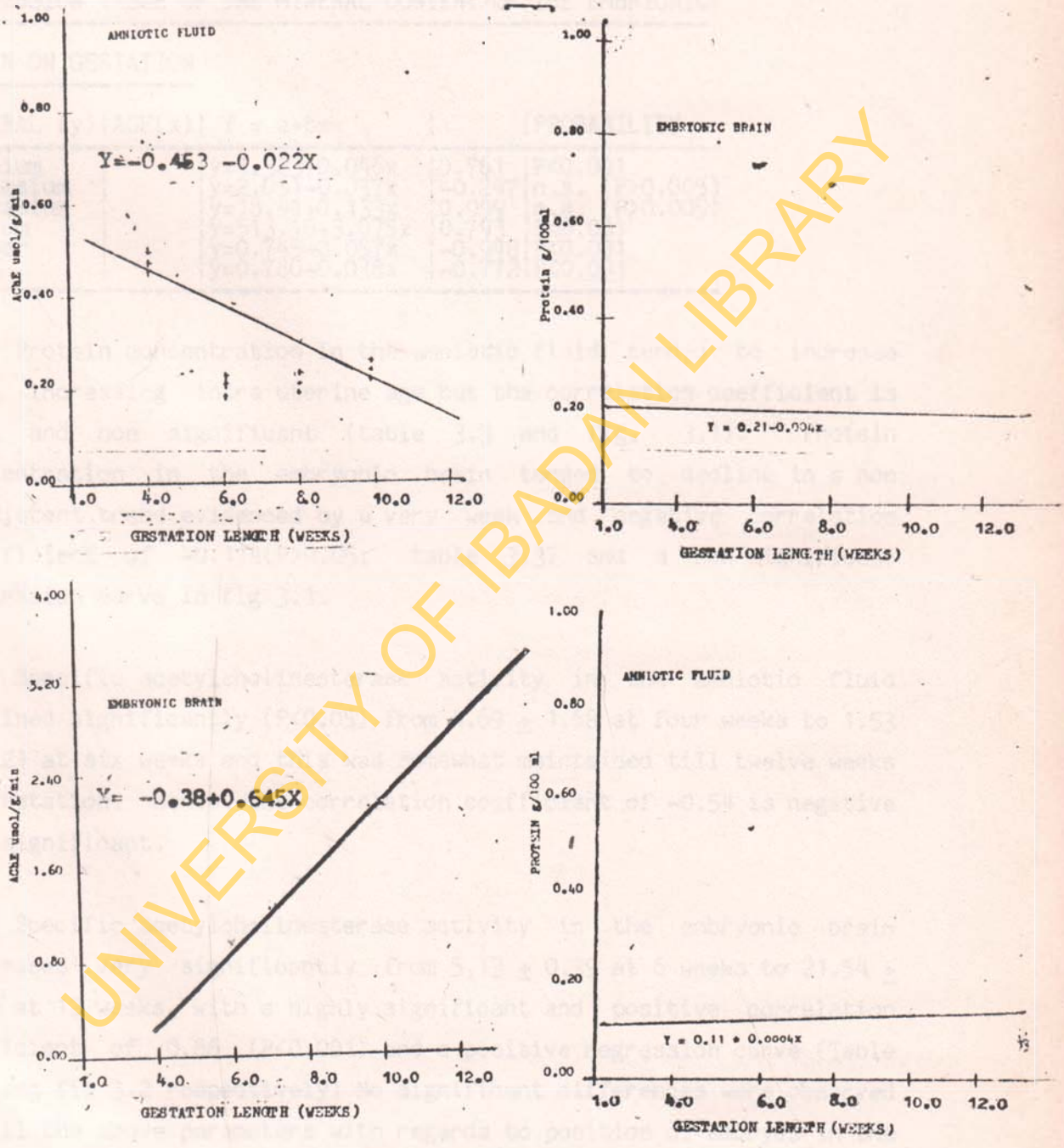


Fig. 3.1. Relationship Between Gestation length and AChE Activities and Total Protein in the Embryonic Brain and Amniotic Fluid.

TABLE 3.5

REGRESSION TABLE OF THE MINERAL CONTENT OF THE EMBRYONIC BRAIN ON GESTATION

MINERAL (y)	AGE(x)	Y = a+bx		PROBABILITY
Calcium		y=1.323+0.046x	0.761	P<0.001
Magnesium		y=2.051-0.037x	-0.247	n.s. (P>0.005)
Potassium		y=18.41+0.133x	0.099	n.s. (P>0.005)
Sodium		y=513.15+3.075x	0.793	P<0.001
Copper		y=0.745-0.057x	-0.948	P<0.001
Zinc		y=0.780-0.038x	-0.772	P<0.001

Protein concentration in the amniotic fluid tended to increase with increasing intra uterine age but the correlation coefficient is weak and non significant (table 3.3 and fig. 3.1). Protein concentration in the embryonic brain tended to decline in a non consistent trend evidenced by a very weak and negative correlation coefficient of -0.114(P>0.05; table 3.3) and a non significant regression curve in fig 3.1.

Specific acetylcholinesterase activity in the amniotic fluid declined significantly (P<0.05) from 4.69 ± 1.68 at four weeks to 1.53 ± 0.21 at six weeks and this was somewhat maintained till twelve weeks of gestation. Hence, the correlation coefficient of -0.54 is negative and significant.

Specific acetylcholinesterase activity in the embryonic brain increases very significantly from 5,13 ± 0.39 at 6 weeks to 21.54 ± 2.60 at 12 weeks, with a highly significant and positive correlation coeficient of 0.86 (P<0.001) and a positive regression curve (Table 3.3 and fig 3.2 respectively) No significant differences were observed in all the above parameters with regards to position of embryos in the uterine horn (P>0.05)

3.8.3. MINERAL PROFILE IN THE FETAL BRAIN DURING GESTATION

Tables 3.4 and 3.5 summarize the mineral profiles of the embryonic brain of the pig with intra uterine age.

There is an increase in calcium content in the embryo brain with gestational age ($r=0.761$, $P<0.001$). With magnesium there was a slight increase in the magnesium content of the embryonic brain from 4 weeks of gestation till about 10 weeks after which it declined. The correlation coefficient is therefore negative but not significant ($r=0.25$, $P>0.05$). Potassium did not exhibit a particularly consistent trend and the correlation coefficient was therefore not significant ($r=0.099$, $P>0.05$). Sodium on the other hand maintained slight increases in concentration with intra-uterine age and was borne out by a positive and significant correlation coefficient ($r=0.793$, $P<0.001$). Copper and Zinc showed steady decline in concentration with intra-uterine age and the correlation coefficients were negative and significant ($r=-0.948$ and -0.772, $P<0.001$, respectively). Fig. 3.3 illustrates there relationships.

3.9 CHANGES IN ACETYLCHOLINESTERASE ACTIVITY AND CATIONS IN THE PORCINE BRAIN AND HYPOPHYSES FROM DAY OLD TO FOUR MONTHS OF AGE

3.9.1 REGIONAL DISTRIBUTION OF AChE IN THE BRAIN

Table 3.6 Shows the general profile of AChE activity, protein concentration and SAChE activities of the brain and hypophyses.

AChE activity was highest in the amygdala, mid-brain and medulla oblongata, lowest in the cerebral cortex and cerebellum but intermediate in the hippocampus, hypothalamus and pons. On the other

hand protein concentration was highest in the cerebellum, pons, medulla oblongata and hypothalamus, lowest in the cerebral cortex and generally stable and medium in the midbrain, hippocampus and amygdala.

Consequently SAChE activity was highest in the midbrain, pons and hypothalamus, lowest in the cerebral cortex and medium in the other brain regions.

3.9.2 HYPOPHYSEAL AChE

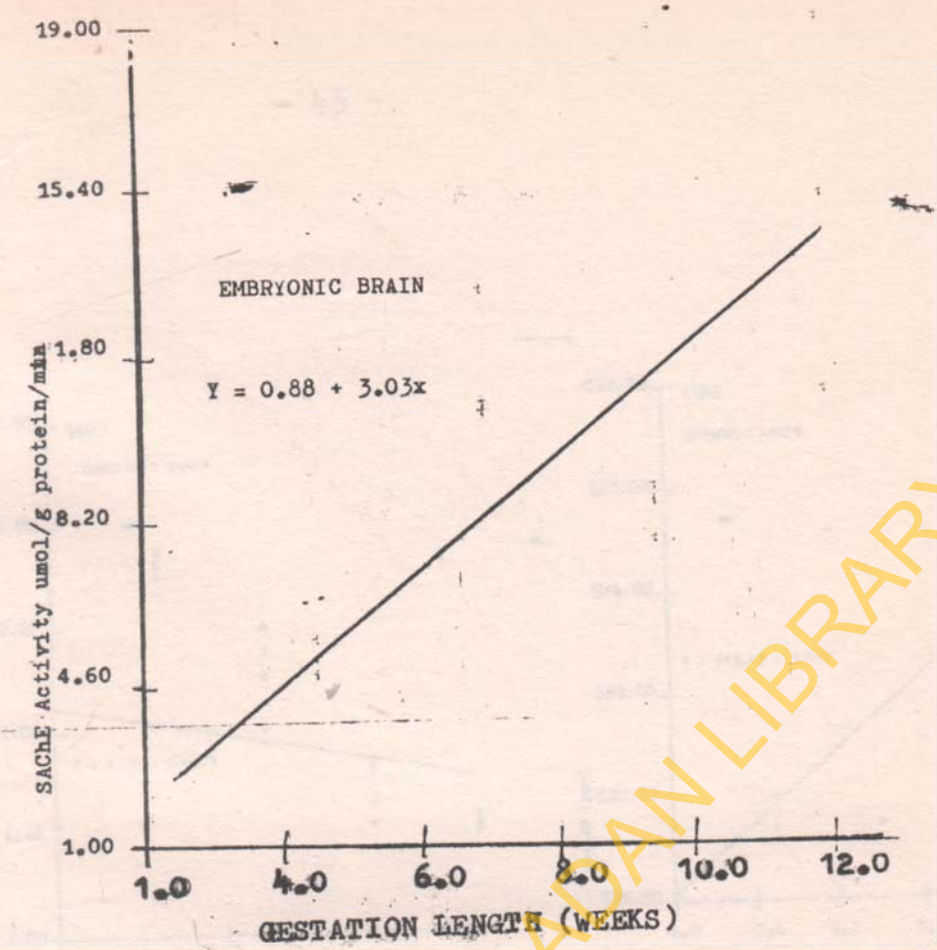
AChE activity in the adenohypophysis was higher than in the neurohypophysis but the difference was not significant ($P>0.05$). Protein concentration was however significantly higher in the adenohypophysis than in the neurohypophysis. ($P<0.05$). SAChE activity was also higher ($P<0.05$) in the adenohypophysis than in the neurohypophysis (table 3.6).

3.9.3 MINERAL PROFILES IN THE BRAIN

Table 3.7 Shows the concentration of minerals in the various brain regions. Calcium, Magnesium, Potassium, Copper and Zinc were more concentrated in the pons, cerebellum, cerebral cortex and medulla oblongata than in the other brain regions. Sodium did not display any particularly consistent trend.

3.9.4 HYPOPHYSEAL MINERAL PROFILES

Calcium, Potassium and Zinc were higher in the adenohypophysis than in the neurohypophysis ($P<0.05$) while Sodium, Magnesium and Copper were similar (table 3.7).



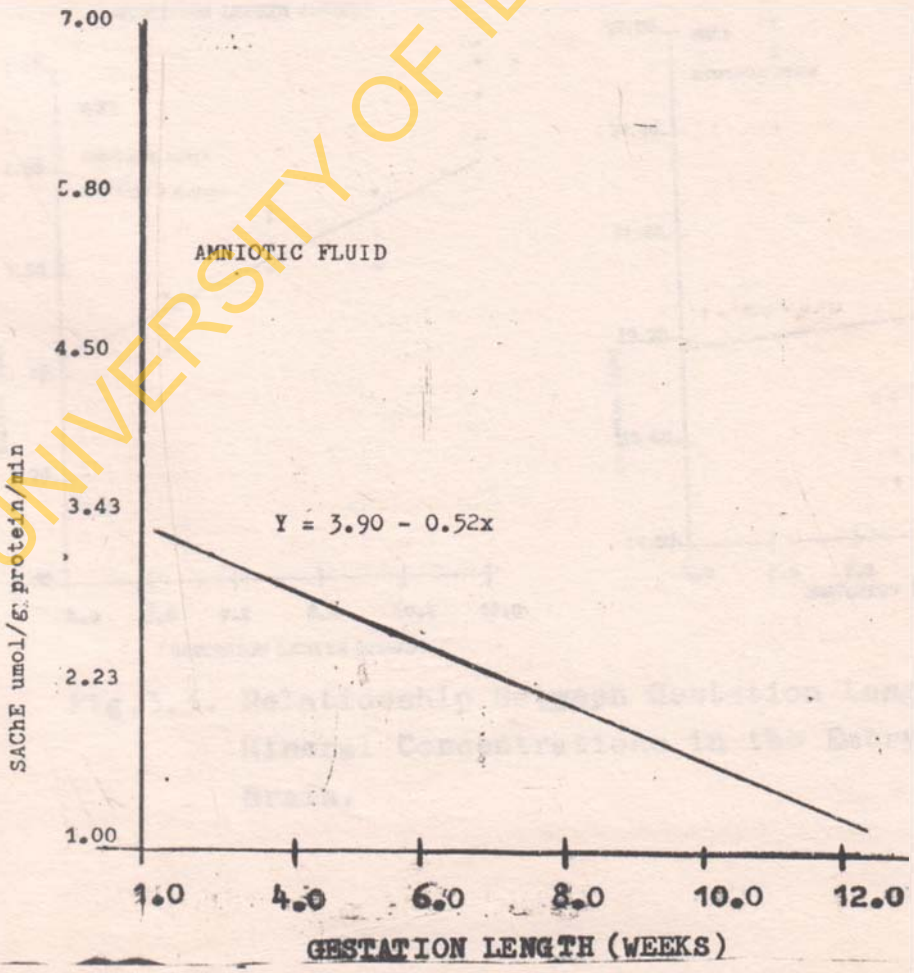


Fig. 3.2. Relationship Between Gestation Length and SAChE Activities in the Embryonic Brain

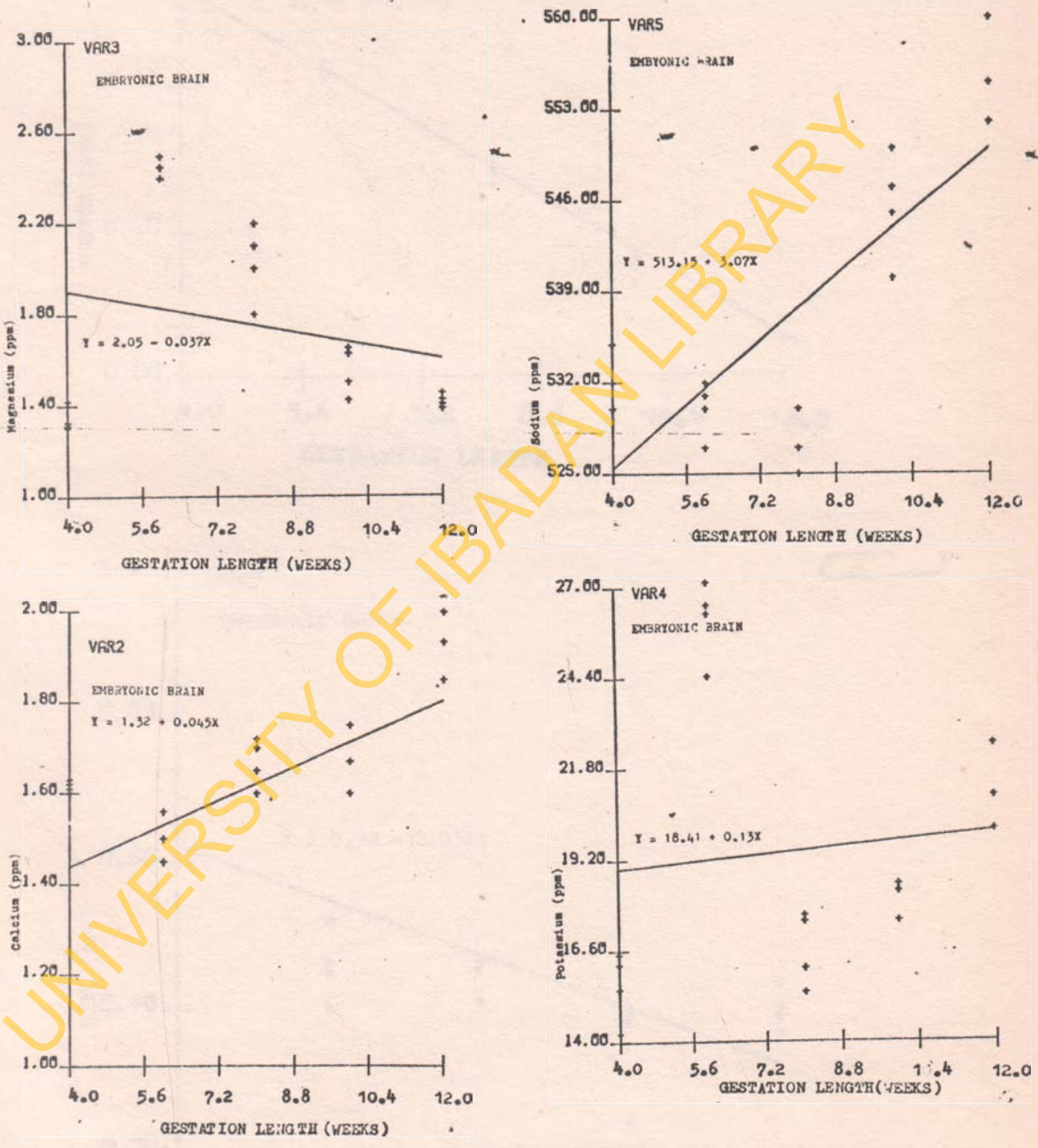
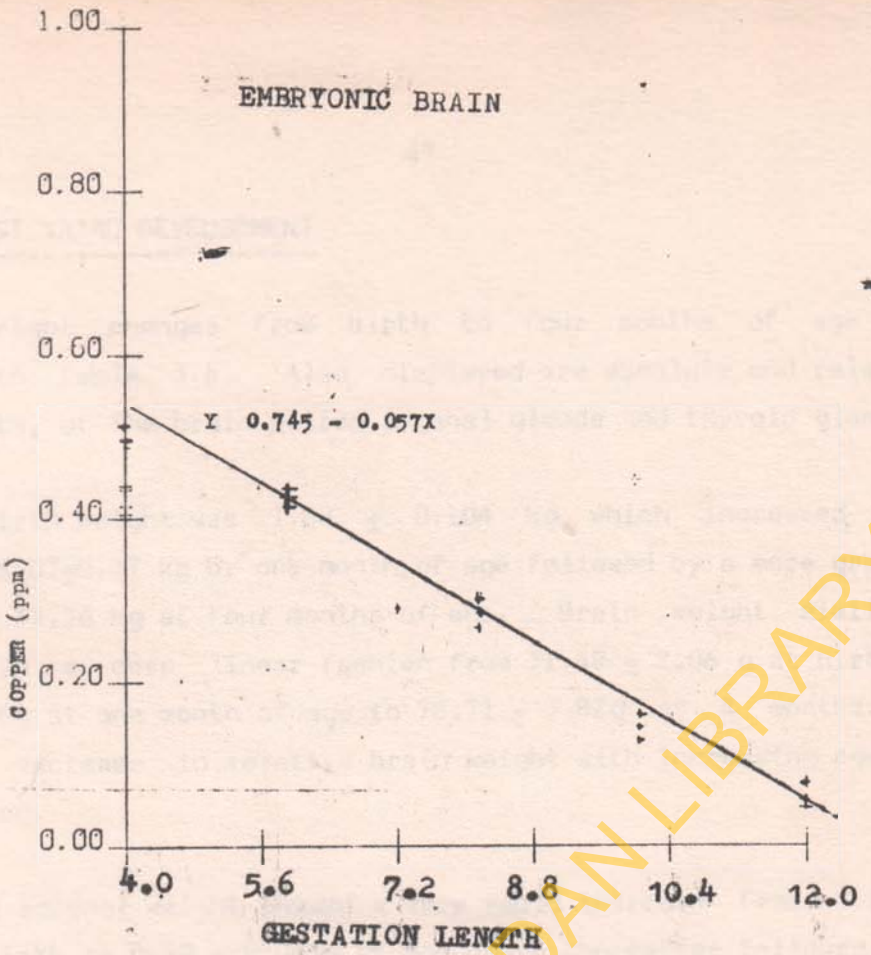


Fig.3.3. Relationship Between Gestation Length and Mineral Concentrations in the Embryonic Brain.



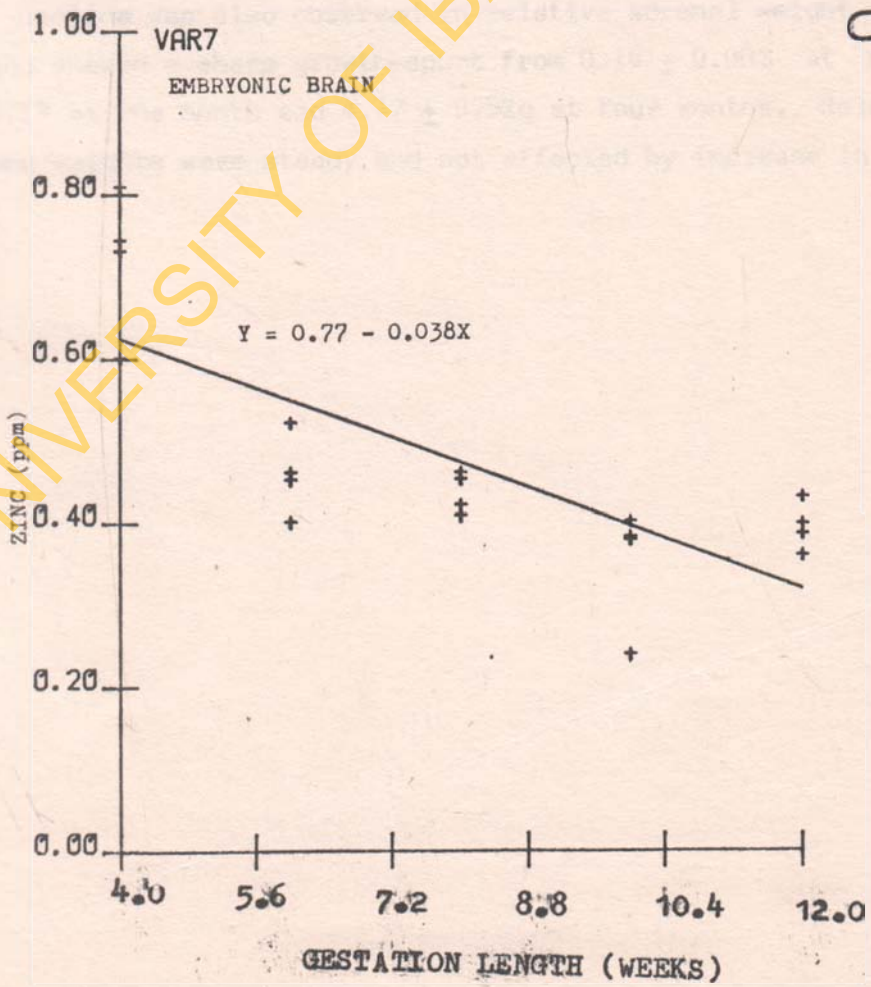


Fig. 3.3(Continued)

3.1.0.1. POST NATAL DEVELOPMENT

Live weight changes from birth to four months of age are displayed in table 3.8. Also displayed are absolute and relative brain weights, of the brain paired adrenal glands and thyroid glands.

Mean birth weight was 1.08 ± 0.104 kg which increased very rapidly to 6.07±0.47 kg by one month of age followed by a more gradual increase to 33.36 kg at four months of age. Brain weight similarly increased in a more linear fashion from 31.48 ± 2.06 g at birth to 50.19 ± 1.76g at one month of age to 78.71 ± 3.82g at 4 months. A consistent decrease in relative brain weight with increasing age was also observed.

Paired adrenal weight showed a very rapid increase from 0.13 ± 0.003g at birth to 0.49 ± 0.063g at one month thereafter followed by a more gradual increase to 1.07 ± 0.03 at four months of age. A consistent decline was also observed in relative adrenal weight. The thyroid gland showed a sharp growth-spurt from 0.18 ± 0.002 at birth to 0.67 ± 0.19 at one month and 4.37 ± 0.52g at four months. Relative thyroid gland weights were steady and not affected by increase in age.

TABLE 3.6

REGIONAL DISTRIBUTION OF AChE ACTIVITY, PROTEIN CONTENT AND SAChE ACTIVITY IN THE BRAIN AND HYPOPHYSES

(means ± S.E.M).

BRAIN REGION	AChE ACTIVITY	PROTEIN CONC.	SAChE ACTIVITY
Pons	4.69 ± 0.75 b	0.30 ± 0.11 a	28.36 ± 12.40 ac
Cerebellum	2.83 ± 0.39 d	0.38 ± 0.19 a	22.03 ± 5.09 d
Amygdala	6.05 ± 1.19 a	0.21 ± 0.06 ab	33.93 ± 7.29 b
Hippocampus	4.06 ± 0.28 c	0.23 ± 0.07 ab	23.92 ± 5.80 d
Hypothalamus	4.85 ± 0.43 b	0.32 ± 0.08 a	26.23 ± 11.82 c
Cerebral cortex	1.56 ± 0.10 e	0.16 ± 0.04 b	11.57 ± 1.95 e
Mid Brain	6.34 ± 0.32 a	0.23 ± 0.03 ab	36.98 ± 10.36 a
Medulla - oblongata	6.43 ± 0.84 a	0.38 ± 0.09 a	25.76 ± 13.28 dc
HYPOPHYSES			
Adenohypophysis	1.43 ± 0.36 a	0.34 ± 0.13 a	10.11 ± 4.96 a
Neurohypophysis	1.09 ± 0.19 a	0.12 ± 0.02 b	8.05 ± 3.12 b

*Values in the same vertical column differently superscripted differ significantly. ($P<0.05$).

TABLE 3.7

REGIONAL DISTRIBUTION OF MINERALS IN THE PORCINE BRAIN AND HYPOPHYSES. (means ± S.E.M)

BRAIN REGIONS	CALCIUM	MAGNESIUM	POTASSIUM
Pons	2.05 ± 0.15^{b}	1.54 ± 0.09^{ac}	28.17 ± 3.29^{a}
Cerebellum	2.42 ± 0.12^{a}	1.57 ± 0.12^{a}	27.16 ± 2.34^{ab}
Amygdala	1.85 ± 0.10^{c}	1.23 ± 0.14^{c}	24.55 ± 2.66^{b}
Hippocampus	1.67 ± 0.12^{d}	1.46 ± 0.09^{bc}	25.00 ± 3.07^{ab}
Hypothalamus	1.88 ± 0.09^{c}	1.52 ± 0.03^{ac}	26.00 ± 2.61^{ab}
Cerebral Cortex	1.66 ± 0.19^{d}	1.36 ± 0.15^{bd}	26.15 ± 2.89^{ab}
Mid Brain	1.84 ± 0.24^{c}	1.29 ± 0.05^{d}	28.18 ± 3.37^{a}
Medulla - Oblongata	2.08 ± 0.26^{b}	1.45 ± 0.03^{b}	25.96 ± 3.56^{ab}
HYPOPHYSES			
Adenohypophysis	2.66 ± 0.26^{a}	2.10 ± 0.04^{a}	21.80 ± 2.59^{a}
Neurohypophysis	2.07 ± 0.23^{b}	1.98 ± 0.25^{a}	15.95 ± 1.43^{b}

BRAIN REGIONS	SODIUM	COPPER	ZINC
Pons	538.09±3.70^{ab}	0.14 ± 0.01^{b}	0.39 ± 0.04^{b}
Cerebellum	545.07±6.62^{ac}	0.16 ± 0.02^{a}	0.49 ± 0.08^{a}
Amygdala	530.90±3.13^{bc}	0.13 ± 0.01^{b}	0.39 ± 0.02^{b}
Hippocampus	529.32±22.87^{b}	0.13 ± 0.02^{b}	0.29 ± 0.02^{c}
Hypothalamus	537.46±4.42^{bc}	0.13 ± 0.02^{b}	0.43 ± 0.05^{b}
Cerebral cortex	534.25±2.81^{bc}	0.14 ± 0.01^{b}	0.45 ± 0.06^{b}
Mid Brain	548.45±6.79^{ac}	0.13 ± 0.01^{b}	0.42 ± 0.07^{b}
Medulla - Oblongata	534.55±3.11^{b}	0.16 ± 0.03^{a}	0.49 ± 0.08^{a}
HYPOPHYSES			
Adenohypophysis	546.00±3.98^{a}	0.21 ± 0.022^{a}	2.20 ± 0.42^{a}
Neurohypophysis	535.12±4.88^{a}	0.22 ± 0.01^{a}	0.48 ± 0.04^{b}

Values in the same vertical column differently superscripted difer significantly ($P<0.05$).

TABLE 3.8 POST-NATAL DEVELOPMENT IN THE PIG.

	Age	SEX			Relative weights %
		Male	Female	mean	
LIVE - WEIGHT(KG)	Day Old	1.12±0.14	1.05±0.72	1.081±0.45 e	
	One Month	6.02±1.10	6.11±0.44	6.07 ±1.02 d	
	Two Months	11.75±2.63	10.75±1.71	11.25±2.12 c	
	3 Months	22.47±1.81	21.05±1.65	21.76±1.63 b	
	4 Months	34.60±1.34	32.10±1.73	33.36±1.14 a	
BRAIN WEIGHT(g)	Day Old	31.65±1.87	31.18±2.18	31.48±2.07 e	3.65±0.06 a
	One Month	51.72±1.03	48.66±2.49	50.19±1.76 d	0.83±0.05 b
	Two Months	69.05±3.65	67.07±4.93	68.06±3.80 c	0.60±0.02 c
	3 Months	71.89±1.77	71.97±2.04	71.93±1.45 b	0.33±0.07 d
	4 Months	81.97±3.82	75.45±3.81	78.71±4.00 a	0.23±0.08 d
PAIRED ADRENAL WEIGHT	Day Old	0.13±0.05	0.15±0.04	0.13±0.05 e	0.01±0.004 a
	One Month	0.58±0.15	0.40±0.06	0.49±0.06 d	0.008±0.0007 b
	Two Months	0.58±0.06	0.61±0.015	0.59±0.04 c	0.005±0.0004 c
	3 Months	0.81±0.05	0.80±0.06	0.81±0.07 b	0.004±0.0006 dc
	4 Months	1.05±0.07	1.08±0.06	1.07±0.03 a	0.003±0.0005 d
THYROID GLAND WT.	Day Old	0.175±0.004	0.18±0.003	0.18±0.002 e	0.02±0.003 a
	One Month	0.73±0.31	0.61±0.13	0.67±0.19 d	0.01±0.006 a
	Two Months	1.40±0.36	1.29±0.26	1.44±0.28 c	0.01±0.005 a
	3 Months	2.38±0.22	2.61±0.30	2.50±0.27 b	0.01±0.006 a
	4 Months	4.26±0.52	4.48±0.52	4.37±0.52 a	0.01±0.007 a

No Sex differences were observed.
Values in the same vertical column bearing different superscripts are significantly different (P<0.05).

3.1.0.2 AGE AND SEX DIFFERENCES IN THE REGIONAU DISTRIBUTION OF AChE ACTIVITY AND CATIONS OF PORCINE BRAINS FROM DAY OUD TO FOUR MONTHS OF AGE

Tables 3.9 and 3.10 show the effect of increasing age and sex on the regional distribution of AChE protein and SAChE in the porcine brain. Table 3.11 shows the various correlation coefficients corresponding to the different brain regions. AChE activity declined significantly ($P<0.05$) with age from day old to four months of age in the pons, hypothalamus, mid brain and medulla oblongata. The correlation coefficients were -0.89($P<0.001$), -0.69($P<0.001$), -0.49($P<0.05$) and 0.81($P<0.001$) respectively and the corresponding regression curves are displayed in figures 3.4 and 3.5. It is worth mentioning that in the brain regions mentioned above, the AChE activities were a bit similar at two and three months of age. The amygdala on the other hand displayed a significant rise in AChE activity with age ($r=0.906$, $P<0.001$), while the Cerebellum and Cerebral cortex displayed non-significant increase in AChE activity with age.

Sex influences were only observed in the Pons, hypothalamus and medulla oblongata (table 3.9) where the males were superior to the females at certain ages ($P<0.05$). No significant differences were observed in the cerebellum, amygdala, hippocampus and midbrain while the cerebral cortex did not exhibit a consistent trend. Age/Sex interaction was evident in the medulla oblongata and pons with the males having significantly higher AChE activities than the females at day old ($P<0.05$).

3.1.0.3 PROTEIN CONCENTRATION

A significant rise in protein content with age from day old to four months of age was observed in all the brain regions. Generally protein content at four months was higher than other age groups while values recorded at two and three months were similar and superior to protein concentrations at day old and one month of age. The various correlation coefficients and regression curves are displayed in table 3.10 and figure 3.5.

Sex differences were not very evident but some age/sex interactions were observed in the amygdala where males had higher protein levels at one to four months of age and in the hippocampus where the four-month males had higher protein levels than the females ($P<0.05$).

3.1.0.4 SAChE ACTIVITY

Significant age differences were observed in the various brain regions.

Table 3.9 shows that SAChE activity was highest at day old followed by a sharp drop by one month of age thereafter declining steadily. The only exceptions were the cerebellum and the amygdala. The cerebellum increased from 16.90 at Day old to 32.83 at 2 months of age declining to 7.53 at four months of age. The amygdala also showed a steady rise from 22.91 at day old to 58.11 at two months followed by a steady decrease to 20.13 at four months.

TABLE 3.9 EFFECT OF SEX ON THE REGIONAL DISTRIBUTION OF AChE ACTIVITY, PROTEIN CONTENTS AND SAChE ACTIVITY OF THE PORCINE BRAIN REGIONS AT DIFFERENT AGES

AGE	BRAIN REGIONS								HYPOTHALAMUS		CEREBRAL -CORTEX		MID BRAIN		MEDULLA -OBLONGATA	
AChE ACTIVITY	PONS		CEREBELLUM		AMYGDALA		HIPPOCAMPUS									
	Male	Female	Male	female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Day Old	7.93	6.84	1.61	1.80	1.99	2.41	3.78	3.85	6.39	5.45*	1.22	1.51*	6.53	6.22	10.38	7.50*
One month	4.55	4.39	3.44	3.95	4.93	4.65	4.05	3.75	6.08	5.17*	1.85	1.96	7.55	6.98	7.45	7.90
Two months	4.85	3.73*	3.15	3.23	8.13	7.27	4.18	4.87	3.61	4.05	1.20	1.55*	6.19	6.03	4.54	5.02
Three months	4.05	4.44	2.87	3.41	7.11	7.11	4.63	4.73	4.08	4.22	1.87	1.26*	6.03	6.02	5.14	5.45
Four months	3.09	3.05	2.43	2.42	8.66	8.35	3.28	3.93	4.74	4.47	1.58	1.58	6.50	5.33	5.12	5.84
S.E.M	0.20		0.19		0.23		0.25		0.19		0.06		0.33		0.23	
PROTEIN																
Day Old	0.09	0.10	0.11	0.09	0.10	0.09	0.09	0.10	0.09	0.09	0.09	0.08	0.09	0.09	0.17	0.09
One month	0.19	0.19	0.14	0.20	0.13*	0.12*	0.12	0.20	0.12	0.28	0.12	0.35	0.11	0.30	0.37	0.46
Two months	0.20	0.23	0.10	0.10	0.19	0.10*	0.23	0.16	0.36	0.37	0.13	0.10	0.33	0.21	0.45	0.22
Three months	0.24	0.25	0.11	0.13	0.30	0.24*	0.23	0.29	0.46	0.44	0.13	0.12	0.23	0.25	0.51	0.58
Four months	0.58	0.51	0.33	0.31	0.45	0.40*	0.57	0.47*	0.50	0.55	0.30	0.31	0.35	0.38	0.69	0.60
S.E.M.	0.01		0.02		0.01		0.02		0.02		0.04		0.03		0.05	
SAChE ACTIVITY																
Day Old	83.58	69.89*	15.02	18.78	20.35	25.42*	41.07	39.58	71.34	60.14*	13.67	18.10*	74.28	70.05*	69.29	82.29
One month	24.28	23.43	25.94	27.88	39.45	39.11	38.46	18.42	49.76	18.47*	15.54	10.70*	67.45	23.51*	20.99	18.98
Two month	23.86	16.46*	32.60	33.06	43.32	72.90*	18.30	32.14	9.88	10.94	9.36	15.04*	18.62	33.236*	10.22	23.24
Three months	17.20	17.85	25.82	27.16	31.21	29.07	20.79	17.00	10.19	9.58	14.56	10.31*	26.78	24.70	10.08	9.43
Four months	5.31	6.04	7.33	7.74	19.24	21.01	5.80	8.52	9.56	8.31	5.28	5.22	18.99	17.66	7.51	9.99
S.E.M	1.77		4.01		2.59		2.87		2.90		0.75		3.95		4.42	

* paired values (male/female) under a brain region and * in an age (in the same horizontal column) group bearing the sign * are significantly different (P<0.05).

TABLE 3.10 AChE ACTIVITY, PROTEIN CONTENT AND SAChE ACTIVITY IN THE PORCINE BRAIN AT DIFFERENT AGES (MEAN + S.E.M.)

	Pons	Cerebellum	Amygdala	Hippocampus	Hypothalamus	Cerebral Cortex	Mid Brain	Medulla Oblongata
AChE ATIVITY								
Day old	7.38±0.14^{a}	1.76±0.18^{b}	2.02±0.09^{d}	3.81±0.14^{b}	5.92±0.16^{a}	1.37±0.02^{c}	6.37±0.12^{b}	8.94±0.32^{a}
One month	4.47±0.09^{b}	3.69±0.27^{a}	4.79±0.13^{c}	3.90±0.21^{b}	5.62±0.19^{a}	1.90±0.05^{a}	7.26±0.32^{a}	7.67±0.15^{b}
Two months	4.29±0.23^{b}	3.19±0.10^{a}	7.70±0.40^{b}	4.52±0.23^{a}	3.83±0.17^{c}	1.38±0.02^{c}	6.11±0.17^{b}	4.78±0.17^{c}
Three months	4.25±0.17^{b}	3.14±0.22^{a}	7.11±0.17^{b}	4.68±0.15^{a}	4.15±0.02^{bc}	1.57±0.05^{b}	6.02±0.151^{b}	5.48±0.17^{c}
Four months	3.08±0.05^{c}	2.43±0.15^{c}	8.50±0.28^{a}	3.60±0.07^{b}	4.61±0.20^{b}	1.58±0.03^{b}	5.92±0.26^{b}	5.48±0.16^{c}

* Values in the same vertical column differenttly superscripted significantly differ (P<0.05)

PROTEIN								
Day old	0.09±0.0004^{d}	0.10±0.01^{c}	0.09±0.001^{d}	0.09±0.002^{d}	0.09±0.002^{e}	0.09±0.003^{c}	0.09±0.02^{d}	0.13±0.06^{e}
One month	0.19±0.005^{c}	0.17±0.01^{b}	0.13±0.011^{c}	0.16±0.02^{c}	0.20±0.003^{d}	0.23±0.17^{b}	0.21±0.01^{c}	0.41±0.06^{c}
Two months	0.21±0.001^{bc}	0.10±0.01^{c}	0.14±0.001^{c}	0.19±0.01^{c}	0.37±0.010^{c}	0.12±0.01^{c}	0.27±0.02^{b}	0.33±0.01^{d}
Three months	0.25±0.01^{b}	0.12±0.01^{c}	0.24±0.01^{b}	0.26±0.02^{b}	0.42±0.01^{b}	0.13±0.005^{c}	0.24±0.01^{bc}	0.55±0.01^{b}
Four months	0.55±0.006^{a}	0.32±0.01^{a}	0.43±0.01^{a}	0.52±0.03^{a}	0.52±0.03^{a}	0.31±0.02^{a}	0.37±0.03^{a}	0.64±0.05^{a}

* values in the same vertical column differently superscripted significantly differ (P<0.05)

SAChE ACTIVITY								
Day old	76.74±1.29^{a}	16.90±1.84^{b}	22.91±1.05^{d}	40.29±0.46^{a}	65.74±2.82^{a}	15.89±0.722^{a}	72.16±4.831^{a}	75.85±8.52^{a}
One month	23.86±0.43^{b}	26.91±3.66^{a}	39.28±2.95^{b}	28.44±2.36^{b}	34.11±1.04^{b}	13.12±1.36^{b}	45.48±0.63^{b}	19.99±0.41^{b}
Two months	20.16±1.04^{b}	32.83±1.90^{a}	58.11±4.43^{a}	25.22±2.70^{b}	10.41±0.48^{c}	12.20±0.97^{b}	25.93±1.49^{c}	16.73±0.23^{b}
Three months	17.53±0.85^{c}	26.49±3.70^{a}	30.14±0.53^{c}	18.90±1.96^{c}	9.89±0.10^{c}	12.44±0.76^{b}	25.74±0.651^{c}	9.76±0.31^{b}
Four months	5.67±0.10^{e}	7.53±0.39^{c}	20.13±0.99^{d}	7.16±0.51^{d}	8.94±0.73^{c}	5.25±0.35^{c}	18.33±1.36^{c}	8.75±0.85^{b}

* values in the same vertical column differently superscripted significantly differ (P<0.05)

TABLE 3.1.1.

REGRESSION TABLE OF AChE PROTEIN AND SAChE IN THE VARIOUS BRAIN REGIONS ON AGE

AChE y	Age	Prediction equation y=a+bx	Simple r	S.E	Sig.
Pons		y=7.346-0.884x	-0.859	0.30	***
Cerebellum		y=2.568+0.088x	0.161	0.39	n.s
Amygdala		y=1.583+1.490x	0.906	0.50	***
Hippocampus		y=4.008+0.176x	0.046	0.37	n.s
Hypothalamus		y=6.077-0.408x	-0.692	0.32	***
Cereberal Cortex		y=1.532+0.008x	0.056	0.07	n.s
Mid Brain		y=6.986-0.215x	-0.494	0.44	*
Medulla Oblongata		y=9.236-0.937x	-0.807	0.41	***
PROTEIN					
Pons		y=0.144+0.053x	0.343	0.20	n.s
Cerebellum		y=0.821-0.145x	0.553	0.17	n.s
Amygala		y=0.029+0.078x	0.904	0.01	***
Hippocampus		y=-0.037+0.088x	0.896	0.04	***
Hypothalamus		y=-0.006+0.106x	0.972	0.02	***
Cerebral Cortex		y=0.033+0.411x	0.727	0.02	***
Mid Brain		y=0.109+0.041x	0.842	0.03	***
Medulla Oblongata		y=0.010+0.125x	0.908	0.07	***
SAChE					
Pons		y=71.890-14.510x	-0.849	1.71	***
Cerebellum		y=27.905-1.957x	-0.279	5.23	n.s
Amygdala		y=38.469-1.513x	-0.150	4.95	n.s
Hippocampus		y=46.526-7.536x	-0.942	3.71	***
Hypothalamus		y=68.929-14.240x	-0.874	2.79	***
Cerebral cortex		y=17.964-2.130x	-0.794	1.79	***
Mid Brain		y=75.247-12.755x	-0.893	4.72	***
Medulla Oblongata		y=68.617-14.287x	-0.781	7.67	***

* P<0.05 ** P<0.01 *** P < 0.001 n.s P>0.05
S.E. standard Error of Estimate

Not surprisingly therefore, highly significant and negative correlation coefficients were recorded for the pons, hippocampus, hypothalamus, cerebral cortex, midbrain, medulla oblongata and very low and insignificant negative correlation coefficients recorded for the cerebellum and amygdala (figure 3.6).

The cerebral cortex as earlier reported recorded a rather low value of 15.89 at day old which was significantly superior ($P<0.05$) to the value recorded at one month. The activity recorded at one month was however similar to values recorded at two and three months but significantly lower than the value recorded at four months of age.

Significant sex effects were observed in the pons, amygdala, hypothalamus, cerebral cortex and midbrain. Males had higher levels than females at one and three months of age in the pons while the exact opposite was observed in the amygdala. In the hypothalamus, cerebral cortex and mid brain SAChE levels were higher in the males at day old and one month of age while at two months of age, the trend was reversed in the cerebral cortex and midbrain.

MINERAL PROFILE

Tables 3.1.2 and 3.1.3 summarize the influence of age and sex on the mineral content of the various brain regions. The regression curves are displayed in figures 3.9 to 3.15. Highly significant age influence was observed in the cation content of the various brain regions.

CALCIUM

With Calcium, slight but significant increases were observed in concentration with age in the Pons, cerebellum, amygdala and midbrain. Table 3.1.4 shows the correlation coefficients of the cation distribution in the various brain regions with age. However significant negative correlations were observed in the hippocampus, hypothalamus and cerebral cortex. A non-significant correlation was observed in the medulla oblongata. This is due to the non-consistent nature of calcium concentration in the medulla oblongata with varying age.

With the exception of the cerebral cortex that shows a significant increase in calcium content of the male brain at four months of age over the female brain, no significant sex influence was discovered.

MAGNESIUM

Slight but significant increases were observed in the pons, cerebellum and midbrain evidenced by the strong and positive correlation coefficients in table 3.1.4 and the regression curves in figures 3.1.1. On the other hand, significant negative decline in magnesium concentration was observed in the hippocampus and cerebral cortex. The amygdala and hypothalamus displayed non-significant negative correlation coefficients while the medulla oblongata showed a rather weak and positive correlation coefficient.

Significant sex influences were observed only in the pons where the females had higher values than the males at day old and four

months of age and also in the hippocampus where the same trend was observed at day old and two months of age but the trend was reversed at three and four months of age.

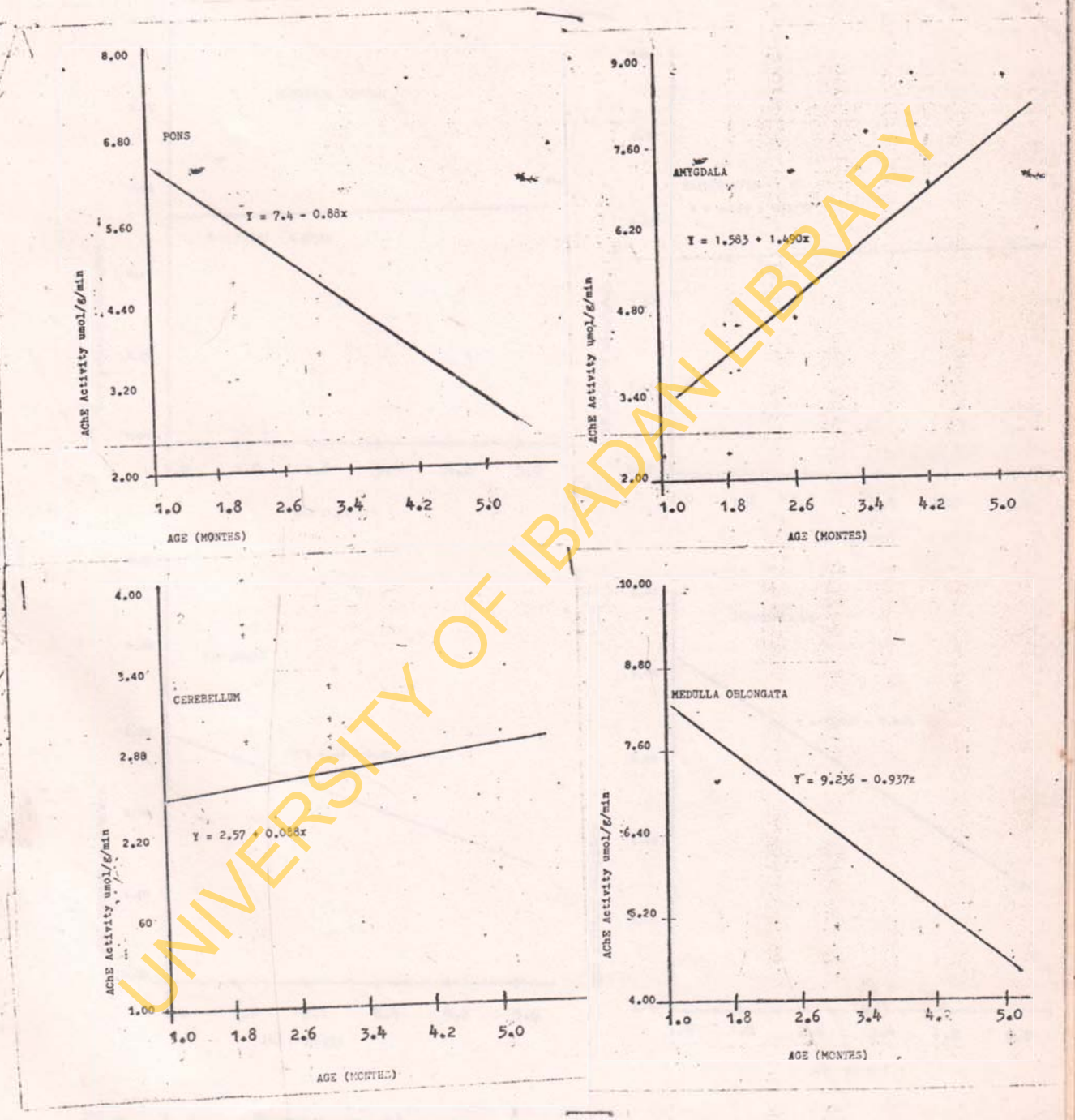
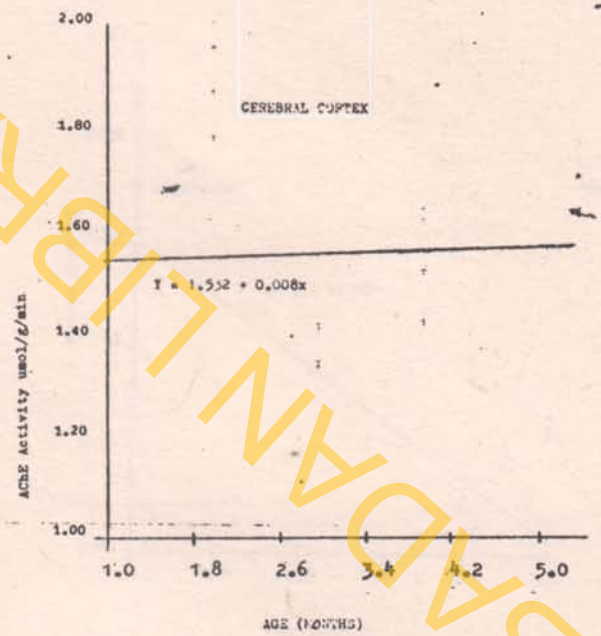
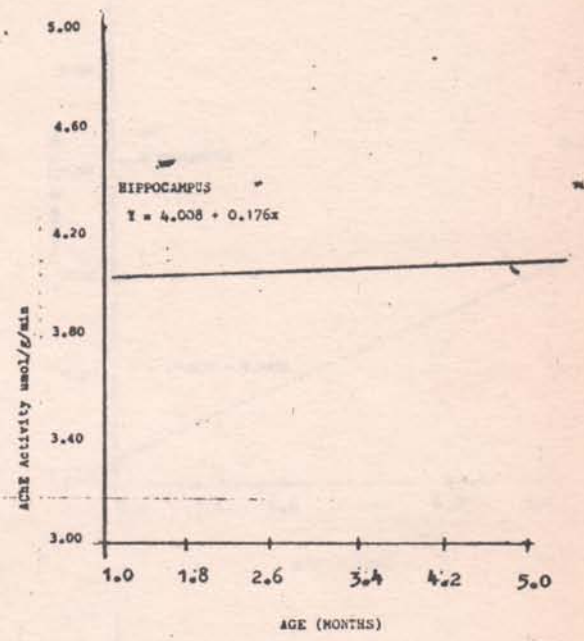
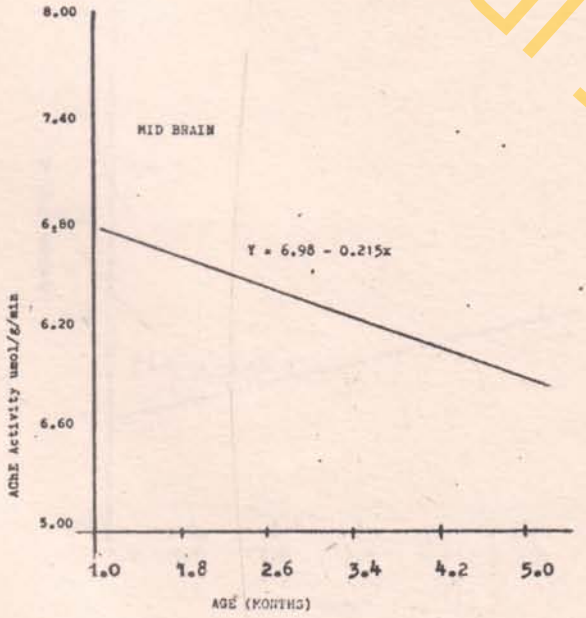


Fig. 3.4. Relationship Between Age And AChE Activities in Different Brain Regions.







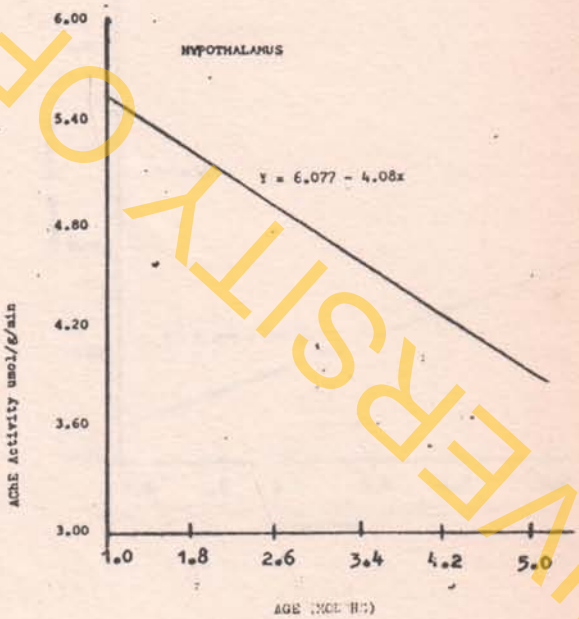


Fig. 3.4. (Continued)

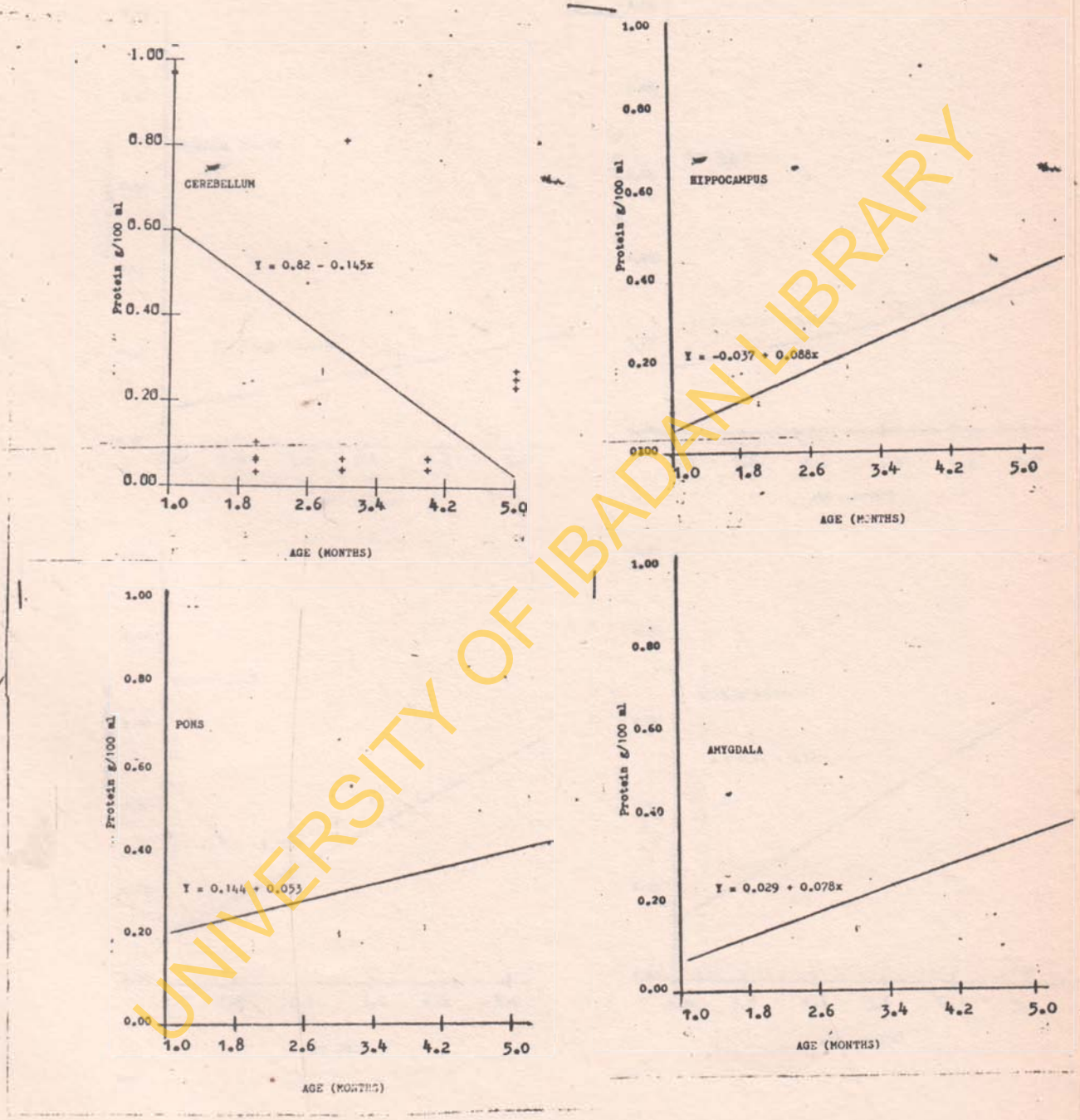


Fig. 3.5. Relationship Between Age and Total Protein in Different Brain Regions.

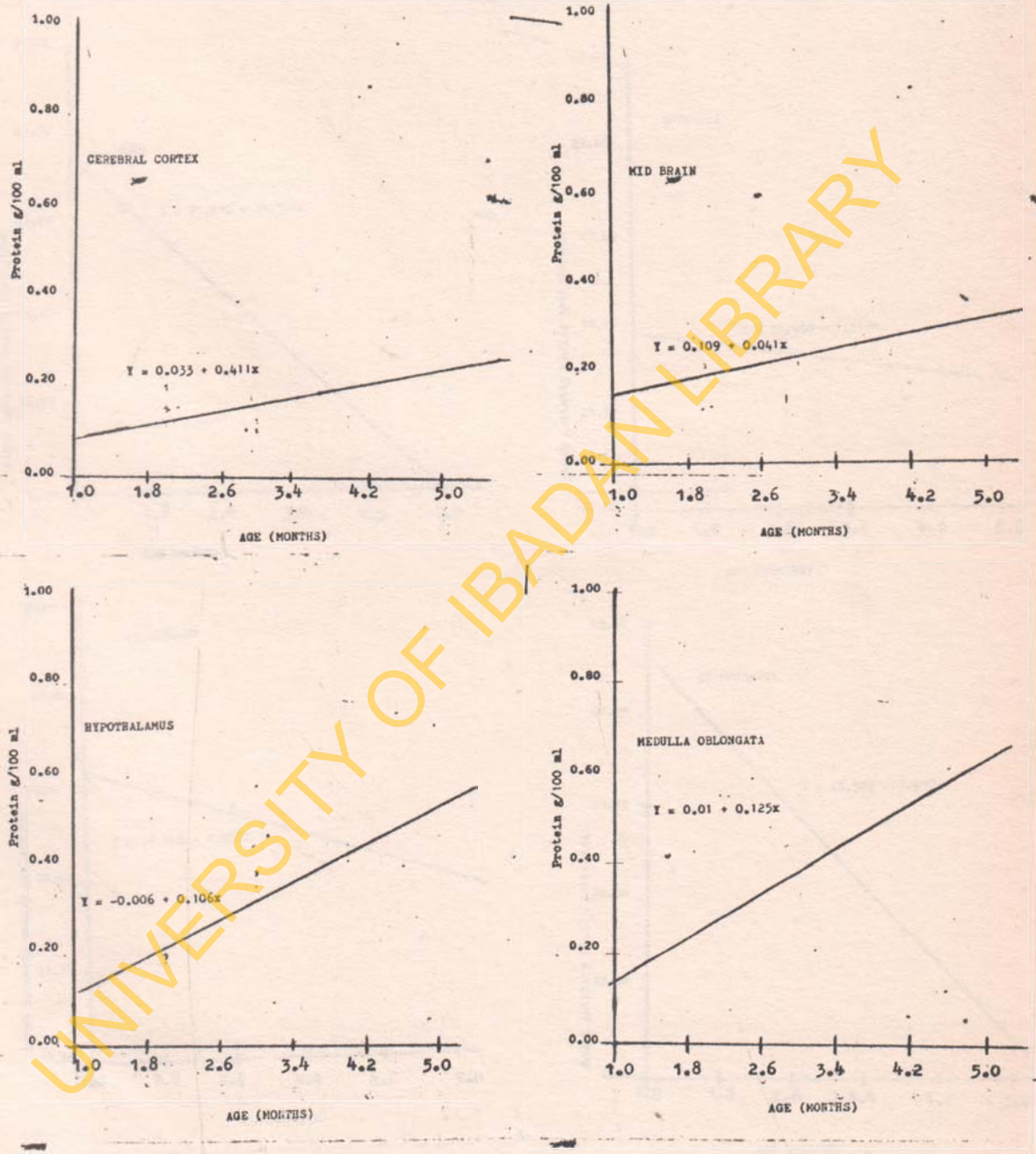


Fig. 3.5. (Continued)

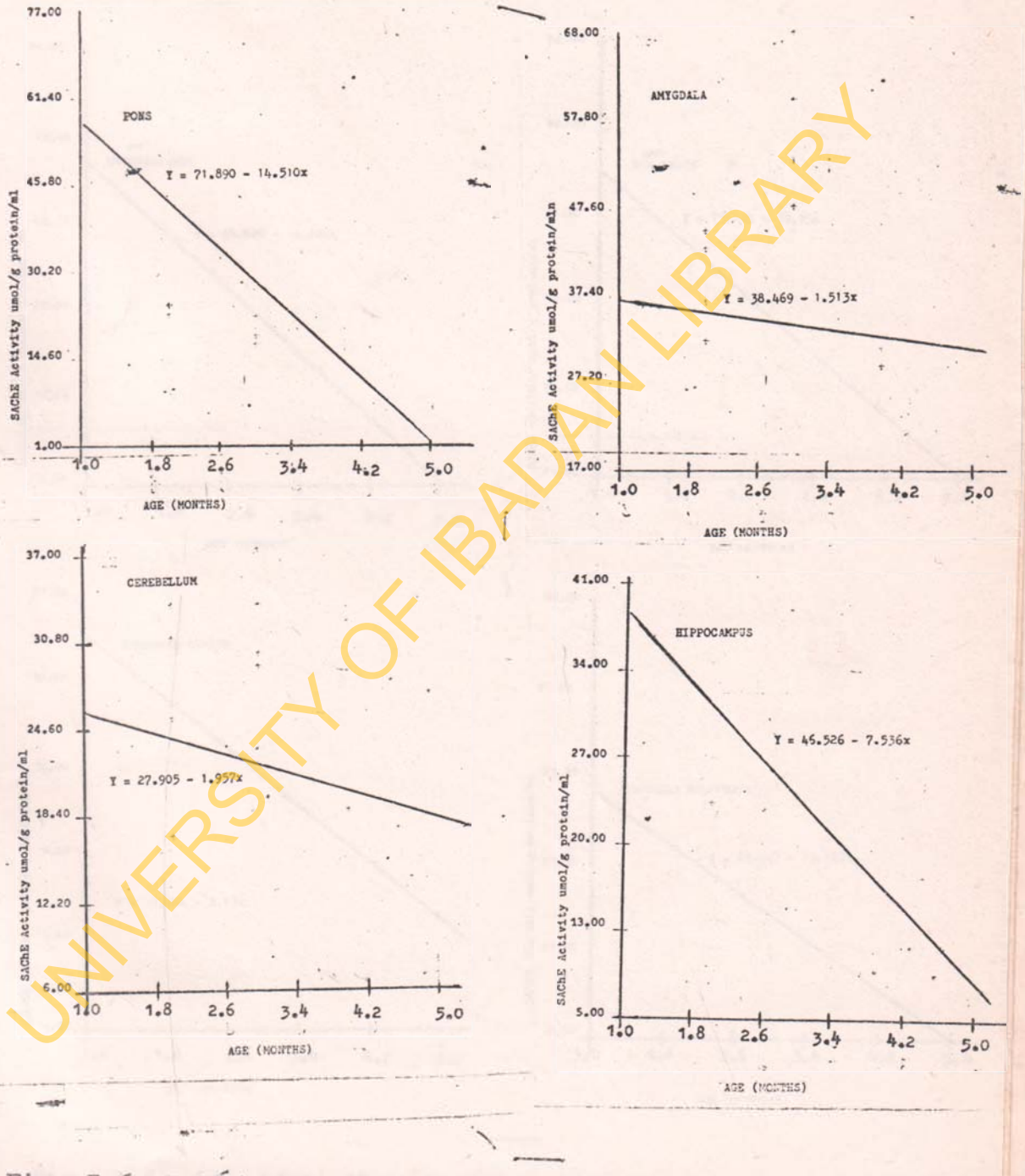


Fig. 3.6. Relationship Between Age and SAChE Activities in Different Brain Regions.

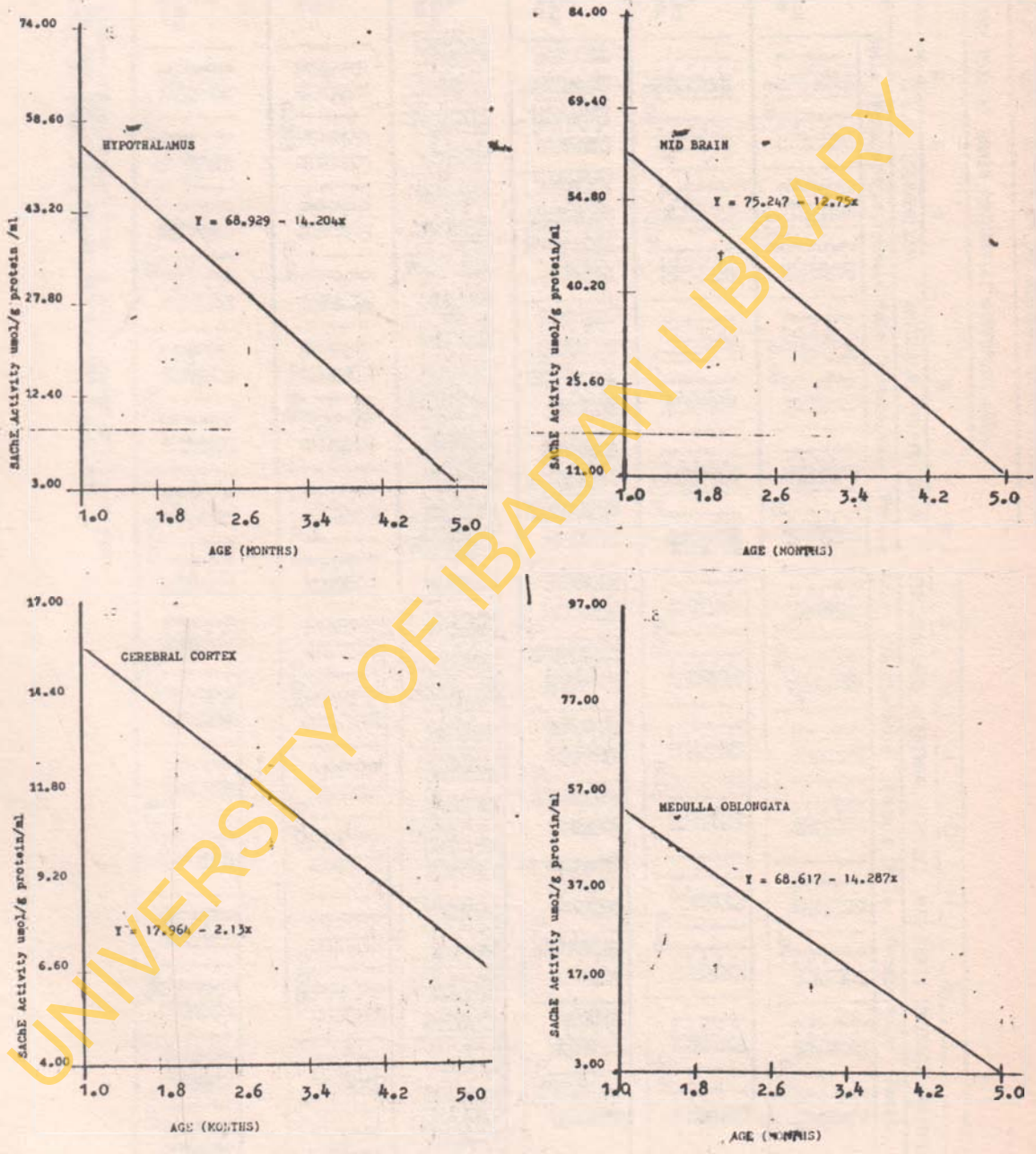


Fig. 3.6. (Continued)

TABLE 3.1.2(a) EFFECT OF SEX ON THE REGIONAL DISTRIBUTION OF CATIONS IN THE PORCINE BRAIN REGIONS AT DIFFERENT AGES.

AGE	BRAIN REGIONS															
CALCIUM	PONS		CEREBELLUM		AMYGDALA		HIPPOCAMPUS		HYPOTHALAMUS		CEREBRAL CORTEX		MID BRAIN		MEDULLA OBLONGATA	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Day old	1.18	1.18	2.02	2.02	1.56	1.53	2.01	1.97	2.10	2.05	2.01	2.07	1.38	1.37	2.92	2.05
One month	1.89	1.83	2.49	2.35	2.01	2.11	1.75	1.83	2.04	2.07	2.14	2.08	1.69	1.64	2.27	2.30
Two months	2.25	2.11	2.50	2.51	1.75	1.63	1.44	1.44	1.87	1.79	1.31	1.142	1.46	1.46	1.74	1.93
Three months	1.82	2.01	2.57	2.56	1.92	1.91	1.09	1.34	1.85	1.80	1.34	1.34	2.10	2.05	2.02	2.16
Four months	2.58	2.53	2.70	2.50	2.05	2.13	1.58	1.60	1.58	1.59*	1.57	1.49	2.67	2.65	2.84	2.79
S.E.M	0.06		0.08		0.06		0.10		0.032		0.03		0.04		0.30	
MAGNESIUM																
Day old	1.43	1.61*	1.24	1.37	1.27	1.20	1.49	1.56*	1.49	1.51	1.65	1.66	1.29	1.29	1.50	1.51
One month	1.30	1.32	1.30	1.30	1.36	1.34	1.49	1.43	1.49	1.52	1.53	1.59	1.22	1.25	1.39	1.38
Two months	1.52	1.47	1.64	1.70	1.16	1.45	1.65	1.70*	1.53	1.60	1.12	1.09	1.20	1.20	1.42	1.38
Three months	1.54	1.55	1.76	1.82	1.21	1.21	1.49	1.42*	1.57	1.54	1.04	1.03	1.31	1.22	1.45	1.53
Four months	1.78	1.92*	1.79	1.80	1.31	1.42	1.22	1.08*	1.46	1.47	1.01	1.01	1.51	1.43	1.52	1.52
S.E.M	0.04		0.04		0.085		0.04		0.03		0.03		0.03		0.02	
POTASSIUM																
Day old	18.87	20.53	20.08	20.53	19.40	18.84	19.87	21.23	20.50	21.87	20.25	20.90	16.52	18.98	16.47	14.81
One month	21.19	21.84	24.25	22.22	18.72	19.89	22.25	20.67	19.63	20.26	32.01	29.25	25.85	24.93	22.07	24.45
Two months	31.32	30.05	30.76	31.20	31.39	31.51	32.28	34.71	31.18	34.35	33.62	35.05	33.75	35.40	35.85	35.75
Three months	34.62	34.76	30.49	30.57	29.36	30.48	30.25	30.87	29.50	30.50	24.00	26.10	35.37	35.30	30.56	31.03
Four months	35.05	34.26	30.81	31.10	22.85	25.10	20.16	20.00	25.35	27.00	20.05	20.62	29.64	30.00	24.36	24.87
S.E.M	0.77		1.00		0.87		0.68		0.39		0.66		0.58		0.53	
SODIUM																
Day old	505.06	533.25	535.62	538.00	520.00	524.25	528.25	538.75	531.75	523.00	534.75	536.75	541.50	547.75	523.75	533.75
One month	538.12	537.00	538.00	536.25	536.25	541.00	541.25	540.00	536.75	536.00	531.25	530.00	545.00	547.50	538.37	537.75
Two months	530.00	530.00	534.25	537.00	527.00	530.00	534.69	536.75	531.25	532.00	527.50	531.75	534.39	532.50	533.25	528.75
Three months	540.50	538.75	545.12	550.00	526.75	532.25	535.00	544.75	533.00	540.50	532.50	542.75	541.25	552.50	540.00	531.25
Four months	551.25	541.50	568.75	568.75	535.00	533.75	552.50	549.25	550.00	555.00	535.50	533.00	573.75	571.25	546.25	538.75
S.E.M	1.77		1.78		3.42		2.78		2.46		2.35		1.95		3.00	
COPPER																
Day old	0.11	0.12	0.10	0.09	0.11	0.10	0.10	0.10	0.09	0.08	0.11	0.10	0.10	0.10	0.09	0.10
One month	0.10	0.10	0.10	0.11	0.10	0.11	0.32	0.57	0.11	0.12	0.12	0.11	0.10	0.10	0.10	0.10
Two months	0.15	0.15	0.21	0.34	0.13	0.13	0.20	0.20	0.11	0.12	0.15	0.15	0.12	0.12	0.20	0.19
Three months	0.15	0.15	0.20	0.20	0.14	0.14	0.14	0.14	0.14	0.13	0.16	0.16	0.14	0.14	0.21	0.20
Four months	0.16	0.17	0.18	0.17	0.17	0.17	0.11	0.12	0.18	0.19	0.17	0.19	0.16	0.17	0.23	0.25
S.E.M.	0.003		0.04		0.005		0.14		0.004		0.004		0.005		0.07	
ZINC																
Day old	0.38	0.39	0.31	0.32	0.40	0.40	0.31	0.33	0.36	0.36	0.42	0.44	0.31	0.31	0.34	0.36
One month	0.34	0.34	0.35	0.44	0.45	0.45	0.33	0.31	0.45	0.42	0.51	0.55	0.31	0.33	0.36	0.39
Two monhs	0.25	0.31*	0.40	0.39	0.30	0.31	0.25	0.26	0.32	0.34	0.27	0.36	0.33	0.32	0.35	0.38
Three months	0.40	0.47*	0.56	0.61	0.31	0.41	0.25	0.23	0.48	0.47	0.32	0.40	0.47	0.51	0.61	0.61
Four months	0.51	0.53	0.80	0.72	0.41	0.41	0.31	0.34	0.58	0.59	0.63	0.59	0.65	0.71	0.77	0.75
S.E.M	0.01		0.02		0.01		0.007		0.01		0.01		0.005		0.01	

* Paired values (male/female) under a brain region and in an age group in the same horizontal column bearing the * sign are significantly different (P<0.05)

TABLE 3.1.3 REGIONAL DISTRIBUTION OF THE MINERAL PROFILE OF PORCINE BRAIN REGIONS AT DIFFERENT AGES. (mean ± S.E.M)

Age	B R A I N R -							
CALCIUM	P O N S	CEREBELLUM	AMYGDALA	HIPPOCAMPUS	HYPOTHALAMUS	CEREBRAL CORTEX	MID BRAIN	MEDULA OBLONGAT
Day old	1.82±0.05^{C}	2.019±0.03^{C}	1.55±0.01^{C}	1.99±0.03^{a}	2.08±0.01^{a}	2.04±0.02^{a}	1.37±0.04^{d}	2.48±0.01^{ab}
One month	1.85±0.03^{c}	2.42 ±0.49^{b}	2.06±0.02^{a}	1.79±0.04^{ab}	2.06±0.02^{a}	2.11±0.006^{a}	1.66±0.02^{c}	2.29±0.04^{ab}
Two months	2.18±0.05^{b}	2.51 ±0.03^{ab}	1.69±0.03^{b}	1.44±0.04^{cd}	1.83±0.02^{b}	1.23±0.05^{d}	1.46±0.03^{d}	1.84±0.02^{b}
Three months	1.92±0.04^{c}	2.56 ±0.04^{a}	1.92±0.03^{a}	1.22±0.02^{d}	1.82±0.01^{b}	1.34±0.02^{c}	2.07±0.03^{b}	2.09±0.03^{b}
Four months	2.56±0.05^{a}	2.60 ±0.09^{a}	2.09±0.05^{a}	1.59±0.06^{bc}	1.58±0.02^{c}	1.53±0.03^{b}	2.66±0.08^{a}	2.82±0.03^{a}
MAGNESIUM								
Day old	1.52±0.01^{b}	1.31 ±0.04^{c}	1.24±0.01^{a}	1.53±0.01^{b}	1.50±0.02^{ab}	1.65±0.06^{a}	1.29±0.04^{b}	1.507±0.03^{a}
One month	1.31±0.06^{c}	1.31 ±0.02^{c}	1.35±0.02^{a}	1.46±0.03^{c}	1.50±0.011	1.56±0.02^{a}	1.23±0.03^{bc}	1.39±0.02^{b}
Two months	1.50±0.02^{b}	1.671±0.03^{b}	1.31±0.007^{a}	1.68±0.02^{a}	1.57±0.05^{a}	1.10±0.03^{b}	1.203±0.03^{c}	1.41±0.017^{b}
Three months	1.54±0.05^{b}	1.79 ±0.02^{a}	1.21±0.01^{a}	1.46±0.02^{c}	1.56±0.02^{a}	1.03±0.02^{b}	1.27±0.01^{bc}	1.49±0.02^{a}
Four months	1.85±0.02^{a}	1.79 ±0.01^{a}	1.36±0.06^{a}	1.15±0.02^{d}	1.47±0.02^{b}	1.01±0.01^{b}	1.47±0.015^{a}	1.52±0.03^{a}
POTASSIUM								
Day old	19.70±0.35^{d}	20.08 ±0.37^{c}	19.12±0.50^{c}	20.55±0.46^{c}	21.19±0.40^{d}	20.57±.0.5^{d}	17.75±0.36^{d}	15.644±0.40^{d}
One month	21.52±0.17^{c}	23.23 ±0.13^{b}	19.30±0.22^{c}	21.46±0.47^{c}	19.95±0.32^{d}	30.63±0.314^{b}	25.39±0.33^{c}	23.26±0.27^{c}
Two months	30.69±0.64^{b}	30.98 ±0.45^{a}	30.95±0.29^{a}	33.50±1.12^{a}	32.77±1.02^{a}	34.34±0.78^{a}	34.58±0.67^{a}	35.80±1.051^{a}
Three months	34.69±0.51^{a}	30.53 ±0.38^{a}	29.92±0.53^{a}	30.56±0.36^{b}	30.00±0.59^{b}	25.05±0.02^{c}	35.34±0.34^{a}	30.79±0.58^{b}
Four months	34.65±0.47^{a}	30.96 ±0.36^{a}	23.98±0.38^{b}	20.08±0.46^{c}	26.17±0.65^{c}	20.34±1.00^{d}	29.82±1.52^{b}	24.62±0.74^{c}
SODIUM								
Day old	534.156±1.32^{bc}	536.81±1.14^{c}	522.12±0.68^{d}	538.50±2.21^{c}	527.35±2.71^{c}	535.75±1.42^{a}	544.62±2.17^{b}	528.75±2.62^{b}
One month	537.56±1.04^{b}	537.00±1.08^{c}	538.62±0.59^{b}	540.62±2.10^{b}	537.37±0.48^{b}	530.62±4.71^{a}	546.25±1.30^{b}	535.06±2.20^{ab}
Two months	530.00±2.70^{c}	535.62±1.38^{c}	528.75±1.55^{c}	535.72±1.89^{bc}	531.62±0.48^{bc}	529.62±1.23^{a}	533.45±0.56^{c}	531.00±1.12^{b}
Three months	539.62±2.02^{b}	547.56±1.03^{b}	529.50±2.37^{c}	539.87±1.44^{b}	536.75±1.34^{a}	537.62±1.35^{a}	546.87±4.27^{b}	535.62±2.80^{ab}
Four months	549.37±1.22^{a}	568.75±2.88^{a}	534.37±1.20^{a}	550.87±2.04^{a}	552.50±1.18^{a}	534.25±1.34^{a}	572.50±1.18^{a}	542.50±1.78^{a}
COPPER								
Day old	0.12±0.001^{c}	0.10±0.01^{c}	0.11±0.24^{d}	0.10±0.001^{b}	0.08±0.006^{d}	0.10±0.002^{c}	0.10±0.002^{d}	0.21±0.005^{a}
One month	0.104±0.001^{d}	0.10±0.002^{c}	0.10±0.001^{d}	0.44±0.003^{a}	0.11±0.006^{c}	0.11±0.003^{c}	0.10±0.003^{d}	0.20±0.002^{a}
Two months	0.15±0.004^{b}	0.273±0.002^{a}	0.13±0.003^{c}	0.21±0.003^{b}	0.11±0.006^{c}	0.15±0.005^{b}	0.12±0.00001^{c}	0.20±0.002^{a}
Three months	0.15±0.001^{b}	0.20±0.001^{ab}	0.14±0.002^{b}	0.14±0.002^{b}	0.13±0.0001^{b}	0.16±0.01^{b}	0.14±0.006^{b}	0.21±0.01^{a}
Four months	0.17±0.002^{a}	0.17±0.003^{bc}	0.17±0.002^{a}	0.11±0.002^{b}	0.19±0.001^{a}	0.18±0.003^{a}	0.17±0.004^{a}	0.24±0.002^{a}
ZINC								
Day old	0.39±0.002^{c}	0.31±0.003^{d}	0.40±0.004^{b}	0.32±0.006^{a}	0.36±0.01^{d}	0.43±0.003^{c}	0.31±0.01^{c}	0.35±0.001^{c}
One month	0.34±0.001^{d}	0.39±0.004^{c}	0.45±0.006^{a}	0.32±0.007^{a}	0.42±0.005^{c}	0.531±0.01^{b}	0.32±0.004^{c}	0.38±0.01^{c}
Two months	0.28±0.014^{e}	0.393±0.01^{c}	0.31±0.01^{d}	0.253±0.004^{b}	0.33±0.01^{e}	0.31 ±0.02^{e}	0.32±0.01^{c}	0.37±0.01^{c}
Three months	0.43±0.001^{b}	0.59±0.01^{b}	0.36±0.01^{c}	0.244±0.004^{b}	0.48±0.002^{b}	0.36 ±0.01^{d}	0.49±0.01^{b}	0.61±0.01^{b}
Four months	0.52±0.007^{a}	0.76±0.02^{a}	0.41±0.002^{b}	0.33±0.006^{a}	0.59±0.01^{a}	0.61 ±0.01^{a}	0.68±0.02^{a}	0.76±0.01^{a}

Values in the same vertical column bearing different superscripts are significantly different (P(0.05).

Table 3.14

REGRESSION TABLE OF MINERAL CONCENTRATION IN THE BRAIN REGIONS OF PIGS ON AGE

Y	X	Prediction Equation Equation	r	S.E.	Significance
CALCIUM					
Pons		y=1.589+0.155x	0.755	0.09	***
Cerebellum		y=2.0222+0.134x	0.805	0.11	***
Amygdala		y=1.616+0.078x	0.563	0.06	**
hippocampus		y=1.922-0.084x	-0.492	0.08	*
hypothalamus		y=2.242-0.122x	-0.943	0.03	***
Cerebral Cortex		y=2.204-0.183x	-0.702	0.06	***
Mid Brain		y=0.948+0.299x	0.881	0.08	***
medulla Oblongata		y=2.054+0.009x	0.026	0.57	n.s
MAGNESIUM					
Pons		y=1.275+0.090x	0.700	0.06	***
Cerebellum		y=1.128+0.148x	0.909	0.06	***
Amygdala		y=1.237-0.003x	-0.016	0.25	n.s
Hippocampus		y=1.677-0.073x	-0.589	0.04	**
Hypothalamus		y=1.539-0.006x	-0.132	0.06	n.s
Cerebral Cortex		y=1.906-0.180x	-0.895	0.06	***
Mid Brain		y=1.147+0.041x	0.549	0.05	*
Medulla Oblongata		y=1.390+0.020x	0.437	0.04	n.s
POTASSIUM					
Pons		y=15.297+4.290x	0.945	0.87	***
Cerebellum		y=18.597+2.855x	0.886	0.74	***
Amygdala		y=18.419+2.043x	0.557	0.81	*
Hippocampus		y=22.332+0.890x	0.210	1.28	n.s
Hypothalamus		y=19.873+2.041x	0.567	1.29	**
Cerebral Cortex		y=27.950+0.601x	-0.151	1.24	n.s
Mid Brain		y=19.197+2.994x	0.645	1.50	**
Medulla Oblongata		y=18.402+2.518x	0.514	1.32	*
SODIUM					
Pons		y=528.175+3.306x	0.648	3.54	**
Cerebellum		y=522.762+7.437x	0.815	3.27	***
Amygdala		y=525.500+1.800x	0.417	2.87	n.s
Hippocampus		y=488.225+13.700x	0.434	42.88	n.s
Hypothalamus		y=522.325+5.025x	0.824	3.000	***
Cerebral cortex		y=531.662+0.862x	0.222	4.87	n.s
Mid Brain					
COPPER					
Pons		y=0.092+0.015x	0.882	0.005	***
Cerebellum		y=0.093+0.023x	0.688	0.01	***
Amygdala		y=0.082+0.017x	0.933	0.005	***
Hippocampus		y=0.106+0.008x	0.283	0.005	n.s
Hypothalamus		y=0.057+0.023x	0.914	0.01	***
Cerebral Cortex		y=0.020+0.079x	0.961	0.01	***
Mid Brain		y=0.075+0.018x	0.949	0.007	***
Medulla Oblongata		y=0.052+0.031x	0.908	0.01	***
ZINC					
Pons		y=0.284+0.036x	0.620	0.02	***
Cerebellum		y=0.160+0.110x	0.944	0.02	***
Amygdala		y=0.406-0.006x	-0.173	0.01	n.s
Hippocampus		y=0.318-0.008x	-0.282	0.01	n.s
Hypothalamus		y=0.282+0.051x	0.790	0.01	***
Cerebral cortex		y=0.385+0.021x	0.253	0.02	n.s
Mid Brain		y=0.167+0.085x	0.892	0.02	***
medulla Oblongata		y=0.189+0.100x	0.904	0.02	***

* P < 0.05 ** P<0.01 *** P<0.001 n.s P>0.05

POTASSIUM

Potassium increased significantly with age in the pons, cerebellum, amygdala, hypothalamus, mid brain and medulla oblongata. The hippocampus showed a non-significant positive correlation coefficient while the cerebral cortex displayed a non-significant negative correlation.

Significant age/sex interaction was observed only in the hypothalamus with the female being superior to the males at two and four months of age.

SODIUM

Sodium increased significantly in the pons, cerebellum, hypothalamus, midbrain, medulla oblongata (as shown by the positive correlation in table 3.14) and non significantly in the amygdala, hippocampus and cerebral cortex.

No significant sex influence was observed except in the hypothalamus where the females were superior to the male at three and four months of age. The male however displayed a higher sodium content at day old than the female.

COPPER

Generally copper increased significantly with age from day old to four months of age. The only exception was the hypothalamus which had a non-significant correlation of 0.283(P>0.05). (Table 1.14 and figure 3.1.4). No significant sex influences were observed.

ZINC

Data in table 3.1.4 Indicate that only the pons, cerebellum, hypothalamus, midbrain and medulla oblongata displayed significant increases in zinc concentration with age. The amygdala and hippocampus displayed a non- significant negative correlation with age while the cerebral cortex on the other hand had a non-significant increase with age.

Significant age sex interactions were observed only in the pons and midbrain where the females had slightly higher levels than the males at one three and four months of age.

3.1.1.3 THE HYPOPHYSES

Tables 3.1.5 and 3.1.6 show the influence of age and sex on AChE activity, protein content and SAChE activity in the hypophyses. The regression curves are also displayed in figures 3.7. and 3.8.

AChE ACTIVITY

In both sexes, AChE activity was higher in the adenohypophysis than the neurohypophysis. Both the adenohypophysis and the neurohypophysis showed a significant decrease in AChE activity with age ($r=-0.99$ and 0.84, respectively ($P<0.001$; table 3.1.9). Significant sex difference was observed only in the adenohypophysis with the female being superior to the male at day old. No significant sex effect was observed in the neurohypophysis.

PROTEIN CONCENTRATION

Protein concentration increased significantly with age in both the adenohypophysis and the neurohypophysis as shown by the highly significant and positive correlation coefficients in table 3.1.7.

No sex influence was observed.

SAChE ACTIVITY

SAChE activity declined significantly with age in the hypophyses. In the adenohypophysis, the decline was fairly steady whereas in the neurohypophysis there was a sharp drop from 20.03 Umole/g protein/min at day old to 7.65 at one month followed by a very steady decline to 2.88 at four months.

The only significant sex influences was observed at day old with the female being superior to the male.

3.1.1.4 MINERAL PROFILE

ADENOHYPOPHYSIS

Calcium, Sodium and copper increased linearly with age evidenced by the positive and significant correlations recorded for these cations (table 3.1.8). Magnesium and potassium did not show a consistent trend resulting in non-significant negative correlations. Zinc content also declined with age but not at a significant rate ($P>0.05$). The only significant sex difference was observed at one month of age when the female piglets had higher sodium content than the male ($P<0.05$).

NEUROHYPOPHYSIS

Zinc and potassium displayed an initial rise in levels from day old to between one and two months of age followed by a decline which was significant in potassium ($r=-0.66$; $P<0.01$, table 3.1.9).

Magnesium and calcium also displayed non-significant negative correlations with age but sodium recorded a highly significant and positive rise with age ($r=0.86$; $P<0.001$). Figure 3.1.6 illustrate the various regression curves obtained.

Significant sex differences were only recorded in calcium, magnesium and Sodium levels. (table 3.1.8). The males were superior to the females at Day old and two months of age with respect to Calcium levels while the reverse was observed with sodium level. Magnesium content in the neurohypophysis of females was also higher than in the males at Day old and One month of age only.

Table 3.1.5 EFFECT OF SEX ON THE DEVELOPMENT OF AChE ACTIVITY, PROTEIN AND SAChE CONTENTS IN THE HYPOPHYSES WITH INCREASING AGE.

	AChE ACTIVITY		PROTEIN CONCENTRATN		SAChE ACTIVITY	
	Male	Female	Male	Female	Male	Female
ADENOHYPOPHYSIS						
Day old	2.03	2.720	0.09	0.09	20.36*	29.49*
One month	2.06	2.08	0.12	0.12	17.81	17.15
Two months	1.19	1.14	0.26	0.30	4.83	3.82
Three months	1.02	1.11	0.45	0.38	2.33	2.94
Four months	0.47	0.46	0.75	0.70	0.78	70.70
S.E.M	0.056		0.053		0.563	
NEUROHYPOPHYSIS						
Day old	1.202	2.19	0.08	0.08	13.91	26.16
One month	1.09	1.06	0.13	0.15	8.29	7.01
Two months	1.19	0.08	0.20	0.21	6.09	3.93
Three months	1.10	0.99	0.19	0.18	5.70	5.43
Four months	0.58	0.65	0.20	0.23	2.88	2.87
S.E.M	0.119		0.009		1.087	

Paired values (male/female) in the same horizontal column and age group bearing the * sign significantly differ ($P<0.05$)

Table 3.1.6 CHANGES IN AChE, PROTEIN AND SAChE CONTENT OF THE HYPOPHYSES WITH INCREASING AGE

	ADENOHYPOPHYSIS	NEUROHYPOPHYSIS
AChE ACTIVITY		
Day old	2.37 ± 0.086^{a}	1.69 ± 0.070^{a}
One month	2.07 ± 0.011^{b}	1.07 ± 0.021^{c}
Two months	1.17 ± 0.071^{c}	0.99 ± 0.103^{b}
Three months	1.06 ± 0.028^{c}	1.05 ± 0.024^{b}
Four months	0.47 ± 0.023^{d}	0.61 ± 0.019^{d}
PROTEIN CONTENT		
Day old	0.09 ± 0.002^{d}	0.08 ± 0.001^{d}
One month	0.12 ± 0.010^{d}	0.14 ± 0.007^{c}
Two months	0.28 ± 0.020^{c}	0.20 ± 0.010^{ab}
Three months	0.42 ± 0.034^{b}	0.19 ± 0.003^{b}
Four months	0.72 ± 0.036^{a}	0.21 ± 0.004^{a}
SAChE ACTIVITY		
Day old	24.93 ± 1.458^{a}	20.03 ± 0.618^{a}
One month	17.48 ± 0.312^{b}	7.65 ± 0.366^{b}
Two months	4.33 ± 0.344^{c}	5.02 ± 0.515^{c}
Three months	2.64 ± 0.129^{d}	5.56 ± 0.098^{c}
Four months	0.78 ± 0.123^{e}	2.87 ± 0.038^{d}

values in the same horizontal column differently superscripted significantly differ ($P<0.05$)

Table 3.1.7. CHANGES IN THE CATIONS CONTENT OF PORCINE HYPOPHYSIS WITH INCREASING AGE

	ADENOHYPOPHYSIS	NEUROHYPOPHYSIS
CALCIUM		
Day old	2.01±0.010^{e}	2.11±0.040^{bc}
One month	2.55±0.032^{c}	2.38±0.035^{a}
Two months	2.39±0.084^{d}	2.15±0.029^{b}
Three months	2.85±0.013^{b}	2.04±0.037^{c}
Four months	3.52±0.067^{a}	2.08±0.045^{bc}
MAGNESIUM		
Day old	2.06±0.012^{bc}	1.92±0.043^{c}
One month	2.20±0.012^{a}	2.42±0.024^{a}
Two months	2.07±0.040^{bc}	2.05±0.000^{b}
Three months	2.02±0.012^{c}	2.02±0.012^{b}
Four months	2.09±0.014^{b}	2.03±0.012^{b}
POTASSIUM		
Day old	19.49±0.308^{b}	15.99±0.266^{d}
One month	31.11±0.460^{a}	19.45±0.221^{a}
Two months	16.88±0.272^{c}	17.22±0.337^{b}
Three months	19.84±0.806^{b}	16.19±0.739^{c}
Four months	20.02±0.881^{b}	10.94±0.235^{e}
SODIUM		
Day old	539.50±1.329^{b}	527.50±1.586^{d}
One month	541.25±3.34^{ab}	531.25±0.851^{a}
Two months	542.62±0.966^{ab}	527.37±0.746^{d}
Three months	547.37±1.225^{a}	539.63±1.676^{b}
Four months	538.75±1.650^{b}	550.63±1.974^{a}
COPPER		
Day old	0.20±0.001^{b}	0.20±0.002^{e}
One month	0.20±0.001^{b}	0.21±0.004^{d}
Two months	0.16±0.006^{c}	0.28±0.105^{a}
Three months	0.22±0.005^{b}	0.23±0.005^{b}
Four months	0.28±0.018^{a}	0.21±0.003^{c}
ZINC		
Day old	0.39±0.017^{b}	0.42±0.008^{c}
One month	0.50±0.012^{a}	0.62±0.005^{a}
Two months	0.24±0.006^{c}	0.41±0.007^{c}
Three months	0.25±0.011^{c}	0.42±0.009^{c}
Four months	0.21±0.003^{d}	0.52±0.008^{b}

values in the same vertical column differently superscripted significantly differ ($P<.05$).

Table 3.1.8 EFFECT OF SEX ON THE MINERAL PROFILE OF PORCINE HYPOPHYSES AT DIFFERENT AGES

	CALCIUM		MAGNESIUM		POTASSIUM	
	Male	Female	Male	Female	Male	Female
ADENOHYPOPHYSIS						
Day old	2.003	2.01	2.03	2.08	19.94	19.05
One month	2.54	2.56	2.24	2.16	30.40	31.94
Two months	2.35	2.40	2.115	2.02	16.06	17.69
Three months	2.896	2.82	2.050	2.00	19.18	20.50
Four months	3.56	3.48	2.13	2.05	20.77	19.26
S.E.M	0.043		0.035		0.512	
NEUROHYPOPHYSIS						
Day old	2.18	2.03*	1.82	2.025*	15.93	16.00
One month	2.40	2.307	2.37	2.46*	19.27	19.64
Two months	2.23*	2.06*	2.03	2.06	17.00	17.44
Three months	2.05	2.02	2.02	2.02	16.57	15.78
Four months	2.10	2.06	2.03	2.04	10.47	11.41
S.E.M	0.031		0.024		0.075	

* paired values (male/female) in the same horizontal column and age group bearing the * sign significantly differ ($P<0.05$)

	SODIUM		COPPER		ZINC	
	Male	Female	Male	Female	Male	Female
ADENOHYPOPHYSIS						
Day old	538.00	541.00	0.19	0.20	0.38	0.41
One month	537.00	545.0*	0.19	0.20	0.5	0.49
Two months	540.50	544.75	0.16	0.17	0.25	0.23
Three months	548.50	546.25	0.22	0.21	0.24	0.26
Four months	560.00	557.50	0.31	0.26	0.21	0.21
S.E.M	2.088		0.012		0.12	
NEUROHYPOPHYSIS						
Day old	520.00	535.0*	0.19	0.21	0.43	0.41
One month	530.75	531.75	0.22	0.21	0.63	0.61
Two months	523.00	531.75*	0.27	0.28	0.42	0.41
Three months	538.00	541.25	0.22	0.23	0.41	0.42
Four months	550.00	551.25	0.21	0.20	0.54	0.51
S.E.M	1.699		0.005		0.009	

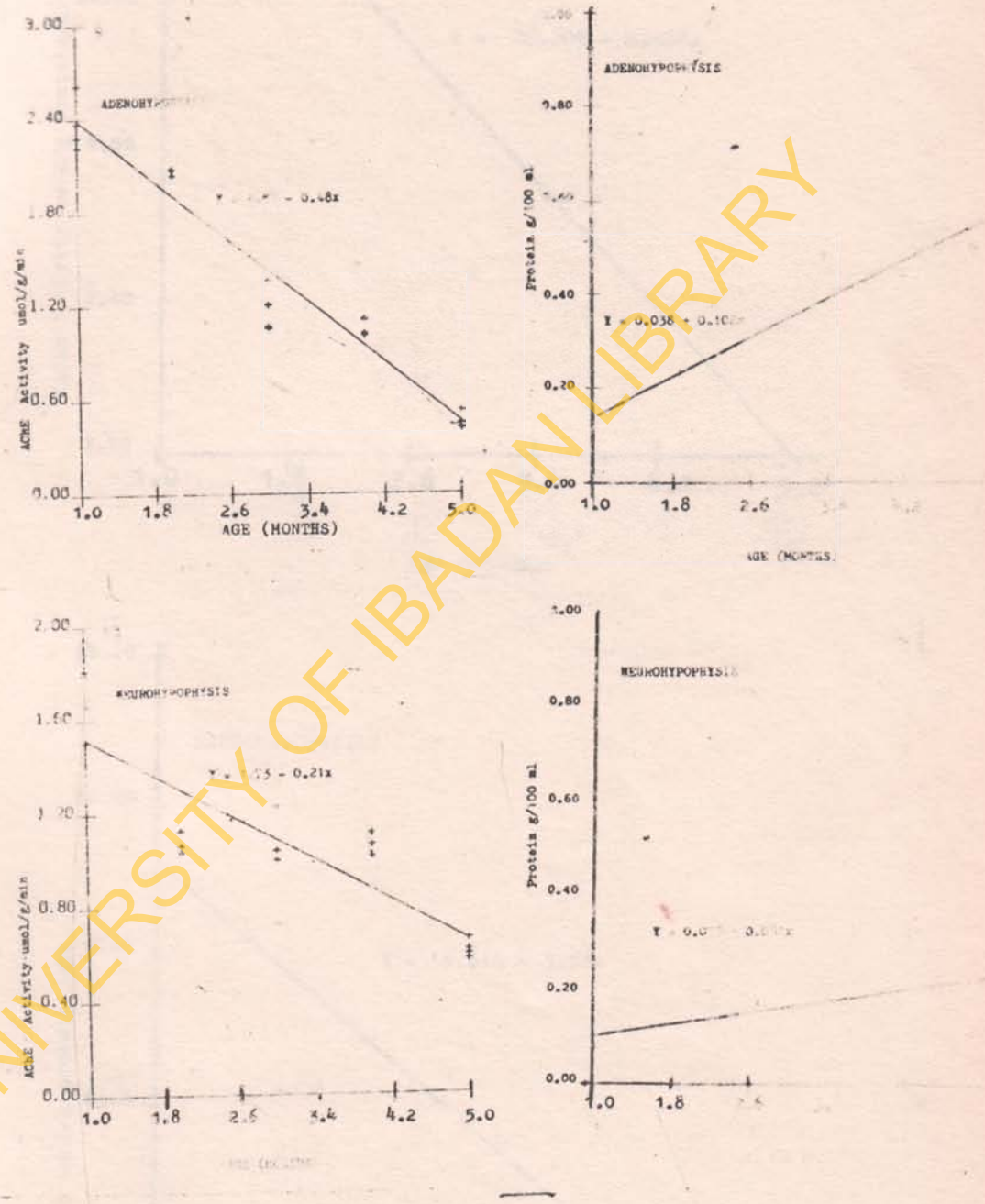
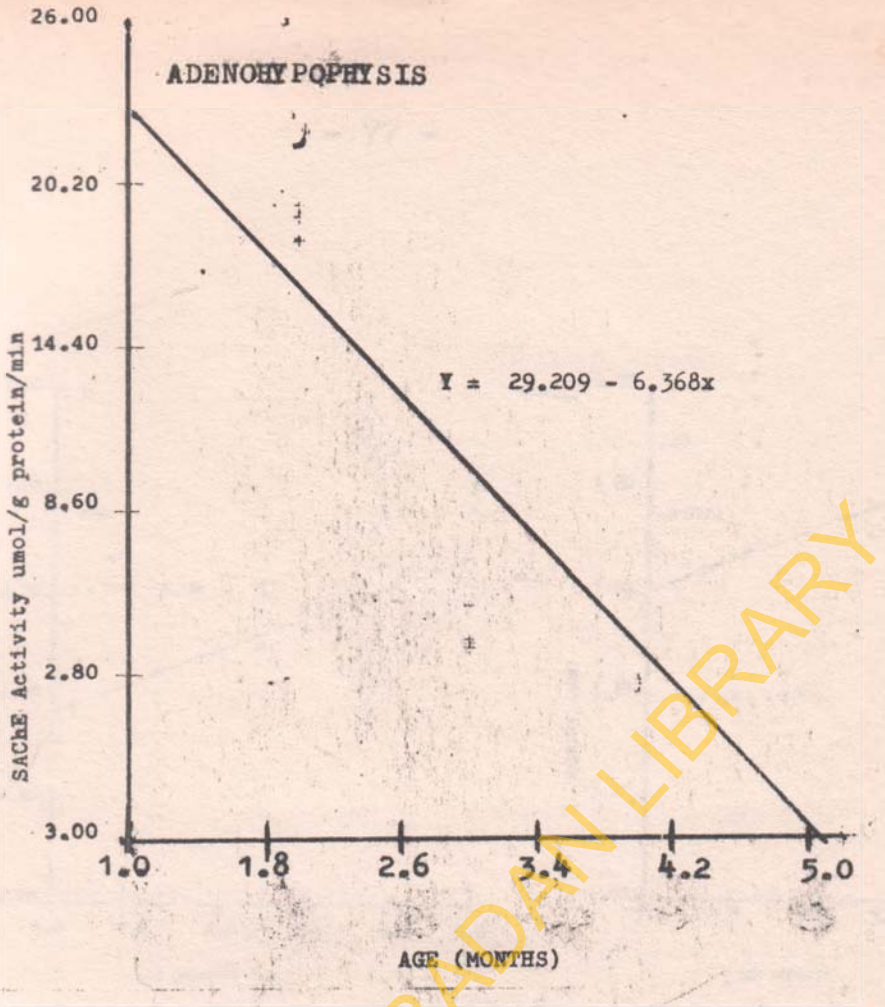


Fig. 3.7. Relationship Between Age and AChE Activities and Total Protein in the Hypophyses.



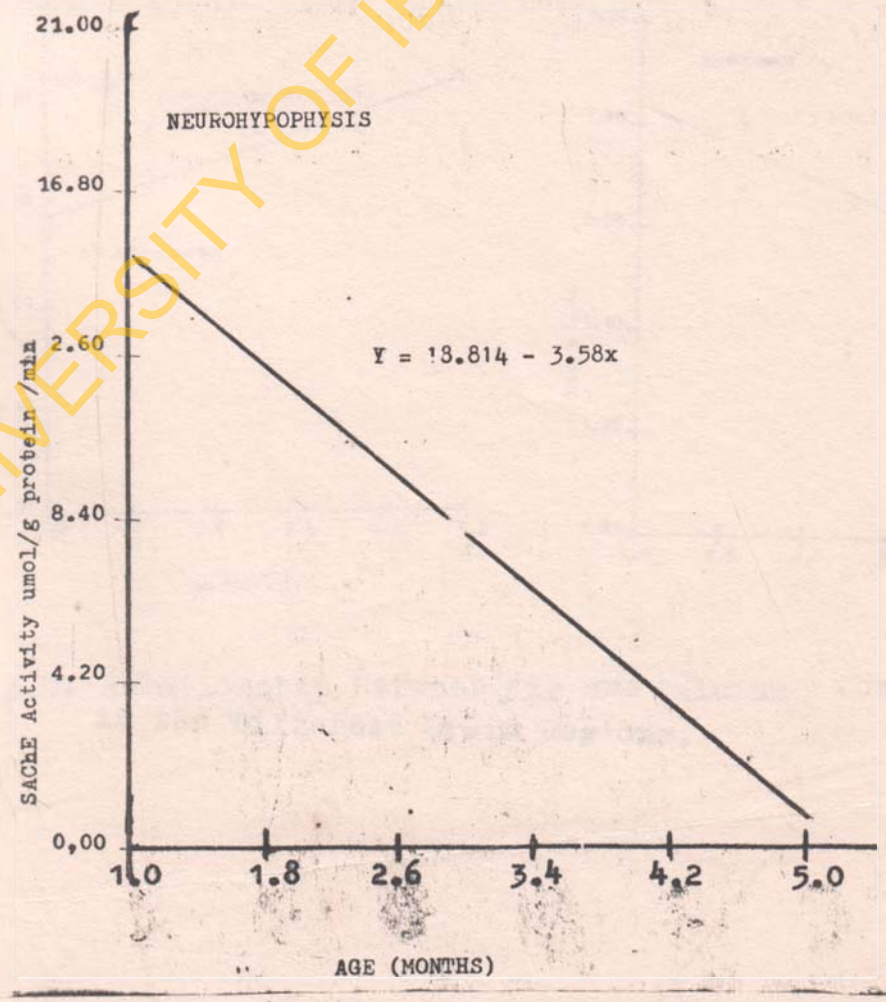


Fig.3.8. Relationship Between Age and SAChE Activities in the Hypophyses.

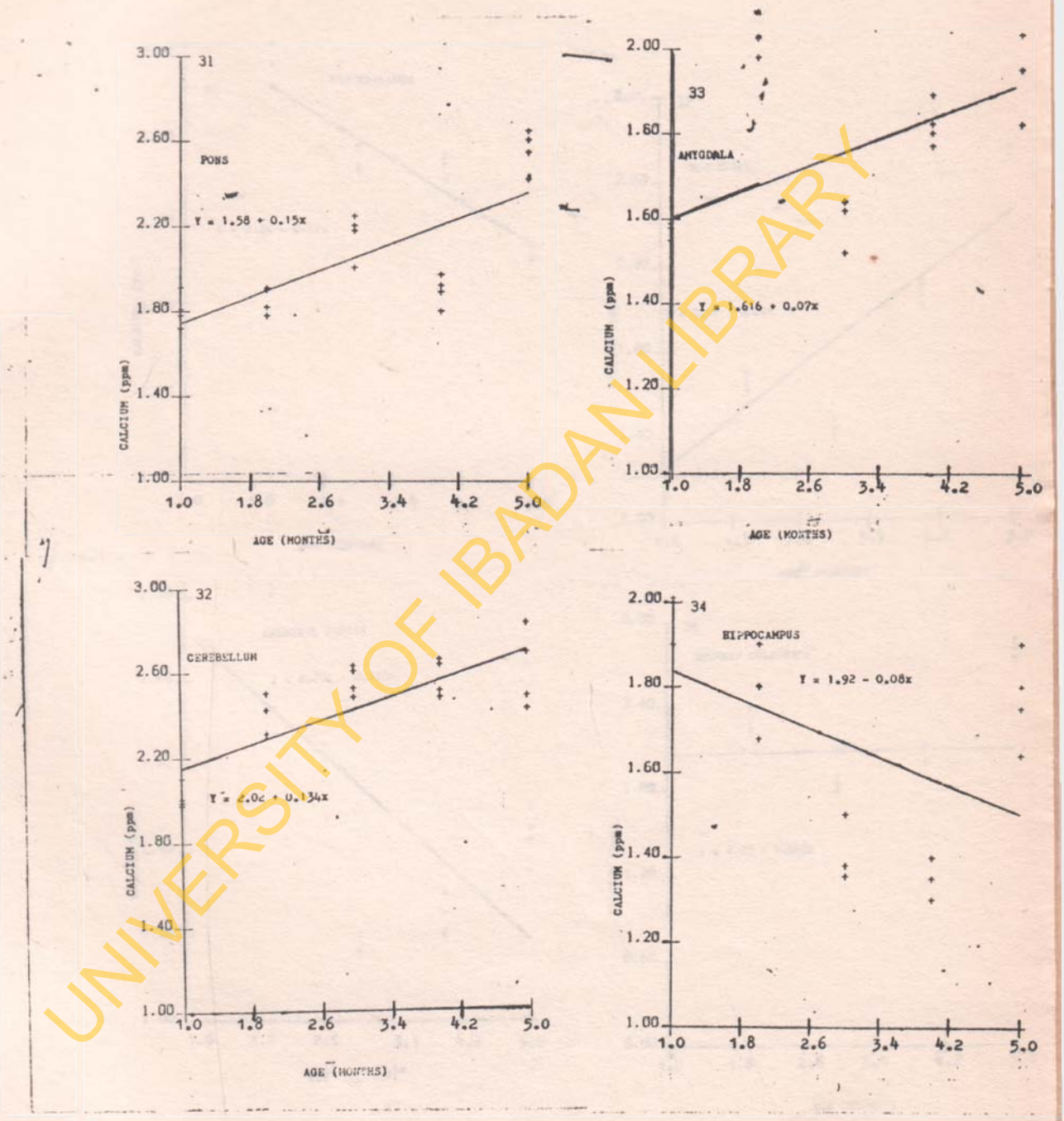


Fig 3.9. Relationship Between Age and Calcium Concentrations in the Different Brain Regions.

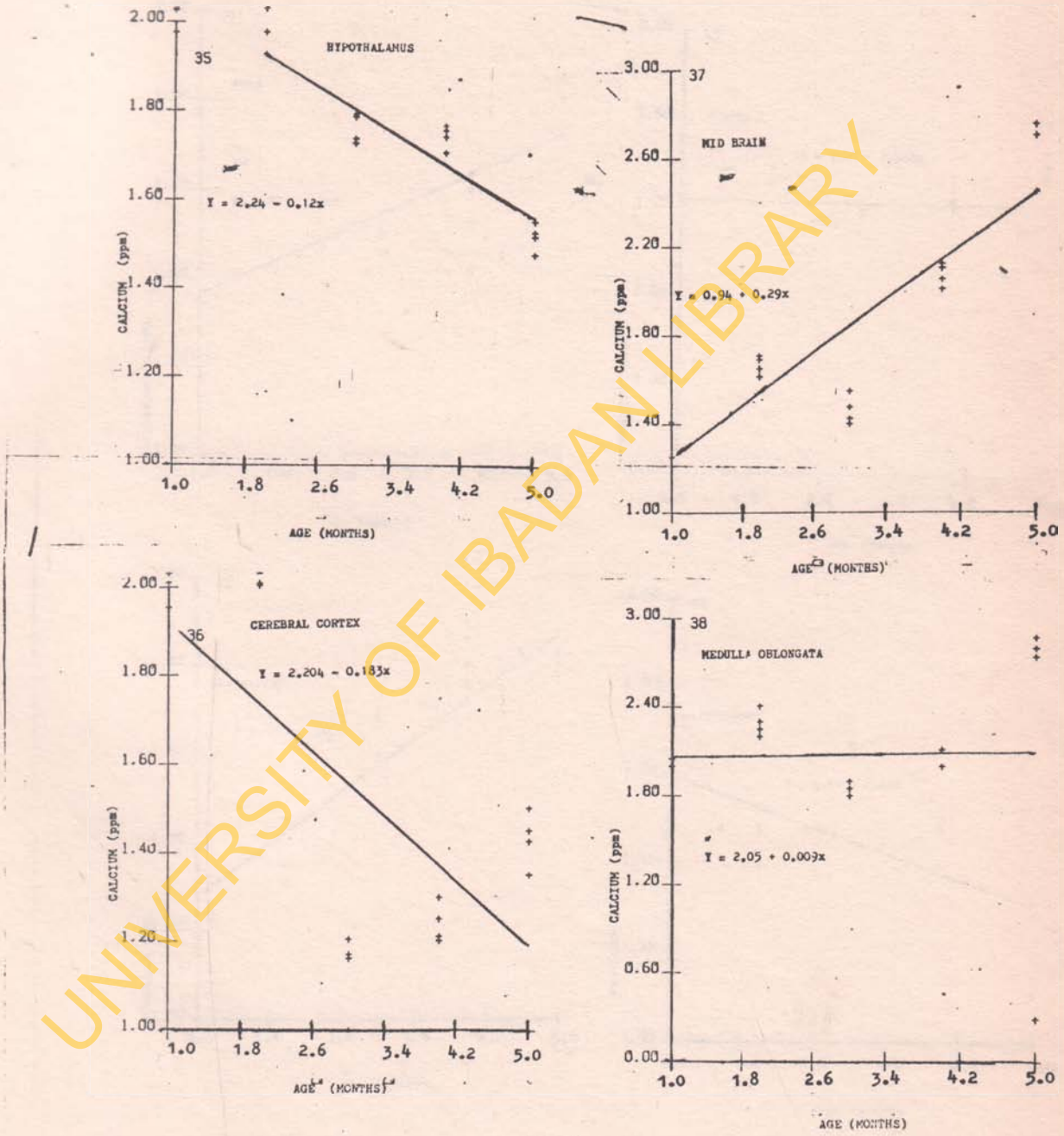


Fig 3.9. (Continued)

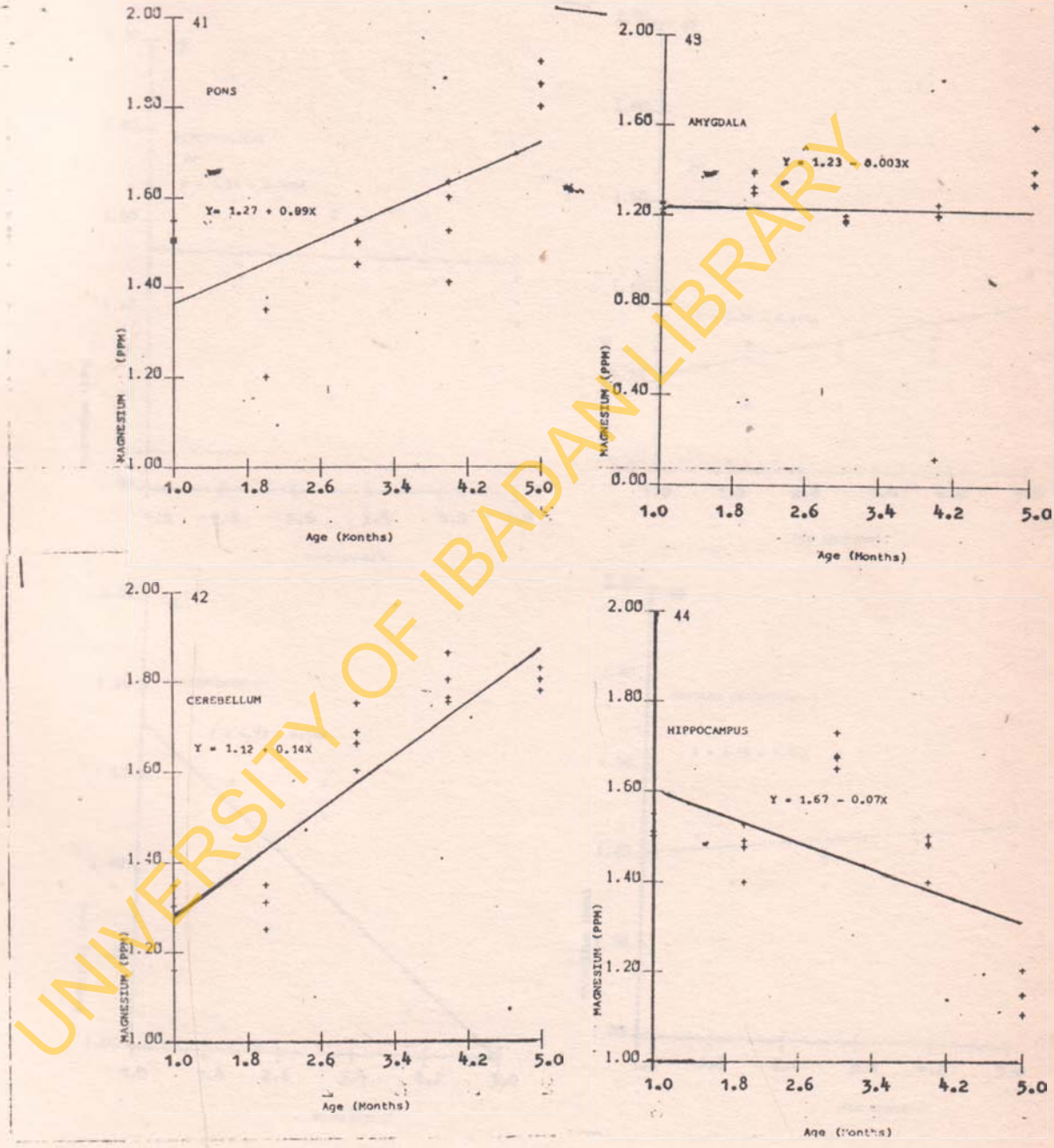


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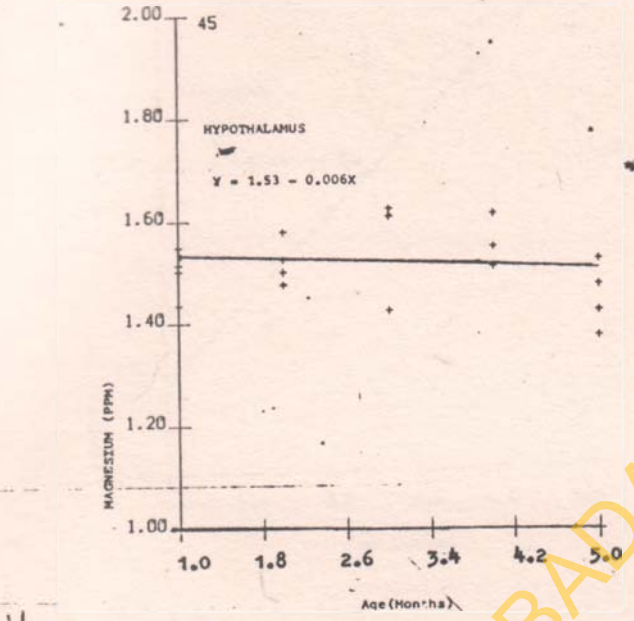
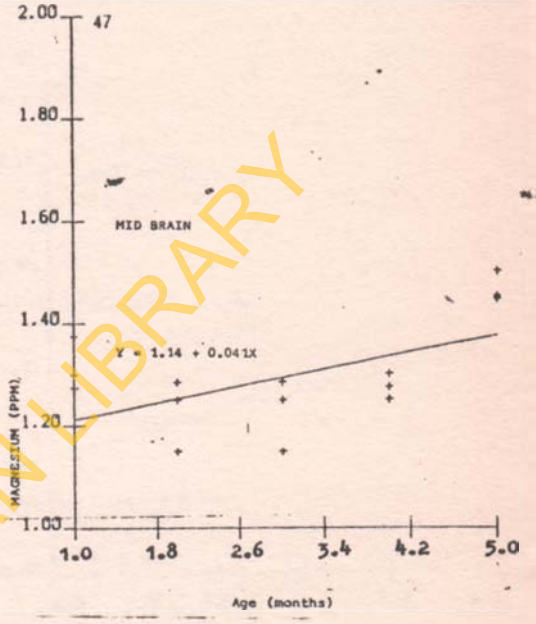
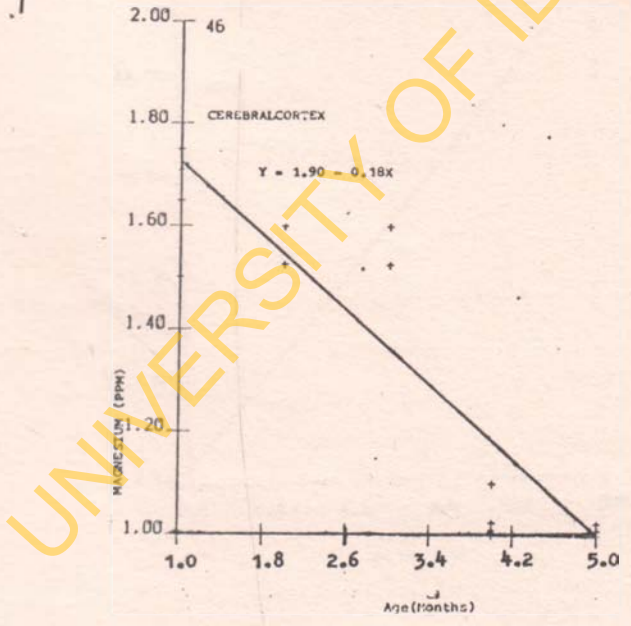
Fig. 3.11. Relationship Between Age and Magnesium Concentrations in the Different Brain Regions.







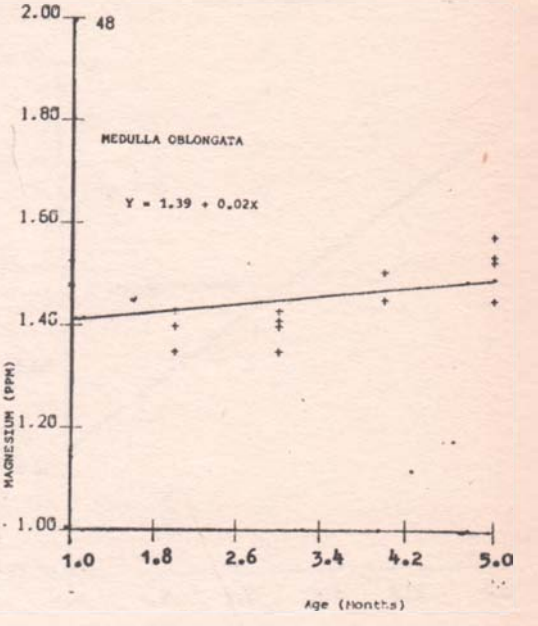


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Fig. 3.11 (Continued)

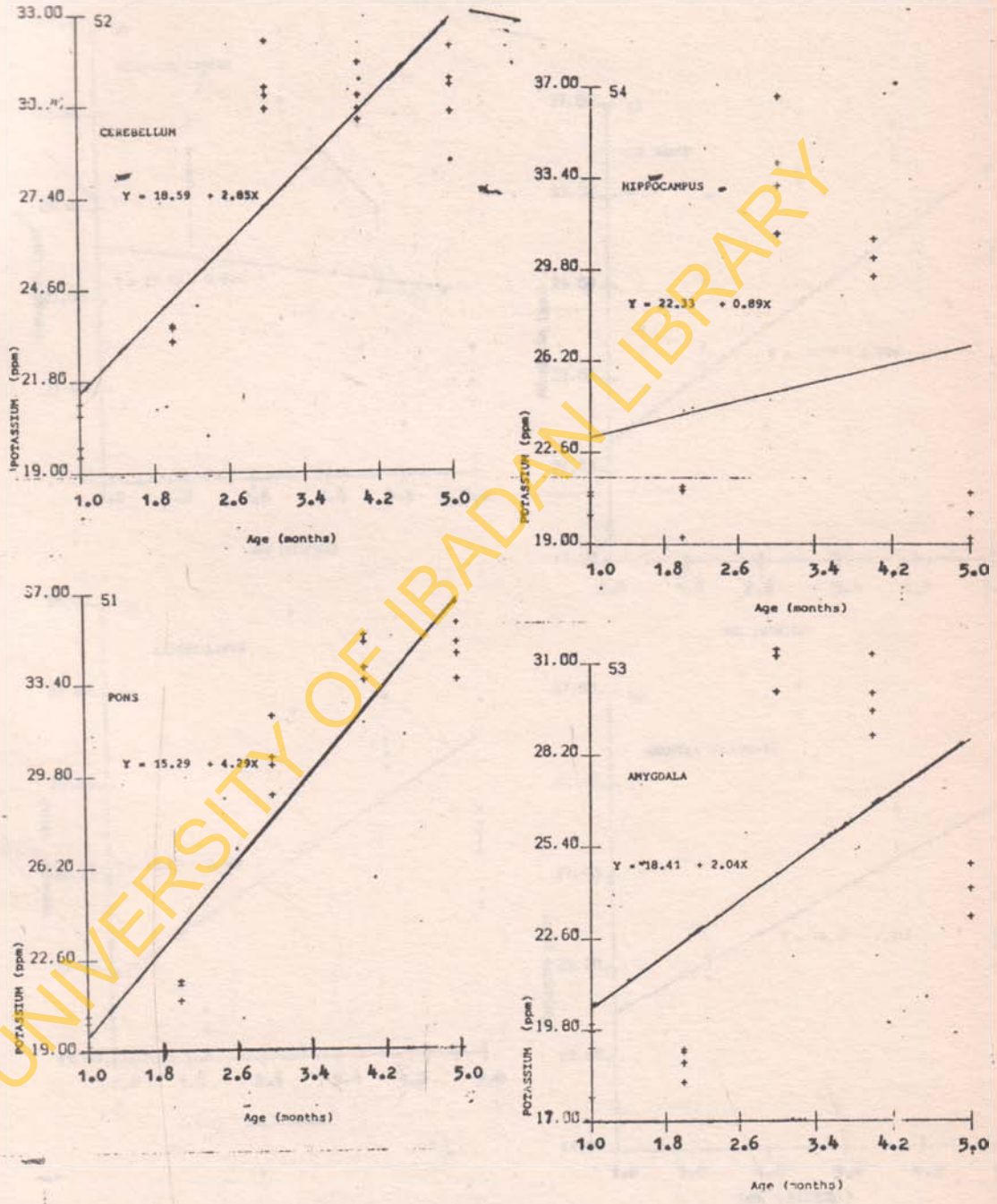


Fig. 3.1.2. Relationship Between Age and Potassium Concentrations in the Different Brain Regions.

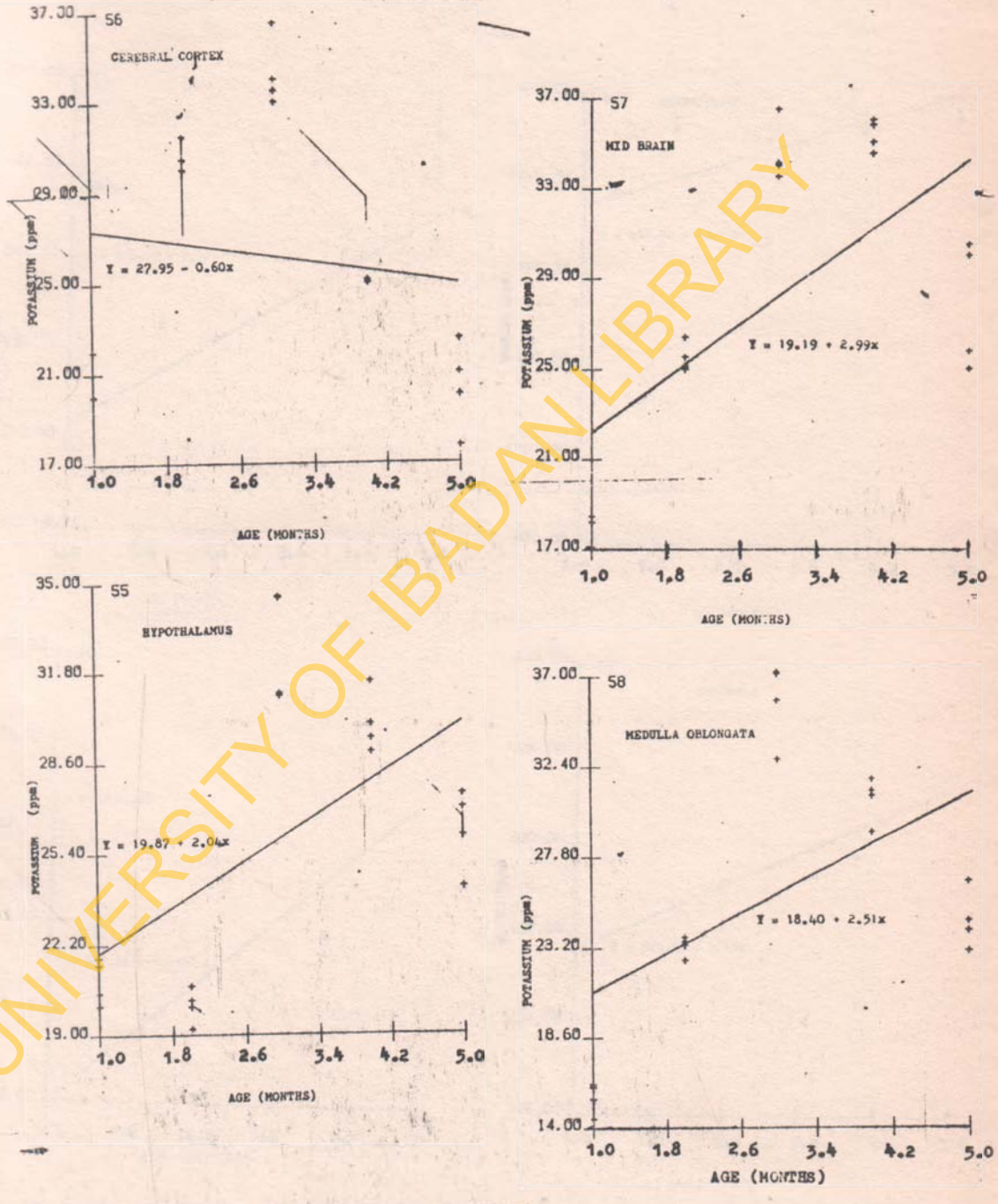


Fig. 3.1.2. (Continued)

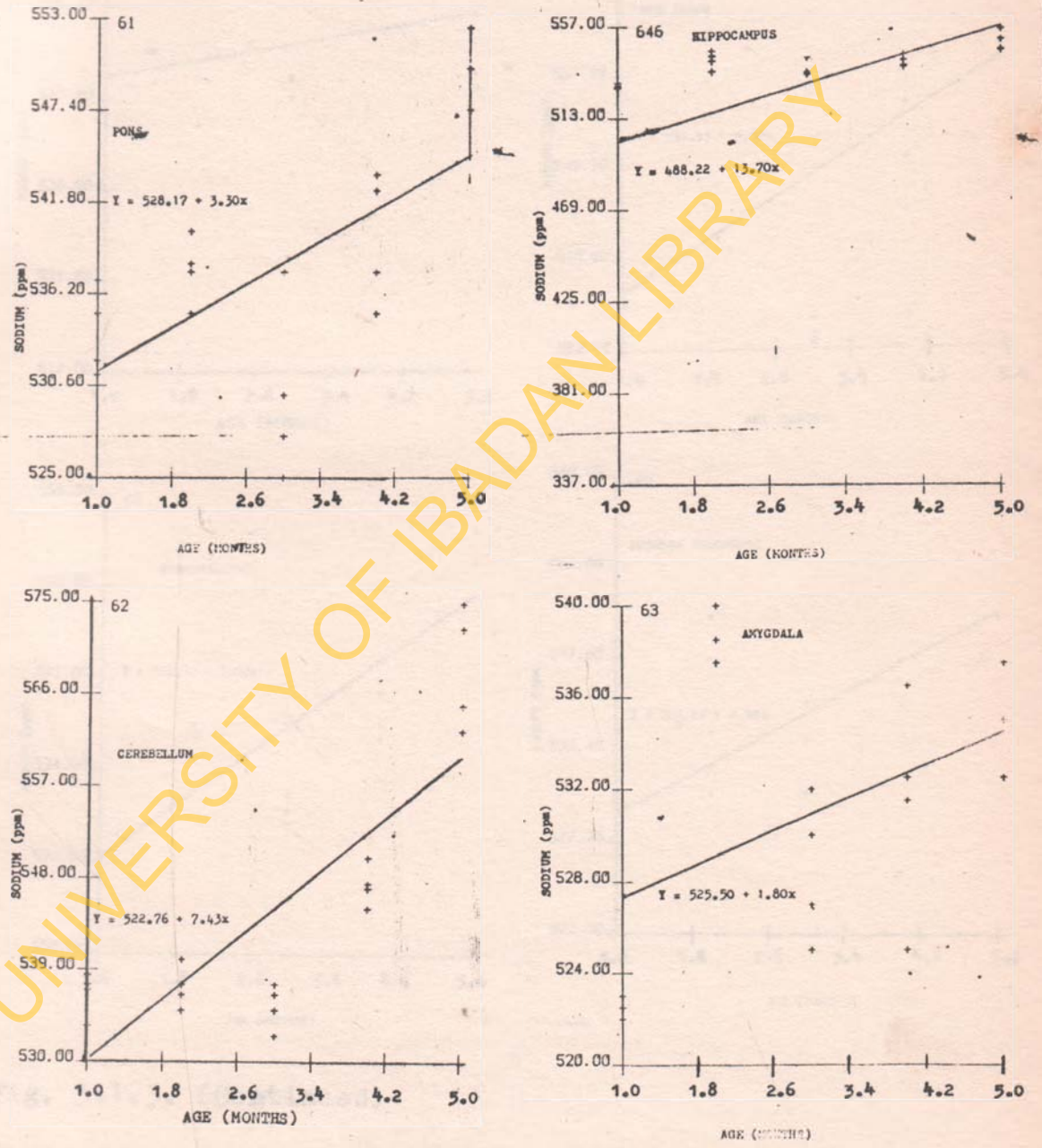


Fig. 3.1.3. Relationship Between Age and Potassium Concentrations in the Different Brain Regions.

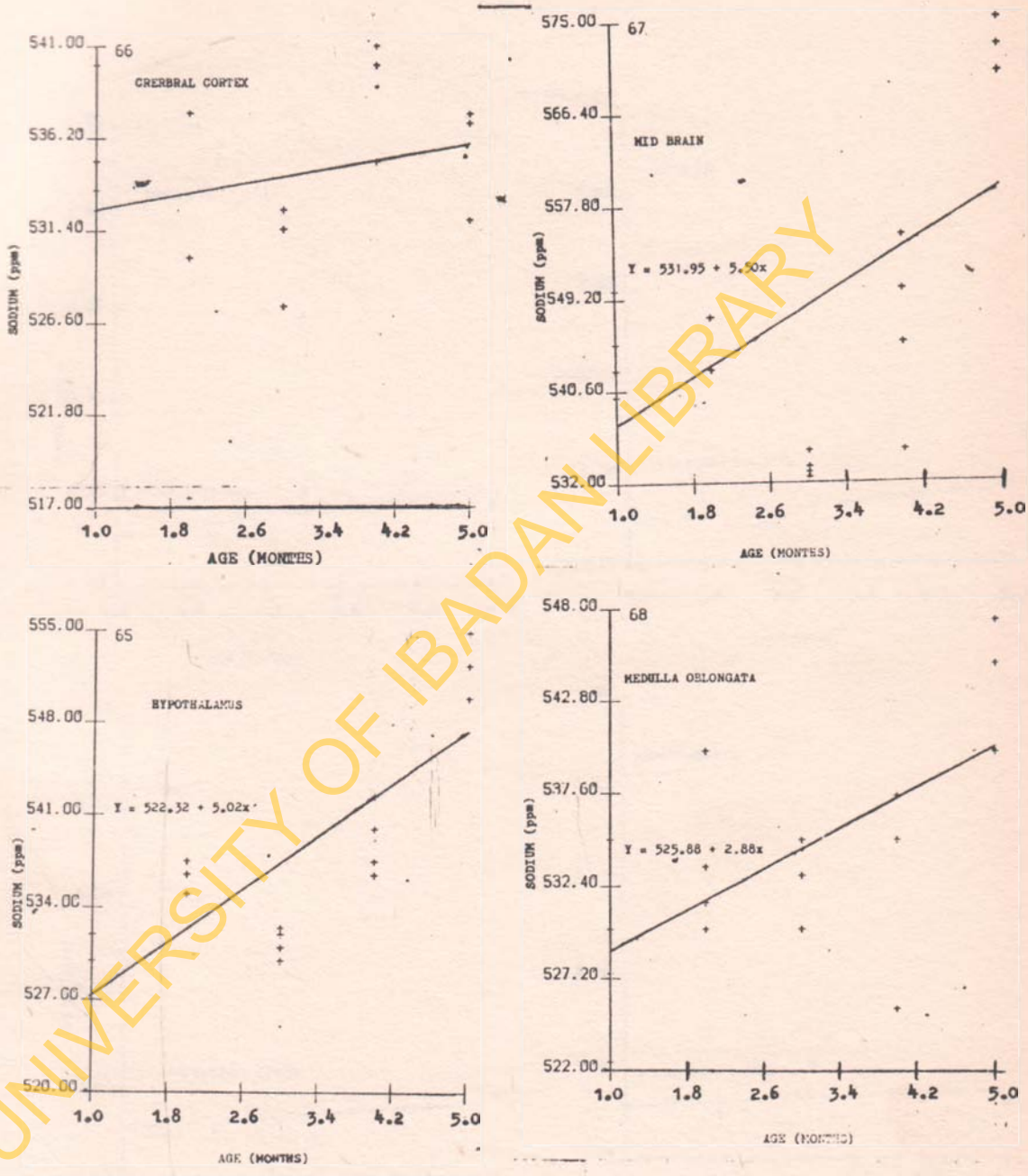


Fig. 3.1.3. (Continued)

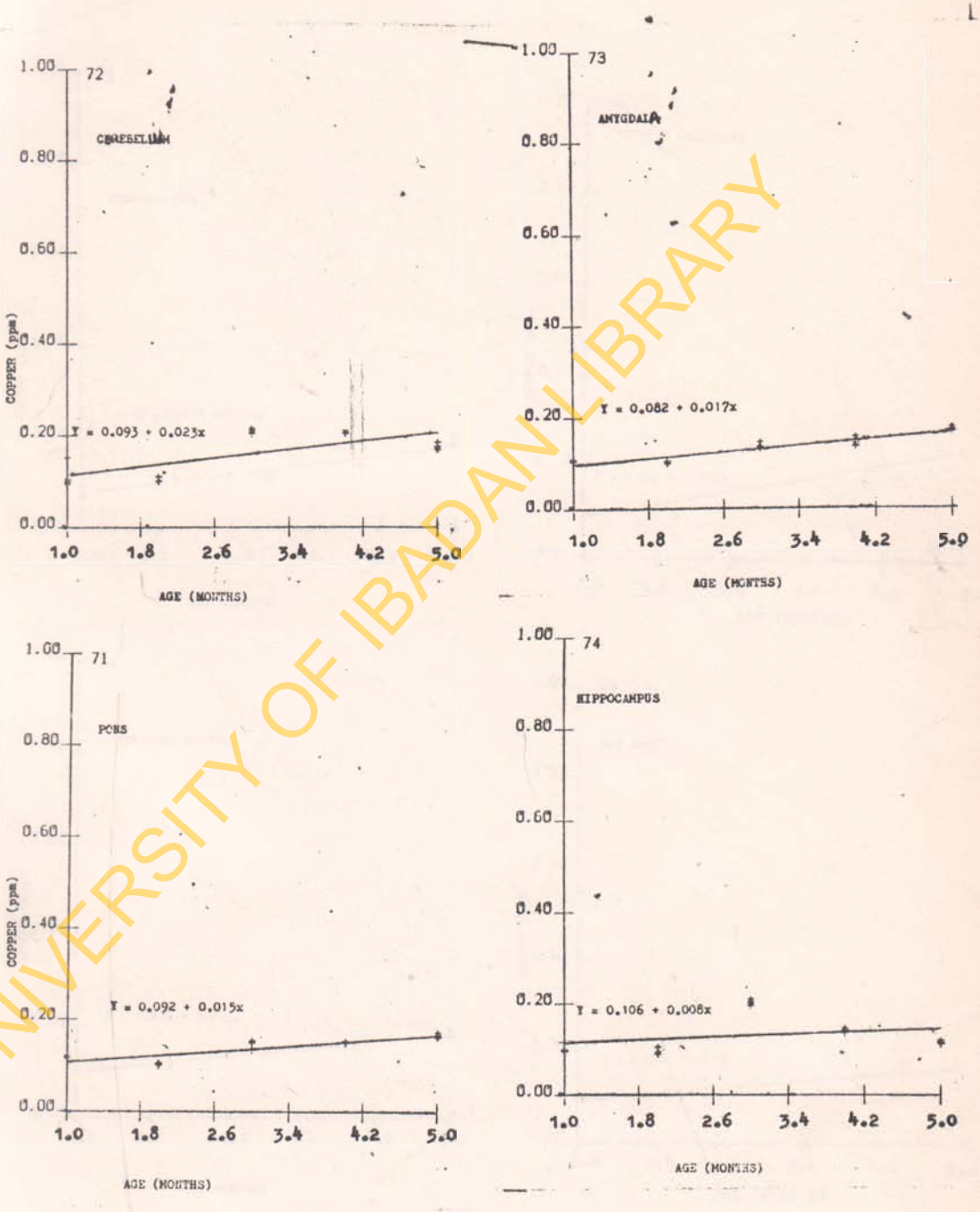


Fig. 3.1.4. Relationship Between Age and Copper Concentrations in the Different Brain Regions.

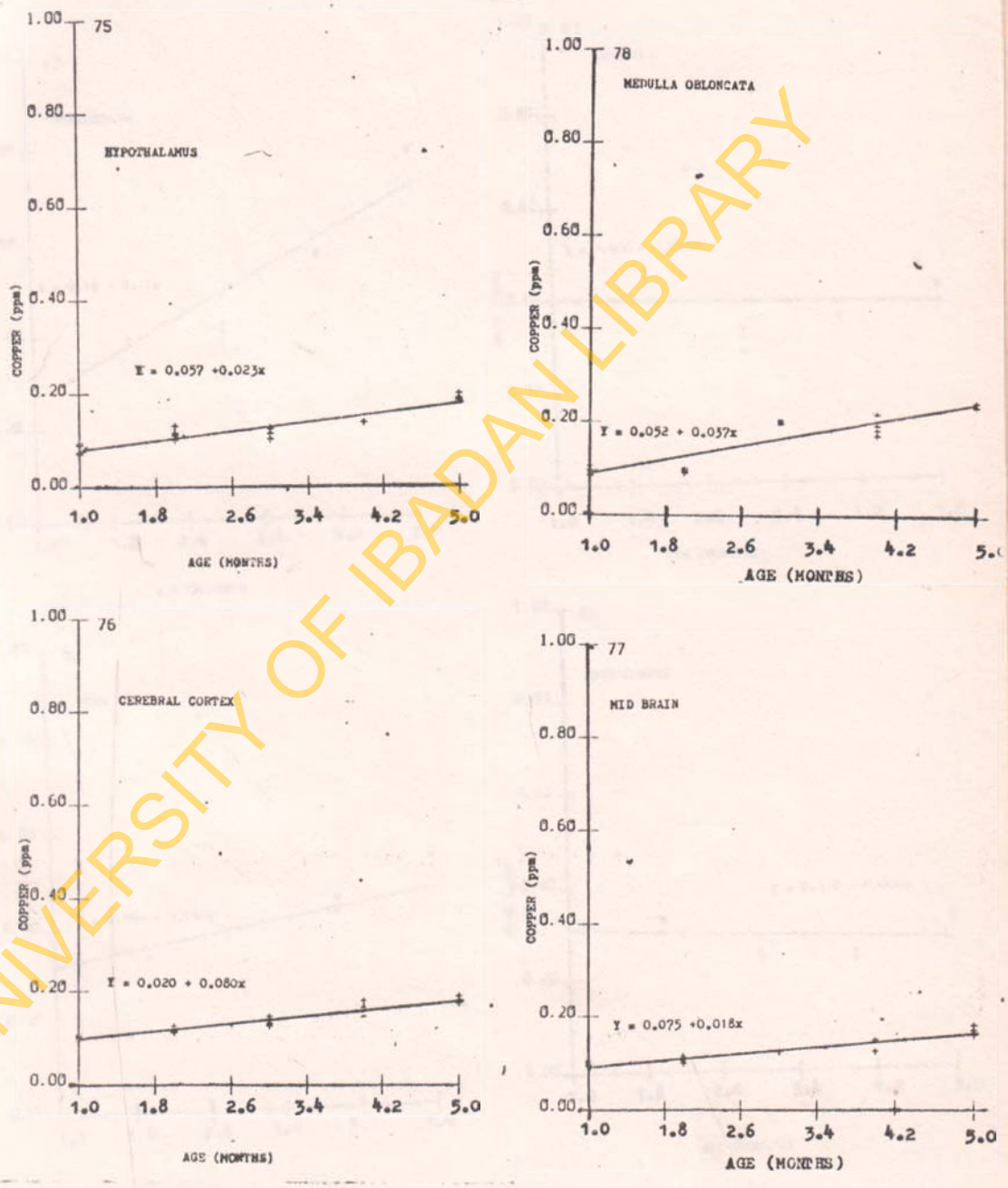


Fig.3.1.4. (Continued)

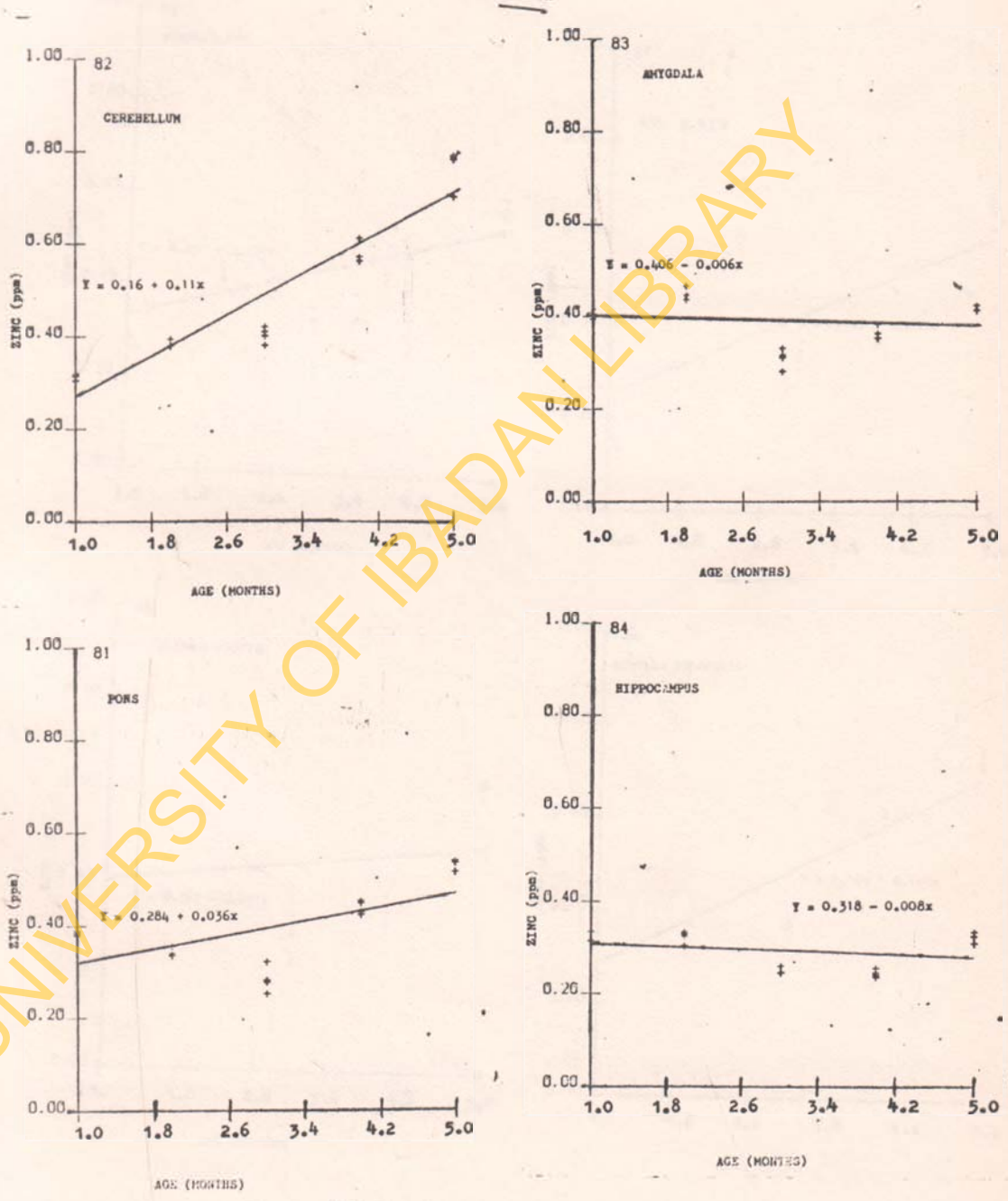


Fig.3.1.5. Relationship Between Age and Zinc Concentrations in the Different Brain Regions.

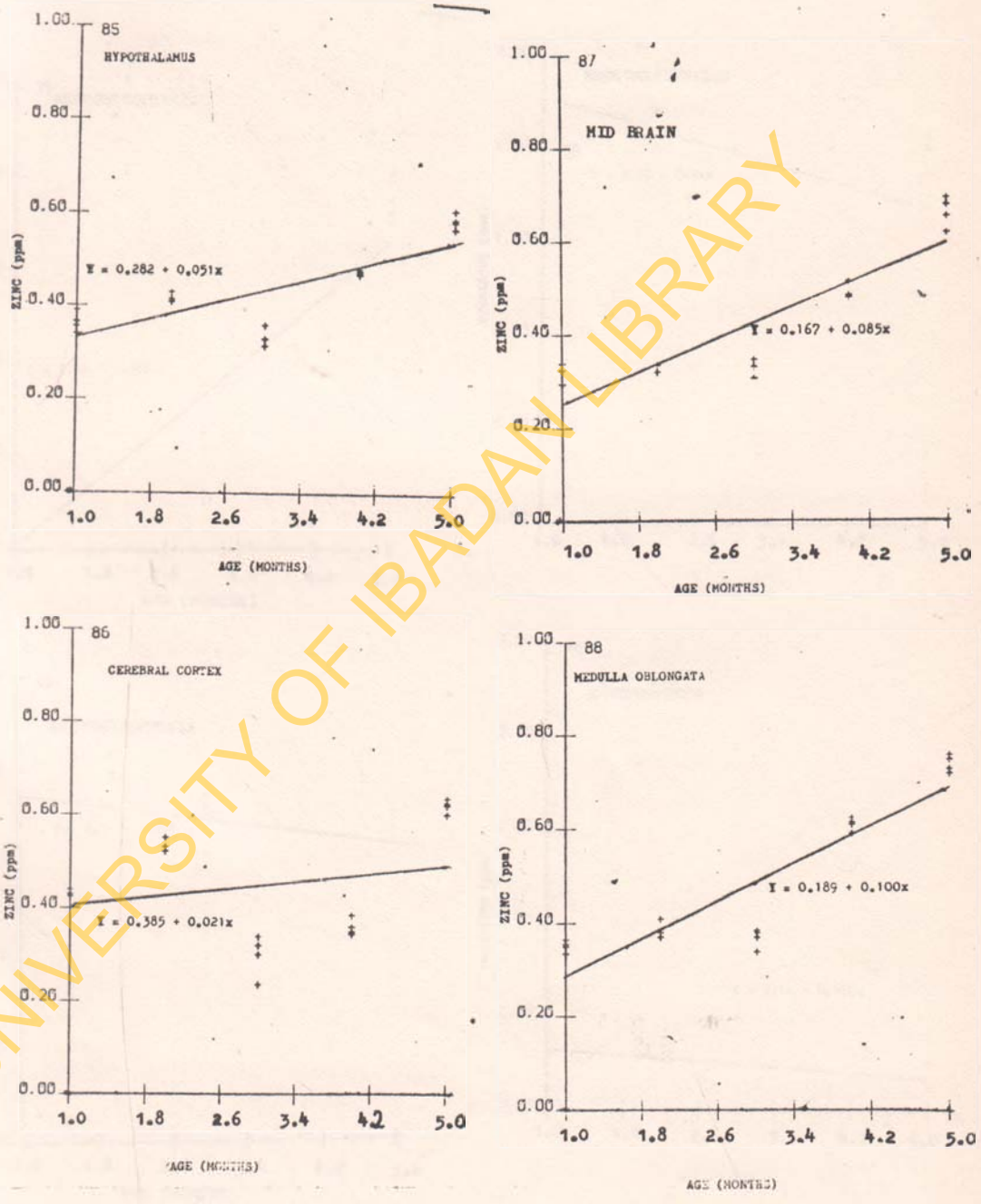


Fig. 3.1.5. (Continued)

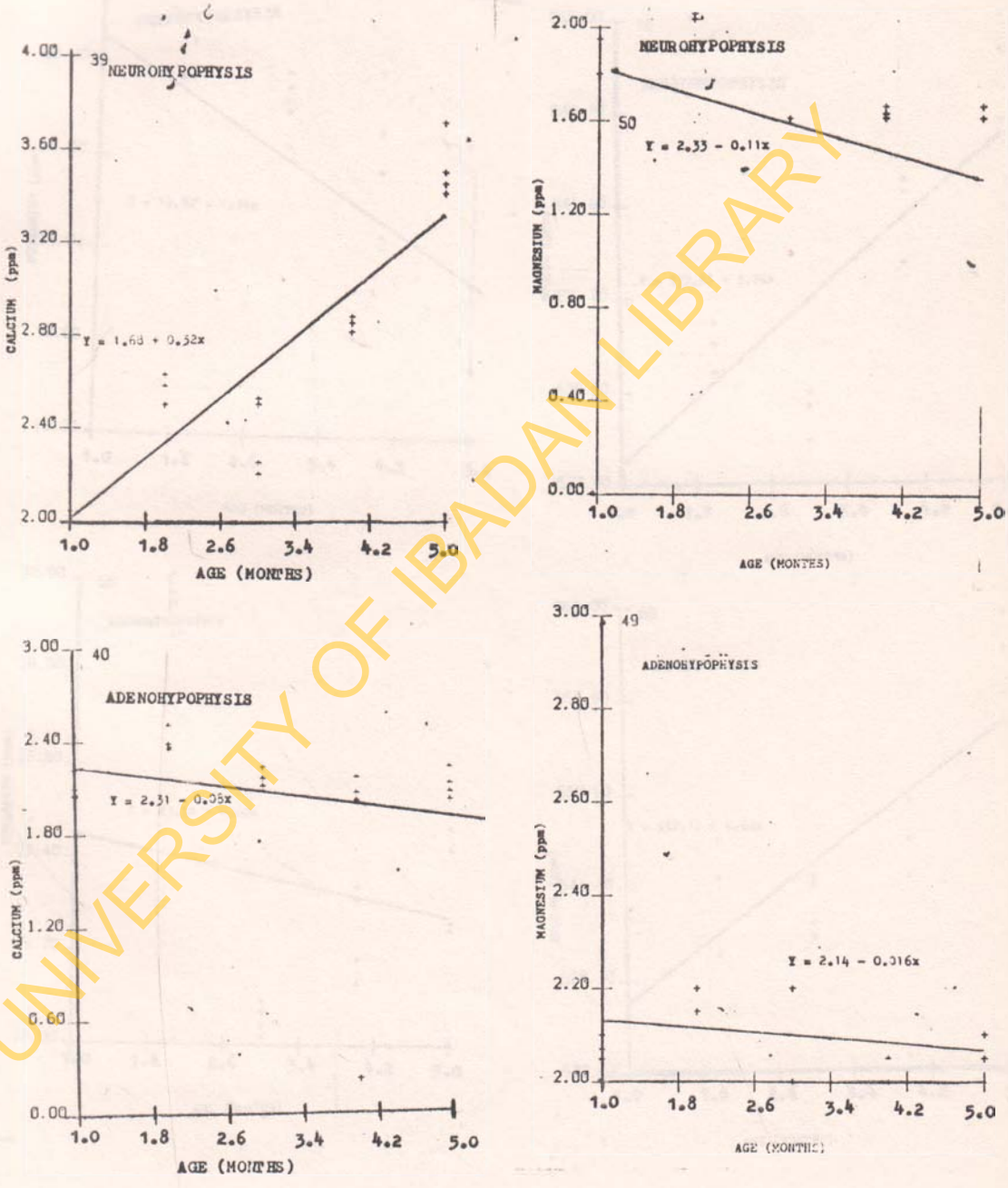
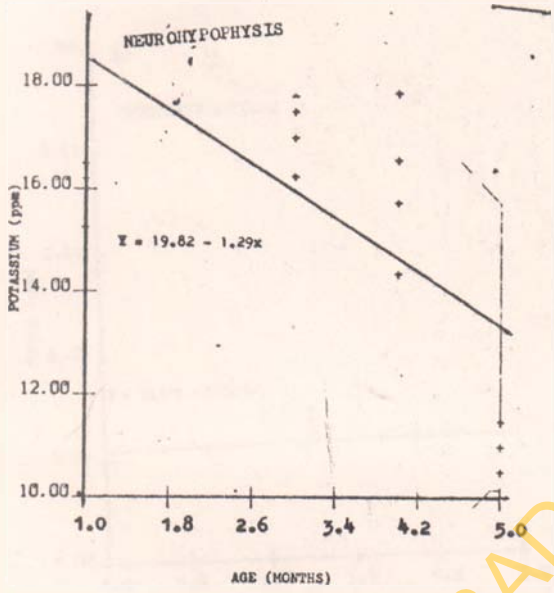
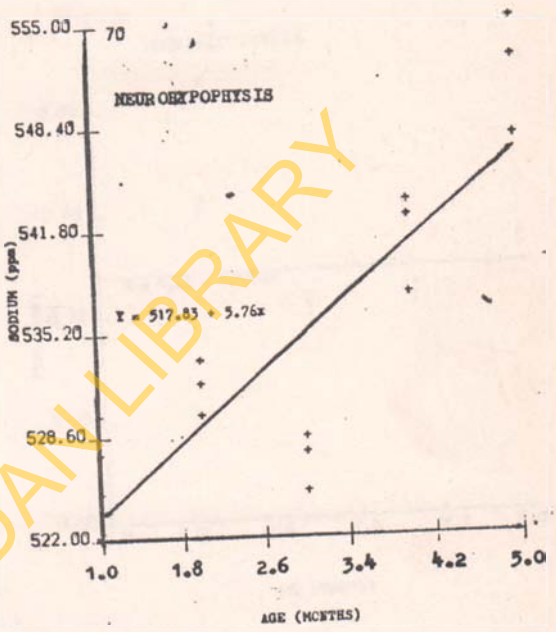
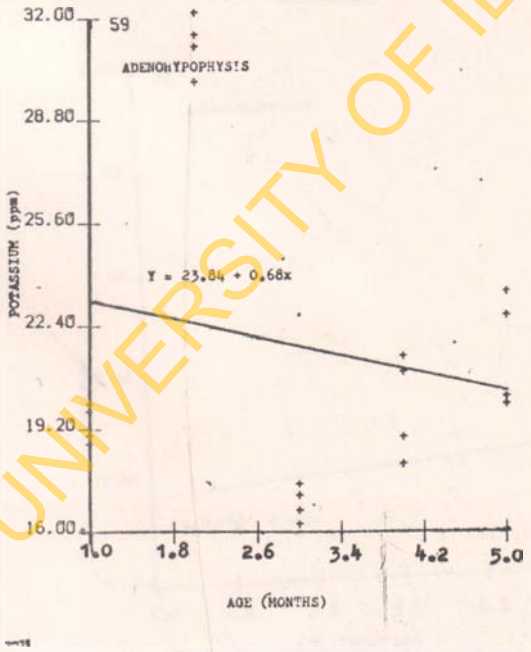
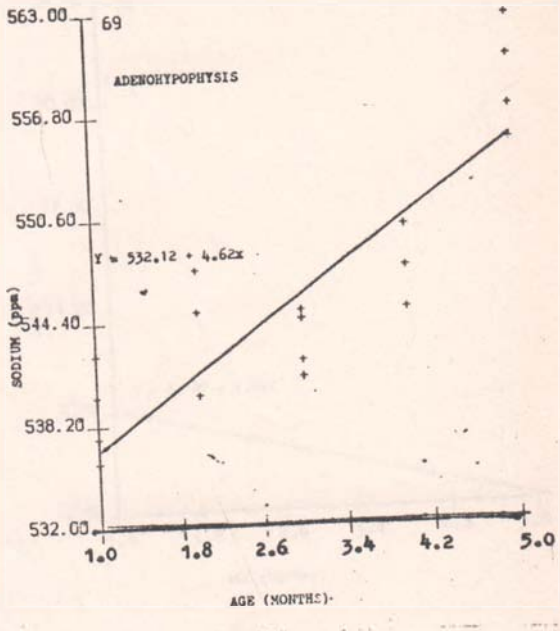


Fig. 3.1.6. Relationship Between Age and Minerals Concentrations in the Hypophyses.









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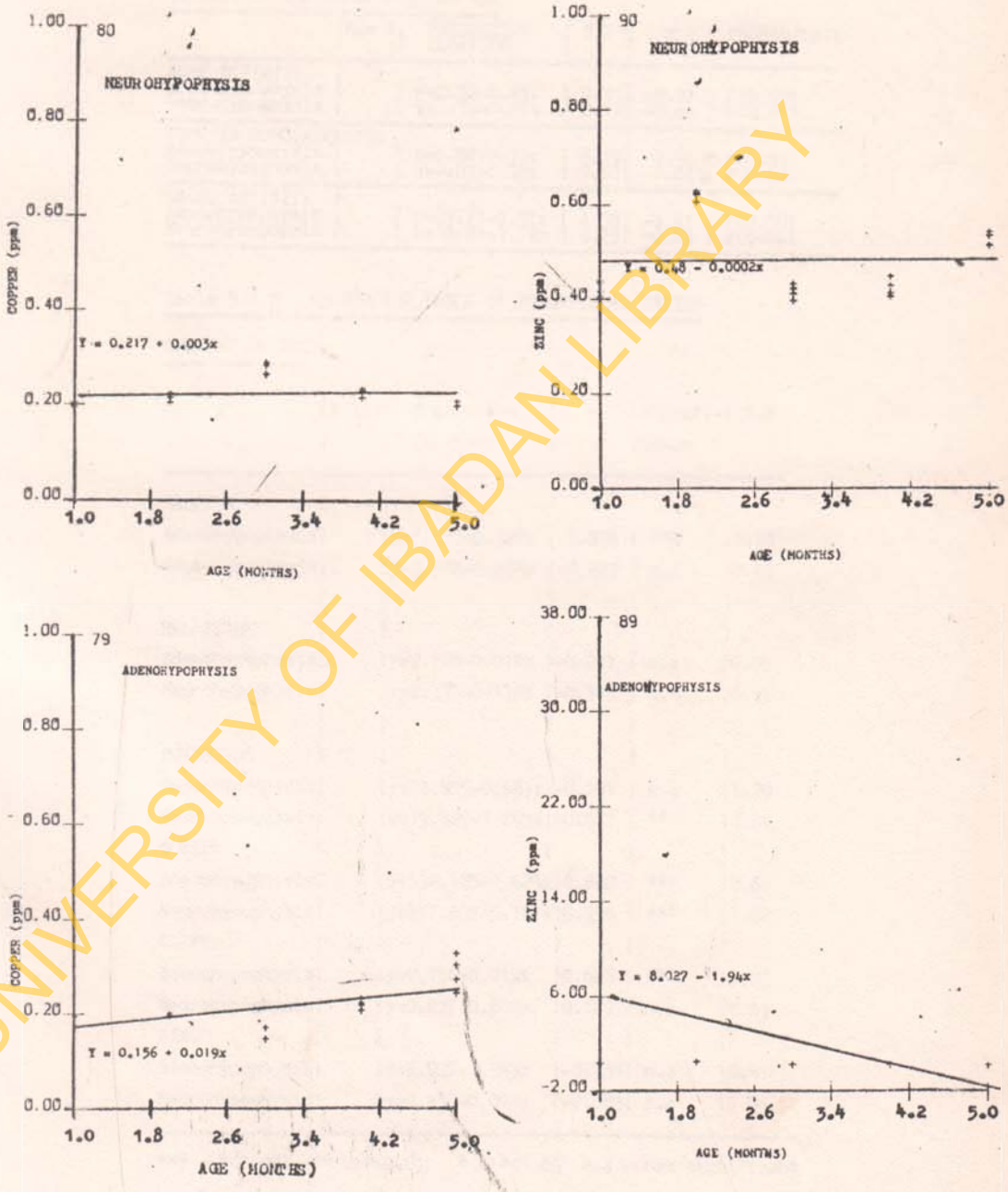

Fig 3.1.6. (COntinued)

TABLE 3.1.9 REGRESSION TABLE OF AChE, PROTEIN AND SAChE CONTENT OF THE HYPOPHYSIS ON AGE

Y	Age X	PREDICTION EQUATION	S.E	r	PROBABILITY
AChE ACTIVITY					
Adenohypophysis		y=2.88-0.48x	0.11	-0.97	P<0.001
Neurohypophysis		y=1.73-0.22x	0.12	-0.84	P<0.001
PROTEIN CONCENTRATION					
Adenohypophysis		y=0.04+0.10x	0.19	0.56	P<0.01
Neurohypophysis		y=0.07+0.03x	0.01	0.88	P<0.001
SAChE ACTIVITY					
Adenohypophysis		y=29.21-6.37x	1.38	-0.93	P<0.001
Neurohypophysis		y=18.81-3.59x	0.79	-0.83	P<0.001

Table 3.1.9 REGRESSION TABLE OF HYPOPHYSEAL CATION CONTENT ON AGE

y	X Age	Prediction Equation	r	signifi-cance	S.E
CALCIUM					
Adenohypophysis		y=1.685+0.326x	0.906	***	0.10
Neurohypophysis		y=2.318-0.083x	-0.263	n.s	0.42
MAGNESIUM					
Adenohypophysis		y=2.146-0.016x	-0.297	n.s	0.05
Neurohypophysis		y=2.335-0.119x	-0.348	n.s	0.45
POTASSIUM					
Adenohypophysis		y=23.845-0.681x	-0.191	n.s	1.20
Neurohypophysis		y=19.829-1.293x	-0.657	**	0.82
SODIUM					
Adenohypophysis		y=532.125+4.625x	0.842	***	3.88
Neurohypophysis		y=517.837+5.762x	0.856	***	2.82
COPPER					
Adenohypophysis		y=0.156+0.019x	0.626	***	0.02
Neurohypophysis		y=0.217+0.003x	0.129	n.s	0.01
ZINC					
Adenohypophysis		y=8.027-1.943x	-0.334	n.s	8.41
Neurohypophysis		y=0.480-0.002x	-0.003	n.s	0.01

*** = P<0.001 ** = P<0.01 * = P<0.05 n.s = not significant

3.2.0 DISCUSSION

ONTOGENETIC DEVELOPMENT

The decline in amniotic fluid volume with age is probably due to the development of the fetuses. As the fetus grows in size, it occupies a larger proportion of the amniotic sac. This also agrees with the works of Meschia, (1955) and Knight et al, (1977). As the amniotic fluid also turns mucoid and hence more viscous during late pregnancy, there is a reduction in volume. An added advantage of such change however, is its increased lubrication and shock-absorbing capacity.

The very drastic increase in reproductive tract weight and embryo weight at 8 weeks of pregnancy is due to the very rapid development at this stage. It also shows that the period between the 6th and 8th weeks must have been very crucial to the sow because of the tremendous development in fetal growth. The very high increase in brain weight shows that complete morphological development of the pig takes place at this period. The rate of growth of the brain also reached its peak at this period.

Results on intra uterine growth at ten weeks indicate more rapid increase in the fetal weight most probably due to increased protein synthesis and bone development. It also supports the view that rapid growth and development in the fetus continues well beyond the tenth week of pregnancy (Moon and Hardy, 1973.)

However there is a very sharp drop in the rate of growth of the brain at this stage which is reflected by the relative brain weight.

The very slight increase in the reproductive tracts and brain

weights at 12 weeks of pregnancy indicate that the fetus had passed its peak of growth. The slightly higher AChE activity observed in the amniotic fluid at 4 weeks of pregnancy may be due to the fact that at that stage of pregnancy, the uterine secretions usually contain some unique proteins induced by progesterone (Knight etal 1973). Their decline thereafter is supposedly due to a natural breakdown of the protein evidenced by the not very consistent nature of the protein concentration of the amniotic fluid. Also since the amniotic fluid merely serves a protective role in supporting the embryo in a liquid environment and preventing the embryonic tissues from damage trauma or pressure from the surounding structures, it plays very little or no nutritive role in the development of the embryo. The negative correlation coefficient of SAChE activity in amniotic fluid with intra uterine age is also expected from the above results.

The increase observed in AChE activity of the pig fetal brain with intra-uterine age may also be due to increase in the brain development of the fetus. The increase may also be linked with the morphological and sexual differentiation of the brain which according to Maclusky et al (1979 a,b) and McEwen (1980) occurs during mid-gestation and shortly before birth. However, the over 100% rise in AChE activity by the 12th week of gestation may be due to the fact that the brain tissues were more developed and of a firmer consistency during that period.

The non-consistent concentrations of protein observed may not necessarily be due to a low protein turnover rate because according to Pfaff and Gregory (1971a), changes in concentration could be due to changed synthesis or changed utilization (breakdown) or both. And indeed, changes in turnover rate can occur without any change in concentration. A lot of authors have authenticated this view (Anton-Tay and Wurtman, 1968).

The specific AChE activity also reflects AChE activity although values more or less fluctuated till the 10th week of gestation. By the 12th week there was a very sharp rise in activity which could also be explained by the possible increase in fetal motility and the firmer consistency of the brain. It also coincides with the period of the development of hormone-receptors in the pig fetal brain.

The birth weights observed closely agrees with the report of Hencken (1959).

The sharp increases in the liveweight, brain weight, the adrenal and thyroid gland weights between birth and one month followed by a lower rate of increases indicate a rapid growth spurt shortly after birth for the first 4 weeks. As the animal was still on milk diet at this stage, the increases must be due to a very high conversion efficiency resulting in high rate of protein synthesis and hence increased tissue deposition. The reduced growth rate after the first month suggests a reduction in the protein turn over rate. The result also complements the work of Davison and Dobbing, (1968) who reported that the establishment of synaptic junction and glial cell multiplication followed by myelinatian in pigs takes place during the period of "brain growth spurt" which occurs during the first five post-natal weeks.

Because of the role of the brain, particularly the hypothalamus, in the growth of the animal through the induced secretion of somatotrophin (STH) by its release-factors, the "brain growth spurt" may also be responsible for the growth spurt body and endocrine glands observed during the same period. Thus it is reasonable to suggest that the rapid development of the brain at this stage of growth would also lead to rapid development of its growth-inducing centres (since they may also be responsible for growth spurt of the brain itself) and

consequently their effect on body growth.

MINERAL PROFILE IN THE FETAL BRAIN

The importance of a good knowledge of the changes taking place in the body composition of pig fetuses particularly for the purpose of correct feeding has been recognized for a long time (Warwick 1928, Mitchel etal 1931, Urbany, 1952 and Pomery, 1960). Although few studies have been made in the mineral content of pig fetus, the increases observed in Calcium content with intra-uterine age agrees with the findings of Mitchell et al (1931). This rise in calcium levels may be indicative of its importance in skeletal development and other physiological functions particularly towards the last trimester of pregnancy.

The non-consistent trend of potassium and the increase in sodium concentration with age are in conflict with the reports of Pomeroy (1960) who found a decrease in sodium content of pig fetuses with intra-uterine age. However it must be noted that their work was done on the whole fetus and not the brain alone as was the case in the present study. The slight rise in potassium and sodium content emphasizes their role in the physiological development of the pig fetus. For instance, Curtis et al(1967) observed a positive relationship between potassium content, lean and protein content of the pig body.

The rise in magnesium levels from four weeks to about 8 weeks of intra-uterine age thereafter followed by a decline closely agreees with the findings of Pomeroy (1960)and Urbany (1952). Who also established that there is a relationship between Sodium ions in the

allantois and the maternal tissue and that the sodium ions are essential for the maintenance of short-circuit current and potential difference across the pig allantoic membranes.

The steady decline in the copper and zinc contents of the embryonic brain with intrauterine age is difficult to explain in the light of available information on ionic changes in the pigs fetus. However, this result may have a role to play in the characteristically low levels of zinc, copper and iron in the piglet blood shortly after parturition. This condition usually result in baby pig anemia and can be treated by injecting with iron and copper-containing compounds.

The general profile of AChE activities in the different brain regions indicate that significant regional differences in activity exist which correspond with the reports of Gerebtzoft (1959) Moudgil and Kanungo, (1973), Owasoyo et al(1979) and Egbunike (1981, 1983). The high AChE activities observed in the amygdala, midbrain and medulla obolongata may be attributed to the fact that there areas are involved in aggressive behaviour such as predatory actions, agressive sexual action, the agonistic behaviours of rage, fight etc (Kang et al 1970). These actions are quick and short-termed, characterized by high nervous stimulation and culmunating in muscular contractions.

The medium activity of AChE in the hypothalamus, hippocampus and pons may be linked with the fact that these regions control basic reflexes concerned with respiration, swallowing, normal sexual behaviour gonadotrphin release and other involuntary actions triggered off by specific stimuli bringing about co-ordinated responses while the constant low activity observed in the cerebral cortex and cerebellum may be a result of the fact that these areas control higher and lower intellectual functions, passive reflex reponses acquired by the body and retained over a long period of time. They are thus sort

of "programmed" into the brain and require very little conscious efforts or nervous stimulation to bring them into play.

Similarly, the high protein concentrations observed in cerebellum, pons, medulla oblongata and hypothalamus and moderate concentrations observed in the mid brain, hippocampus and amygadala could be linked with the corresponding enzyme activities because the enzyme is glycoprotein in nature and a high enzyme activity usually connotes a relatively high protein synthesis. The low protein concentration observed in the cerebral cortex could also be attributed to this view.

It is therefore not surprising that specific acetylcholinesterase activity was highest in the mid brain, pons and hypothalamus, moderate in the medulla oblongata, hippocampus, amygdala and cerebellum and lowest in the cortex. This is evident from the fact that since the specific AChE lacitivity is a function of the protein concentration, a low AChE activity coupled with a low protein concentration would ultimately result in a low SAChE activity. The very low AChE activities observed in the hypophyses compared to the brain could be attributed to the fibrous, relatively small size of the gland and the histologocal differences between them and the brain. There is also the fact that the glands are more secretory than nervous in nature depending more on secretory cells than neuronal cells and thus require less nervous stimulation to function since it depends to a large extent on its feed-back mechanism for hormonal regulation.

The higher protein concentrations observed in the adenohypophyses over the neurohypophyses may be due to its more secretary nature expecially since it has a direct anatomical link (via portal veins) with the hypothalamus. This increased cellular secretory activity over the neurohypophysis may also explain the increase in SAChE

activity in the adenohypophysis over the neurohypophysis.

MINERAL PROFILE

The fairly higher levels of calcium, zinc and potassium in the adenohypophyses may enhance the organ in its growth-promoting function. In addition, since the minerals enhance protein synthesis by promoting increased replication of DNA neuromuscular functions and electrolyte balance, they presumably contribute to the higher protein concentration observed in the adenohypophyses. As calcium is needed for the release of AChE at neuromuscular junctions (Rahamimmof, 1976), it may also be responsible for the slightly higher AChE activity obsered in the adenohypophysis over the neurohypophysis.

AGE AND SEX DIFFERENCES IN THE BRAIN REGIONS

That AChE activity in the brain regions is age-dependent is well known (Moudgil and Kanungo, 1973). The significant decline observed in AChE activities in the pons, hypothalamus, midbrain and medulla oblongata with increasing age followed a pattern characterictic of the pig and suggests that these brain regions had reached their maximum development with respect to the neurons at the period of birth or shortly thereafter. This view is further supported by the fact that these brain regions control the behaviour and activities of the pig shortly after birth and do not require a latent or learning" period for the animal to perform the relevant functions. This hypothesis is further supported by the report of (Colenbrander et al 1978) who observed a rise in serum testosterone level in the pig from birth till the 3rd week of age with a decline thereafter probably accompanied by biochemical and morphologic changes. It will be recalled too that the hypothalamus is involved in the release of gonadotrophins by the adenohypophysis and thus indirectly controls testosterone secretion by

the testis. The decline in testosterone level of the blood may be a result of the declining AChE activity in the hypothalamus. Other workers (Schreff et al, 1980) have also observed a decrease in brain AChE activity with age in the rat and according to Curtis et al (1967) birth weights do not necessarily correlate with chemical maturity and the age-related studies of Ascheim (1976), Mills and Mahesh (1978) also indicated that the endocrine system is involved in the ageing process.

The similar and moderate AChE activities observed in these brain regions at two and three months of age may also be linked to the developmental status of the piglets since at the phase of growth, the animal does not involve itself in marked sexual and behavioural activities.

It is also known that after birth, neurons do not possess the ability to regenerate and this strongly supports the hypothesis of a decline in neuronal activity with age.

The slight but non-significant increases in AChE activity observed in the cerebral cortex and cerebellum suggest that since the pig is still in the learning phase, a higher activity of AChE at this state may facilitate the learning process. This view is supported by the works of Kovacs (1971) and Moudgil and Kanungo (1973) who also observed an increase in the AChE activity of the rat cerebral cortex and cerebellum followed later by a decrease. In these two regions the highest rates reached at one month of age were followed by a decrease.

The generally higher AChE activities associated with the male brain regions such as pons, hypothalamus and medulla oblongata agree with the observation of Egbunike (1983) and may be a result of the higher behavioural activities of the males compared to the females.

In addition, since the release of gonodotrophins and sex steroids in the female is cyclic and in the male continuous, it may be reasonable to suggest that the hypothalamus of the male animal is more active than the hypothalamus of its female counterpart. This observation may also be a result of the sexual dimorphic nature of the brain as reported by Arnold (1980) and the organizational sex-difference in the brain (Raisman and Field, 1971, 1973). Moreover, Greenough et al (1977) detected sex-differences in the topographical distribution of Golgi-stained dendrites in the hamsters while Ryan and Arnold (1979) observed sexual differences in topographical distributions of AChE and catocholamines in the brain.

The increase in protein concentrations of the brain regions with increasing age may be due to a combination of factors such as nutrition, hormones as well as environment. For instance rapid growth rates were observed in both the live weights, brain weights as well as other endocrine organ weights in the pigs with age. This obviously involves increased protein synthesis and tissue deposition. As the brain, particularly the hypothalamus, controls growth through its induction of STH secretion, a higher activity of the hypothalamus should naturally involve increased RNA synthesis. It is also known that at this early phase of growth, the pig brain receives a large supply of blood and this may increase the metabolic rate of the brain. Kovacs(1971) also reported that the rate of protein synthesis in rat brain varies with age and that a rise in the early phase of growth is eventually followed by a decrease in later life.

The fairly similar protein levels recorded at two and three months of age agree with the observation on AChE activity at that period. Although the sex differences observed in the amygdala and hippocampus could not be easily explained especially with respect to AChE activity, it is possible that these differences may be due to

sex-differences in the size of nuclei in neurons in the pre optic area and the hypothalamus and hippocampus as reported by Staudt and Dorner, (1976) especially during the brain growth spurt period which occurs during the first five post natal weeks (Davison and Dobbing, 1968). The very high SAChE activities observed in the brain regions at birth coincides with the very rapid growth of the brain and endocrine organs at that period. Moreover, the rather low protein concentration at this phase of growth further contributed to these high SAChE activities recorded.

The progressive decline in SAChE activity with age may be a result of increase in protein concentrations of the brain regions concerned coupled with a decline in AChE activities. The deviations from this trend observed in the cerebellum, amygdala and cerebral cortex which recorded an initial rise in SAChE activity from birth to about two months followed by a decline may be due to the fact that these animals as reported earlier were still in the learning phase up til about three months of age.

The increased SAChE activities observed in the male pons, hypothalamus and midbrain may be due to increased activity of the centres concerned with control of sexual behaviour since the release and exposure of the male brain to sexual steroids and gonadotrophins in the male is rather constant.

The significant positive correlations observed in the calcium, magnesium, potassium and sodium, copper and zinc concentrations in the pons, cerebellum, medulla oblongata and midbrain indicates increase in brain electrolytes with increasing age or body weight. This result is supported by the positive cerrelations observed by Curtis etal(1967) between potassium concentration and protein. It also reinforces the validity of their suggestion that body electrolyte concentrations

correlates positively with developmental maturity.

A look at the role played by these minerals in the brain indicates that they enhance growth, through increased RNA synthesis and utilization of amino acids. Some of them particularly calcium and zinc are involved in the transmission of nervous impulses and sexual maturity (Sandstead et al 1967). Also the brain regions where these increases occured are centres responsible for cruder basic reflexes such as swallowing, respiration, locomotion, co-ordination and maintenance of balance. Thus these are centres that involve active participation of the brain.

The negative and at times significant correlations observed in calcium and magnesium content of the hippocampus, hypothalamus and cerebral cortex suggest that these regions with the exception of the hypothalamus are not directly responsible for growth and thus take very little part in active endocrine control. More over, the cerebral cortex with its characteristically low AChE activities and not very consistent protein concentrations stands out as a centre more or less involved in high mental and sensory activities which are all the time part of the animal's in-built responses. However, sodium, potassium, copper and zinc correlate positively and significantly with age in the hypothalamus which underscores the exceptional role of the hypothalamus in the overal metabolism of the body.

The amygdala, while not having particularly consistent concentrations of magnesium, sodium and zinc showed remarkably high increases in calcium, copper and potassium concentrations with age which reflects on the role of the amygdala on its modulating influence on the hypothalamic hypophysial system for the secretions of certain trophic hormones (Koikegami et al, 1953).

The negative and significant correlations in AChE activities in the hypophyses with age may be a consequence of reduced acetylcholine release in the adenohypophysis which would result in decrease in AChE activity. The decreased ACh release may be a result of ACh inhibition by the circulating gonadal steroids exerting a negative feed back response on gonadotrophin secretion by the pituitary gonadotrophs. The decline in AChE activity observed in the neurohypophysis may be related to its role in being the source of oxytocin and vasopressin which are hormones that mimmick the action of ACh.

Thus it is not unlikely that the secretions of the neurohormones may result in a decreased rate of ACh secretion so as not to evoke muscular contrations beyond a tolerable level. The possible suppression of ACh release in both hypophyses may thus require large and continous amounts of AChE which would ultimately result in its rapid depletion from the synaptic junctions.

The higher AChE activity observed in the female adenohypophysis over the male may be related to the rate of gonadotrophin secretion and may also be a pointer to a possible sexual differentiation of the adenohypophyseal gonadotrophs. This is evidently a hypothesis worth further investigation.

The increased protein concentrations in the hypophyses with age may be directly linked with the increased growth rate of the brain and the associated organs of the body while the steady decline in SAChE activities with age observed in the hypophyses may be directly related to the decreased rate of AChE activity and increased protein concentration in the organ.

The higher SAChE activities recorded in the female hypophyses over the males may also be due to a possible sexual dimorphism in the

nuclei of the hypophyseal secretory cells.

Another interesting point to note is the concomitant decrease in AChE and SAChE activities in the hypothalamus coupled with sex-differences. Thus the hypothalamic-hypophyseal axis may be implicated in the low enzyme activities observed in these organs.

The highly significant positive correlations observed in calcium, sodium and copper concentrations in the adenohypophyses with age and the lack of a consistent trend in magnesium, potassium and Zinc levels indicate that the minerals that are more essential to growth correlated well with age. The exception is zinc which cannot be readily explained.

The result may also be explained by the fact that calcium and sodium tend to antagonize magnesium and potassium by competing with them for active sites on enzyme systems (Dixon and Webb, 1961) although the reasons for this are not very clear.

Potassium showed a negative and significant correlation while sodium displayed a positive and significant correlation with age in the neurohypophysis. This may also be explained by the sodium-potassium interrelationship or reciprocity. All the other minerals showed non- significant correlations.

CHAPTER IV

EFFECT OF AGE AT ORCHIDECTOMY AND TESTOSTERONE ON PORCINE BEHAVIOUR AND BRAIN AND HYPOPHYSEAL PHYSIOLOGY

4.1.1 **INTRODUCTION**

Among other functions, the testes secrete testosterone, which is known to have diverse influences on the male vertebrate: viz:- The action of androgens (e.g. testosterone) is evident on the sexual characteristics of the male. Androgens also possess the following biological activities in the male:

1) They stimulate spermatogenesis in the hypophysectomized animal and hasten the onset of spermatogenesis in the seasonal breeders.

2) They prolong the life span of epididymal sperm. Sperm motility lasts for approximately thirty days in the guinea pig following castration whereas androgen treatment will increase sperm viability to the normal period of seventy days.

3) They promote growth, development and secretory activities of the accessory sexual organs such as the prostate, vesicular glands, bulbo urethral gland, vas diferens, cowper's gland, penis and scrotum.

4) They stimulate sexual behaviour and libido in the male.

5) They induce nitrogen retention distinct from its action on the reproductive tract. In other words, testosterone possesses protein anabolic activity which involves the total organism. Androgens stimulate growth and have also been associated with nitrogen balance by facilitating nitrogen retention which is vital for protein anabolism (Kochakian, 1946).

6) They also influence the intensity and frequency of sexual bahaviour and aggression in adult male vertebrates (Guhl, 1961, Hart, 1974 and Young, 1961).

Because they are sensitive to androgens, these behaviours are co-ordinated to some degree with each other, with related physiological and morphological changes and with developmental secretion. Hence, this study was designed to evaluate the effects of castration at various ages with or without restosterone replacement in boars.

Sexual differentiation is a phenomenon among mammals and birds for which distinct critical periods exist in early brain development. There also seems to be a link between sex differences in brain structure and sex differences in neuroendocrine function and behaviour (McEwen, 1980). A possible reason for sexual dimorphism in the brain may also be due to sex differences in gene products subject to genomic regulation by steriod-receptor complexes e.g. the same enzyme may be regulated at a different rate in say the male hypothalamus and the female hypothalamus. Another reason may be due to differences in the inducibility of a gene product such as occurs in estrogen induction of progestin receptors (Moguilevsky and Raynard, 1979).

4.1.2 **LITERATURE REVIEW**

The age-dependence of the hypothalamic pituitary-gonadal axis has been well established (Masafumi et al., 1981). Also well known is the age-dependence of steriod hormone receptors in the brain (Cidlowski and Muldoon, 1976). This is partly why most of the conflicting reports on the effects of castration depend on the age at which the operation was carried; whether pre-pubertally or post pubertally.

Thus Yahr and Coquelin (1980), while not being able to distinctly explain why pre- versus post-pubertal castration produced different effects on aggression in male gerbils, discovered that differences in age at

castration can help to explain why prepubertally-castrated males and intact females were about twice as aggressive as post-pubertally castrated males. Anisko et al (1973) also observed increased aggressiveness of pre-pubertal gerbil castrates over the normal males.

Androgens act on the hypothalamus and the pituitary gland to exert a feed-back system of gonadotropin secretion. In recent years, receptors for sex steriod hormones have been found in the brain and the pituitary glands of rats suggesting that they perform important role in this feed-back control system (Cidlowski and Muldoon, 1976). Thus castration increases metabolism at the hypophyses and hyhothalamus (Roy and Laumas, 1969).

The interaction between hormone synthesis and protein anabolism is made more evident by Martini (1973) who discovered that inhibition of protein synthesis interferes with the synthesis of releasing factors in the rat hypothalamus. He further found that the cerebral cortex and amygdala are not androgen-sensitive whereas the hypothalamus (particularly the median eminence) and the adenohypophysis are androgen-sensitive.

Although not much work has been done on dose-response relationships between testosterone administration and sexual bahaviour, Yahr et al (1979) found that the castration-eliminated sexual activity in male gerbils was prevented by large injections of testosterone propionate. Mating behaviour, indexed by mounting, intromission and ejaculation, was reinstated.

Sodersten et al (1980) also confirmed earlier reports that sexual behaviour persists for some time after abrupt testosterone withdrawal i.e. castration. They also reported that blood titre levels of testosterone in male rats is normally higher than that needed for maintenance of sexual behaviour. Davidson (1977) remarked that castration of sexually

experienced rats is usually followed by a gradual decline in sexual bahaviour whereas castration increased plasma Luteinizing hormone (LH) within 12 hours (Nansel et al, 1969) followed by a further progressive increase over a 3 week period (Damassa et al., 1976; Gay and Haugen, 1977).

The prolonged response of the brain mechanisms controlling sexual behaviour and possibly LH secretion to androgen withdrawal is at variance with the prompt response of the accessory sexual glands which are androgen-sensitive. Both the central and the peripheral responses can be prevented by testosterone treatment (Sodersten et al., 1980). It has also been suggested that castration results in a gradual desensitization of the brain and that as a consequence, more testosterone is needed for restoration of the behaviour in long-term castrates than acutely castrated rats (Davidson, 1972). Furthermore, the level of testosterone stimulation needed for induction of the behaviour in castrated sexually inactive rats over a brief period of time is lower than the serum testosterone present in normal males.

The specificity of testosterone for maintenance of sexual behaviour was also confirmed by Sodersten (1973) and Paup et al (1974) who discovered that subcutaneous injections of estradiol benzoate stimulated the display of mounts and intromissions by castrated rats but failed to induce ejaculations partly due to the inability of the estradiol benzoate to stimulate the peripheral androgen-sensitive sexual organs, particularly the penis. The importance of adequate androgenic stimulation of the penis for sexual behaviour was demonstrated by Beach and Levinson (1950).

Meyerson (1964) postulated that brain monoamines mediate the effects of gonadal hormones on sexual behaviour in the female rat. Brain monoamines inhibition facilitate the induction of sexual behaviour by

testosterone in castrated rats (Mayerson, 1964; Malmenas, 1973). Christensen and Clemens (1974) established that testosterone acts on the hypothalamus to induce sexual behaviour. The hippocampus and septum have also been suggested to be target areas for testosterone (Fuxe et al., 1978 Kohler et al., 1978). Other results indicate that differences in the behaviour of adult rats are related to differences in the perinatal milieu (Dohler and Wuttke, 1965; Pang et al., 1979; Goldman, 1978). Hece the presence of testicuar secretions during th first ten days of life facilitates the display of ejaculations by male rats in adulthood and inhibits display of female behaviour.

Hupp et al (1961) had long ago observed that castration however produced some changes that are not reversible by testosterone administration alone except when combined with adequate doses of estrogen. They therefore suggested a synergistic effect of testosterone and estrogen on the secretory activity of the various accessory sex glands of the boar. However, Booth (1980) found that prepubertally-castrated boars receiving testosterone injection had increased weights of accessory glands, increased zinc and fructose levels in the seminal vescles and raised plasma androgens with full restoration of mating behvaiour.

It has also been established that apart from having receptors for testosterone, the brain also contains enzyme, responsible for the conversion of testostorone to estradiol. Halasz (1969) also suggested that in the male, the hypothalamus is sufficient to maintain near-normal testicular activity without afferent input.

In a more interesting experiment, Kang et al (1970) found that stimulation of the genital afferent nerves and injections of gonadal hormones into adult rhesus monkey reulted in electrical responses in the

hippocampus and limbic structures which are involved in satiety and "need" state of sex drive. A direct evidence of the presence of receptor systems was established by Lisk (1962), Michael and Scot (1964) and Harris and Michael (1969) who placed testoterone in the brain of a castrated male rat and found it sufficient to stimulate mating behaviour.

According to Eisenfeld and Axelrod (1965, 1966), Kato and Villee (1967b, McGuire and Lisk (1968) and McEwen and Pfaff (1970), testosterone-3H also tends to be retained by cells of limbic hypothalamus system which includes the septum, amygdala, hippocampus, with a limited retention capacity by the medial preoptic area. Pfaff and Pfaffmann (1969a) also added that testosterone injected into castrated male rats influences the spontaneous activity and the responses to peripheral stimili of individual neurons in the preoptic area, olfactory bulb and mesencephalic reticular formation. Also reported is the fact that the absolute magnitude of a neuron's respnse to an individual odour is androgen-sensitive but the relative magnitude of a neuron's responses to different odours (measured by a different trial response analysis) is not androgen-sensitive (Pfaff and Pfaffman, 1969b, Pfaff and Gregory, 1971a).

Castration has been known to increase monoamine (Norepinephrine) levels in the anterior pituitary (Stefano et al., 1965). These levels are decreased again following hormonal theraphy. Some other workers (Wurtman, 1968) found that castration increased the whole brain turnover rates of norepinephrine even through there were no large changes in norepinephrine concentration. Fuxe and Hokfelt (1969a, b) found increased in the utilization of dopamine in the median eminence after low doses of pituitary or testosterone in castrated rats. Moguilevsky et al., (1966) observed changes

in hypothalamic oxidative and anaerobic metabolism following sex hormone variations.

Sutherland and Gorski (1970) also found that drugs which inhibit and rogenization at the level of the brain such as barbiturates induce lower enzymes capable of inactivating plasma steroids. This supposes that androgens suppress liver enzymes capable of inactivating plasma steroids and suggests an elevation of plasma steroids on androgen treatment. Moguilevky et al., (1966) further observed that endogenous oxygen uptake and anaerobic metabolism in the hypothalamus was significantly depressed in male rats by castration.

Androgens may also prevent development of a serotogenic system in the brain or induce the development of another system antogonistic to the serotogenic system (Ladosky and Gaziri, 1970). Gorski, (1973) also advanced the view that androgens alter fundamental neurochemical processes, perhaps protein synthesis within the suprachiasmatic region and this in some way prevents the cyclic release of Gonadotropin (GTH) in the adult. Further, androgen deficiency during a critical hypothalamic differentiation phase results in predominantly female organization of the brain (Dorner, 1969). Thus male homosexuality could be completely and permanently prevented by a single androgen injection administered during the hypothalamic differentiation phase. This indicates an androgen prophylaxis of neuroendocrine-conditioned male sexuality. Dorner (1973) concluded by suggesting that changes in the androgen and/or estrogen levels during the hypothalamic organization phase may results in permanent disorders of gonadal function and/or sexual behaviour during the post-pubertal hypothalamic functional phase. Therefore in gonadal males, androgen

deficiency during the hypothalamic differentiation period leads to post-pubertal hypo- or even homosexuality.

It has been shown by several authors that neurons in the anterior preoptic area involved in LH release may pass through the basal hypothalamus (Eveett, 1964); Halasz and Gorski, 1967). They also found that a gross lesion of the median eminence and the adjacent area of the tuberal regions results in gonadal atrophy and a reduction of pituitary LH synthesis.

Green et al (1957) also induced hypersexuality in the monkey and cat with ablations of the pyriform cortex or amygdala while MacLean (1970a, b) reported intensive grooming and penile erection following the after-discharges induced by electrical stimulation of the hippocampus in the rat, cat, and monkey. The limbic system has also been established to influence gonadotropin secretion, the stimuli arriving from the amydgala being facilitatory and those of the hippocampus inhibitory (Koikegami et al., 1954; Bunn and Everett, 1957; Velase and Taleisnik 1969a, b).

Although contradictory results exist in literature concerning the influence of the mid brain on gonadrotropin secretion, a dual role of the midbrain has been described for the control of corticotropin hormone release (Mangili et al., 1966, Fortier 1966), but even in that case the lack of specific organized systems is evident.

Injection of androgens into castrated mammals prevents the atrophy of penis, scrotum, prostrate, seminal vesicles, epididymis and bulbo urethral glands which normally follows removal of the testes, and the increase in weight of the prostrate of the castrated rat after androgen treatment is the basis of a method of testosterone bioassay.

4.1.3 **PORCINE PLASMA TESTOTERONE**

Although dose-response relationship is not well established several workers had determined the testosterone levels in the boar. Andresen (1976) observed an increase in plasma testosterone level from 2.5 ng/ml plasma at 100 days of age to 5.5 ng/ml plasma at 156 days of age followed by a fall of 2.7 ng/ml at 213 to 226 days of age. These determinations were based on the fact that boars reach adolscence between 110 to 125 days old and become sexually mature between six and seven months. Gray et al., (1971) similarly established a rise in the testosterone level in boars from 15.9 ug/100 ml bolld at three months of age to 27.0 lug/100 ml blood at six months of age followed by a drop to 11.6 ug/100 ml at nine months of age. More recently Booth and Baldwim (1980) observed average testosterone levels of 1.27-6.28 ug/ml in boars.

Various dosages of testosterone have been adminstered to castrated boars. Joshi and Raeside (1973) administered a weekly im injection of 37.5 mg/boar followed by 75 mg for another six weeks. Hupp et al (1961), administering deep intramuscular injection of testosterone propionate in sesame oil to 24 months old boars gave dose leves of 7.5, 37.5 and 187.5 ug/lb body weight for three separate periods. The hormone was aministered in each period three times weekly. After castration, each boar received an initial dose of 7.5 ug/lb body weight to be increased to the next level if the castrate failed to serve during a two week interval at this level. Bidner et al (1973) deviated from this mode of administration by including 2.2 mg methyl-testosterone per kg of ration fed to pigs lbetween 23 and 90 kg body weight.

In all these instances it could be seen that all dosages were far higher than the physiologic level of testosterone in the boar blood.

4.1.4 POSSIBLE MECHANISMS OF HORMONAL ACTIONS

Several mechanisms for hormone action have been postulated. It has been demonstrated that a number of different hormones act on the active transport across cell membrane. This has been shown for insulin with regard to the transport of glucose into the cell and STH and androgens with regard to the transport of amino acids. A current hypothesis is that the hormones act as gene-activators leading to the production of messenger- RNA and induced enzyme synthesis (Karlson and Sekeris, 1966).

The general effects produced by the action of hormones in the living organisms may be classified as follows:

(1) Morphogenesis,

(2) Maintennce of physilogical events.

The somatotrophic (STH) hormone is an outstanding hormone with regards to morphogenetic action where the overall growth of the organism reflects the action of this hormone.

The morphogenetic action of steroid hormones on the reproductive tract is seen in the growth of the uterus following treatment with estradiol and of the prostate following treatment with testosterone.

The main argument is that hormones exert a large portion of their actions through the activation or inhibition of enzyme systems. There is evidence that many hormones bring about their intracellular effects through the mediation of a second messenger that has been shown to be 3',5' cyclic adenosine monophosphate (cylic AMP) (Sutherland et al., 1968).

Through the action of adenyl cyclase, adenosine triphosphate (ATP) is converted to cyclic AMP, which then acts within the effector cell to produce the appropriate hormonal response. Cyclic AMP has now been established as a second messenger that mediates some of the effects of

quite a number of hormones: ACTH on the adrenal cortex, TSH on the thyroid, vasopressin on the kidney, epinerphrine and glucagon on the liver, LH on bovine corpus luteum and Interstitial cells stimulating hormone (ICSH) on the testis which directly stimulates testosterone production. Hormones may also alter the permeability of the cell membrane or the membranes of intracellular organelles. In this way, the hormone could influence the movement of materials into the cells or between subsellular structures and thus condition the rate of some biochemical sequence (Levine and Goldstein, 1955). The cell membrane acts as a barrier and prevents the free entry of some materials but the hormone such as steroids that promotes the synthesis of proteins act at this cell surface to facilitate the entrance of amino acids into the intracellular pool.

There is a growing interest in the possibility that some hormones activate specific genes (DNA), thus promoting the transcription of new kinds of M-RNA which then code for the synthesis of specific proteins at the ribosomal level.

Hormones in circulation are frequently bound to specific carrier proteins and are continuously subjected to enzymatic inactivation or destruction in such organs as the liver and kidneys.

4.1.5 THE RECEPTOR MECHANISMS

A lot of mention has been made about steroid receptors in the brain and the gonads. It is therefore pertinent to review the concept of hormonal receptors.

The advent of hormone labelling has amply demonstrated that particular hormones are selectively concentrated by specific target cells and tissues; for example, estrogens by the uterus, androgens by the male

accessory sex glands and FSH and LH by the gonads. In general, the tissues that respond most profoundly show the highest uptake of the hormone and retain it longest. The mechanism of action of a hormone involves its interaction with receptor sites of the target cells and the chain of intracellular events that eventually leads to the organismal adjustments generally regarded as the effects of the hormone.

The receptor hypothesis therefore states that every target cell has specific sites that bind particular hormones and some progress has been made in identifying and characterizing the macromolecules which serve the cells in this capacity.

There seems to be two major types of hormone receptor sites: those on the cell surface associated with plasma membrane and others within the cell either as part of the internal membrane structure of present in the cytosol itself. It has also been found that in many cases, the receptor is or involves a protein (Sutherland, 1972).

4.1.6 THE ROLE OF MINERALS ON TESTOSTERONE METABOLISM

The possible role of minerals and vitamins in reproduction has been the object of studies by some researchers in recent years. Calcium has for a long time been linked with protein synthesis (Kunerth and Pitman, 1939) and the growth and parathyroid hormones have also been implicated in the increase observed in the absorption of calcium ions in the young amimal. In addition, a high level of potassium ions is associated with a high rate of ATP depletion in conditions of high metabolic rate because ATP is the metabolic substrate for the energy-dependent transmembrane transport of potassium and sodium ions.

The role of zinc in protein synthesis has also been well established (Lieberman et al., 1963). Zinc also plays a significant role in sexual maturation (Sandstead et al., 1973) and is important for the function of many enzymes (Mikac-Devic, 1970). Prasad and Oberleas (1974) found that since deficiency in rats depresses zinc content of the testis, hones, muscles, oesophagus and kidney. Dietery zinc deficiency has also been known to result in decreases in most organ weights such as liver, lungs, testis whereas the kidneys and adrenal glands increase in weight (Prasad and Oberleas, 1974). The condition also depresses the activities of many zinc-dependent enzymes.

Since one of the most prominent effects of zinc deficiency is growth retardation, one may conclude that the protein content of the cell is being decreased concomittantly. Thus Fullis (1958), Millar et al (1958) and Barney et al (1968) observed marked atrophy of the testis in zinc deficient animals. Zinc is also involved in carbohydrate and lipid metabolism.

Potassium, along with sodium, plays a major role in electroylte balance. While sodium functions extracellularly, potassium functions intracellularly. Their deficiency retards growth and lowers the utilization of digested proteins. Sodium is also involved in fat deposition and testicular development (Orent-Keiles et al., 1937). Low potassium in diets causes slow growth and sterility (Orent-Keiles and McCollum, 1942). Sodium and potassium are also antagonistic to calcium and magnesium. Thus high levels of potassium disturb metabolism of magnesium. Testosterone improves calcium and phosphorus balance (Reifenstein and Albright, 1947).

Magnesium is an enzyme activator for many enzyme systems involved in the metabolism of carbohydrates, proteins and fats. Copper plays a vital role in the synthesis and proper functioning of several oxidative enzymes necessary for normal metabolism. It's deficiency leads to lesions in the brain stem and spinal cord and poor growth.

4.1.7 ROLE OF THE HYPOPHYSEAL - ADRENAL - THYROID - GONADAL AXIS IN ANDROGEN METABLOSM

The importance of the hypothalamus in the maintenance of pituitary functions has already been discussed. In recent years, much evidence has accumulated to show that impairment of pituitary, thyroid and adrenal functions have serious effects on normal reproductive behaviour in mammals. The thyroid hormone influences reproduction and fertility by helping to maintain the pituitary-hypophyseal-gondal relationship and also by affecting the metabolic pool of nitrogen and available energy, thus allowing the proper growth of tissues of the reproductive system and the growing embryo. Thus hypothyroidism impairs testicular development in young animals and delays sexual maturity and in certain cases abolishes testosterone production.

Some studies (Samperex et al., 1969) have indicated that the hypophyses have a much higher capacity to bind androgen molecules than the hypothalamus and that castration considerably activates the transformation of testosterone into dihydrotestosterone (DHT) in the hypophyses and that the rate of conversion increases with the length of time after castration (Martini, 1973).

This conversion is also activated by castration at the hypothalamic level. This suggests that testoterone must be converted into "active" metabolite before initiating the feedback responses mentined earlier and also reinforces the conclusion that both the anterior pituitary and hypothalamus are the sites on which androgen exerts its feedback effect on gonadotropin secretion. It is therefore probable that the pituitary plays a more important role in this process than the hypothalamus. It is interesting to note that following castration, a higher uptake of radioactive testosterone has been reported to occur both in the anterior pituitary and in the hypothalamus (Roy and Laumans, 1969).

In addition, among the large number of steroids found in the adrenal cortex are sex hormones which include androgens, estrogens and progresterone-like substances. The amount of androgens produced by the andrenal cortex has been known to increase after castration, but the level is not sufficient to restore normal reproductive functions.

4.1.8 **MATERIALS AND METHODS**

Fifty-one male Large White pigs housed, fed and provided water as described earlier were used. They were randomly divided into four groups as follows according to the age at which castration was done:

Group 1: Orchidectomy as performed at one week of age.

Group 2: Orchidectomy as performed at 3-4 months of age.

Group 3: Orchidectomy as performed at 5-6 months of age.

Group 4: Orchidectomy as performed at 7-8 months of age.

Orchidectomy was performed by open surgery according to the method of Berge and Westhues (1966).

Each group therefore comprised (a) intact control boards (IC), (b) orchidectomized boars treated with 1 ml of corn oil containing 25 mg of testosterone enanthate intramuscularly (equivalent to 18.0 mg testosterone) (OT) and (c) orchidectomized control boars (OC) which were orchidectomizedand given intramuscularly 1 ml of corn oil.

The injections were administered at 0900-1000 hours every Monday for five weeks. 24 hours after the last injection all the animals were slaughtered and their brains and hypophyses quickly removed, disseted and processed as described earlier.

4.1.9 **BEHAVIOURAL TESTS**

24 hours after the second testosterone administration, all animals in grups 2, 3 and 4 were regularly introduced to cycling gilts and the following parameters studied and respectively scored as follows:

(a) **SEXUAL BEHVIOUR**

Parameter	Scores
(i) Courtship	1
(ii) Mounting	2
(iii) Intromission	3
(iv) Ejaculation	4

(b) **AGONISTIC BEHAVIOUR**

Parameter	Scores
Investigative behviour	1
Strutting	2
Restless/Slashing	3
Violent/Biting	4

Scoring was based on the behaviour of each animal within a five-minute period.

STATISTICAL ANALYSIS

The data were subjected to multifactor analyses of variance and the treatment means compared by the least significant difference method of Steel and Torrie (1960).

4.2.0 EFFECT OF PRE-WEANING CASTRATION WITH OR WITHOUT TESTOSTERONE ON PORCINE BRAIN AND HYPOPHYSEAL AChE ACTIVITY TOTAL PROTEIN AND SAChE ACTIVITY

The results are summarised in Table 4.1.1.

Preweaning castration without testosterone replacement depressed AChE levels ($P<0.05$) in the amygdala, hypothalamus, midbrain and medulla oblongata while testosterone therapy restored AChE activity to normal levels in only the amygdala. The AChE content in the pons of OC and IC animals were similarly inferior to that of OT animals ($P<0.05$).

AChE activity in the cerebellum and hippocampus of OT animals was higher than in OC animals but not IC animals which however did not differ significantly from the other controls. AChE in the cerebral cortex and hypothalamus was depressed by orchidectomy with or without testosterone while in the mesencephalon and medulla, the enzyme was partially restored and enhanced, respectively.

In the adenohypophysis AChE activity was similar in the OT and IC but inferior to that in the OC animals while in the neurohypophysis, the testosterone therapy elevated the enzyme level beyond that of the IC but not that of the OC. The control animals were however similar.

TABLE 4.1.1. THE EFFECT OF PREWEANING CASTRATION WITH OR WITHOUT TESTOSTERONE ON ACETYLCHOLINESTERASE ACTIVITY IN THE PIG.

BRAIN REGIONS	ANIMAL GROUPS: ORCHIDECTOMIZED With testosterone	ORCHIDECTOMIZED Without testosterone	Intact control
a) *Acetylcholinesterase activity (µmole/g/min)			
Pons	4.874 ± 0.274a	4.258 ± 0.311b	4.184 ± 0.123b
Cerebellum	3.284 ± 0.187a	2.513 ± 0.402b	2.970 ± 0.081ab
Amygdala	5.899 ± 0.337a	3.594 ± 0.180b	6.058 ± 0.062a
Hippocampus	4.979 ± 0.226a	4.092 ± 0.414b	4.491 ± 0.376ab
Hypothalamus	4.153 ± 0.135b	4.572 ± 0.357b	5.737 ± 0.172a
Cerebral cortex	1.286 ± 0.032b	1.398 ± 0.021b	1.723 ± 0.107a
Mid brain	7.493 ± 0.273b	4.470 ± 0.211c	8.374 ± 0.235a
Medulla oblongata	7.479 ± 0.353a	4.601 ± 0.154c	6.999 ± 0.094
Adenohypophysis	0.561 ± 0.053b	1.116 ± 0.074 a	0.356 ± 0.028b
Neurohypophsis	1.124 ± 0.063a	0.890 ± 0.034ab	0.515 ± 0.002b
Grand mean	4.113 ± 2.528	3.150 ± 1.524	4.141 ± 2.731
b) ** Protein concentration (g/100ml)	ORCHIDECTOMIZED With testosterone	ORCHIDECTOMIZED Without testosterone	Intact control
Pons	1.360 ± 0.035a	0.449 ± 0.014b	1.394 ± 0.086 a
Cerebellum	1.206 ± 0.044a	0.157 ± 0.008c	0.934 ± 0.061b
Amygdala	1.128 ± 0.045a	0.246 ± 0.007c	0.610 ± 0.033b
Hippocampus	1.058 ± 0.028a	0.173 ± 0.007c	0.791 ± 0.037b
Hypothalamus	1.101 ± 0.028a	0.773 ± 0.010b	0.989 ± 0.050a
Cerebral	1.094 ± 0.044a	0.139 ± 0.010b	0.966 ± 0.027a
Mid brain	1.264 ± 0.084a	0.468 ± 0.029b	1.091 ± 0.020a
Medulla oblongata	1.514 ± 0.104a	0.420 ± 0.009c	1.166 ± 0.028a
Adenohypophysis	1.059 ± 0.031a	0.239 ± 0.008b	1.033 ± 0.051a
Neurohypophysis	1.096 ± 0.058 a	0.250 ± 0.025b	1.037 ± 0.028a
Grand mean	1.188 ± 0.150	0.331 ± 0.198	1.005 ± 0.209
c) ***Specific acetylcholinesterase activity (µmole/gprotein/min)	ORCHIDECTOMIZED With testosterone	ORCHIDECTOMIZED Without testosterone	Intact control
Pons	3.576 ± 0.577b	9.490 ± 0.657a	3.051 ± 0.212b
Cerebellum	2.739 ± 0.203c	15.974 ± 2.424a	3.248 ± 0.282b
Amydgala	5.313 ± 0.450c	14.690 ± 0.869a	10.047 ± 0.557b
Hippocampus	4.718 ± 0.121b	23.786 ± 2.470a	5.734 ± 0.561b
Hypothalamus	3.770 ± 0.097c	25.818 ± 1.988a	5.845 ± 0.262b
Cerebral cortex	1.094 ± 0.100b	10.370 ± 0.985a	1.782 ± 0.097b
Mid brain	6.020 ± 0.394b	9.782 ± 0.679a	7.678 ± 0.227b
Medulla Oblongata	4.980 ± 0.198b	10.934 ± 0.546a	6.012 ± 0.114b
Adenohypophysis	0.537 ± 0.063b	4.684 ± 0.358a	0.348 ± 0.031b
Neurohypophysis	1.036 ± 0.081b	3.667 ± 0.305a	0.498 ± 0.012b
Grand mean	3.378 ± 1.957	12.924 ± 7.320	4.434 ± 3.175

Castration without testosterone significantly depressed protein levels in all brain regions. In addition, protein levels in the OT animals and the IC were similar in the pons, hypothalamus, cerebral cortex and midbrain while in the cerebellum, amygdala, hippocampus and medulla oblongata of the OT animals, protein levels were significantly enhanced. Protein concentration in both hypophyses of OT and IC animals were similar and significantly superior to the OC animals ($P<0.05$).

SAChE activity in the OT and IC animals was similar and significantly inferior ($P<0.05$) to that in the OC animals in the pons, cerebellum, hippocampus, cerebral cortex, midbrain and medulla. SAChE activity was enhanced in the amygdala and hypothalamus of the OC animals than the IC which in turn had higher levels than the OT animals. Both the OT and IC animals have similar hypophyseal SAChE activity which were significantly lower than the OC animals.

4.2.1 MINERAL PROFILE IN THE BRAIN AND HYPOPHYSES

The effects of the various treatments are summarized in Table 4.1.2.

CALCIUM

Calcium levels were similar in the OT and OC animals and superior to those in the IC animals in the pons and cerebral cortex while the reverse occurred in the hippocampus, hypothalamus and medulla oblongata. Calcium was much enhanced in the cerebellum, amygdala and mid-brain of the OC animals while much lower levels were observed in the IC and OT animals ($P<0.05$). In the adenohypophysis, the OC and the IC animals had similar calcium levels but inferior to the OT animals. In the neurohypophysis, the

IC and OC animals had similar calcium levels which were superior to the OT animals.

MAGNESIUM

In the different brain regions, magnesium levels in the OT and OC animals were similar and superior to the IC animals in the pons and amygdala. In the medulla oblongata, the OC animals and the IC had similar magnesium levels which were superior to their OT counterparts.

In the cerebral cortex, the OT animals and control had similar magnesium levels significantly ($P<0.05$) higher than the OC animals. The other brain regions viz: the hypothalamus, hippocampus and the cerebral cortex did not show a particularly consistent trend.

In both hypophyses, the OC animals had significantly higher magnesium levels than the other two groups ($P<0.05$) which were similar to each other.

ZINC

Zinc was depressed in the hippocampus, hypothalamus and medulla oblongata of the OC animals while it was considerably enhanced and moderate in the OT and IC animals, respectively. Highest zinc levels were recorded in the cerebral cortex and midbrain of the OC animals, medium in the OT and least in the IC groups.

Zinc was depressed in the cerebellum and amygdala of the IC animals, moderate in the OC animals and considerably enhanced in the OT animals.

TABLE 4.1.2 EFFECT OF PRE-WEANING CASTRATION AND TESTOSTERONE ON THE *MINERAL PROFILE IN THE BRAIN AND HYPOPHYSES OF PIGS.

	ANIMAL GROUPS		
	ORCHIDECTOMIZED		
a) CALCIUM	With testosterone	Without testosterone	Intact control
Pons	1.689 ± 0.175 a	1.796 ± 0.058 a	1.374 ± 0.021 b
Cerebellum	2.061 ± 0.025 a	2.172 ± 0.059 ab	1.883 ± 0.050 b
Amygdala	1.534 ± 0.035 b	2.214 ± 0.028 a	1.760 ± 0.033 b
Hippocampus	2.670 ± 0.051 b	1.474 ± 0.031 b	2.053 ± 0.032 a
Hypothalamus	1.480 ± 0.030 b	1.679 ± 0.108 b	2.180 ± 0.031 a
Cerebral cortex	1.772 ± 0.034aa	1.880 ± 0.022 a	1.406 ± 0.004 b
Mid brain	1.264 ± 0.031 c	1.690 ± 0.038 b	2.246 ± 0.062 a
Medulla oblongata	1.638 ± 0.059 b	1.645 ± 0.034 b	2.530 ± 0.025 a
Adenohypophysis	2.350 ± 0.045 a	2.089 ± 0.010 b	2.053 ± 0.030 b
Neurohypophysis	2.070 ± 0.019 b	2.420 ± 0.067 a	2.516 ± 0.098 a
Grand mean	1.753 ± 0.323	1.906 ± 0.303	2.001 ± 0.403
b) MAGNESIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	1.368 ± 0.020 a	1.362 ± 0.034 a	1.258 ± 0.017 b
Cerebellum	1.341 ± 0.013 ab	1.406 ± 0.013 a	1.305 ± 0.013 b
Amygdala	1.361 ± 0.029 a	1.342 ± 0.007 a	1.196 ± 0.004 b
Hippocampus	1.270 ± 0.008 c	1.380 ± 0.009 b	1.490 ± 0.043 a
Hypothalamus	1.370 ± 0.021 a	1.227 ± 0.03 b	1.010 ± 0.058 c
Cerebral cortex	1.542 ± 0.065 a	1.484 ± 0.020 ab	1.422 ± 0.063 b
Mid brain	1.326 ± 0.045 a	1.188 ± 0.027 b	1.397 ± 0.005 a
Medulla oblongata	1.390 ± 0.045 b	1.491 ± 0.068 a	1.539 ± 0.008
Adenohypophysis	1.257 ± 0.008 b	1.528 ± 0.004 a	1.325 ± 0.015 b
Neurohypophysis	0.901 ± 0.060 b	1.189 ± 0.005 a	1.005 ± 0.035 b
Grand mean	1.313 ± 0.164	1.360 ± 0.125	1.295 ± 0.183
c) ZINC	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	0.326 ± 0.006 c	0.382 ± 0.004 b	0.437 ± 0.008 a
Cerebellum	0.473 ± 0.010 a	0.378 ± 0.012 b	0.313 ± 0.006 c
Amygdala	0.581 ± 0.012 a	0.410 ± 0.006 c	0.308 ± 0.003 b
Hippocampus	0.472 ± 0.007 a	0.262 ± 0.006 c	0.308 ± 0.003 b
Hypothalamus	0.464 ± 0.006 a	0.287 ± 0.004 c	0.350 ± 0.003 b
Cerebral cortex	0.319 ± 0.001 b	0.364 ± 0.003 a	0.251 ± 0.008 c
Mid brain	0.302 ± 0.002 b	0.416 ± 0.006 a	0.242 ± 0.006 c
Medulla oblongata	0.482 ± 0.005 a	0.336 ± 0.008 c	0.402 ± 0.005 b
Adenohypophysis	0.454 ± 0.002 b	0.507 ± 0.003 a	0.520 ± 0.003 a
Neurohypophysis	0.409 ± 0.002 b	0.273 ± 0.005 c	0.474 ± 0.008 a
Grand mean	0.428 ± 0.089	0.359 ± 0.083	0.371 ± 0.093

Values in the same horizontal column differently superscripted differ significantly ($P < 0.05$)

Values are means ± S.E.M.

*Values in parts per million (ppm).

TABLE 4.1.2 Continued

BRAIN REGIONS	ANIMAL GROUPS		
f) POTASSIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	22.800 ± 0.860 a	25.415 ± 1.187 a	22.592 ± 0.094 a
Cerebellum	25.455 ± 1.645 a	25.400 ± 1.596 a	22.5 5 ± 0.779 a
Amygdala	27.200 ± 2.482 a	18.100 ± 2.190 b	21.000 ± 1.129 b
Hippocampus	18.750 ± 2.46 b	24.700 ± 0.952 a	21.000 ± 1.346 a
Hypothalamus	23.000 ± 1.658 a	22.070 ± 0.898 a	22.204 ± 0.647 a
Cerebral cortex	27.946 ± 1.416 a	27.465 ± 1.377 a	27.540 ± 1.418 a
Mid brain	21.710 ± 1.514 a	21.445 ± 0.896 a	21.976 ± 0.664 a
Medulla oblongata	22.000 ± 1.458 a	25.929 ± 0.839 a	25.868 ± 1.232 a
Adenohypophysis	27.550 ± 3.186 ab	29.123 ± 1.220 a	24.803 ± 1.996 b
Neurohypophysis	20.915 ± 0.551 ab	18.046 ± 0.973 b	24.130 ± 1.188 a
Grand mean	23.733 ± 3.136	23.769 ± 3.748	24.377 ± 3.092
e) SODIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	528.960 ± 2.430 a	530.650 ± 0.970 a	518. 00 ± 1.176 a
Cerebellum	531.000 ± 4.848 a	528.350 ± 1.288 a	525.000 ± 3.688 a
Amygdala	515. ± 5.701 a	531.200 ± 1.268 a	522.750 ± 2.694 a
Hippocampus	532.00 ± 4.062 a	531.900 ± 1.208 a	523.000 ± 6.879 a
Hypothalamus	529.000 ± 5.788 a	534.700 ± 2.502 a	520.600 ± 1.517 a
Cerebral cortex	518.700 ± 1.364 a	521.355 ± 0.847 a	514.200 ± 3.397 a
Mid brain	514.320 ± 3.747 a	521.450 ± 2.488 a	532.400 ± 2.502 a
Medulla oblongata	523.200 ± 0.931 a	521.620 ± 1.071 a	572.800 ± 3.007 a
Adenohypophysis	536.900 ± 1.806 a	519.000 ± 7.314 a	531.000 ± 2.214 a
Neurohypophysis	521.800 ± 4.934 a	539.400 ± 2.182 a	520.400 ± 3.010 a
Grand mean	524.992 ± 7.512	528.462 ± 6.443	523.553 ± 6. 52 a
f) COPPER	ORCHIDECTOMIZED		
	With testosterone	Without stestosterone	Intact control
Pons	0.120 ± 0.001 b	0.126 ± 0.006 b	0.153 ± 0.007 a
Cerebellum	0.154 ± 0.003 a	0.153 ± 0.008 a	0.163 ± 0.003 a
Amygdala	0.170 ± 0.001 a	0.149 ± 0.007 b	0.098 ± 0.004 c
Hippocampus	0,124 ± 0.003 c	0.152 ± 0.005 b	0.179 ± 0.003 a
Hypothalamus	0.162 ± 0.010 b	0.145 ± 0.008 c	0.191 ± 0.007 a
Cerebral cortex	0.160 ± 0.002 a	0.122 ± 0.003 b	0.172 ± 0.005 a
Mid brain	0.144 ± 0.008 b	0.127 ± 0.002 c	0.205 ± 0.007 a
Medulla oblongata	0.148 ± 0.008 b	0.128 ± 0.001 c	0.170 ± 0.004 a
Adenohypophysis	0,260 ± 0.008 c	0.280 ± 0.007 b	0.302 ± 0.010 a
Neurohypophysis	0,268 ± 0.004 a	0.145 ± 0.003 b	0.265 ± 0.012 a
Grand mean	0.171 ± 0.051	0.153 ± 0.046	0.189 ± 0.057

Values in the same horizontal column differently superscripted differ significantly ($P < 0.05$)

Values are means ± S.E.M.

In the adenohypophysis, both the OC and IC groups had similar levels which were higher than the OT groups ($P<0.05$). In the neurohypophysis, highest levels were observed in the IC animals with the OC animals recording the lowest level while the OT animals showed a slightly higher value ($P<0.05$).

POTASSIUM

Potassium levels in the three treatment groups were similar in all the brain regions except in the amygdala where potassium was significantly depressed in the OC animals and in the hippocampus where the OC animals had higher levels than the other two groups.

In the adenohypophysis, both the OT and OC animals had similar potassium content but potassium is considerably more enhanced ($P<0.05$) in the OC than the IC animals. In the neurohypophysis, the OC animals had similar level with the OT animals but inferior to the IC animals. The IC group was however similar to their OT counterparts.

SODIUM

There was no significant ($P>0.05$) treatment effect on both brain and hypophyseal sodium levels.

COPPER

Copper was significantly depressed in the medulla, midbrain, cerebral cortex, hypothalamus and pons of the OC animals. No significant changes were observed in the cerebellum but in the hippocampus, the OT animals had depressed copper levels while moderate and considerabLy enhanced levels were observed in the OC and the IC animals, respectively.

Copper was lowest in the amygdala of the IC animals, medium in the OC animals and highest in the OT group.

In the adenohypophysis, the highest copper level was recorded in the IC animals ($P<0.05$) followed by the OC group and least in the OT group. In the neurohypophyses, testosterone therapy restored copper levels to the same level as found in the intact control while orchidectomy significantly depressed it.

4.2.2 EFFECT OF PRE- VERSUS POST PUBERTAL CASTRATION WITH OR WITHOUT TESTOSTERONE THERAPY ON SEXUAL BAHAVIOUR IN MALE PIGS

Each group of experimental animals was tested with intact and mature sows and the response noted. Table 4.1.2.

CASTRATED PIGS WITHOUT REPLACEMENT THERAPHY

The prepubertally castrated pigs, i.e. those castrated at three months of age were very timid and lacked the urge to go for the females. It was also observed that when the females discovered this docility in the pigs, they became very aggressive and chased the barrows all over the pen, biting and slashing at them all the way.

After about 3 weeks of constant exposure to the females, about 60% of them became fairly aggressive and repelled the attack of the females. However, only about 20% attempted mating while the remaining 80% showed apparent disinterest in the coutship process.

The pigs castrated between 5-6 months of age and 7-8 months of age were fairly aggressive towards the female within 10 seconds of exposure but still lacked of interest in mating. They were however more interested in

Table 4.1.2.1

PARAMETERS OF AGGRESSION AND SEXUAL BEHAVIOUR DISLAYED BY PRE-VERSUS POST-PUBERTALLY CASTRTED MALE PIGS COMPARED TO GONADALLY INTACT MALES

	Pre-pubertally Castrated Males	Post-pubertally Castrated Males	Intact Males	Castrates Treated with Testosterone
ALL SUBJECTS (Total number tested)	12	24	36	36
Number of animals observed to be:				
Investigative	4	2	30	18
Strutting	2	4	15	15
Restless	7	5	32	26
Violent	1	13	25	23
Aggression index	2.36 ± 0.97	3.21 ± 1.02	2.50 ± 1.16	2.66 ± 1.11
Main parameter exhibited	Strutting/ Restless	Restless/ Violent	Strutting/ Violent	Strutting/ Restless
SEXUAL BEHAVIOUR Number of animals displayed:				
Courtship	3	18	34	20
Mounting	2	4	32	18
Intromission	-	-	5	-
Ejaculation	-	-	5	-
Behaviour index	1.40 ± 0.49	1.18 ± 0.38	1.75 ± 0.85	1.47 ± 0.50
Main behaviour exhibited	Courtship/ Mounting	Courtship/ Mounting	Courtship/ Mounting	Courtship/ Mounting

agonistic behaviour such as strutting, slashing and biting (see Table 4.1.2).

CASTRATED PIGS WITH TESTOSTERONE REPLACEMENT THERAPY

All the three treated groups showed marked interest in the female whether on heat or not by the third week of testosterone therapy. However, the interest was more aggressive initially without about 65% of them becoming very aggressive within 10 seconds of contact with the females and displayed characteristic agonistic behaviour. By the fourth week of treatment about 50% were attempting mating within 30 seconds of contact with the female while over 80% of them still displayed aggressive behaviour by the end of the fifth week with 60% attempting mating.

INTACT BOARS

Above 90% of the intact controls attempted to mate when exposed to females. They displayed characteristic boar sexual behaviour by sniffing the urinogenital area of the females and following them around. In some cases when the females urinated, the male would sniff the urine perhaps trying to discern a particular odour which may indicate receptivity by the sow or not, depending on her estrus status.

This courtship period normally lasted for a few seconds and the boars attempted to mount within 5 seconds of entry into the female pens.

Females that were not on heat and which restricted mounting were vigorously pursued and attacked. They too fought back.

It should also be noted that the sows accepted the intact boars more readily than they did the castrates.

4.2.3 EFFECT OF ORCHIDECTOMY AT 3-4 MONTHS OF AGE AND TESTOSTERONE ON THE PIG BRAIN AND HYPOPHYSEAL PHYSIOLOGY

Table 4.1.3 summarizes the effects of orchidectomy with and without testosterone on the acetylcholinesterase activity, specific acetylcholinsterase activity, and protein content of the porcine brain and hypophyses.

Castration without testosterone significantly depressed AChE in the pons, cerebellum, hippocampus, hypothalamus and medulla oblongata while replacement therapy boosted activity to above normal levels in the same regions except the hypothalamus.

The cerebral cortex, midbrain and amygdala were not significantly affected by orchidectomy but the OT animals had considerably enhanced activity in the amygdala and midbrain, cerebellum, pons, amygdala, hippocampus, hypothalamus and medulla oblongata than the IC animals. AChE activity in the adenohypophysis of the OC and IC animals were similar and inferior to the OT group. However, no significant differences ($P>0.05$) were observed between the OT and the OC animals. In, addition no significant differences were observed in the neurohypophysis.

Castration without replacement therapy similarly depressed protein levels in the pons, cerebellum and medulla oblongata ($P<0.05$) while in the OT animals it was elevated more than the IC animals In the pons, cerebellum, amygdala, hippocampus and midbrain. No significant effects were observed in the hypophyses. SAChE activity was significantly depressed in the amygdala, hippocampus, hypothalamus, cerebral cortex and midbrain of the OT animals and elevated in the OC and IC animals. SAChE activities in the hippocampus, cerebral cortex and midbrain of both the OC and IC were similar.

TABLE 4.1.3 THE EFFECT OF ORCHIDECTOMIZED WITH OR WITHOUT TESTOSTERONE AT 3-4 MONTHS OF AGE ON PROCINE BRAIN AND HYPOPHYSEAL PHYSIOLOGY.

BRAIN REGIONS	ANIMAL GROUPS		
a) *ACETYLCHOLINESTERASE ACTIVITY	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	7.310 ± 0.363 a	2.465 ± 0.256 c	4.217 ± 0.170 b
Cerebellum	4.300 ± 0.180 a	2.209 ± 0.085 c	2.981 ± 0.103 b
Amygdala	5.960 ± 0.300 a	5.022 ± 0.190 b	4.793 ± 0.114 b
Hippocampus	6.468 ± 0.176 a	3.395 ± 0.059 c	4.565 ± 0.286 b
Hypothalamus	6.059 ± 0.196 a	4.138 ± 0.379 b	5.826 ± 0.161 a
Cerebral cortex	1.843 ± 0.086 a	1.640 ± 0.057 a	1.774 ± 0.037 a
Mid brain	9.663 ± 0.956 a	7.460 ± 0.113 b	7.222 ± 0.111 b
Medulla oblongata	9.516 ± 0.245 a	5.257 ± 0.283 c	6.193 ± 0.327 b
Adenohypophysis	1.858 ± 0.023 a	1.418 ± 0.032 ab	0.861 ± 0.054 b
Neurohypophysis	1.146 ± 0.044 a	1.075 ± 0.025 a	1.151 ± 0.020 a
Grand mean	5.412 ± 3.076	3.408 ± 2.050	3.955 ± 2.199
b) **PROTEIN CONCENTRATION	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	1.021 ± 0.086 a	0.572 ± 0.109 b	0.965 ± 0.157 a
Cerebellum	0.799 ± 0.070 a	0.328 ± 0.040 c	0.560 ± 0.087 b
Amygdala	0.462 ± 0.056 a	0.127 ± 0.008 b	0.168 ± 0.008 b
Hippocampus	0.907 ± 0.088 a	0.177 ± -.032 b	0.198 ± 0.012 b
Hypothalamus	0.409 ± 0.043 a	0.147n± 0.005 b	0.263 ± 0.019 ab
Cerebral cortex	0.236 ± 0.015 a	0.097 ± 0.006 a	0.136 ± 0.013 a
Mid brain	0.932 ± 0.104 a	0.274 ± 0.025 b	0.273 ± 0.038 b
Medulla oblongata	0.474 ± 0.023 a	0.257 ± 0.020 b	0.468 ± 0.025 a
Adenohypophysis	0.117 ± 0.003 a	0.175 ± 0.010 a	0.183 ± 0.020 a
Neurohypophysis	0.104 ± 0.004 a	0.189 ± 0.005 a	0.088 ± 0.005 a
Grand mean	0.546 ± 0.346	0.234 ± 0.138	0.330 ± 0.267
c) SPECIFIC ACETYLCHOLINE-STERASE ACTIVITY	ORCHIDECTOMIZED		
Pons	7.276 ± 0.490 a	4.732 ± 0.897 a	4.653 ± 0.605 a
Cerebellum	5.544 ± 0.686 a	7.151 ± 1.219 a	5.787 ± 1.126 a
Amygdala	13.686 ± 2.279 c	40.403 ± 4.381 a	28.639 ± 1.525 b
Hippocampus	7.278 ± 0.520 b	20.744 ± 3.386 a	23.390 ± 2.037 a
Hypothalamus	15.467 ± 2.029 c	28.067 ± 2.002 a	22.449 ± 1.325 b
Cerebral cortex	7.841 ± 0.198 b	16.974 ± 0.726 a	13.547 ± 1.343 a
Mid brain	10.547 ± 1.122 b	27.770 ± 2.177 a	27.941 ± 3.601 a
Medulla oblongata	20.147 ± 0.839 a	20.557 ± 0.815 a	13.315 ± 0.835 b
Adenohypophysis	15.933 ± 0.646 a	8.203 ± 0.651 b	5.762 ± 1.318 b
Neurohypophysis	11.102 ± 0.284 a	5.691 ± 0.070 b	13.141 ± 0.876 a
Grand mean	11.483 ± 4.720	18.029 ± 11.809	15.862 ± 9.175

Values in the same horizontal column different superscripted differ significantly ($P < 0.05$)

Values are means ± S.E.M.
* AChE activity in μmole/g/minc; ** Total protein in g/100 ml
*** SAChE activity in μmole/g/protein/min.

The OC animals however had higher activities than the IC in the amygdala and hypothalamus.

The pons and cerebellum were not significantly affected ($P>0.05$) and SAChE activity in the OC and OT animals were similar and superior to the IC.

In the adenohypophyses, SAChE activity was significantly higher ($P<0.05$) in the OT animals than either the OC and IC groups which were similar. SAChe activity in the neurohypophyses of both the OT and IC were similar and superior to the OC animals.

4.2.4 MINERAL PROFILE IN THE BRAIN AND HYPOPHYSIS

Table 4.1.4 summarizes the effects of the treatments on the brain and hypophyseal mineral content.

Castration without testosterone significantly depressed calcium levels in the pons, cerebellum, amygdala, hypothalamus and midbrain ($P<0.05$). Higher calcium levels were observed in the medulla oblongata, midbrain, cerebral cortex and hippocampus in the IC than in the OT group while a reverse of this trend was observed in the pons. Calcium levels in the cerebellum and hypothalamus of both the IC and OT animals were similar. Calcium was significantly depressed in the adenohypophysis of the OC animals and partially restored in the OT group. In the neurohypophysis, similar calcium levels were observed in the OC and OT animals but the OC animals had significantly lower level than the IC animals ($P<0.05$).

MAGNESIUM

Magnesium levels were relatively stable in the cerebellum and hypothalamus. In the medulla oblongata and amygdala, the OC animals had significantly lower ($P<0.05$) levels than the IC and OT animals whereas in the hippocampus and cerebral cortex, the untreated castrates had higher magnesium levels than either of the other two groups. Magnesium was highest in the adenohypophysis of the OT group, medium in the OC and low in the IC animals. In the neurohypophysis, the OT and IC had similar levels which were superior to that in the OC group.

ZINC

Castration without testosterone significantly reduced zinc level in all brain regions except the midbrain where no significant treatment effects were observed. Testosterone partially restored zinc level in the cerebellum, amygdala, hippocampus, hypothalamus, cerebral cortex and medulla oblongata.

Zinc level in the pons of OC and OT animals were similar and significantly higher than the IC animals.

POTASSIUM

Castration without testosterone significantly depressed potassium level in all brain regions while testosterone partially restored it. The IC animals also had higher levels than the OT animals in the medulla oblongata, cerebral cortex, hypothalamus and pons while similar levels were observed in the amygdala and midbrain. Castration without testosterone significantly depressed potassium level in both hypophyses and testosterone partially enhanced it in the adenohypophsis but not in the neurohypophysis.

TABLE 4.1.4: EFFECT OF ORCHIDECTOMY AT 3-4 MONTHS OF AGE AND TESTOSTERONE ON THE *MINERAL PROFILE OF THE PORCINE BRAIN AND HYPOPHYSIS.

BRAIN REGIONS	ANIMAL GROUPS		
	ORCHIDECTOMIZED		
a) CALCIUM	With testoterone	Without testosterone	Intact control
Pons	2.200 ± 0.041 a	0.925 ± 0.048 c	1.375 ± 0.025 b
Cerebellum	2.175 ± 0.085 a	1.000 ± 0.071 b	2.325 ± 0.085 a
Amygdala	0.995 ± 0.067 b	0.400 ± 0.041 c	1.385 ± 0.075 a
Hippocampus	1.125 ± 0.025 b	1.025 ± 0.075 b	2.925 ± 0.138 a
Hypothalamus	1.525 ± 0.063 a	1.100 ± 0.082 b	1.362 ± 0.029 a
Cerebral cortex	1.025 ± 0.025 b	1.100 ± 0.025 c	2.225 ± 0.335 a
Mid brain	1.575 ± 0.085 b	0.825 ± 0.025 cc	2.125 ± 0.048 a
Medulla oblongata	1.062 ± 0.025 b	1.000 ± 0.041 b	2.975 ± 0.144 a
Adenohypophysis	1.890 ± 0.012 b	0.895 ± 0.005 c	2.605 ± 0.066 a
Neurohypophysis	1.287 ± 0.031 ab	1.125 ± 0.048 b	1.375 ± 0.025 a
Grand mean	1.486 ± 0.047	0.939 ± 0.213	2.068 ± 0.655
b) MAGNESIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	2.375 ± 0.025 a	2.137 ± 0.024 b	1.887 ± 0.012 c
Cerebellum	2.000 ± 0.025 a	2.037 ± 0.028 a	2.027 ± 0.024 a
Amygdala	2.305 ± 0.051 b	1.094 ± 0.073 c	2.550 ± 0.096 a
Hippocampus	1.180 ± 0.029 b	1.782 ± 0.064 a	1.212 ± 0.093 b
Hypothalamus	2.375 ± 0.048 a	2.265 ± 0.057 a	2.362 ± 0.037 a
Cerebral cortex	1.725 ± 0.183 b	2.145 ± 0.048 a	1.187 ± 0.031 c
Mid brain	1.895 ± 0.021 a	1.555 ± 0.012 a	1.282 ± 0.022 b
Medulla oblongata	2.385 ± 0.053 a	1.975 ± 0.085 b	2.407 ± 0.067 a
Adenohypophysis	2.030 ± 0.024 b	1.730 ± 0.043 b	1.525 ± 0.032 c
Neurohypophysis	1.725 ± 0.041 b	2.075 ± 0.025 a	1.830 ± 0.024 b
Grand mean	2.002 ± 0.369	1.879 ± 0.351	1.827 ± 0.513
c) ZINC	ORCHIDECTOMIZED		
Pons	0.538 ± 0.023 a	0.602 ± 0.022 a	0.288 ± 0.007 b
Cerebellum	0.462 ± 0.018 a	0.258 ± 0.020 b	0.530 ± 0.009 a
Amygdala	0.304 ± 0.009 a	0.190 ± 0.005 b	0.311 ± 0.007 a
Hippocampus	0.360 ± 0.026 b	0.156 ± 0.017 c	0.505 ± 0.040 a
Hypothalamus	0.256 ± 0.005 b	0.139 ± 0.006 c	0.520 ± 0.031 a
Cerebral cortex	0.250 ± 0.018 b	0.231 ± 0.045 b	0.513 ± 0.052 a
Mid brain	0.182 ± 0.014 a	0.162 ± 0.025 a	0.220 ± 0.011 a
Medulla oblongata	0.259 ± 0.020 b	0.148 ± 0.016 c	0.625 ± 0.058 a
Adenohypophysis	0.234 ± 0.012 b	0.113 ± 0.004 c	0.692 ± 0.040 a
Neurohypophysis	0.229 ± 0.011 a	0.168 ± 0.001 a	0.220 ± 0.008 a
Grand mean	0.308 ± 0.113	0.217 ± 0.142	0.442 ± 0.169

Values in the same horizontal column differently superscripted differ significantly ($P < 0.05$)

Values are means ± S.E.M.

*Values in parts per million (ppm)

TABLE 4.1.4 (CONTINUED)

BRAIN REGIONS	ANIMAL GROUPS		
d) POTASSIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	8.300 ± 0.644 b	6.000 ± 0.637 b	27.275 ± 1.051 a
Cerebellum	22.375 ± 1.028 b	14.250 ± 0.750 c	30.375 ± 1.068 a
Amygdala	20.625 ± 1.307 a	8.375 ± 0.591 b	20.125 ± 0.876 a
Hippocampus	17.750 ± 1.028 a	5.625 ± 0.625 c	11.500 ± 0.612 b
Hypothalamus	21.250 ± 0.722 b	7.625 ± 1.068 c	31.750 ± 1.181 a
Cerebral cortex	7.625 ± 0.314 c	11.875 ± 1.181 b	22.250 ± 1.031 a
Mid brain	14.000 ± 0.657 a	7.375 ± 1.021 b	16.875 ± 0.718 a
Medulla oblongata	17.500 ± 1.021 b	10.500 ± 1.028 c	28.375 ± 0.479 a
Adenohypophysis	9.625 ± 0.375 b	5.875 ± 0.975 c	25.875 ± 1.808 a
Neurohypophysis	9.125 ± 0.987 b	7.625 ± 1.028 b	14.375 ± 0.473 a
Grand mean	14.817 ± 5.795	8.512 ± 2.842	22.877 ± 6.986
e) SODIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	520.500 ± 2.102 a	453.750 ± 5.543 b	520.000 ± 4.082 a
Cerebellum	528.750 ± 4.732 a	316.250 ± 2.394 b	526.250 ± 5.543 a
Amygdala	516.250 ± 3.146 a	514.250 ± 4.049 a	508.000 ± 1.779 a
Hippocampus	538.125 ± 1.779 a	522.500 ± 1.031 a	545.000 ± 4.083 a
Hypothalamus	235.000 ± 20.104 b	303.000 ± 12.069 b	536.250 ± 3.750 a
Cerebral cortex	526.250 ± 6.575 a	540.000 ± 3.535 a	536.875 ± 5.340 a
Mid brain	541.815 ± 1.197 a	506.750 ± 2.360 b	551.750 ± 3.119 a
Medulla oblongata	493.750 ± 7.465 b	537.500 ± 4.330 a	522.500 ± 4.787 ab
Adenohypophysis	546.750 ± 1.974 a	276.125 ± 5.354 b	554.250 ± 2.834 a
Neurohypophysis	265.000 ± 17.104 b	523.750 ± 3.750 a	517.375 ± 2.626 a
Grand mean	471.220 ± 117.153	449.387	531.819 ± 15.47
e) COPPER	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	0.262 ± 0.005 a	0.139 ± 0.070 a	0.112 ± 0.011 a
Cerebellum	0.155 ± 0.005 a	0.118 ± 0.003 a	0.095 ± 0.005 a
Amygdala	0.367 ± 0.013 b	0.179 ± 0.008 b	0.602 ± 0.051 a
Hippocampus	0.144 ± 0.004 a	0.048 ± 0.011 a	0.179 ± 0.005 a
Hypothalamus	0.084 ± 0.003 a	0.050 ± 0.002 a	0.084 ± 0.003 a
Cerebral cortex	0.157 ± 0.025 a	0.063 ± 0.003 a	0.212 ± 0.014 a
Mid brain	0.060 ± 0.005 a	0.070 ± 0.001 a	0.251 ± 0.010 a
Medulla oblongata	0.080 ± 0.002 a	0.046 ± 0.005 a	0.118 ± 0.006 a
Adenohypophysis	0.087 ± 0.005 a	0.120 ± 0.076 a	0.105 ± 0.003 a
Neurohypophysis	0.090 ± 0.006 a	0.200 ± 0.014 a	0.162 ± 0.005 a
Grand mean	0.144 ± 0.085	0.103 ± 0.056	0.192 ± 0.154

Values in the same horizontal column differently superscripted differ significantly ($P < 0.05$)

Values are means ± S.E.M.

SODIUM

The OC animals had significantly depressed sodium levels in the pons, cerebellum, hypothalamus and midbrain while testosterone restored the level to normal. The other brain regions were not significantly affected ($P>0.05$).

Castration without testosterone significantly depressed sodium level in the adenohypophysis compared with the IC and OT animals which were however similar.

COPPER

No significant effects were observed in the pons, cerebellum, hippocampus, hypothalamus, cerebral cortex and medulla. In the midbrain and amygdala, the OC and OT animals were similar and inferior to the IC animals ($P<0.05$).

No significant effects were observed in both hypophyses.

4.3.0 EFFECT OF ORCHIDECTOMY AT 5-6 MONTHS OF AGE WITH OR WITHOUT TESTOSTERONE ON THE PORCINE BRAIN AND HYPOPHYSEAL ACETYLCHOLINESTERASE LEVELS

Table 4.1.5 summarizes lthe effect of orchidectomy at 5-6 months of age with or without testosterone on the porcine brain and hypophyseal acetylcholinesterase activity. AChE activity is depressed in the pons, cerebellum, amygdala, hypothalamus and medulla oblongata of the OC animals while testosterone considerably enhanced it in the amygdala, hippocampus, hypothalamus and medulla oblongata. The cerebral cortex and the hypophyses were unaffected.

TABLE 4.1.5: THE EFFECT OF ORCHIDECTOMY AT 5-6 MONTHS OF AGE AND TESTOSTERONE ON FROCINE BRAIN AND HYPOPHYSEAL PHYSIOLOGY

BRAIN REGIONS	ANIMAL GROUPS		
a) *ACETYLCHOLINESTERASE ACTIVITY	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	3.429 ± 0.106 a	3.325 ± 0.084 a	4.914 ± 0.112 a
Cerebellum	2.805 ± 0.078 a	2.037 ± 0.028 b	2.700 ± 0.227 a
Amygdala	6.097 ± 0.079 a	3.548 ± 0.379 c	4.571 ± 0.128 b
Hippocampus	5.174 ± 0.226 a	3.519 ± 0.276 b	3.638 ± 0.143 b
Hypothalamus	7.051 ± 0.404 a	3.220 ± 0.329 c	3.800 ± 0.209 b
Cerebral cortex	1.944 ± 0.030 a	1.466 ± 0.089 a	1.826 ± 0.055 a
Mid brain	6.416 ± 0.336 a	6.677 ± 0.305 a	5.358 ± 0.301 b
Medulla oblongata	5.158 ± 0.421 a	4.162 ± 0.248 b	4.552 ± 0.374 ab
Adenohypophysis	1.478 ± 0.090 a	1.205 ± 0.050 a	1.092 ± 0.026 a
Neurohypophysis	1.144 ± 0.041 a	1.261 ± 0.021 a	0.971 ± 0.028 a
Grand mean	4.069 ± 2.178	3.042 ± 1.670	3.342 ± 1.606
b) **PROTEIN CONCENTRATION	ORCHIDECTOMIZED		
Pons	1.301 ± 0.014 b	0.494 ± 0.006 a	0.488 ± 0.012 a
Cerebellum	0.411 ± 0.059 a	0.117 ± 0.004 b	0.336 ± 0.012 a
Amygdala	0.290 ± 0.027 a	0.116 ± 0.003 b	0.194 ± 0.012 ab
Hippocampus	0.343 ± 0.040 a	0.179 ± 0.008 b	0.226 ± 0.006 ab
Hypothalamus	0.490 ± 0.036 a	0.258 ± 0.020 b	0.294 ± 0.007 b
Cerebral cortex	0.249 ± 0.028 a	0.156 ± 0.005 ab	0.118 ± 0.002 b
Mid brain	0.621 ± 0.002 a	0.185 ± 0.008 b	0.618 ± 0.038 a
Medulla oblongata	0.872 ± 0.021 b	0.287 ± 0.013 c	1.403 ± 0.218 a
Adenohypophysis	0.225 ± 0.011 a	0.155 ± 0.001 a	0.166 ± 0.009 a
Neurohypophysis	0.146 ± 0.009 a	0.097 ± 0.003 a	0.102 ± 0.004 a
Grand mean	0.495 ± 0.355	0.204 ± 0.118	0.394 ± 0.390
c) ***SPECIFIC ACETYLCHOLINESTERASE ACTIVITY	ORCHIDECTOMIZED		
Pons	2.635 ± 0.070 c	6.732 ± 0.198 b	10.073 ± 0.138 a
Cerebellum	7.253 ± 1.081 b	17.519 ± 0.840 a	8.403 ± 1.260 b
Amygdala	21.698 ± 0.682 b	27.005 ± 4.860 a	23.972 ± 2.054 ab
Hippocampus	15.238 ± 0.908 b	19.695 ± 1.156 a	16.135 ± 0.974
Hypothalamus	14.647 ± 1.414 a	12.762 ± 1.675 a	12.896 ± 0.496 a
Cerebral xcortex	8.052 ± 0.838 b	9.335 ± 0.414 b	15.482 ± 0.552 a
Mid brain	10.350 ± 0.646 b	36.297 ± 1.950 a	8.844 ± 1.027 b
Medulla oblongata	5.957 ± 0.031 b	14.663 ± 1.413 a	3.430 ± 0.487 b
Adenohypophysis	6.553 ± 0.201 a	7.799 ± 0.239 a	6.645 ± 0.436 a
Neurohypophysis	7.848 ± 0.245	13.001 ± 0.411	9.527 ± 0.497
Grand mean	10.023 ± 5.622	16.481 ± 9.233	11.541 ± 5.832

Values in the same horizontal column differently superscripted differ significantly ($P < 0.05$)
Values are means ± S.E.M.
* AChE activity in μmole/g/min
** Total protein in g/100 ml
*** SAChE activity in μmole/g protein/min

Orchidectomy significantly reduced protein levels in the cerebellum, midbrain and medulla oblongata with testosterone therapy significantly enhancing protein levels in all the brain regions. No significant differences were observed between the IC and the OC animals in the pons, amygdala, hippocampus, hypothalamus and cerebral cortex. No significant differences were observed in the hypophyses.

SAChE activities were highest in the cerebellum, hippocampus, midbrain and medulla oblongata of the OC animals but similar in both the IC and OT animals. The hypothalamus was however not affected. Castration with or without testosterone significantly depressed SAChE activity in the pons and cerebral cortex.

No significant effects were observed in the adenohypophysis while in the neurohypophysis, the IC and OT animals had similar levels which were inferior to the OC animals.

4.3.1 MINERAL PROFILE IN THE BRAIN AND HYPOPHYSES CALCIUM

Calcium is significantly depressed in the OC animals in the amygdala, medulla, hippocampus and hypothalamus and restored by testosterone in the OT animals. (Table 4.1.6).

The midbrain, cerebral cortex, pons and cerebellum were not significantly affected ($P>0.05$).

No significant effects were observed in the adenohypophysis while in the neurohypophysis the OC animals had depressed calcium level which was partially restored by testosterone treatment in the OT animals.

TABLE 4.1.6: EFFECT OF ORCHIDECTOMY AT 5-6 MONTHS OF AGE AND TESTOSTERONE ON THE *MINERAL PROFILE OF THE PORCINE BRAIN AND HYPOPHYSES.

BRAIN REGIONS	ANIMAL GROUPS		
a) CALCIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	1.027 ± 0.024 a	1.050 ± 0.029 a	2.007 ± 0.005 a
Cerebellum	0.890 ± 0.024 a	1.237 ± 0.024 a	1.740 ± 0.048 a
Amygdala	1.230 ± 0.012 ab	0.992 ± 0.064 b	2.008 ± 0.403 a
Hippocampus	1.512 ± 0.031 ab	1.150 ± 0.096 b	2.325 ± 0.071 a
Hypothalamus	2.150 ± 0.031 a	0.847 ± 0.050 b	2.716 ± 0.041 a
Cerebral cortex	1.587 ± 0.031 a	1.075 ± 0.050 a	1.6 ? ± 0.020 a
Mid brain	1.026 ± 0.015 a.	1.320 ± 0.012 a	1.692 ± 0.040 a
Medulla oblongata	2.292 ± 0.084 a	0.826 ± 0.244 b	3.062 ± 0.242 a
Adenohypophysis	1.792 ± 0.015 a	2.075 ± 0.050 a	2.630 ± 0.051 a
Neurohypophysis	2.330 ± 0.047 ab	1.837 ± 0.024 b	2.887 ± 0.088 a
Grand mean	1.587 ± 0.537	1.241 ± 0.411	2.272 ± 0.524
b) MAGNESIUM	ORCHIDE.TOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	1.282 ± 0.048 c	2.393 ± 0.025 a	1.605 ± 0.021 b
Cerebellum	1.180 ± 0.027 c	2.485 ± 0.062 a	2.051 ± 0.030 b
Amygdala	1.275 ± 0.027 c	2.425 ± 0.033 a	2.068 ± 0.021 b
Hippocampus	1.351 ± 0.018 b	2.480 ± 0.063 a	1.485 ± 0.023 b
Hypothalamus	1.810 ± 0.077 b	1.435 ± 0.050 c	2.014 ± 0.003 a
Cerebral cortex	1.249 ± 0.056 c	2.372 ± 0.070 a	1.612 ± 0.041 b
Mid brain	1.460 ± 0.029 b	2.220 ± 0.039 a	1.250 ± 0.025 c
Medulla oblongata	1.582 ± 0.014 c	2.117 ± 0.046 a	1.935 ± 0.025 b
Adenohypophysis	1.871 ± 0.055 a	1.625 ± 0.085	1.950 ± 0.029 a
Neurohypophysis	1.674 ± 0.097 c	2.367 ± 0.035 a	1.956 ± 0.037 b
Grand mean	1.473 ± 0.247	2.192 ± 0.369	1.793 ± 0.283
c) ZINC	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	0.264 ± 0.005 b	0.198 ± 0.009 c	0.355 ± 0.010 a
Cerebellum	0.164 ± 0.005 c	0.402 ± 0.008 a	0.336 ± 0.014 b
Amygdala	0.216 ± 0.003 b	0.190 ± 0.007a	0.162 ± 0.015 a
Hippocampus	0.335 ± 0.003 b	0.407 ± 0.005 a	0.332 ± 0.025 a
Hypothalamus	0.301 ± 0.004 b	0.148 ± 0.004 c	0.541 ± 0.015 a
Cerebral cortex	0.189 ± 0.009 c	0.309 ± 0.005 a	0.242 ± 0.001 b
Mid brain	0.417 ± 0.002 b	0.249 ± 0.005 c	0.543 ± 0.020 a
Medulla oblongata	0.347 ± 0.144 b	0.250 ± 0.006 c	0.578 ± 0.016 a
Adenohypophysis	0.363 ± 0.005 b	0.262 ± 0.012 c	0.429 ± 0.012 a
Neurohypophysis	0.256 ± 0.008 b	0.187 ± 0.012 c	0.301 ± 0.007 a
Grand mean	0.276 ± 0.094	0.260 ± 0.089	0.382 ± 0.138

Values in the same horizontal column differently superscripted differ significantly ($P < 0.05$)

Values are means ± S.E.M.

*Values in parts per million (ppm).

TABLE 4.1.6 (CONTINUED) -

BRAIN REGIONS	ANIMAL GROUPS		
d) POTASSIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	30.250 ± 1.181 a	18.500 ± 0.515 c	25.625 ± 1.028 b
Cerebellum	25.500 ± 0.500 a	19.875 ± 0.921 b	24.625 ± 0.866 a
Amygdala	21.062 ± 0.472 a	15.625 ± 0.625 b	20.625 ± 0.851 a
Hippocampus	27.500 ± 0.353 b	21.000 ± 0.613 c	31.000 ± 0.577 a
Hypothalamus	26.880 ± 0.375 b	20.625 ± 1.197 c	35.500 ± 0.500 a
Cerebral cortex	25.937 ± 0.926 a	11.430 ± 0.743 b	28.875 ± 0.479 a
Mid brain	32.700 ± 1.051 a	17.300 ± 0.829 b	34.500 ± 0.500 a
Medulla oblongata	25.375 ± 0.878 a	15.375 ± 0.944 c	22.625 ± 0.625 b
Adenohypophysis	22.625 ± 0.462 b	11.500 ± 0.645 c	25.000 ± 0.816
Neurohypophysis	21.750 ± 0.479 a	11.425 ± 0.472 b	21.500 ± 0.645 a
Grand mean	25.952 ± 3.667	16.265 ± 3.819	26.987 ± 5.266
e) SODIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	543.000 ± 1.205 a	506.250 ± 5.543 b	536.750 ± 2.609 a
Cerebellum	536.875 ± 1.541 a	191.250 ± 10.282 b	534.250 ± 4.049 a
Amygdala	529.250 ± 2.689 a	331.250 ± 11.250 b	531.250 ± 1.250 a
Hippocampus	532.750 ± 1.031 a	269.375 ± 8.315 b	541.750 ± 1.181 a
Hypothalamus	537.500 ± 5.204 a	270.500 ± 13.823 b	545.500 ± 4.406 a
Cerebral cortex	524.250 ± 5.813 a	242.500 ± 5.95 b	538.750 ± 2.839 a
Mid brain	544.750 ± 5.186 a	537.500 ± 3.227 a	539.250 ± 1.493 a
Medulla oblongata	530.000 ± 2.041 ab	515.000 ± 4.082 b	543.000 ± 1.225 a
Adenohypophysis	530.000 ± 1.041 a	303.750 ± 4.732 b	529.250 ± 1.493
Neurohypophysis	432.500 ± 1.041 a	523.000 ± 6.178 a	531.750 ± 1.181 a
Grand mean	534.087 ± 6.416	369.037 ± 135.470	537.150 ± 5.446
f) COPPER	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	0.098 ± 0.001 a	0.057 ± 0.002 a	0.149 ± 0.007 a
Cerebellum	0.282 ± 0.002 a	0.195 ± 0.003 ab	0.109 ± 0.003 b
Amygdala	0.103 ± 0.004 ab	0.085 ± 0.003 b	0.218 ± 0.009 a
Hippocampus	0.094 ± 0.006 a	0.049 ± 0.003 a	0.151 ± 0.010 a
Hypothalamus	0.087 ± 0.002 a	0.131 ± 0.006 a	0.099 ± 0.003 a
Cerebral cortex	0.068 ± 0.002 a	0.055 ± 0.005 a	0.101 ± 0.004 a
Mid brain	0.068 ± 0.007 a	0.035 ± 0.003 a	0.116 ± 0.004 a
Medulla oblongata	0.127 ± 0.008 ab	0.060 ± 0.004 b	0.203 ± 0.002 a
Adenohypophysis	0.077 ± 0.006 a	0.154 ± 0.008 a	0.155 ± 0.402 a
Neurohypophysis	0.101 ± 0.001 ab	0.048 ± 0.003 b	0.175 ± 0.006 a
Grand mean	0.110 ± 0.063	0.087 ± 0.054	0.148 ± 0.042

Values in the same horizontal column differently superscripted differ significantly ($P < 0.05$)

Values are means ± S.E.M.

MAGNESIUM

Magnesium levels were highest in the pons, cerebellum, amygdala, hippocampus, cerebral cortex, midbrain and medulla oblongata of the OC animals, moderate and lowest in the IC and OT animals respectively. However, in the hypothalamus castration depressed magnesium level and was only partially restored in the OT animals.

Magnesium levels in the adenohypophysis of the IC and OT animals were similar and superior to the OC animals. In the neurohypophysis, the OC animals had considerably higher magnesium level than either the IC or OT groups.

ZINC

Castration significantly depressed zinc levels in the pons, hypothalamus, midbrain and medulla oblongata and was partially enhanced by testosterone treatment. However OC animals had higher zinc levels in the cerebellum, and cerebral cortex than either the IC or OT groups. The IC animals in turn had higher levels than the other two groups in all the brain regions except the hippocampus.

Castration depressed zinc content in both hypophyses and was partially restored by testosterone treatment.

POTASSIUM

Potassium was significantly reduced by castration in all the brain regions ($P<0.05$). However the OT animals had higher levels than the IC animals in the pons and medulla oblongata while the reverse occurred in the hippocampus and hypothalamus. Similar levels were recorded in the

cerebellum, amygdala, cerebral cortex and midbrain of both the IC and OT animals.

SODIUM

The IC and OT animals were similar and significantly superior to the OC animals in all the brain regions except the midbrain where no significant differences were observed.

No significant differences were observed in the neurohypophysis while in the adenohypophysis, castration depressed sodium content and was restored by testosterone therapy.

COPPER

No significant differences ($P<0.05$) were observed in the pons, hippocampus, hypothalamus, cerebral cortex and midbrain.

In the amygdala and medulla oblongata, the OC animals were similar to the OT animals but significantly inferior to the IC group ($P>0.05$).

No significant differences were recorded in the adenohypophysis but in the neurohypophysis, the OC animals were inferior to the IC group but similar to the OT animals.

4.4.0 EFFECT OF ORCHIDECTOMY AT 7-8 MONTHS OF AGE AND TESTOSTERONE ON PROCINE BRAIN AND HYPOPHYSES

Table 4.1.7 summarizes the effect of orchidectomy at 7-8 months of age and testosterone on porcine brain and hypophyseal physiology.

No significant differences were observed in AChE activity in the cerebral cortex and neurohypophysis. AChE activities in the amygdala, hypothalamus and adenohypophysis of the IC and OC animals were similar and

TABLE 4.1.7: THE EFFECT OF ORCHIDECTOMY AT 7-8 MONTHS OF AGE AND TESTOSTERONE ON PROCINE BRAIN AND HYPOPHYSEAL PHYSIOLOGY.

BRAIN REGIONS	ANIMAL GOUPS		
a) *ACETYLCHOLINESTERASE ACTIVITY	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	3.360 ± 0.129 a	4.163 ± 0.133 ab	3.050 ± 0.168 b
Cerebellum	2.970 ± 0.019 a	2.233 ± 0.388 b	3.021 ± 0.014 a
Amygdala	5.992 ± 0.176 a	5.201 ± 0.215 b	5.393 ± 0.555 b
Nippocampus	4.779 ± 0.234 b	6.481 ± 0.437 a	5.123 ± 0.961 b
Hypothalamus	5.047 ± 0.051 a	4.163 ± 0.246 b	4.465 ± 0.681 b
Cerebral cortex	1.761 ± 0.009 a	1.711 ± 0.026 a	1.669 ± 0.037 a
Mid brain	8.492 ± 0.446 b	9.472 ± 0.662 a	9.826 ± 0.520 a
Medulla oblongata	7.902 ± 0.163 b	6.705 ± 0.351 c	9.635 ± 0.412 a
Adenohypophysis	1.599 ± 0.142 a	1.138 ± 0.136 b	1.008 ± 0.077 b
Neurohypophysis	1.044 ± 0.074 a	1.439 ± 0.057 a	1.348 ± 0.053 a
Grand mean	4.295 ±2.608	4.270 ± 2.734	4.348 ± 2.937
b) ** PROTEIN CONCENTRATION	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	0.695 ± 0.016 a	0.586 ± 0.032 b	0.547 ± 0.031 b
Cerebellum	0.186 ± 0.010 a	0.099 ± 0.009 b	0.135 ± 0.010 b
Amygdala	0.808 ± 0.031 a	0.135 ± 0.011 b	0.157 ± 0.011 b
Hippocampus	0.418 ± 0.038 a	0.141 ± 0.008 c	0.365 ± 0.021 b
Hypothalamus	0.312 ± 0.009 a	0.122 ± 0.003 c	0.246 ± 0.005 b
Cerebral cortex	0.220 ± 0.007 a	0.074 ± 0.004 c	0.128 ± 0.001 b
Mid brain	0.284 ± 0.004 a	0.194 ± 0.004 c	0.236 ± 0.008 b
Medulla oblongata	0.437 ± 0.005 a	0.179 ± 0.004 c	0.338 ± 0.024 b
Adenohypophysis	0.231 ± 0.006 a	0.260 ± 0.004 b	0.418 ± 0.013 a
Neurohypophysis	0.349 ± 0.028 a	0.089 ± 0.005 c	0.158 ± 0.007 b
Grand mean	0.394 ± 0.207	0.188 ± 0.150	0.276 ± 0.138
c) ***SPECIFIC ACETYLCHO-LINESTERASEAACTIVITY	ORCHIDECTOMONIZED With testosterone	Without testosterone	Intact control
Pons	4,842 ± 0.141 a	7.171 ± 0.406 a	5.622 ± 0.355 a
Cerebellum	16.147 ± 1.286 a	20.357 ± 1.411 a	22.807 ± 1.762 a
Amygdala	7.458 ± 0.393 b	39.198 ± 3.199 a	35.126 ± 3.593 a
Hippocampus	11.755 ± 1.202 b	46.330 ± 3.462 a	11.993 ± 1.207 b
Hypothalamus	16.244 ± 0.591 b	34.013 ± 1.542 a	18.198 ± 0.939 b
Cerebral cortex	7.978 ± 0.489 b	23.212 ± 1.155 a	13.081 ± 0.167 b
Mid brain	29.930 ± 0.611 b	48.762 ± 1.583 a	41.837 ± 2.416 a
Medulla oblongata	18.097 ± 0.490 b	37.416 ± 0.714 a	25.819 ± 1.516 b
Adenohypopphysis	6.928 ± 0.404 a	4.377 ± 0.231 a	2.414 ± 0.094 a
Neurohypophysis	2.999 ± 0.111 b	16.169 ± 0.850 a	7.203 ± 0.242 b
Grand mean	12.251 ± 8.808	27.700 ± 15.739	18.404 ± 12.986

Values in the same horizontal column differently superscripted differ significantly ($P < 0.05$)
Values are means ± S.E.M.
* AChE activity in umole/g/min.
**Total protein in g/100 ml
*** SAChE activity in umole/protein/min.

inferior to the OT animals. Contrariwise AChE activites in the midbrain of the OC and IC animals were similar and superior to the OT animals.

Castration significantly depressed AChE activity in the medulla oblongata and cerebellum and was partially restored by testosterone. However in the pons and hippocampus, AChE activites in the OT and IC animals were similar and inferior to the OC animals.

Castration significantly depressed protein levels in the hippocampus, hypothalamus, cerebral cortex, midbrain and medulla oblongata while testosterone considerably enhanced it in OT above IC animals in all the brain regions. However protein levels in the pons, cerebellum and amygdala of the OC animals were similar to the IC but inferior to the OT groups.

Castration also significantly depressed protein content in both hypophyses but was considerably enhanced by testosterone in the neurohypophysis of OT animals.

No significant differences were observed in the SAChE activites in the pons and cerebellum, whereas in the amygdala and midbrain the OC animals were similar to the IC group but superior to the OT group. SAChE activites in the hippocampus, hypothalamus, cerebral cortex and medulla oblongata of the IC animals were similar to the OT animals but inferior to the OC group.

No significant differences were obseved in the adenohypophysis ($P>0.05$). In the neurohypophysis however, the OC animals had higher levels than either the OT or IC groups which were similar.

4.4.1 MINERAL PROFILE IN THE BRAIN AND HYPOPHYSES

The results are displayed in Table 4.1.8.

CALCIUM

The cerebral cortex, midbrain and medulla oblongata were not significantly affected (P>0.05). Calcium levels in the pons, cerebellum, hippocampus and hypothalamus of the OC and OT groups were similar and significantly inferior to the IC animals (P<0.05). In the amygdala, calcium was depressed by castration and partially resotred by testosterone. Calcium levels were depressed by orchidectomy in the hypophyses but partially restored by testosterone in the neurohypophysis.

MAGNESIUM

Results obtained on magnesium concentration did not follow any consistent pattern.

OC animals were higher than OT animals but inferior to the IC in the amygdala and hippocampus while the control was inferior to the OC group in the hypothalamus. In the cerebellum, midbrain and medulla oblongata, magnesium levels in the OC and OT animals were similar but inferior to the IC (P<0.05). In the pons and the cerebral cortex, the OC animals were similar to the IC but superior to the OT animals (P<0.05).

In the adenohypophysis, both the IC and OC animals were similar and superior to the OT animals. In the neurohypophysis, the highest level was recorded in the OT animals while the OC and IC were moderate and low respectively.

TABLE 4.1.8: EFFECT OF ORCHIDECTOMY AT 7-8 MONTHS OF AGE AND TESTOSTERONE ON THE *MINERAL PROFILE OF THE PROCINE BRAIN AND HYPOPHYSES.

BRAIN REGIONS	ANIMAL GROUPS		
a) CALCIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	1.292 ± 0.015 b	1.050 ± 0.025 b	2.705 ± 0.095 a
Cerebellum	0.920 ± 0.071 b	0.832 ± 0.031 b	1.592 ± 0.032 a
Amygdala	1.492 ± 0.022 b	0.997 ± 0.002 c	2.945 ± 0.214 a
Hippocampus	1.260 ± 0.029 b	0.975 ± 0.025 c	3.044 ± 0.367 a
Hypothalamus	1.076 ± 0.025 b	1.227 ± 0.477 b	2.148 ± 0.090 a
Cerebelum cortex	1.191 ± 0.028 a	1.037 ± 0.043 a	1.219 ± 0.050 a
Mid brain	1.442 ± 0.067 a	1.164 ± 0.038 a	1.405 ± 0.068 a
Medulla oblongata	1.027 ± 0.027 a	1.090 ± 0.032 a	1.230 ± 0.092 a
Adenohypophysis	1.972 ± 0.032 b	2.042 ± 0.026 b	3.531 ± 0.670 a
Neurohypophysis	1.750 ± 0.020 b	1.137 ± 0.062 c	2.580 ± 0.058 a
Grand mean	1.342 ± 0.329	1.150 ± 0.329	2.240 ± 0.839
b) MAGNESIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	1.385 ± 0.031 b	2.130 ± 0.077 a	2.131 ± 0.084 a
Cerebellum	1.397 ± 0.027 b	1.330 ± 0.037 b	2.105 ± 0.061 a
Amygdala	1.560 ± 0.089 c	1.895 ± 0.038 b	2.523 ± 0.036 a
Hippocampus	1.505 ± 0.013 c	1.930 ± 0.054 b	2.272 ± 0.130 a
Hypothalamus	1.621 ± 0.046 b	2.308 ± 0.106 a	1.279 ± 0.096 a
Cerebral cortex	1.815 ± 0.050 b	2.088 ± 0.046 ab	2.170 ± 0.088 a
Mid brain	1.795 ± 0.083 b	1.922 ± 0.034 b	2.277 ± 0.066 a
Medulla oblongata	1.820 ± 0.067 b	1.910 ± 0.065 b	2.405 ± 0.142 a
Adenohypophysis	1.745 ± 0.026 b	2.241 ± 0.049 a	2.290 ± 0.105 a
Neurohypophysis	1.830 ± 0.030 a	1.428 ± 0.069 b	1.073 ± 0.044 c
Grand mean	1.647 ± 0.177	1.915 ±0.316	2.053 ± 0.481
c) ZINC	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	0.350 ± 0.019 b	0.165 ± 0.027 c	0.460 ± 0.051 a
Cerebellum	0.242 ± 0.025 a	0.222 ± 0.015 a	0.183 ± 0.013 a
Amygdala	0.392 ± 0.015 b	0.305 ± 0.010 b	0.401 ± 0.008 a
Hippocampus	1.267 ± 0.027 a	0.220 ± 0.022 c	0.511 ± 0.039 b
Hypothalamus	0.161 ± 0.012 b	0.246 ± 0.021 a	0.292 ± 0.014 a
Cerebral cortex	0.251 ± 0.012 b	0.192 ± 0.010 b	0.390 ± 0.012 a
Mid brain	0.297 ± 0.019 a	0.164 ± 0.013 b	0.352 ± 0.205 a
Medulla oblongata	0.125 ± 0.004 c	0.225 ± 0.011 b	0.309 ± 0.034 a
Adenohypophysis	0.223 ± 0.012 c	0.287 ± 0.054 b	0.410 ± 0.027 a
Neurohypophysis	0.202 ± 0.008 a	0.155 ± 0.011 ab	0.132 ± 0.007 b
Grand mean	0.351 ± 0.332	0.212 ± 0.051	0.364 ± 0.128

Values in the same horizontal column differently superscripted differ significantly ($P < 0.05$)

Values are means ± S.E.M.

*Values in parts per million (ppm)

TABLE 4.1.8 (CONTINED)

BRAIN REGIONS	ANIMAL GROUPS		
d) POTASSIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	37.375 ± 1.650 a	10.812 ± 0.571 c	15.500 ± 2.102 b
Cerebellum	29.500 ± 1.658 a	6.822 ± 1.265 c	36.625 ± 1.559 b
Amygdala	19.625 ± 2.340 b	7.642 ± 0.657 c	24.500 ± 0.866 a
Hippocampus	31.250 ± 3.146 b	11.737 ± 0.694 c	37.000 ± 1.860 a
Hypothalamus	24.875 ± 0.921 b	10.500 ± 1.041 c	30.375 ± 4.100 a
Cerebral cortex	24.375 ± 1.315 b	6.625 ± 0.545 c	38.875 ± 1.712 a
Mid brain	20.125 ± 1.712 a	6.187 ± 0.825 b	10.375 ± 0.629 b
Adenohypophysis	19.775 ± 0.746 b	6.875 ± 0.746 c	24.500 ± 2.02 a
Neurohypophysis	11.950 ± 0.500 a	7.250 ± 0.968 b	15.125 ± 0.718 a
Grand mean	24.225 ± 7.147	8.219 ± 2.006	25.350 ± 10.114
e) SODIUM	ORCHIDECTOMIZED		
	With testosterone	Without testosterone	Intact control
Pons	540.000 ± 4.082 a	531.250 ± 2.394 a	541.250 ± 3.750 a
Cerebellum	535.000 ± 7.901 a	171.562 ± 6.684 b	527.500 ± 9.682 a
Amygdala	535.500 ± 2.466 a	196.250 ± 6.384 b	528.500 ± 2.217 a
Hippocampus	510.500 ± 6.076 a	325.500 ± 10.218 b	536.500 ± 5.56 a
Hypothalamus	512.500 ± 8.339 a	410.500 ± 13.175 b	497.500 ± 7.906 a
Cerebral cortex	536.250 ± 12.311 a	528.000 ± 5.307 a	540.500 ± 3.571 a
Mid brain	507.500 ± 9.682 a	317.500 ± 8.292 b	520.000 ± 6.124 a
Medulla oblongata	533.750 ± 1.181 a	216.750 ± 6.237 b	528.000 ± 2.858 a
Adenohypophysis	508.750 ± 6.884 b	550.000 ± 3.540 a	543.750 ± 1.250 b
Neurohypophysis	526.250 ± 8.260 a	403.250 ± 3.065 c	503.000 ± 8.888 b
Grand mean	524.55 ± 13.185	365.056 ± 142.850	526.650 ± 15.824
f) COPPER	ORCHIDECTOMIZED		
	With testosterone	Without tesosterone	Intact control
Pons	0.097 ± 0.008 a	0.030 ± 0.003 b	0.095 ± 0.006 a
Cerebellum	0.081 ± 0.007 a	0.061 ± 0.006 a	0.016 ± 0.012 a
Amygdala	0.084 ± 0.006 a	0.061 ± 0.010 a	0.087 ± 0.008 a
Hippocampus	0.106 ± 0.002 a	0.055 ± 0.007 b	0.067 ± 0.008 b
Hypothalamus	0.107 ± 0.002 a	0.061 ± 0.002 b	0.070 ± 0.009 b
Cerebral cortex	0.077 ± 0.001 a	0.094 ± 0.004 a	0.072 ± 0.006 a
Mid brain	0.076 ± 0.004 a	0.041 ± 0.002 b	0.080 ± 0.003 a
Medulla oblongata	0.081 ± 0.001 b	0.056 ± 0.008 c	0.110 ± 0.010 a
Adenohypophysis	0.063 ± 0.003 b	0.049 ± 0.004 b	0.135 ± 0.010 a
Neurohypophysis	0.085 ± 0.011 b	0.049 ± 0.005 b	0.127 ± 0.011 a
Grand mean	0.086 ± 0.014	0.056 ± 0.017	0.090 ± 0.026

Values in the same horizontal column differently superscripted differ significantly ($P < 0.05$)

Values are means ± S.E.M.

ZINC

Castration significantly depressed zinc in the cerebral cortex, midbrain, medulla oblongata, hippocampus, amygdala, pons and partially restored by testosterone in the same regions except the hippocampus, where it was enhanced considerably. No significant effects were observed in the cerebellum and the neurohypophysis whereas in the adenohypophysis both the OC and OT animals were inferior to the IC animals.

POTASSIUM

Castration significantly depressed potassium levels in all the brain regions and hypophyses ($P<0.05$) except the medulla oblongata and testosterone treatment exhibited a partially restorative effect.

SODIUM

With the exception of the pons and cerebral cortex where no significant differnces were detected, castration significantly lowered sodium levels in the other regions and were restored by testosterone treatment.

In the adenohypophysis, both the OC and IC animals were similar and superior to the OT while in the neurohypophysis, castration significantly lowered sodium levels while testosterone had a restorative effect.

COPPER

No significant differences were observed in the cerebellum, amygdala and cerebral cortex ($P>0.05$).

In the pons and midbrain, both the IC and OT were similar and superior to the OC while in the hippocampus and hypothalamus, the OC and IC were similar and significanlty inferior to the OT animals ($P<0.05$).

Castration also significantly depressed copper levels in both hypophyses ($P<0.05$) and testosterone therapy failed to exert a significant restorative effect.

DISCUSSION

4.5.0 ORCHIDECTOMY AND TESTOSTERONE THERAPY ON SEXUAL BEHAVIOUR IN BOARS

The apparent lack of interest of the orchidectomized boars in mating behaviour suggests that orchidectomy must have largely diminished sexual response in such boars. This confirms earlier reports by Davidson (1972) that castation usually abolishes sexual behaviour. The fact that a few percentage of the boars still attempted mating also suggests that sexual behaviour still persists for some time after castration probably due to increased plasma luteinizing hormone secretion (Nansel et al., 1979).

The increased aggressive behaviour observed in boars orchidectomized post pubertally over the pre-pubertal castrates tend to oppose the report by Anisko et al (1977) that castration increases aggression in pre-pubertal male gerbils. However they performed their experiment on pre-pubertal males whereas in this study, the post-pubertal castrates were more aggressive than the pre-pubertal castrates. This study also conflicts with the research by Yahr and Coquelin (1980) that pre-pubertal males were more aggressive than post-pubertal males. These differences might be due to specie differences because it is known that copulation in the pigs may be more violent than in some other species of

animals particularly the gerbils which were used for the Yahr and Coquelin experiments.

This study therefore suggests that post-pubertally castrated boars might be able to withstand the responses due to androgen withdrawal (e.g. atrophy of the secondary sexual organs) more than pre-pubertal castrates. It also indicates that the age at castration affects the behaviour of the animal.

The ability of testosterone to partially restore sexual behaviour lends credence to the view that androgen therapy if started early enough restores sexual behaviour in castrated males. This is more probably achieved through the growth-promoting functions of androgen in stimulating the development of the accessory sexual organs and restoration of sexual behaviour and libido in the male.

4.5.1 AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY IN THE BRAIN REGIONS

The results indicate that AChE activities are highly depressed by castration in most of the brain regions of the four groups of animals used- and restored by replacement therapy. Of particular interest is the fact that AChE activities were depressed constantly in the hypothalamus, midbrain, medulla oblongata and pons in all the age groups except the pons which was unaffected in the pons of preweaning castrates and the hypothalamus in the animals castrated at 7-8 months of age.

These results thus support the view that the hypothalamic-pituitary gonadal axis may be age-dependent (Masafumi et al., 1981).

The resistance of the hypothalamus to testoterone withdrawal at puberty may also be due to the fact that the hypothalamus through aromatization may induce the conversion of estradiol to testosterone (McEwen et al 1980). This suggestion is further strengthened by the report of McEwen and Pfaff (1970) that estradiol is concentrated in the limbic hypothalamic system and tends to be retained by cells in the same regions as testosterone^{3}H.

It is also noteworthy that AChE activities are depressed by castration in androgen-sensitive areas of the hippocampus, hypothalamus, and midbrain and relatively unaffected in the cerebral cortex of all the age groups studied. This discovery is of particular interest since according to Martini (1973), the cerebral cortex is not androgen-sensitive. It is pertinent to note that the functions performed by the cerebral cortex involve higher mental activities not necessarily induced by sexual stimuli.

The fairly higher levels of AChE activities in the testerone-treated groups above the control also suggests that the amount of testosterone administered may be slightly more than normally occurs in the serum of normal males. This may therefore increase brain-induced sexual behaviour and activity of the androgen-receptor cells in the androgen-sensitive areas of the brain. These actions would definitely be reflected by more normal activity culminating in increased release of AChE.

The decline in the protein concentration by castration in all the brain regions of the pre-weaning castrates may be a result of the absence of the protein anabolic activity of testosterone. Also since testosterone is implicated in the release of somatotrophic hormone and therefore functions in morphogenesis, it is not surprising that testosterone deprivation at a very early and critical period of growth may lead to

impaired protein synthesis and possible inactivation of enzyme systems involved with growth (Sutherland et al., 1968).

In the other age groups, the pons, cerebellum, medulla oblongata, midbrain and hypothalamus had their protein levels significantly reduced by castration and this may also be due to increased nitrogen excretion after castration (Roy and Laumas, 1969). Gorski, (1973) also advanced the view that androgen withdrawal may alter fundamental neurochemical processes such as protein synthesis within the hypothalamus and this may in some way prevent the cyclic release of the gonadotrophins in the adult.

The observed suppression of protein synthesis in parts of the brain by testosterone withdrawal confirms this hypothesis. Bass et al., (1970) provided a link between protein synthesis and brain development when they discovered that pigs maintained post-natally on protein restricted diet had severe growth retardation and imparied brain development. The cerebral cortex was however relatively unaffected.

The present study also indicates that the cerebral cortex and the hippocampus except at 5-6 months were also relatively unaffected. This apart from being due to reasons connected with the functional capacity of the cerebral cortex may also be due to the fact that the cortex has been established as the most metabolically active region in the animal brain and is directly related to ACh synthesis. Such a vital role may make it relatively resistant to abuse and thus helps the animal to maintain its coordination and retain its higher mental functions in the face of stress.

The fairly high specific acetylcholinesterase activities recorded in most brain regions of the testostrone-deprived animals may be directly linked to the very low protein concentrations of those regions which normally results in high SAChE activity. The repercussion is that higher

than normal activity occurs in the neurons in an attempt to maintain the normal physiologic functions of the animal. When this is viewed against the very low protein content and AChE activities of the regions, neural fatigue may easily occur in such animals. This would readily reduce major enzyme activities and results in poor body growth.

Although Williamson and Payne (1975) reported that castration of pigs facilitates management and prevents indiscriminate mating, no effects were observed on flavour, odour or tenderness of the meat. On the other hand, they found intact boars to be better converters of food than castrates.

4.5.2 MINERAL PROFILE IN THE BRAIN REGIONS

With the exception of the pre-weaners where calcium levels were not consistent in the brain regions of treated and untreated castrates versus the control, calcium levels were generally depressed by castration and restored by replacement therapy in the pons, cerebellum, amygdala, hypothalamus, hippocampus and midbrain (3 months castrate) and medulla oblongata (5 months castrates). The cerebral cortex was unaffected. This trend could be explained by the decline in rate of protein synthesis caused by castration and which invariable slows down the rate of calcium absorption from diet. This would also lead to decreased calcium retention in the brain. The results thus confirms the work of Kunerth and Pitman (1939) who have linked calcium ions retention with protein synthesis.

Also Collier and Ilson (1971) report that calcium ions are necessary for the release of ACh at neuromuscular junctions and their necessity for the transmission of nerve impulses also lends evidence to the

depletion of calcium from the critical brain regions involved with active behaviour and growth.

This also explains the steady concentrations observed in the cerebral cortex since AChE activities in the cortex were unaffected by testosterone withdrawal.

A fairly inconsistent trend was observed with magnesium content of the brain regions in the various age groups.

The pre-weaning castrates did not exhibit any consistency in magnesium concentration compared to the controls or the T-injected groups, and like the calcium levels may be due to the fact that these piglets were still actively growing and as such their brains would be very active. This would imply increase in metabolic turn-over rates of major metabolites in the brain and may account for the inconsistency in the mineral concentrations of the brain regions of the animals.

The rise in magnesium levels in the cerebral cortex and midbrain of the animals castrated at 3 months of age and the amygdala, hippocampus, pons, cerebral cortex, cerebellum, midbrain and medulla oblongata of those castrated at 5-6 months of age indicates a possible compensation for the calcium depletion observed in most of the brain regions mentioned. This view is the fact supported by report that alkaline earth metals often compete with one another and in particular, a number of magnesium-activated enzymes are inhibited by calcium ions while the calcium-activated myosin adenosine triphosphate is inhibited by magnesium ions (Dixon and Webb, 1961).

In the animals orchidectomized at 7-8 months animals, the cerebral cortex was unaffected by testosterone withdrawal which is in line with earlier reports on the stability of the metabolic pathways in the cerebral cortex.

The depression of sodium and potassium levels by castration and their subsequent restoration by T-treatment is an indication of their vital roles in neuromuscular functions and utilization of proteins and energy. It also supports the view that sodium concentrations are affected by the level of potassium in the medium because the two act in concert to maintain electrolyte balance. Hence a disturbance of the membrane permeability of the brain which may be brought about by impaired protein synthesis as a consequence of testosterone withdrawal (Levine and Goldstein, 1955) would definitely result in disturbed movement of materials (particularly ions and amino acids) in and out of the cells and the sub cellular structures and thus impair the rate of biochemical sequence-through the blood-brain barrier.

This argument is further supported by the depression of endogenous oxygen uptake and oxidative and anaerobic metabolism of the hypothalamus following castration observed by (Mogvilevsky et al., 1966) which would interfere with normal functioning of the brain.

With the exception of the 3-4 months group that could not show any consistent trend, castration also depressed copper levels in brain regions such as midbrain, hypothalamus, pons and medulla oblongata which are areas concerned with sexual development and active behaviour.

The lack of effect of T-withdrawal on cerebral cortex, amygdala and cerebellum of the 7-8 months group may stem from the earlier report that the amygdala and cerebral cortex are not testosternone-sensitive and are

thus relatively stable to testosterone-withdrawal especially when castration is performed post-pubertally.

The depression of zinc levels by castration in the hippocampus, hypothalamus and medulla oblongata and its elevation in the cerebral cortex (except inthe 7-8 months castrate) indicates that the depression occurs in regions that control growth which probably depend on zinc-dependent enzyme systems while the elevation in the cortex is an attempt to prevent brain malformations and abnomalities usually associated with a deficiency of zinc.

Although there is no direct evidence that zinc is an activator of the AChE hydrolytic systems, the metal is known to be a component of several enzyme systems and plays a role in sexual maturity. The report of Fullis (1958) indicates that zinc deficiency brings about atrophy of the testis which resembles the testicular atrophy associated with castration.

It should also be mentioned that in many of these brain regions, and with most of the minerals studied, T-injection on castrates while elevating the mineral levels significantly above the levels observed in the untreated castrates was not quite able to bring the levels above those observed in the control animals. The reason for this is not very clear and may suggest that other metabolites apart from testosterone in the intact pig contribute to mineral metabolism in the brain.

It is therefore clear from all the foregoing that castration renders the brain to a lot of abuse and disrupts the normal functioning of the brain. Other probable deleterious effects of castration are impaired brain protein synthesis and electrolyte balance.

Since the brain controls body coordination and growth processes, any condition which disturbs the metabolic pathways of the various enzymatic processes in the brain should be avoided.

It is thus clear, that castration whether performed pre or post - pubertally has very little if any advantages on the animal. On the other hand, it lowers the performance, stress adaptability and impairs endocrine balance of the animal.

The only consolation appears to be the striking and welcome stability and resistance of the cerebral cortex which is the most active region of the brain to hormol abuse.

4.5.3 **AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY HYPOPHYSES**

The inability of castration to significantly influence AChE activities in the hypophyses may be due to the feed back response of the hypophyses to lowered serum testosterone brought about by castration. In addition, studies have indicated that the hypophyses have a very high capacity to bind adrogen molecules (Samperez et al 1969) and castration considerably activates the transformation of testosterone into dihydrotestosterone (DHT) in the hypophyses (Martini, 1973). McEwen (1978a,b) has also shown that 5 DHT is one of the major brain metabolites involved in brain AChE sexual differentiation. Hence, it is very probable that castration and its attendant elevation of LH production by the hypophyses results in increased activity of the hypophyseal gonadotrophs which helps in maintaining AChE activity at near normal levels.

Although a number of workers have deomostrated that brain AChE activity is associated with overall metabolic condition or resting metabolic rate (RMR) (Ling, 1970) and that AChE activity was lowered in the

hypothalamus of thyroid-deficient animals (Geel and Timirasm 1967), the present study failed to show any significant influence of castration on AChE activity in the Pituitary gland. The reason for this may be that the absence of testosterone in protein anabolism and the growth promoting metabolic pathways may enchance augmented secretion of TSH by the pituitary through the feed-back systems which would maintain normal secretion of the thyroid gland hormones and thus sustain the calorigenic and protein anabolic activities of the thyroid gland.

The decline in the protein concentrations in the hypophyses following castration and the restoration effect of testosterone shows that castration or testosterone withdrawal has an effect on the hypothalamic-hypophyseal axis. Thus the increased nitrogen excretion and disturbance of nitrogen balance associated with testosterone withdrawal may have profound effects on the amino-acid metabolism at the cellular level in the pituitary gland.

The increase in SAChE acitivities in the hypophyses of castrated pigs is a direct reflection of the decreased protein levels in those organs which ultimately results in higher than normal SAChE activities and which may lead to cellular fatigue.

4.5.4 MINERAL PROFILE IN THE HYPOPHYSES

The depression of calcium in the adenohypophysis and neurohypophysis of the castrated animals and inability of testosterone therapy to fully restore calcium levels to normal may be linked to the decline in the protein concentrations of the hypophyses of the castrated animals.

Although growth-rate studies were not carried out on the animals, it is not unlikely that the impaired protein synthesis and consequent disturbed absorption of calcium may have serious implications on growth. As was mentioned earlier, the result that testosterone therapy is unable to completely restore the mineral levels suggests that the testis apart from producing testosterone probably produced some other metabolities which enhance clacium and other mineral balance in the brain. Another attractive hypothesis is that the products of the testis may function to maintain normal endocrine status of the animal which testosterone injections alone would not do.

The elevation of magnesium by castration particularly in the neurohypophysis and the adenohypophysis (the pre-weaning castrates) is a further proof of magnesium acting to compensate for calcium depletion or decreased absorption by the adenohypophysis.

The elevation of magnesium by castration may also be due to the increased activity of the hypophyses of the castrated animal which would invariably require magnesium ions as a co-enzyme of the phosphorylated transferase concerned with metabolic processes which may be facilitated to compensate for the effects of castration.

Although sodium levels in the pre-weaners were not consistent and did not show any defined pattern irrespective of treatment, generally, castration depressed sodium and potassium levels in both hypophyses and this may be linked to the synergistic nature of the two minerals in maintaining electrolyte balance. It is also not surprising that a condition that leads to protein depletion is attended by lowered sodium and potassium levels.

Copper and zinc ions were similarly depressed by castration although the zinc levels of the neurohypophyses were relatively stable especially in the older animals. In both cases too, testosterone therapy restored copper and zinc levels but not to the same levels as the controls. This further supports the suggestion of certain extra testosterone metabolites in the testis contributing to the mineral metabolism of the hypophyses.

The decline in zinc and copper levels by castration may be related to their roles in body growth and sexual maturation.

It is interesting to note that in most of the cases where depression of mineral levels were observed, the adenohypophysis is invariably involved. This may be due to the fact that the adenohyphysis is more associated with the hypothalamus since it receives most of its gonadotrophin-release-inhibiting factors through the hypothalamic portal vein. It is therefore probable that conditions which disturb the normal functioning of the hypothalamus also have some effect on the hypophyses.

CHAPTER FIVE

EFFECT OF OVERIECTOMY WITH ESTRADIOL OR PROGESTERONE ON PORCINE BRAIN AND HYPOPHYSEAL PHYSIOLOGY

INTRODUCTION

5.1.0 SECRETIONS OF THE OVARY

The ovary possesses two functions: The production of ova and the production of hormones. These hormones are estrogens, progestogens and relaxin. The first two are the female sex hormones and are steroids in nature. Relaxin is a polypetide and is active towards the end of gestation. The two estrogenic steroids secreted by the ovary are estradiol and estrone. Like androgens, estrogens are not stored in the body but are removed through inactivation by the liver and elimination in both urine and faeces. Approximately 10% of blood estrogens are eliminated and the remainder inactivated.

Stimulation of estrogen release from the ovary is under the control of the gonadotropins from the anterior pituitary gland. Hence, hypophysectomy of the female is followed by atrophy of the reproductive tract and all structures dependent on the presence of estrogens.

According to current concepts, a feedback mechanism operates whereby the level of FSH and LH is controlled by the concentration of estradiol and progesterone in the blood. Very low levels of estradiol appear to stimulate FSH release which in conjunction with LH causes marked increase in estradiol release. An increase in the blood estradiol level eventually acts back on the pituitary inhibiting further, the release of FSH and hence, a drop in estradiol occurs. Thus excessive quantities of FSH are produced following ovariectomy and decrease in FSH following estradiol injection.

5.1.1 **FUNCTIONS OF ESTROGENS**

(1) The hormone stimulates growth and secretory activity of structures receptive to the hormone and acts in concert with other hormones especially progesterone and relaxin to elicit normal reproductive functions.

(2) Estrogens stimulate marked growth of the uterus resulting in increase in the mass of both the endometrium and myometrium. Estrogen administration to ovariectomized females has striking effects on the uterus. The effect on uterine growth is preceded by various alterations in tissue composition and enzyme activity (turner and Bagnara, 1975). An early change occuring in the uterus within an hour or so after estrogen administration is an increased blood supply associated with increased permeability of the uterine capillaries (Szego and Sloan, 1961).

This is accompanied by an uptake of water and electrolytes by the uterine tissue and within four hours both aerobic and anaerobic glycolyses are elevated. The inhibition of water by uterine tissues results in marked increase in uterine weight and accelerated incorporation of C^{14} amino acids into the uterine tissues followed by cellular proliferation (Szego and Lawson, 1964). Other effects include increase in the RNA content, rates of respiration and glycolysis in the uterus.

(3) Estrogens stimulate uterine contractility by increasing both the amplitude and rate of contration.

(4) Estrogens also stimulate growth and muscular activity of the oviducts. In addition, they stimulate growth and development of the mammary duct system in all species and both the duct and alveolar systems in some species.

(5) They also stimulate loosening of the Pubic symphysis and the increase in size of the interpubic ligament.

(6) Although ovulation is a consequence of the effects of pituitary gonadotropins, heat, sexual receptivity and other psychic manifestations are probably brought about by the ovarian hormones acting through the central nervous system.

(7) Although estrogen injections alone may induce sexual receptivity in the rat, full mating behaviour generally depends upon both estrogens and progesterone. However, progesterone is usually required in small amounts.

Thus Timiras (1971) proposed that estrogens act as "organizer" of behavioural activity during critical periods of brain development because the hormone increases RNA synthesis in emrbryonic neural tissue. Vernadakis (1973) also observed increased RNA synthesis, increased cellular activity and increase in cell number, specifically of glial cells, by estradiol. Estrogen also induce enlargement (hypertrophy) of the adenohypophysis (Schreiber, 1973).

The mode of action is supposed to be direct (Lisk, 1967b) because the adenohypophysis behaves like a target tissue for estrogens (Eisenfeld and Axelrod, 1966). They also induce thyrozine binding by adenophypohyseal proteins (Schreiber et al, 1970 a,b). The above effects may be reversed by simultaneous treatment of the animal with estrogens and thyroxine, a classic feed back effect.

PROGESTOGENS

Progesterone is the most prevalent, naturally occuring progestogen and is secreted mainly by the lutein cells of the corpus Luteium. This hormone is also secreted by the placenta while some amounts have been isolated from the adrenal gland.

Like other steroids, progesterone is not stored in the body. It is either rapidly utilized or excreted and so is present in low concentrations in the body tissues.

FUNCTIONS: The action of progesterone is difficult to separate from that of other hormones particularly estrogens. This is due to the fact that progesterone normally acts in conjunction with estrogens and other steroids and produces few specific effects when active alone.

Generally estrogens primarily promote growth processes whereas progestogens encourage tissue differentiation.

Other major functions include:

(1) The induction of the formation of a secretory endometrium in a uterus previously sensitized by estrogens. This is characterized by increases in mucosal thickness, increased coiling of the glands, edema of the stroma and presence of glycogen droplets in the glandular cells.

(2) Progesterone inhibits spontaneous uterine motility and the response of the myometrium to oxytocin.

(3) Progesterone in conjunction with estrogen induces growth of the lobule alveolar system of the mammary gland.

(4) Progesterone is necessary for the maintenance of gestation.

(5) Progesterone acts synergistically with estrogen to induce behavioural estrus in the female.

(6) Progesterone induces ovulation in the cow, bird, rat and rabbit but also inhibits ovulation when given chronically.

5.1.1 LITERATURE REVIEW

The nervous system of mammals possesses groups of cells which release hormones.

For some time now, the output mechanisms of these neuroedocrine cells have been under considerable attention. The cells are able to respond to circulating steroids by either a decrease or an increase of the membrane potential (Schade' and Van Wilgenburg, 1970).

The involvement of the nervous system and the hypophyseal gonadal axis in reproduction has been extensively reviewed in previous chapters with more emphasis on the male animal.

The main role in the regulation of anterior pituitary function is played by the hypothalamic-hypophysisotrophic factors or hormones (Guillemin, 1964, 1967, Schally et al, 1968, Campbell, 1970). These factors are either releasing factors or inhibiting factors whose primary effect is the release or reduction of anterior pituitary hormones into the blood, i.e. their secretion and not their increased synthesis. At the moment, it is now well established that the hypothalamic-hypophysiotrophic hormones directly influence biosynthesis of the anterior pituitary hormones (Schally et al, 1977).

A number of brain regions have been implicated in gonadal function and sexual behaviour. Kluver and Bucy (1939) and Green et al (1969), induced hypersexaulity in monkeys and cats with ablations of the pyriform cortex or amygdala. In addition, electrical stimulation of the amygdala led to ovulation in the rabbit (Koikegami et al, 1953) and in the light-induced constant estrous rat (Bunn and Everett, 1957), while stimulation of the hippocampus blocked spontaneous ovulation in the rat (Velasco and Taleinsnik, 1969) and slightly facilitated the induction of ovulation in

the rabbit (Kawakami et al, 1966a, Kawakami et al, 1967). Bilateral lesions of the hippocampus or amygdala in the adult rat altered the estrus cycle (Koikegami et al 1960, Koikegami, 1964) while the destruction of the amygdala in the immature rat induced precocious puberty (Elwers and Critchlow, 1960, 1961). On the other hand, destruction of the hippocampus delayed the onset of puberty (Riss, 1958, Riss et al 1963). In 1970, Koves and Halasz demonstrated that the neural trigger for ovulation is located in the medial preoptic area.

Kawakami et al (1970b) found that the effect of sex steroids on the electrical activity of the hypothalamus differed depending on the timing of administration of steroids either in castrated or cyclic rats. Injection of estrogen caused a rise in the multiple unit acitivity in the dorsal hippocampus, amygdala and hypothalamus. Administration of progesterone did not show particularly striking results.

Kawakami et al (1973) also found that stimulation of medial preoptic area, amygdala and hippocampus resulted in increased FSH and LH release by the adenohypophysis in prepuberal rats. A lesion of the anterior hypothalamus has been made to induce vaginal estrus (Dey 1941, Flerko and Bardos, 1960). This proves that the cyclic secretion of gonadotropic hormones is controlled by the anterior and preoptic area of the hypothalamus.

In the anteior preoptic area, a feed back centre for ovarian steroids exists controlling the cyclic discharge of gonadotropic hormones (Flerko and Bardos, 1961, Terasawa and Sawyer, 1970). While stimulation of medial preoptic area in mature rats induced increased level of serum FSH and LH in the blood, stimiulation of the hippocampus alone inhibited the elevation of serum LH level. This fact suggested that the hippocampus

stimulation inhibited the release of FSH. Thus the hippocampus may participate in the gonadotropin secretion dominating the hypothalamus.

Also, stimulation of the midbrain can evoke both facilitatory and inhibitory influences on gonadotropin secretion. Thus, stimulation of the midbrain inhibits some neurons and activates those of the hypothalamic ventromedial nucleus (Tsubokawa and Sutin, 1963) and the posterior hypothalamus. Stimulation of the amygdala also facilitates gonadotropin secretion. It has also been found that the midbrain projects into the amygdala (Machne and Segundo, 1950).

Anatomical stuidies have demonstrated that the midbrain is linked with the hippocampus by a pathway which permits reciprocal influences between these two structures thus forming a limbic forebrain-midbrain circuit (Nauta, 1958).

This circuit therefore explains the involvement of hippocampus in the inbhibition of gonadotropin secretion after midbrain stimulation (Velasco and Taleisnik, 1969b). These two antagonistic systems subserve the transmission from the periphery to the hypothalamus of most of the sensory stimuli affecting gonadotropin secretion. The midbrain thus acts as a central station for the distribution of flow of afferent impulses to the hypothalamus or to the limbic system but also modulates the magnitude of sensory imputs according to the information received from upper neural structures and the prevailing hormonal background.

It has already been mentioned in preceeding chapters that during the sex differentiation of the brain, the production by the neonatal testes of androgen is the factor which determines the course of brain development, suggesting that the neonatal is more sensitive to androgen than ovarian steroids. The sex differntiation of the brain is made more evident by the

fact that the male animal will most usually desplay sexual behaviour when presented with a receptive female whereas reproductive activity of the female is cyclic.

Ovulation which is the key to the estrous cycle of the female is brought about by the neural activation of the pituitary gland in response to circulating levels of ovarian estrogen. Because of the dependence of this brain function upon estrogen, one might assume that the sex difference in reproductive activity is due to the presence of a functional ovary in the female and its absence in the male. However, when an ovarian graft is transplanted into the castrated rat, ovulation does not occur. Even in the presence of ovarian steroids, the brain of the male rat cannot bring about ovulation. With respect to the pattern of pituitary gland secretion, the ability of the brain of the female rat to regulate the cyclic surge of gonadotropic hormone responsible for ovulation accounts for this fundamental sex difference. It has already been established (Gorski, 1971a) that neurons within the medial basal hypothalamus and the arcuate nucleus are responsible for follicular growth and estrogen secretion but cannot independently bring about ovulation.

In the female ovulation is regulated by the preoptic anterior hypothalamus (Halasz and Gorski, 1967, Koves and Halasz, 1970) and Studies have indicated that this region binds to labelled estrogen (Pfaff, 1968, Zigmond and McEwen, 1970). Thus intraphypothalamic infusion of estradiol benzoate (EB) induces anovulatory persistent estrus in the rat (Wagner et al 1966, Sutherland and Gorski, 1970).

It is already known that one major functional result of sex steroid effect on brain tissue is the alteration of pituitary gonadotropin release and investigation show that radioactive estradiol-17B is highly

concentrated in the ventromedial hypothalamus, preoptic area, amydgala and septum and less concentrated in other brain areas such as cortex. This regional distribution has been determined both from scintillation counting of dissected brain regions (Eisenfeld and Axelrod, 1965, Green et al 1969, Kato and Villee, 1967a, McEwen and Pfaff, 1970) and from autoradiographic description of estrogen concentration following systemic injection (Michael, 1965, Pfaff, 1968a).

Estradiol concentrations have also been established in the limbic hypothalamic system which also includes the hippocampus. Estradiol-^{3}H tended to be retained by cells in the same region as testosterone-3H (McEwen and Pfaff, 1970). They also observed low estradiol uptake capacity in many structures outside the limbic hypothalamic system.

According to Moguilevsky and Raynard, (1979), a possible reason for sexual dimorphism in the brain may also be due to differences in the inducibility of a gene product such as occurs in the estrogen induction of progestin receptors. A similar situation is in the estrogen induction of choline acetyltransferase in the preoptic area (Luine et al, 1975). As is already known, changes involved in sexual differntiation are supposed to be located in the hypothalamus and the preoptic area (Ifft, 1972).

In addition, study by Kato et al (1968) indicates an increase in the hypothalamic estrogen receptor concentrations during sexual maturation. There are also indications that estrogens and androgens act on the hypothalamus and pituitary gland to exert a feed back system of gonadotropin secretion.

Baum and Schietlen (1979) observed highest estradiol binding in the pituitary followied by the hypothalamus, midbrain, amygdala and cerebral cortex. Although not much data is available on dose-response relationship,

Willson et al (1979) recorded estrogen concenstration in gilts of 6.8 to 10.2 ng/ml plasma.

Average plasma volumes of progesterone were recorded at 2 ng/ml in non pregnant gilts and between 7 to 25 ng/ml during pregnancy. Estrogen level during pregnancy was somewhat stable at 7 ng/ml (George et al, 1978).

5.1.2 EFFECT OF OVARIAN STEROIDS ON OVAIECTOMIZED GILTS

Immediately after ovariectomy, serum LH increased significantly while FSH and prolactin did not show any appreciable change. Progesterone administered to ewes ovariectomized during pregnancy showed increased volume of allantoic fluid whereas estradiol benzoate prevented excessive accummulation of allantoic fluid. Sensitivity of the brain to ovarian steroids depends on the neonatal treatment. For example, McEwen (1980) observed that neonatal treatment of female rats with testosterone reduces adult sensitivity to estradiol with respect to a variety of estrogen-dependent neuroendocrine and behavioural parameters.

A similar observation was recorded for adult sensititivity to progesterone. After ovariectomy, the oviduct, uterus, vagina and mammary glands atrophy and may be largely revived through adequate estrogen therapy. Even in the adult human female, the menstrual cycle is interrupted in the absence of estrogenic hormones.

Estrogen administration on ovariectomized rats induced the inhibition of hydrocortisone-mediated suppression of the compensatory ACTH secretion and may be interpreted as an interference of the two steroids on common receptive sites at both the hypothalamic and the pituitary levels (Tallian, 1973).

Also changes in corticotropin releasing factor content of the hypothalamus was observed. This suggests an increased FSH and TSH secretion which plays a role in the increase of the pituitary-adrenal axis function which is mediated through an increase in plasma corticosterone binding capacity (Fortier et al, 1970). Ross et al 1971) demonstrated that progesterone rapidly facilitates lordosis behaviour in ovariectomized rats primed with estradiol benzoate in the mesencephalic reticular formation.

Kawakami et al (1970a,b) in a pioneering work described sudden increases in multi-unit activity in the medial basal hypothalamus on the afternoon of proestrus which are susceptible to change by ovariectomy and estrogen or progesterone administration. BarraClough and Cross (1963) had earlier described the effects of injected progesterone on responses of individual hypothalamic neurons to peripheral stimuli.

Tach et al (1972) found that estradiol injection of ovariectomized rats increased mitotic division and RNA synathesis in the rat uterus whereas progesterone injection tended to block these estradiol-dependent effects.

Effects of ovarian steroids on hypothalamic monoamine levels have also been reported. Norepinephrine levels increase in the anterior hypothalamus after ovariectomy (Stefano et al 1965) and are decreased again following estrogen and progesterone treatment (Donoso and Stefano, 1967). In the normal female rat, they are minimum at estrus after a peak at proestrus. These changes in norephinephrine levels complement the results of Kobayashi et al (1963) who found that hypothalamic monoamine oxidase (MAO) activity increased after ovariectomy, decreased again after treatment with estrogen and was highest in proestrus.

After ovariectomy, hypothalamic choline acetylase changes were the

reciprocal of MAO changes (Kobayashi et al, 1963). The above findings were more or less confirmed by Zolovick et al (1963).

Anton-Tay and Wurtman (1968) found that ovariectomy increased the whole brain turnover rates of norephinepherine even though there were no drastic changes in norephinephrine concentration.

Since it is known that drugs which deplete the brain of monoamines (in particular of norepinephrine) block ovulation and that sex steroids alter monoamine levels (Coppola et al, 1965), it is possible that estradiol and progesterone regulate ovulation through the alteration of the levels of norephinephrine and other monoamines. Gonadal hormones have also been known to play an important role in behavioural changes which are in part mediated through cholinergic elements of the central nervous system (Oliverio et al 1973). Iramain et al (1979) discovered that orchidectomy plus estradiol administration decreased AChE activity in the rat cerebral cortex and mesencephalon while it was increased in the amygdala. These workers also observed diminished AChE activity in the adenohypophysis of orchidectomized rats. Nayeemunisa (1976) reported that the hormone may influence protein synthesis as manifested in decreased protein content and AChE levels in the rat brain on in vivo administration of progesterone.

Moudgil and Kanungo, (1973) reported that 17-B-estradiol induced AChE activity in rat brain while ovariectomy decreased AChE activity in the cerebral hemisphere. The increase in AChE acitivity after estradiol administration is supposed to be due to an increase in the transcription of M-RNA which increases the synthesis of the enzyme. For example, Hansel (1959) showed a decrease in the level of estrogen in women with old age and this may affect the level of AChE activity of the brain and thereby alter the behavioural pattern of the female.

AChE is involved in the feed back control of Luteinizing hormone secretion (Florindo and Martini, 1975) and behavioural changes which are in part mediated through cholinergic central mechanisms and are influenced by steroid hormones (Olivero et al., 1973, Lindstrom, 1975).

5.1.3 SOME METABOLIC EFFECTS OF OVARIAN STEROIDS ON MINERAL METABOLISM

Not much information is available on the interaction between ovarian steroids and mineral metabolism in the animal body. However, since estrogens are involved in protein synthesis and a deficiency of some cations such as zinc results in decreased DNA synthesis (which reduces protein syntesis), it is not too presumptious to assume that the mineral may also enhance the normal functioning of the enzyme. Estrogens also stimulate retention of water, sodium, calcium and nitrogen. Estrogen increases zinc accumulation (Gunn and Gould, 1956) and induces increased zinc incorporation in the dorsoolateral lobe of castrate rats (Muntzing et al, 1977).

5.1.4 MATERIALS AND METHODS

Twelve Large White gilts housed, fed and provided water as described earlier were used. They were randomly assigned to 3 equal groups: Ovariectomized and treated with estradiol (OE), ovariectomized and treated with progesterone (OP) and sham operated (SO). OE and OP gilts were bilaterally ovariectomized while SO gilts were sham operated and all the animals were allowed to recover for four weeks. OE gilts were thereafter given 5 daily intramuscular injections each of 3 mg of estradiol valerate (E_2) in 1 ml of corn oil between 0900-1000 hours. OP gilts were similarly treated with 20 mg of hydroxyprogesterone caproate in 1 ml of

corn oil while the sham operated gilts received the corn oil only. Ovariectomy was performed through a mid-ventral incision according to the method of Berge and Westhues, (1966).

24 hours after the last injection, all the animals were slaughtered and their brains and hypophyses quickly removed, dissected and processed as described earlier.

5.1.5 STATISTICAL ANALYSES

The Data were subjected to multifactor analyses of variance as described earlier.

RESULTS

5.2.0 EFFECT OF OVARIECTOMY WITH ESTRADIOL OR PROGEESTERONE ON BRAIN AND HYPOPHYSEAL AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY

The effects of the treatments on AChE activity, total protein and SAChE activity are summarized in Table 5.1

AChE acitivity was significantly and similarly depressed ($P>0.05$) in the cerebellum, amygdala and hippocampus by estrogen or progesterone treatment alone. In the midbrain, however, progesterone was more potent in depressing the enzyme activity. On the other hand, estrogen was potent in enhancing the AChE activity ($P<0.05$) in the pons, cerebral cortex and medulla oblongata. In the cerebral cortex, AChE was depressed by progesterone alone but unaffected by estrogen. AChE activity in the adenohypophysis were similar in both the sham operated and the OE gilts but inferior to the OP animals. No significant differences were observed in

TABLE 5.1.1: EFFECT OF OVARIECTOMY WITH ESTRADIOL OR PROGESTERONE THERAPY ON PROCINE BRAIN AND HYPOPHYSEAL PHYSIOLOGY.

BRAIN REGIONS	ANIMAL GROUPS		
a) ACETYLCHOLINESTERASE ACTIVITY*	Sham operated	OVARIECTOMIZED	
		With estradiol	With progesterone
Pons	3.052 ± 0.284 c	4.623 ± 0.058 a	3.508 ± 0.081 b
Cerebellum	4.553 ± 0.080 a	2.878 ± 0.160 b	2.918 ± 0.070 b
Amygdala	9.114 ± 0.492 a	5.447 ± 0.203 b	5.060 ± 0.73 b
Hippocampus	4.326 ± 0.245 a	3.421 ± 0.185 b	3.123 ± 0.049 b
Hypothalamus	4.469 ± 0.071 a	4.428 ± 0.093 a	3.914 ± 0.043 b
Cerebral cortex	1.614 ± 0.089 a	1.292 ± 0.016 ab	1.007 ± 0.014 b
Mid brain	7.530 ± 0.259 a	5.550 ± 0.276 b	4.335 ± 0.467 c
Medulla oblongata	5.447 ± 0.163 a	5.043 ± 0.102 a	3.842 ± 0.184 b
Adenohypophysis	0.509 ± 0.057 b	0.548 ± 0.046 b	1.005 ± 0.049 a
Neurohypophysis	1.059 ± 0.027 a	0.931 ± 0.007 a	1.041 ± 0.024 a
Grand mean	4.167 ± 2.758	3.416 ± 1.915	2.975 ± 1.447
b) PROTEIN CONCENTRATION **	Sham operated	OVARIECTOMIZED	
		With estradiol	With progestorone
Pons	0.571 ± 0.024 a	0.512 ± 0.017 b	0.548 ± 0.014 ab
Cerebellum	0.363 ± 0.023 a	0.261 ± 0.013 c	0.311 ± 0.017 b
Amygdala	0.356 ± 0.013 a	0.220 ± 0.005 b	0.170 ± 0.019 c
Hippocampus	0.329 ± 0.007 b	0.414 ± 0.021 a	0.267 ± 0.007 c
Hypothalamus	0.382 ± 0.015 a	0.304 ± 0.033 b	0.231 ± 0.042 c
Cerebral cortex	0.356 ± 0.020 a	0.280 ± 0.002 b	0.296 ± 0.009 ab
Mid brain	0.338 ± 0.004 a	0.253 ± 0.010 b	0.239 ± 0.010 b
Medulla oblongata	0.573 ± 0.003 c	0.698 ± 0.009 a	0.624 ± 0.018 b
Adenohypophysis	0.319 ± 0.006 a	0.244 ± 0.007 b	0.179 ± 0.006 c
Neurohypophysis	0.222 ± 0.010 a	0.227 ± 0.015 a	0.172 ± 0.004 b
Grand mean	0.379 ± 0.110	0.341 ± 0.156	0.304 ± 0.158
c) SPECIFIC ACETYLCHOLINESTERASE ACTIVITY***	Stam operated	OVARIETCTOMIZED	
		With estradiol	With progesterone
Pons	5.385 ± 0.637 b	9.051 ± 0.315 a	7.145 ± 0.199 ab
Cerebellum	12.719 ± 0.922 a	11.213 ± 1.192 a	9.466 ± 0.562 a
Amygdala	25.781 ± 1.591 b	24.811 ± 1.137 b	30.808 ± 3.387 a
Hippocampus	13.133 ± 0.626 a	8.322 ± 0.543 b	11.714 ± 0.501 a
Hypothalamus	11.756 ± 0.503 c	15.202 ± 2.016 b	19.401 ± 4.651 a
Cerebral cortex	4.807 ± 0.149 a	4.621 ± 0.070 a	3.245 ± 0.123 a
Mid brain	22.290 ± 0.748 a	22.060 ± 1.386 a	18.101 ± 1.608
Medulla oblongata	9.514 ± 0.318 a	7.232 ± 0.168 ab	6.166 ± 0.322 b
Adenohypophysis	1.559 ± 0.169 b	2.239 ± 0.132 b	5.611 ± 0.170 a
Neurohypophysis	4.786 ± 0.190 a	4.153 ± 0.317 a	6.057 ± 0.108 a
Grand mean	11.173 ± 7.842	10.890 ± 7.602	11.771 ± 8.577

Values in the same horizontal column bearing different superscripts differ significantly ($P < 0.05$)
Values are means ± S.E.M.
* AChE activity in mole/g/min
** Total protein in g/100 ml
*** SACHE activity in mmole/g protein/min.

the neurohypophysis ($P>0.05$).

Total protein was significantly depressed ($P<0.05$) by estrogen and progesterone in the cerebellum, amygdala, hypothalamus and midbrain. However, estrogen was more potent in depressing total protein in the cerebellum while progesterone was more potent in depressing total protein in the amygdala, hippocampus, hypothalamus, and midbrain. In the cerebral cortex and pons, total protein was depressed by estrogen but unaffected by progesterone. In the medulla oblongata, both estrogen and progesterone considerably enhanced total protein content with estrogen being superior to progesterone.

In the adenohypophysis, total protein was significatnly depressed by estrogen and progesterone alone ($P<0.05$) with estrogen being superior to progesterone.

In the neurohypophysis, total protein in the SO and OE animals were similar and superior to the OP animals. No significant differences were observed in the SAChE activities in the cerebellum, cerebral cortex and nuerophypophysis ($P>0.05$).

In the pons, SAChE acitivity was enhanced by estradiol but unaffected by progesterone while in the adenohypophysis and amygdala, SAChE activities were similar in the SO and OE animals but inferior to the OP animals.

In the midbrain and medulla oblongata SAChE acitivity was depressed by progesterone ($P<0.05$) but unaffected by estrogen, while in the hippocampus SAChE activity was depressed by estrogen but unaffected by progesterone. Both estrogen and progesterone significantly enhanced SAChE activities in the hypothalamus but progesterone was more significantly potent than estrogen.

5.2.1 EFFECT OF OVARIECTOMY WITH ESTRADIOL OR PROGESTERONE ON THE MINERAL PROFILE OF THE PORCINE BRAIN AND HYPOPHYSES

CALCIUM

The results are summarized in Tables 5.1.2 and 5.1.3 Calcium in the pons and adenohypophysis of OE and OP animals were similar and superior to the sham operated animals. Similarly, both estrogen and progesterone significantly elevated calcium content in the cerebellum but estradiol was more potent than progesterone. In the amygdala and hippocampus, calcium levels in the OE and OP animals were similar but inferior to the SO animals.

Calcium was enhanced by progesterone in the hypothalamus and midbrain but unaffected by estrogen. Estrogen and Progesterone significantly enhanced calcium levels in the neurohypophysis with progesterone being more potent than estrogen ($P<0.05$). No significant changes were observed in the medulla oblongata ($P>0.05$).

MAGNESIUM

Both estradiol and progesterone significantly enhanced magnesium levels ($P<0.05$) in the pons, medulla oblongata, adenohypophysis and neurohypophysis. However, in each case, estradiol was significantly more potent than progesterone ($P<0.05$).

In the Amygdala and midbrain, progesterone significanlty elevated magnesium levels but was unaffected by estrogen. In the hippocampus and cerebral cortex, magnesium was depressed by estrogen but unaffected by progesterone. In the cerebellum and hypothalamus, magnesium was significantly depressed by estradiol and enhanced by progesterone.

TABLE 5.1.2: EFFECT OF OVARIECTOMY WITH ESTRADIOL OR PROGESTORINE THERAPY ON THE *CALCIUM, MAGENSSIUM AND ZINC LEVELS IN THE PROCINE BRAIN AND HYPOPHYSIS.

BRAIN REGIONS	ANIMAL GROUPS		
a) CALCIUM	Sham operated	OVARIECTOMIZED	
		With estradiol	With progesterone
Pons	1.005 ± 0.021 b	1.406 ± 0.021 a	1.229 ± 0.010 a
Cerebellum	0.952 ± 0.022 c	1.662 ± 0.012 a	1.474 ± 0.010 b
Amygdala	1.545 ± 0.026 a	1.200 ± 0.057 b	1.105 ± 0.069 b
Hippocampus	1.312 ± 0.031 a	1.024 ± 0.015 b	1.115 ± 0.066 b
Hypothalamus	1.400 ± 0.074 b	1.562 ± 0.085 ab	1.639 ± 0.052 a
Cerebral cortex	1.306 ± 0.059 a	1.337 ± 0.059 a	1.102 ± 0.071 b
Mid brain	1.250 ± 0.064 b	1.200 ± 0.082 b	1.500 ± 0.074 a
Medulla oblongata	0.987 ± 0.047 a	1.100 ± 0.057 a	1.124 ± 0.076 a
Adenohypophysis	2.042 ± 0.478 a	2.087 ± 0.043 a	1.626 ± 0.053 b
Neurohypophysis	1.631 ± 0.028 c	2.055 ± 0.025 b	2.220 ± 0.092 a
Grand mean	1.339 ± 0.328	1.459 ± 0.369	1.413 ± 0.358
b) MAGNESIUM	Sham operated	OVRAIECTOMIZED	
		With estradiol	With progesterone
Pons	1.295 ± 0.015 c	1.471 ± 0.013 b	1.751 ± 0.002 a
Cerebellum	1.493 ± 0.136 b	1.397 ± 0.003 c	2.001 ± 0.003 a
Amygdala	1.481 ± 0.003 b	1.500 ± 0.002 b	2.021 ± 0.004 a
Hippocampus	1.914 ± 0.013 a	1.580 ± 0.005 b	2.059 ± 0.020 a
Hypothalamus	1.540 ± 0.005 b	1.483 ± 0.007 c	2.046 ± 0.029 a
Cerebral cortex	1.546 ± 0.003 a	1.423 ± 0.003 a	1.536 ± 0.036 a
Mid brain	1.526 ± 0.003 b	1.482 ± 0.003 b	2.133 ± 0.024 a
Medulla oblongata	1.396 ± 0.004 c	1.571 ± 0.010 b	1.912 ± 0.024 a
Adenohypophysis	1.476 ± 0.003 c	1.914 ± 0.042 b	2.282 ± 0.007 a
Neurohypophysis	1.343 ± 0.017 c	1.712 ± 0.020 b	2.124 ± 0.031 a
Grand mean	1.501 ± 0.168	1.553 ± 0.155	1.987 ± 0.211
c) ZINC	Sham operated	OVARIECTOMOZED	
		With estradiol	With progesterone
Pons	0.987 ± 0.052 a	0.668 ± 0.022 b	0.549 ± 0.020 c
Cerebellum	0.961 ± 0.012 b	0.697 ± 0.002 c	1.089 ± 0.010 a
Amygdala	0.529 ± 0.010 c	0.602 ± 0.015 b	0.952 ± 0.060 a
Hippocampus	0.242 ± 0.017 b	0.702 ± 0.014 a	0.748 ± 0.010 a
Hypothalamus	0.687 ± 0.036 b	0.797 ± 0.012 a	0.779 ± 0.024 a
Cerebral cortex	0.922 ± 0.005 a	0.734 ± 0.030 b	0.592 ± 0.008 c
Mid brain	1.047 ± 0.006 a	0.576 ± 0.007 b	0.604 ± 0.008 b
Medulla oblongata	1.037 ± 0.011 a	0.677 ± 0.014 c	0.796 ± 0.019 b
Adenohypophysis	0.457 ± 0.022 c	0.813 ± 0.006 b	1.169 ± 0.003 a
Neurohypophysis	0.797 ± 0.038 b	1.110 ± 0.003 a	1.146 ± 0.004 a
Grand mean	0.766 ± 0.278	0.738 ± 0.150	0.842 ± 0.234

Values in the same horizontal column bearing different superscripts differ significantly ($P < 0.05$)

Values are means ± S.E.M.

*Values are in parts per million (ppm).

TABLE 5.1.3: EFFECT OF OVARIECTOMY WITH ESTRADIOL OR PROGESTERONE THERAPY ON THE *POTASSIUM, SODIUM AND COPPER LEVELS IN PROCINE BRAIN AND HYPOPHYSES.

BRAIN REGIONS	ANIMAL GROUPS		
d) POTASSIUM	Sham operated	OVARIECTOMIZED	
		With estradiol	With progesterone
Pons	37.980 ± 2.059 a	27.461 ± 1.465 b	23.025 ± 0.842 c
Cerebellum	33.293 ± 1.137 a	25.663 ± 0.546 b	26.861 ± 0.627 b
Amygdala	42.175 ± 0.934 a	27.807 ± 0.476 b	25.000 ± 0.876 b
Hippocampus	33.637 ± 1.492 a	26.901 ± 0.689 b	25.000 ± 0.456 b
Hypothalamus	26.186 ± 1.881 a	26.187 ± 0.857 a	20.609 ± 0.963 b
Cerebral cortex	30.325 ± 2.093 a	28.612 ± 1.310 a	27.890 ± 0.656 a
Mid brain	33.476 ± 0.427 a	30.500 ± 0.612 a	31.317 ± 1.807 a
Medulla oblongata	32.300 ± 0.397 a	30.375 ± 0.898 a	25.250 ± 1.031 b
Adenohypophysis	28.050 ± 0.733 b	32.250 ± 0.722 a	35.025 ± 0.953 a
Neurohypophysis	22.027 ± 0.711 b	28.250 ± 0.616 a	17.394 ± 1.066 c
Grand mean	32.045 ± 5.765	28.401 ± 2.083	25.737 ± 5.032
e) SODIUM	Sham operated	OVARIECTOMIZED	
		With estradiol	With progesterone
Pons	508.750 ± 4.270 a	535.000 ± 4.082 a	528.250 ± 3.370 a
Cerebellum	527.875 ± 1.443 a	525.000 ± 5.400 a	529.250 ± 1.051 a
Amygdala	539.375 ± 2.135 a	529.750 ± 1.051 a	527.625 ± 7.733 a
Hippocampus	537.500 ± 3.227 a	531.250 ± 2.394 a	532.112 ± 0.616 a
Hypothalamus	537.750 ± 1.534 a	530.125 ± 0.935 a	536.375 ± 2.286 a
Cerebral cortex	541.875 ± 2.394 a	526.875 ± 9.093 a	532.500 ± 8.292 a
Mid brain	541.375 ± 1.250 a	526.250 ± 4.270 a	545.000 ± 3.536 a
Medulla oblongata	542.000 ± 3.227 a	529.375 ± 2.095 a	517.500 ± 8.780 a
Adenohypophysis	517.375 ± 2.996 a	533.375 ± 1.061 a	545.000 ± 2.041 a
Neurohypophysis	538.750 ± 4.270 a	532.850 ± 6.631 a	542.625 ± 2.500 a
Grand mean	533.262 ± 11.567	529.835 ± 3.278	533.624 ± 8.787
f) COPPER	Sham operated	OVARIECTOMIZED	
		With estradiol	With progesterone
Pons	0.191 ± 0.004 a	0.087 ± 0.005 b	0.080 ± 0.007 b
Cerebellum	0.122 ± 0.003 b	0.130 ± 0.009 b	0.202 ± 0.008 a
Amygdala	0.120 ± 0.008 a	0.076 ± 0.004 b	0.087 ± 0.004 b
Hippocampus	0.136 ± 0.002 b	0.156 ± 0.009 a	0.084 ± 0.001 c
Hypothalamus	0.142 ± 0.004 a	0.135 ± 0.008 a	0.096 ± 0.002 b
Cerebral cortex	0.147 ± 0.003 a	0.101 ± 0.009 b	0.082 ± 0.001 b
Mid brain	0.126 ± 0.002 a	0.119 ± 0.001 a	0.096 ± 0.003 b
Medulla oblongata	0.124 ± 0.003 a	0.092 ± 0.002 b	0.085 ± 0.003 b
Adenohypophysis	0.173 ± 0.006 a	0.087 ± 0.004 c	0.139 ± 0.010 b
Neurohypophysis	0.126 ± 0.012 a	0.104 ± 0.004 b	0.074 ± 0.402 c
Grand mean	0.141 ± 0.024	0.109 ± 0.026	0.102 ± 0.039

Values in the same horizontal column bearing different superscripts differ significantly ($P < 0.05$)

Values are means ± S.E.M.

*Values are in parts per million (ppm).

ZINC

Estrogen and progesterone significantly depressed zinc levels in the pons and cerebral cortex. However, in each case, progresterone was more potent in depressing zinc than estrogen.

Zinc levels in the OE and OP animals were similar and superior to the sham operated animals in the neurohypophysis, hippocampus and hypothalamus.

In the amygdala and adenohypophysis, zinc levels were significantly enhanced by estradiol and progesterone but the OP animals were superior to the OE animals.

Estrogen and progesterone depressed zinc levels in the midbrain and medulla oblongata, however, the medulla of OE animals was inferior to the OP animals. In the cerebellum, estradiol significantly depressed zinc level ($P<0.05$) but was considerably enhanced by progesterone.

POTASSIUM

Both estrogen or progesterone treatment alone depressed potassium level in the pons ($P<0.05$) but the OP animals were inferior to the OE animals. In the cerebellum, Amygdala and hippocampus, potassium levels in the OE and OP animals were similar and inferior to the SO animals ($P<0.05$). In the hypothalamus and medulla oblongata, the SO animals and OE animals were similar and superior to the SO animals ($P<0.05$). In the neurohypophysis, estrogen injection along significantly enhanced potassium level ($P<0.05$) while progesterone depressed it.

No significant changes were observed in the cerebral cortex.

SODIUM

No significant differences were observed in the brain and hypophyseal sodium levels ($P>0.05$).

COPPER

Copper levels were significantly depressed by estrogen or progesterone treatment alone in the pons, amygdala, cerebral cortex and medulla oblongata ($P<0.05$). In the hypothalamus and midbrain, SO and OE animals were similar and superior to progesterone-treated animals.

In the cerebellum, the SO and OE animals were similar and inferior to OP animals; while in the hippocampus, copper levels were significantly enhanced and depressed by estradiol and progesterone respectively ($P<0.05$).

In the hypophyses, estrogen or progesterone treatment alone significantly depressed copper levels ($P<0.05$) but in the adenohypophysis, the OE animals were inferior to the OP animals whereas in the neurohypophysis, the OE animals were superior to the OP animals.

5.3.0 DISCUSSION

The depression of AChE activities in the pons, cerebellum, amygdala, hippocampus and midbrain of bilaterally ovariectomized gilts irrespective of steroid treatment is in line with the reports of Pfaff (1968), Eisenfeld and Axelrod, (1975) who have linked these brain regions with steroid metabolism by neural cells.

It also lends credence to the observation of Iramain et al (1979) that orchidectomy plus estradiol administration decreased AChE activity in the rat cerebral cortex. Moudgil and Kanungo (1973) also observed a

decrease in the AChE activity of the rat cerebral hemisphere following ovariectomy.

The rise in AChE activity in the pons, hypothalamus, cerebral cortex, midbrain and medulla oblongata of the Estradiol-treated gilts over the progesterone treated animals implies that estrogen has a more facilitatory role on nervous transmission than progesterone.

Since AChE activity has been known to enhance adaptability, Estradiol may also be more useful in allowing the animal to adjust favourably to changes brought about by gonadal steroids withdrawal in the female.

The decline in AChE activity upon gonadal steroids withdrawal in the female also emphasizes the necessity of these hormones in maintaining normal metabolic functions of the brain.

Hence ovariectomy may possibly lead to impairment of brain activities. The ability of the hypothalamus, midbrain, pons and medulla oblongata to respond more to estradiol treatment than progesterone also supports the report of Kawakami et al (1970b) that estrogens cause a rise in the multiple unit activity in the dorsal hippocampus, amygdala and hypothalamus while progesterone did not show appreciable results. However, it must be mentioned that in the present study, progesterone and estradiol injections had similar effects in the cerebellum, amygdala and hippocampus. The reason for this may be due to the dual purpose function of the midbrain which is known to exert both facilitatory and inhibitory effects on gonadotrophin secretion.

In addition, stimulation of the midbrain may activate the neurons of the hypothalamic ventromedial nucleus (Tsubokawa and Sutin, 1963) and

posterior hypothalamus while at the same time inhibiting the hippocampus and amygdala in gonadotrophin secretion (Velasco and Taleisnik, 1969b).

This reciprocity of the midbrain in gonadotrophin release may thus explain the higher AChE activity induced by Estradiol in the midbrain and hypothalamus over the amygdala and hippocampus.

While it is known that the hypothalamus is responsible for follicular growth and estrogen secretion and also regulates ovulation (Halasz and Gorski, 1967, Koves and Halasz, 1970). Other studies have shown that stimulation of the hippocampus may in fact block ovulation in the rat (Velasco and Taleisnik 1969). A further evidence of the fairly antagonistic nature of the hippocampal neurons to the hypothalamus comes from the report of Terasawa and Sawyer (1970) and Flerko, and Bardos, (1961) that while stimulation of the medial preoptic area of the hypothalamus in the rat increased serum FSH and LH levels in the blood, stimulation of the hippocampus alone inhibited the elevation ofserum LH level and suggests that the hippocampus stimulation inhibited the neural surge of the ovulatory hormone and facilitated the release of FSH.

This is not to say that the hippocampus is antagonistic to behavioural estrus or gonadal function in the female because Riss et al (1963) have demonstrated that destruction of the hippocampus delayed onset of puberty in the rat.

It has also been shown by Nauta, (1958) that the anatomical connections of the brain regions contribute largely to these striking and related differences in their functions. Thus it has been established that the neurons of the midbrain projects to the amygdala and are linked with the hippocampus by a bi-directional medial fore-brain bundle which permits reciprocal influences between the regions with the midbrain acting as a

central station for the distribution of flow of afferent impulses to the hypothalamus or to the limbic system and modulates the magnitude of sensory inputs according to the information received from upper neural structures and the prevailing hormonal background.

The lack of difference in AChE activity in the cerbral cortex and medulla oblongata of the sham ovariectomized and Estradiol treated gilts is a reflection of the steroid receptivity and sensitivity of these structures. Thus while it is known that estradiol is highly concentrated in the hypothalamus, preoptic area, amygdala and septum (Pfaff, 1968), very low concentrations have been found in the cortex.

Another possible reason for the AChE activity induction by estradiol is the discovery that estrogen induces choline acetyltransferase in the preoptic area (Luine et al, 1975) and AChE is also known to be involved in the feedback control of LH secretion which is elevated in the serum after ovariectomy and depressed by estradiol administration.

The apparent potency of estradiol over progesterone may be due to the fact that estradiol when administered alone to ovariectomized animals may be able to restore near normal gonadal functions probably due to its ability to induce progestin receptors or to make use of the small amounts of progesterone secreted by the adrenal gland to elicit the appropriate responses. Progesterone on the other hand when administered alone tends to behave as an anti-estrogen unless the target tissues are previously sensitized by estrogens.

The higher protein concentrations observed in the sham ovariectomized gilts than the other bilaterally ovariectomized gilts in the cerebellum, amygdala, hypothalamus and midbrain supports the earlier suggestion that these regions play profound roles in sexual development and

maturation of the animal and hence require the two major female gonadal steroids estrogen and progesterone working together synergistically to maintain sexual behaviour.

The higher protein concentrations observed in the estradiol-treated castrates over their progesterone treated counterparts in the amygdala, hippocampus, hypothalamus and medulla oblongata implies higher protein synthesis induced by estradiol since it is known that estradiol promotes body tissue growth presumably due to an increase in the transcription of M-RNA. Chan and O'Malley (1978) opined that exogenous estradiol causes functional changes in target-tissues involving an initial hormone specific receptor interaction ultimately leading to enhanced protein synthesis and increase in the activities of specific enzymes.

Progesterone contrariwise is reported to facilitate protein catabolism. Hence since the hypothalamus, amygdala and hippocampus have been identified as receptor sites of estradiol, it is hardly surprising that increased protein synthesis is enhanced in the regions. Another possible evidence is the increase in blood flow (hypermia) which follows estrogen administration after ovariectomy. Concurrent with this is an increased permeability of the capillaries (Szego and Sloan, 1961). Although these changes take place in the uterus, it is not unlikely that they are manifested in the brain too. Thus an increased permeability of the capillaries would allow more metabolites particularly enzymes and amino acids plus the necessary ions to escape through the blood-brain barrier into the brain cell membranes which is the primary site of protein synthesis.

The similarly in protein concentration of the cerebral cortex and midbrain of both groups may arise from the earlier suggestion that the

cortex is not a major site of estradiol metabolism while the midbrain is known to have a dual "carriageway" link with the limbic system and the hypothalamus. In addition, the cortex is less associated with sexual behaviour and more with learning and memory functions.

The reason for the higher protein concentration induced by progesterone in the cerebellum is not very clear and may need further investigation.

The higher SAChE activities observed in the brain regions of progesterone treated castrates is a reflection of the depressed protein concentration of the regions and their lowered AChE activities. These depressions would ultimately result in elevated SAChE activities implying a compensatory attempt by the neural cells to offset the metabolic inbalance brought about by changes accompanying ovariectomy and progesterone administration.

5.3.1 MINERAL PROFILE IN THE BRAIN

The results indicate that ovariectomy depressed calcium levels in the amygdala and hippocampus irrespective of hormonal treatment while it elevated calcium in the pons and cerebellum. Progesterone treatment alone elevated calcium in the midbrain and hypothalamus and depressed it in the cortex. This trend closely reflects the inhibitory role of the midbrain on the hippocampus and amygdala.

The mechanism whereby calcium is depressed by ovariectomy and either progesterone or estradiol administration cannot be readily explained in the light of available information but a likely possibility is an interference with some enzyme activated systems in the brain.

It is well established that the ouput mechanisms of neuroednocrine cells respond to circulating steroids by either a decrease or an increase

in the membrane potential of the cells which are principally determined by the electrolyte balance between sodium and potassium. It is therefore possible that the lack of effect of steroid withdrawal on sodium concentration of the brain regions is a result of an increase in turnover rates of sodium to maintain fairly normal concentrations of the ions.

However, the results show that while estradiol facilitated potassium ions retention in the medulla oblongata, pons and hypothalamus than progesterone administration, similar levels were observed in the cerebellum, amygdala, hippocampus, midbrain and cerebral cortex. This suggests that estradiol facilitates mineral retention by the brain cells in response to their role in protein synthesis. It also shows that estradiol has a higher capacity to increase cell permeability through increased blood flow than progesterone.

The elevated magnesium level induced by progesterone administration on ovariectomized gilts in most of the brain regions is indicative of the compensatory role magnesium tends to play during calcium depletion. It also suggest possible progesterone induction of magnesium which may be required in the several magnesium activated enzyme metabolic pathways required for maintenance of neuromuscular activities. It also suggests possile antagonism between magnesium and calcium.

The depressed copper levels in the ovariectomized gilts is also indicative of the impaired mineral retention of the brain cells consequent upon ovarian steroids withdrawal. The slightly higher copper levels in the estradiol-treated castrates suggests increased blood flow to the affected brain regions resulting in passage of more metabolites across the blood-brain barrier to the brain cells.

The effect of ovariectomy and ovarian steroids therapy on brain

zinc levels were not very consistent and further study is recommended to further elucidate the role of ovarian steroids metabolism on zinc retention.

Suffice to say however, that estradiol and progesterone treated castrates had higher zinc levels than the sham ovariectomized gilts in the hippocampus and hypothalamus which is in agreement with the reports that estrogens increases zinc accumulation (Gunn and Gould, 1956) and induced increased zinc incorporation in the dorsoventral lobe of the castrate rats (Muntzing et al, 1977).

5.3.2 AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY IN THE HYPOPHYSES

No treatment effects were observed in the AChE activity of the neurophypophysis which suggest a considerable amount of tolerance to ovarian steroids withdrawal. It also indicates that the positive feed back mechanisms that normally follow ovariectomy such as LH and FSH surge did not influence the activity of the gland.

However, the adenohypophysis which is the site of FSH and LH release showed increased AChE activity in progesterone-treated castrates. The restorative effect of estradiol in AChE activity in the adenohypophysis of the ovariectomized gilts confirms the observation that the LH and FSH surge in the serum following ovariectomy can be diminished largely by estradiol administration while progesterone has a very limited effect.

Thus the adenohypophysis of the estradiol-treated ovariectomized gilt is able to respond to the negativefeed back effect of exogenous estradiol administration thereby allowing the gland to function normally. This is also another evidence of the sensitivity of the hypothalamic-

hypophyseal-gonadal axis to estrogen administration.

The decreased protein concentrations of the hypophyses of the progesterone-treated, ovariectomized gilts and the concommitant restorative effect of estradiol on protein synthesis lends credence to the presumption that progesterone is protein catabolic while estrogen is protein anabolic.

While the SAChE activities in the neurohypophysis were unaffected by the treatments, the adenohypophysis of the progesterone-treated ovariectomized gilts recorded higher levels than the other two groups which attracts the earlier explanation of an attempt to compensatte for decreased protein synthesis resulting in increased SAChE activity.

5.3.3 MINERAL PROFILE IN THE HYPOPHYSES

The inability of progesterone to maintain normal calcium levels in the adenohypophysis of the ovariectormized gilts suggests that the protein catabolic action of progesterone also facilitates decreased calcium retention. The restoration of calcium level to normal by estradiol in the ovariectomized gilts may be linked to the role of estradiol (Florindo and Martini, 1975), and calcium ions in the release of ACh at neuromuscular junctions (Rahamimmof, 1976). In addition, increased calcium absorption is known to favour increased protein synthesis and the utilization of amino acids.

The increased retention of calcium in the neurohypophysis of the progesterone-treated ovariectomized gilts may probably have a link with the accumulation of fluids observed in the uterus of progesterone-treated, ovariectomized sows during pregnancy and the inhibition of the oxytocic effect of neurohypophysis. (Alexander and Williams, 1968).

Magnesium as usual is induced by progesterone treatment and lowest

in the hypophyses of the sham ovariectomized gilts. This again suggests a possible role for progesterone in magnesium metabolism which has to be investigated further.

Potassium is highest in the adenohypophysis of the ovariectomized groups of animals than in the sham ovariectomized group while in the neurohypophyses, the progesterone injected group had the least concentration. Possible reason for this may be due to cations interaction and possible antagonism between potassium, calcium and magnesium.

Sodium on the other hand remained relatively stable indicating its role in electrolyte balance and osmotic regulation of the body fluids of the animal.

The lowering of copper levels by ovariectomy and the fairly restorative effects of estradiol confirms the role of copper in red blood cells formation and the synthesis and activation of several oxidative enzymes necessary for the normal metabolism of the pig. Complement to this is the increased blood flow associated with estrogens which may readily result in increased copper retention by the hypophyses.

The higher levels of zinc in the hypophyses of the ovarian steroids treated ovariectomized gilts may be due to the regenerative action of zinc on body tissues and a compensatory increase of the mineral in an attempt to reduce the degenerative changes characterizing ovariectomy. Another possible evidence for this function is the known fact that zinc deficiency or impaired zinc metabolism in the rat body results in atropy of the sex organs and brain malformations (Fullis, 1958, Hurley, 1974) which are effects also manifested by gonadal steroid withdrawal. Thus the elevation of zinc in the hypohyses of the ovariectomized ovarian steroids-treated gilts may be a feed back response to attenuate the effects of ovariectomy.

CHAPTER SIX

EFFECTS OF TESTOSTERONE INJECTION ON THE AChE ACTIVITY AND CATIONS IN THE BRAIN AND HYPOPHYSES OF GILTS

CHAPTER SIX

6.1 EFFECTS OF TESTOSTERONE INJECTION ON THE AChE ACTIVITY AND CATIONS IN THE BRAIN AND HYPOPHYSES OF GILTS

6.1.1 INTRODUCTION

Preceeding chapters have dealt rather exhaustively on the role of the gonadal hormones in reproductive behaviour. How this behaviour is mediated by the nervous system has also received considerable attention.

Evidences abound that the presence or absence of perinatal androgens appears to determine irreversibly the nature of adult sexual behaviour shown by rats and other rodents whereas perinatal ovarian secretions do not appear to play such a role (Gorski, 1973, Arnold, 1980). Thus genetic females injected with a single dose of androgen around the time of birth can be permanently masculinized behaviourally as well as defeminized. That is, such a genetic female will show typical male behaviours more frequently and typical female behaviours less frequently at adulthood compared to a normal female. Similarly, males deprived of androgens by castration at birth will be defeminized and demasculinized (Arnold, 1980).

It therefore appears that androgens, when administered to female animals act as anti-estrogens.

6.1.2 LITERATURE REVIEW

The concept of organizational effects of hormones suggests that Sex differences exist in the brain and workers have shown differential receptivity to gonadal steroids by certain brain regions. That is, some

regions in the male brain are different in their functions, steroid receptivity and probably structure from the same brain regions of female animals (Nottebohn and Arnold, 1976).

Thus Pfaff (1970) reported that the effect of testosterone on male behaviour in male rats can be mimicked using testosterone injections in females. McEwen, (1970) noted that neonatal treatment of female rats with testosterone reduces adult sensitivity to estradiol with respect to a variety of estrogen-dependent neuroendocrine and behavioural parameters.

Indications also show that neonatal androgenization may influence adult sensitivity to progesterone as some male rats exhibit somewhat the same reduced hormone sensitivity as neonatally androgenized females.

Testosterone injected into gilts in the early and middle of the luteal phase of the estrous cycle blocked ovulation but did not at the end of the phase. Also, the treated gilts were in estrus longer (4.0 to 6.8 days) than the control (2 days) (Ciro and Torres, 1974).

The work of Clemens et al (1969, 1970) shows that while a normal female rat shows lordosis behaviour after ovariectomy and priming with estradiol benzoate and progesterone, lordosis behaviour in the androgenized female is suppressed. Further evidence is provided by Gorski (1968) who reported that when as little as 10 ug testosterone propionate is administered to the one or two-day-old female rat ovulation is prevented but when such females are tested for lordosis behaviour after ovariectomy and replacement therapy with both estradiol benzoate and progesterone, they display normal levels of female behaviour (Clemens et al 1969).

Flerko and Mess (1968), McGuire and Lisk (1968) observed that testosterone administration on intact females decreases estradiol uptake by the hypothalamus. However, information is lacking on dose-response

relationship on androgenization of the female and induced male behaviour.

A striking anomaly observed by Gorski (1973) was the fact that androgenized female rats displayed significantly higher lordosis quotient following estradiol benzoate treatment than control females. However, Gorski was quick to add that the dose of testosterone propionate, the age at injection, probably the age at testing, the hormonal replacement and conditions under which the tests are conducted are critical factors which affect the results obtained. Thus 10 ug testosterone propionate given to the 6-day-old female was much less effective in inducing anovulatory persistent estrus than the same dose given on day 4.

A critical question on the mechanism of androgenization is whether the androgen merely prevents the maturation or development of the fully functional cyclic regulatory system or it induces changes which actually masculinize the brain. Ladosky and Gaziri (1970) reported that brain levels of serotonin increases significantly in the female and that testosterone antagonizes the serotonergic system. Another evidence was provided by Dorner (1971) that an absolute independence of the "Sex-specific" brain differentiation from the genetic sex exists. Thus, a complete inversion of sexual behaviour after post pubertal androgen activation was observed between male and female rats following androgen deficiency in the males and androgen overdosage in the females during the hypothalamic differentiation period. Dorner also observed that prepubertal administration of androgen to female rats resulted in a normal or approximately normal cyclic ovarian function after puberty. However, these females displayed predominantly heretotypical (i.e. homosexual) behaviour. This suggests a partial chronological dissociation in the differentiation of hypothalamic sex and mating centres. On the other hand, such

testosterone treatment during the hypothalamic organization phase results in a predominantly male differentiation of these centres and acyclic pituitary gonadotropin secretion coupled with hypo or homo-sexuality in post pubertal life.

Another example of the anti-estrogenic effect of androgen is its inhibition of the estrogen-induced hypertrophy of the anterior pituitary and also the inhibition of the thyroxine-binding capacity of anterior pituitary proteins (Schreiber, 1973).

It is also suggested that estrogens and androgens compete for identical protein binding sites in the anterior pituitary. This suggestion is confirmed by the discovery by Gorski et al (1968) of a morphological sex difference in the suprachiasmatic nuclei of the hypothalamus (SCN) and the findings of Moore (1978) that neonatal testosterone treatment of females disrupts the periodic release of LH in the same way lesions of the SCN do.

The similarities between the effects of perinatal testosterone treatment and SCN lesions on the disruption of LH release in female led to the speculation that testosterone treatment may affect the function of the SCN and other brain areas that affect female sexual behaviour such as the frontal cortex, hippocampus and Septum (Sodersten et al, 1980). Emmens and Miller (1969) therefore advanced the hypothesis that testosterone belongs to the anti-estrogens which do not inhibit or reduce estrogen concentration in the target tissues, but exert their influence in some unknown way.

6.2.1 MATERIALS AND METHODS

Ten 4-month-old Large White gilts raised and maintained at the physiology unit of the university of Ibadan farm were sacrificed for this study. The animals were housed and managed as described in preceeding chapters.

The gilts (28 to 35 kg body weight) were injected intramuscularly with 1 ml of corn oil containing 25mg of Testosterone enanthate (equivalent to 18.0mg testosterone).

The injections were administered every Wednesday at 0900-1000 hours and repeated every Wednesday for five weeks.

The other group of gilts (CG) received the oil vehicle only at the same time and rate as TE gilts.

24 hours after the fifth and last injection, all the gilts were sacrificed and their brains and hypohyses quickly removed and processed as described earlier.

6.2.2 STATISTICAL ANALYSIS

All results were subjected to statistical analyses as described in preceeding chapters.

RESULTS

6.3.1 EFFECT OF TESTOSTERONE INJECTION ON THE AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY OF THE BRAIN AND HYPOPHYSES OF GILTS.

The results are summarized in Table 6.1. Testosterone injection significantly depressed AChE activity in all the brain regions ($P<0.05$). However, no significant differences were observed in the hypophyses ($P>0.05$).

TABLE 6.1. EFFECT OF TESTOSTERONE INJECTION ON THE AChE ACTIVITY, TOTAL PROTEI AND SAChE ACTIVITY IN THE BRAIN AND HYPOPHYSES OF GILTS.***

(a) *AChE ACTIVITY BRAIN REGIONS	ANIMAL GROUPS	
	TESTOSTERONE-INJECTED GILTS	CONTROL GILTS
Pons	2.870 ± 0.139^{b}	4.868 ± 0.156^{a}
Cerebellum	2.424 ± 0.193^{b}	3.829 ± 0.165^{a}
Amygdala	2.385 ± 0.177^{b}	4.079 ± 0.062^{a}
Hippocampus	2.519 ± 0.213^{b}	6.153 ± 0.227^{a}
Hypothalamus	3.658 ± 0.162^{b}	4.895 ± 0.212^{a}
Cerebral cortex	1.343 ± 0.059^{b}	1.861 ± 0.070^{a}
Mid Brain	5.760 ± 0.231^{b}	10.130 ± 0.363^{a}
Medulla Oblongate	6.061 ± 0.127^{b}	6.921 ± 0.124^{a}
Adenohypophysis	0.445 ± 0.020^{a}	0.710 ± 0.031^{a}
Neurohypophysis	1.292 ± 0.050^{a}	1.455 ± 0.031^{a}
GRAND MEAN	2.876 ± 0.735	4.490 ± 1.130^{a}

(b) **TOTAL PROTEIN BRAIN REGIONS.	ANIMAL GROUPS	
	TESTOSTERONE-INJECTED GILTS	CONTROL GILTS
Pons	0.464 ± 0.019^{a}	0.340 ± 0.023^{b}
Cerebellum	0.264 ± 0.015^{b}	0.638 ± 0.071^{a}
Amygdala	0.300 ± 0.006^{a}	0.322 ± 0.007^{a}
Hippocampus	0.252 ± 0.011^{a}	0.331 ± 0.007^{a}
Hypothalamus	0.346 ± 0.025^{b}	0.767 ± 0.080^{a}
Cerebral cortex	0.188 ± 0.004^{b}	0.332 ± 0.004^{a}
Mid Brain	0.601 ± 0.004^{a}	0.602 ± 0.064^{a}
Medulla Oblongata	0.543 ± 0.022^{b}	1.037 ± 0.030^{a}
Adenohypophysis	0.169 ± 0.004^{a}	0.179 ± 0.003^{a}
Neurohypophysis	0.126 ± 0.009^{a}	0.076 ± 0.004^{a}
GRAND MEAN	0.325 ± 0.065^{b}	0.462 ± 0.117^{a}

(c) ***SAChE ACTIVITY BRAIN REGIONS.	ANIMAL GROUPS	
	TESTOSTERONE-INJECTED GILTS	CONTROL GILTS
Pons	6.185 ± 0.126^{b}	14.477 ± 0.560^{a}
Cerebellum	9.414 ± 1.286^{a}	6.359 ± 0.974^{b}
Amygdala	7.995 ± 0.703^{b}	12.696 ± 0.316^{a}
Hippocampus	10.147 ± 1.096^{b}	18.667 ± 0.936^{a}
Hypothalamus	10.800 ± 0.089^{a}	6.725 ± 0.912^{b}
Cerebral cortex	7.182 ± 0.360^{a}	5.618 ± 0.252^{a}
Mid Brain	9.597 ± 0.416^{b}	17.763 ± 2.477^{a}
Medulla Oblongata	11.248 ± 0.513^{a}	6.706 ± 0.289^{b}
Adenohypophysis	3.527 ± 0.100^{a}	3.979 ± 0.222^{a}
Neurohypophysis	10.390 ± 0.680^{b}	19.312 ± 0.695^{a}
GRAND MEAN	8.648 ± 0.971^{a}	11.230 ± 2.402^{b}

Values in the same horizontal column differently sup cripted differ significantly (P>0.05).

*AchE Activity in µmole/g/min.
**Total Protein in g/100 ml.
***AchE Activity in µmole/g protein/min.
****Values are means ± standard Error of the mean.

Testosterone injection further depressed total protein levels in the cerebellum, hypothalamus, cerebral cortex, medulla oblongata, and elevated it in the pons ($P<0.05$). No significant differences were observed in the amygdala, hippocampos, midbrain, adenohypophysis and the neurohypophysis, ($P>0.05$).

The control gilts (CG) had significantly higher SAChE activities than the TE gilts in the pons, amygdala, hippocampus, mid brain neurohypophysis and inferior to the TE gilts in the Cerebellum, hypothalamus and medulla oblongata ($P<0.05$). No significant differences were observed in the Cerebral cortex and the Adenohypophysis ($P>0.05$).

6.3.2 EFFECT OF TESTOSTERONE INJECTION ON THE MINERAL PROFILE IN THE BRAIN AND HYPOPHYSES OF GILTS.

The results are summarized in Tables 6.2 and 6.3.

CALCIUM

Testosterone injected gilts exhibited depressed Calcium levels in all the brain regions and the hypophyses ($P<0.05$).

MAGNESIUM

Unlike the trend in calcium, testosterone administration on gilts significantly elevated magnesium levels in all the brain regions and hypophyses ($P<0.05$).

ZINC

In a similar trend with magnesium levels, testosterone administration also raised magnesium levels in intact gilts above the controls ($P<0.05$) in all the brain regions and the hypophyses.

TABLE 6.2: EFFECT OF TESTOSTERONE INJECTION ON THE *CALCIUM, MAGNESIUM AND ZINC LEVELS IN THE BRAIN AND HYPOPHYSES OF GILTS**

(a) CALCIUM BRAIN REGIONS.	ANIMAL GROUPS	
	TESTOTERONE-INJECTED GILTS	CONTROL GILTS
Pons	0.880 ± 0.025[b]	2.640 ± 0.075[a]
Cerebellum	0.826 ± 0.010[b]	3.040 ± 0.081[a]
Amygdala	0.900 ± 0.016[b]	2.980 ± 0.222[a]
Hippocampus	1.680 ± 0.025[b]	3.090 ± 0.181[a]
Hypothalamus	0.901 ± 0.016[b]	1.860 ± 0.108[a]
Cerebral cortex	0.911 ± 0.019[b]	2.020 ± 0.058[a]
Mid Brain	1.081 ± 0.020[b]	2.920 ± 0.159[a]
Medulla Oblongata	1.281 ± 0.038[b]	2.580 ± 0.116[a]
Adenohypophysis	1.340 ± 0.080[b]	2.160 ± 0.070[a]
Neurohypophysis	2.260 ± 0.060[b]	3.220 ± 0.080[a]
GRAND MEAN	1.206 ± 0.184[b]	2.651 ± 0.194

(b) MAGNESIUM BRAIN REGIONS.	ANIMAL GROUPS	
	TESTOSTERONE-INJECTED GILTS	CONTROL GILTS
Pons	2.625 ± 0.080[a]	2.270 ± 0.040[b]
Cerebellum	2.534 ± 0.040[a]	2.292 ± 0.004[b]
Amygdala	2.655 ± 0.070[a]	2.312 ± 0.040[b]
Hippocampus	2.753 ± 0.400[a]	2.200 ± 0.040[b]
Hypothalamus	2.737 ± 0.030[a]	2.129 ± 0.080[b]
Cerebral cortex	2.705 ± 0.080[a]	2.281 ± 0.022[b]
Mid Brain	2.590 ± 0.147[a]	2.304 ± 0.30[b]
Medulla Oblongata	2.818 ± 0.100[a]	2.256 ± 0.090[b]
Adenohypophysis	2.536 ± 0.070[a]	2.297 ± 0.030[b]
Neurohypophysis	2.246 ± 0.063[a]	2.366 ± 0.030[a]
GRAND MEAN	2.620 ± 0.064[a]	2.271 ± 0.026[b]

(c) ZINC BRAIN REGIONS	ANIMAL GROUPS	
	TESTOSTERONE-INJECTED GILTS	CONTROL GILTS.
Pons	1.396 ± 0.018[a]	1.098 ± 0.040[b]
Cerebellum	1.211 ± 0.003[a]	1.136 ± 0.050[a]
Amygdala	0.869 ± 0.020[a]	0.614 ± 0. 04[b]
Hippocampus	0.903 ± 0.004[a]	0.528 ± 0.020[b]
Hypothalamus	0.896 ± 0.206[a]	0.619 ± 0.107[b]
Cerebral cortex	1.270 ± 0.004[a]	1.101 ± 0.608[b]
Mid Brain	0.956 ± 0.031[a]	0.608 ± 0.020[b]
Medulla Oblongata	1.261 ± 0.040[a]	1.006 ± 0.020[b]
Adenohypophysis	0.931 ± 0.050[a]	0.678 ± 0.030[b]
Neurohypophysis	1.458 ± 0.040[a]	1.127 ± 0.018[b]
GRAND MEAN	1.115 ± 0.091[a]	0.851 ± 0.104[b]

Values on the same horizontal line bearing different superscripts differ significantly (P>0.05).

*Values are in parts per million (ppm).
**Values are means ± standard error of the mean.

TABLE 6.3: EFFECT OF TESTOSTERONE INJECTION ON THE POTASSIUM, SODIUM AND COPPER LEVELS IN THE BRAIN AND HYPOPHYSES OF GILTS**

(d) POTASSIUM BRAIN REGIONS.	ANIMAL GROUPS	
	TESTOSTERONE-INJECTED GILTS	CONTROL GILTS
Pons	25.900 ± 1.950[a]	19.000 ± 1.844[b]
Cerebellum	13.500 ± 1.498[a]	9.800 ± 1.297[b]
Amygdala	20.100 ± 0.900[a]	7.210 ± 0.917[b]
Hippocampus	21.600 ± 1.476[a]	13.874 ± 1.136[b]
Hypothalamus	23.240 ± 0.850[a]	9.970 ± 0.422[b]
Cerebral cortex	20.000 ± 1.581[a]	10.250 ± 0.268[b]
Mid Brain	23.500 ± 1.207[a]	12.500 ± 0.805[b]
Medulla Oblongata	12.400 ± 0.796[a]	7.400 ± 0.331[b]
Adenohypophysis	19.960 ± 0.984[a]	5.200 ± 0.860[b]
Neurohypophysis	16.900 ± 1.536[a]	12.000 ± 0.741[b]
GRAND MEAN.	19.71 ± 1.733[a]	10.720 ± 1.570[b]

(e) SODIUM BRAIN REGIONS	ANIMAL GROUPS	
	TESTOSTERONE-INJECTED GILTS	CONTROL GILTS
Pons	549.000 ± 2.449[a]	553.000 ± 3.391[a]
Cerebellum	539.000 ± 2.449[a]	543.200 ± 3.246[a]
Amygdala	547.000 ± 2.549[a]	512.000 ± 4.062[b]
Hippocampus	525.000 ± 3.536[a]	533.000 ± 2.000[a]
Hypothalamus	529.000 ± 5.100[b]	547.000 ± 5.148[a]
Cerebral cortex	533.000 ± 5.612[b]	548.000 ± 3.391[a]
Mid Brain	531.400 ± 2.821[b]	553.000 ± 3.742[a]
Medulla Oblongata	520.500 ± 1.631[a]	509.000 ± 3.317[b]
Adenohypophysis	543. 00 ± 4.070[a]	540.000 ± 3.162[a]
Neurohypophisis	532.000 ± 6.042[a]	528.000 ± 2.549[a]
GRAND MEAN	534.900 ± 3.752[a]	536.620 ± 6.373[a]

(f) COPPER BRAIN REGIONS	ANIMAL GROUPS	
	TESTOSTERONE-INJECTED GILTS	CONTROL GILTS
Pons	0.168 ± 0.007[a]	0.100 ± 0.003[a]
Cerebellum	0.130 ± 0.004[a]	0.104 ± 0.005[a]
Amygdala	0.162 ± 0.008[a]	0.164 ± 0.004[a]
Hippocampus	0.134 ± 0.002[a]	0.187 ± 0.004[a]
Hypothalamus	0.190 ± 0.003[a]	0.102 ± 0.004[a]
Cerebral Cortex	0.184 ± 0.007[a]	0.104 ± 0.002[a]
Mid Brain	0.128 ± 0.006[a]	0.126 ± 0.006[a]
Medulla Oblongata	0.160 ± 0.008[a]	0.126 ± 0.004[a]
Adenohypophysis	0.124 ± 0.004[b]	0.640 ± 0.004[a]
Neurohypophysis	0.187 ± 0.003[b]	0.631 ± 0.003[a]
GRAND MEAN.	0.157 ± 0.010[b]	0.228 ± 0.086[a]

Values in the same horizontal line bearing different superscripts differ significantly (P>0.05).

*Values are in parts per million (ppm)
**Values are means ± standard Error of the means.

POTASSIUM

Potassium levels in the testosterone-injected gilts were significantly superior to their control counterparts in all the brain regions and hypophyses ($P<0.05$).

SODIUM

Testosterone administration caused a significant rise ($P<0.05$) in the sodium levels observed in the medulla oblongata, amygdala and a significant decrease in the sodium levels in the cerebral cortex, mid-brain and hypothalamus ($P<0.05$). No significant changes were observed in the pons, cerebellum, hippocampus, adenohypophysis and neurohypophysis ($P>0.05$).

COPPER

No significant treatment effects were observed in all the brain regions ($P>0.05$). However, the copper levels of the hypophyses of the testosterone-injected gilts were inferior to the intact controls ($P<0.05$).

6.4.1 **DISCUSSION**

That androgens behave as anti-estrogens in the female has been well established (Arnold, 1980) and their role in the inversion of female behaviour when administered during the hypothalamic differentiation period has been well described by Dorner (1971).

Thus the depression of AChE activities in the brain regions of testosterone-treated gilts confirms the inhibitory role of testosterone on the development of the cholinergic system in the female.

Ladosky and Gaziri (1970) had already established that testosterone inhibits the increase in brain levels of serotonin in females. The present study thus lends credence to the view that testosterone may be involved in some or the whole family of neurohumoral transmitters including acetylcholine, norepinephrine, dopamine and Serotonin.

The anti-estrogenic effect of testosterone is further supported by the discovery by schreiber (1973) that estrogens and androgens compete for identical protein binding sites in the anterior pituitary.

The report of Moore (1978) that perinatal treatment of females with testosterone mimmicks the effects of lesions of the suprachiasmatic nuclei of the hypothalamus characterized by a disruption of the LH release enhances the speculation that testosterone affects other brain areas such as the frontal and entorhinal cortex, hippocampus and septum. The present study also confirms this speculation.

It is therefore not surprising that testosterone also blocked ovulation in gilts (Ciro and Torres, 1974) and decreased estradiol uptake by the hypothalamus (Flerko and Mess, 1969, McGuire and Lisk, 1969). The foregoing argument may be extended to explain the depression of protein levels in the Cerebellum, Cerebral cortex, hypothalamus and medulla oblongata of testosterone injected gilts.

Such result is a reflection of the fact that testosterone injection into the female reduces the sensitivity of the hypothalamus to estradiol and disturbs the family of estrogen-dependent neuroendocrine and behavioural parameters such as M-RNA and DNA Synthesis, respiratory and glycolytic rate of the brain and the enzyme-synthesis pathways.

The lowering of SAChE activities of testosterone injected gilts also suggests the inability of the brain cells to compensate for the decline in AChE activity and protein concentrations through increased metabolic and turnover rates. This indicates that testosterone may, apart from blocking the uptake of estradiol by brain cells, actually destroy or seriously impair the cellular integrity of the cells thereby reducing the functional capacity of the brain.

The consequences of such a condition in the animal would be very grave and would probably include a very slow or complete destruction of the ability to respond to appropriate stimuli, slow growth and cessation of reproductive functions and a possible lack of co-ordination.

6.4.2 MINERAL PROFILE IN THE BRAIN REGIONS

The depression of calcium levels in Testosterone injected gilts suggest a possible disruption of enzyme systems necessary for transmission of impulses and which are calcium-dependent. The lowered calcium levels would also impair the release of ACh as observed in the study and may have serious implications for skeletal development and utilization of dietary amino acids.

It thus appears that the depression of calcium levels partly contributed to the lowered protein concentrations observed in the brain regions. This condition may therefore very easily put the animal in a negative nitrogen balance, reduced feed intake and depressed feed conversion efficiency.

Paradoxically, magnesium levels were elevated in the brain regions of the testosterone-treated gilts and probably indicates an attempt to replace or compensate for the depletion of calcium ions especially in the maintenance of the magnesium-dependent metabolic processes involving fats, proteins and carbohydrates. It is however very doubtful if magnesium ions can functionally replace calcium despite the fact that they are both divalent. On the other hand, they may even compete with each other and the magnesium ion-activated enzyme systems may in fact be inhibited by calcium (Dixon and Webb, 1961). Thus the high levels of magnesium observed in the brain of the testosterone-injected gilts may be due to the inability of the brain cells to efficiently utilize the mineral. The relative stability of the brain sodium and copper levels to testosterone injection supports the importance of sodium in maintenance of electrolyte balance and osmotic regulation of the body fluids and copper in the synthesis of red blood cells and iron mobilization. This implies that the brain tries to resist the probable degenerative changes induced by testosterone treatment by using the extra cellular sodium to act as a buffer system and the copper in maintaining oxidative enzymes necessary for the metabolism of the animal.

The elevated potassium and zinc levels may not be easily explained in the light of the preceeding discussion because according to Muntzing et al., (1977) estrogens induce the increased zinc incorporation in the dorsolateral lobe of castrate rats and by its anti-estrogenic activity, testosterone would be expected to depress zinc levels in the brain. However a possible explanation of this anomaly is that the brain being a very sensitive and well protected organ may posses certain mechanisms which tend to protect it from excessive abuse by a disturbance of the metabolic

systems. It is therefore probable that zinc, by virtue of its vital role in metabolism, sexual development and protein synthesis, is retained by the brain cells or allowed to pass in through the blood-brain barrier. It is however also possible that the disruption of the membrane permeability of the brain cells and the blood-brain barrier contribute to these anomalies.

6.4.3 AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY IN THE HYPOPHYSES

The possible influence of testosterone administration on the hypophyses of intact gilts is described by Dorner (1971) that testosterone treatment during the hypothalamic organization phase results in a predominantly male differentiation of these centres and acyclic pituitary gonadotrophin secretion coupled with hypo- or homosexuality in post pubertal life. Schreiber, (1973) also reported that testosterone inhibits the estrogen-induced hypertrophy of the thyroxine-binding capacity of the anterior pituitary proteins.

There is still a further suggestion of identical protein binding sites in the anterior pituitary for estrogens and androgens. The study therefore suggests that testosterone-injected gilts would exhibit impaired sexual behaviour.

However in the present study, the above suggestion could not be confirmed as testosterone injection had no effect on the AChE activity, protein concentration and SAChE activity of the adenohypophyses. Likewise in the neurohypophysis, AChE activity, and protein concentration were unaffected by testosterone injection but SAChE activity was depressed in the neurohypophysis of the testosterone injected gilts.

The present results imply that the disburbance of the estradiol binding sites of the hypothalamus by testosterone would interfere with the release of FSH and LH by the pituitary. However, the lack of effect on the pituitary AChE activity and protein levels suggest that the target cells of testosterone in the female hypothalamus are the only ovarian-steriods receptive areas. This presumably allows the other areas involved in the secretion of releasing and inhibiting factors (the hypophysiotrophic neurohormones) which regulate the release of other hypophyseal hormones to function normally. This view is supported by Martini (1973) who implied in his brilliant work that the hypothalamus contains specific neurons and sites for the production of specific hypophysiotrophic neurohormones.

Thus while testosterone injection of gilts may interfere with the functioning of the hypophysial gonadrotrophs, other cells such as the somatotrophs, lactotrophs, corticotrophs and the thyrotrophs may continue to function at least for sometime.

6.4.4 MINERAL PROFILE IN THE HYPOPHYSES

The depression of calcium and copper levels with the concomitant elevation of magnesium, potassium and zinc in the adenohypophysis may be due to a possible interference in the cellular activity of the gonadotrophs resulting in decreased retention of calcium and copper ions presumably resulting from changes in the enzyme systems involved in oxidative metabolism and transmission of nervous impluses.

The same trend was observed in the neurohypophysis except that magnesium and sodium levels were not affected by testosterone treatment.

This trend may also be due to changes in the transmission of nervous impulses and the calcium activated release of ACh at nerve endings in view of the anatomical connection of the neurohypophysis with the hypothalamus.

The foregoing therefore indicates that testosterone administration on gilts has potentially unfavourable consequences on the animal by its ability to interfere with endocrine functions and enzyme metabolic pathways and has no discernable advantages at the moment. However further studies are necessary to elucidate the direct mechanism by which testosterone exhibits these manifestations in the female animal.

CHAPTER SEVEN

INFLUENCE OF HEAT STRESS AND WATER DEPRIVATION ON PORCINE BRAIN AND HYPOPHYSEAL ACETYLCHOLINESTERASE AND CATIONS

INTRODUCTION

7.1.1 STRESS

Psychologically, stres implies a number of factors, viz:

(1) Some external object which excites it.

(2) Specific feelings characteristic of particular emotions and

(3) The emotion tends to find expression in some characteristic action.

Accompanying the feelings and the motor activities there are (4) certain physiological states in which the autonomic nervous and the endocrine systems play an important part and finally there is often (5) a pre-existing physiological state which is necessary if the appetite or emotional needs is to be experienced. This is most obvious in the case of hunger, thirst and sexual impulse.

7.1.2 HEAT STRESS: ITS EFFECT ON BODY METABOLISM

After prolonged exertion in hot surroundings, the normal rise in body temperature with profuse sweating can be followed by rapid onset of coma, convultions and finally death. Areas affected usually include the floor of the third ventricle or the pons.

The hypothalamus also affects heat regulation, peripheral vasoconstriction, vasodilation and sweating (Grossman, 1960, 1962).

The ability to lose heat by sweating decreases in the following order: Man, horse, camel, cattle, sheep, goat, pig, cats and chicken. Conversely, the ability to lose heat by panting increases in roughly the same order.

7.1.3 WATER DEPRIVATION: EFFECTS ON BODY METABOLISM

Shortage of water causes more immediate and more intolerable distress than shortage of food. Thirst creates a 'dry-sensation' of the mouth and craving for fluid rapidly becomes compelling. As time goes on, the dryness of the mouth increases, production of saliva decreases and finally ceases. Swallowing of food becomes impossible. This is finally followed by delirium and death within a day or two in a dry climate or a little longer in a moist environment (Bell et al., 1972).

Normally, the intake of water in food and drink is so regulated that it balances the loss of water in urine, faeces, sweat and breadth. The body weight and amount of water in the body therefore remains constant. When the rate of water loss is greater than the rate of water replacement, dehydration of the body results and there is a reduction in physical efficiency.

LITERATURE REVIEW

7.1.4 HEAT STRESS

Heat stress is generally a combined effects of temperature and humidity. Air humidity is important because evaporation is inhibited by the reduction in water vapor pressure gradient between the lungs and the air. Studies of the effect of humidity level on the well being of the pig indicate that body heat loss by ventilation becomes increasingly difficult as the humidity level is raised (Ingram, 1965).

It must also be noted that in carrying out its varied functions, blood must maintain homeostasis and homoiothermy in the organism.

A shift in the temperature of the environment beyond a certain range can be expected to bring about gradual quantitative and qualitative changes in certain blood constituents. The reactions which can be observed vary according to the nature, duration and intensity of the climatic stimulus and follow the pattern of the general adaptation syndrome i.e. shock, adpatation and exhaustion.

In the pig, at times of exogenous physical stress e.g. hyperthermy, blood volume increases owing to mobilization of reserves (Steinhardt and Studzinski, 1967). Heat stress is also usually accompanied by a decline in red blood cell count. However, long term heat stress at a low or moderate level seems to lead to haemoconcentration as a result of fluid loss.

The pig is at particular disadvantage under short-term heat stress. Although it can achieve an increase in circulating blood volume and thus lose heat by vasodilation, its weak cardiovascular system is not able under continued stress to circulate the enlarged blood quantity quickely enough thereby ensuring the transport of oxygen and carbon-dioxide.

A rise in ambient temperature leads to increased respiratory activity and a condition of respiratory alkalosis. An increase in metabolic activity causes a rise in the presence of acids or acidosis from which the organism must be protected.

An increase in physical stress creates an acid surplus due to formation of lactate, metabolic and respiratory acidosis which may exhaust the bicarbonate buffer system of the blood resulting in a fall of pH.

The detrimental effect of high ambient temperatures on the growth processes of pigs or sexual development, spermatogenesis and embryonic survival is based on a number of physiological interactions which animal production research has been trying to unravel for many years.

Heat stress during gestation is also known to reduce birth weights in rats and sheep (Cartwright and Thwaites, 1967, Benson and Morsis, 1971, Brown et al., 1977). Heat stress during gestation also reduced placental weight (Alexander and Williams, 1971) and uterine blood flow (Oakes et al., 1976).

Heat stress in swine reduces food intake (Nichols et al., 1980) while sprinkling with water increased food intake and average daily gain. Heat stress exerts a myriad of effects on body functions viz, reproduction, metabolism etc. Hence heat stress affects protein concentration in the blood of the pig via interaction with blood volume. An increase in blood volume arising from vasodilation leads to a drop in total protein concentration as a consequence of haemodilution (Yanga, 1972).

Heat stress also disturbs body metabolism by increasing blood suger levels (Tewes et al., 1981). Heat stress is also known to disturb the activity of some endocrine glands chief among which are the thyroid, hypophyses and the adrenal glands. Thus Brooks et al., (1962) and Yousef et al., (1967) observed a reduction in thyroid function in heat stressed cattle and sheep and an impairment of endocrine dynamics in cattle (Collier et al., 1982).

Acclimatization to raise environmental temperatures is accompanied by a decrease in the basal metabolism (Bedrak et al., 1971) and a lowered concentration of thyroxine and thyrotrophin (Tal and Sulman, 1973) while TRH content of the hypothalamus stays constant (Bedrak et al., 1980).

While Bedrak et al., (1980) observed an increase in the activity of enzymes associated with steriod metabolism with increasing temperature of incubation, they recorded concomitant decline in serum testosterone in heat acclimatized rats. Their explanation is based on the assumption that heat

stress presumably brings about an increase in the rate of androgen catabolism by the liver and kidney. The same workers also observed decreased sperm production and impaired integrity of sertoli cells in heat exposed rats. The results were confirmed by Egbunike and Dede, (1980) who observed that short-term exposure of boars to tropical sunlight resulted in a drop in sperm production and increased sperm abnormalities.

The works of Bedrak et al., (1971), Sod-Moriah and Bedrak, (1976) further suggest that the lowered basal metabolism and increased urinary excretion of steroids induced by heat stress are due to lowered thyroidal function coupled with an increased secretion of ACTH and an increase in the rate of steroid inactivation. In spite of the relatively scanty information on the effect of heat stress on pigs, the impairment of thyroid function seems to be the area that is continuously attracting attention. The exposure of warm blooded animals to cold environments increase thyroid function. The stress-induced inhibition of TSH secretion and elevated ACTH level could arise as a result of a competition between the corticotropin-releasing factor (CRF) and Thyrotropin releasing factor (TRF) at the hypothalamic level so that enhanced release of one principle would necessarily depress the release of the other (Fortier, 1973). Another possibility could involve a competition of a similar type at the pituitary level between ACTH and TSH secretion, the two processes being inversely related, so that stimulation or inhibition of one would have the opposite effect on the other.

It is also presumed that the dorsal hippocampus exerts opposite influences on TSH and ACTH secretions and may be involved, in association with other components of the limbic system, in the stress-induced shift of these secretory activities. This is due to the inhibitory nature of the

hippocampus on the pituitary-adrenocortical system (Endoczi and Lissak 1962; Kawakami et al., 1968).

Thus according to de Wied (1973) the pituitary-adrenal axis is the system "par excellence" of homeostasis and is responsible for the relative freedom which higher organisms exhibit in a constantly changing environment. Stress not only results in the discharge of ACTH from the adenohypophysis but also in the release of vasopressin from the posterior hypophyses. This close relationship between the vasopressor and adrenocorticotropic response to stress led to the hypothesis that vasopressin may be responsible for the release of ACTH.

Another striking evidence is the efficacy with which vasopressin induces the release of ACTH, and the inhibition of ACTH release in animals with extensive lesions in the median eminence of the hypothalamus which at the same time causes diabetes insipidus as the result of the destruction of the hypothalamic-neurohypophyseal connections (McCann, 1957, de Wied et al., 1958).

7.1.5 WATER DEPRIVATION

The sensation of thirst is presumably produced by an increased osmotic pressure of the fluid in cells and calculations based on experiments in man with hypertonic saline show that thirst is produced when about 1 percent of the intracellular water of the body has passed into the extracellular space. The osmoregulator mechanism for antidiuretic hormone (ADH) release and the central thirst mechanism may not be identical. The drinking effect may thus be due to a stimulation of nervous elements specifically sensitive to an elevated sodium chloride concentration of the

internal evironment. The thirst mechanism is therefore probably stituated more posteriorly than the osmoregulators.

Other symptoms of water deprivation as described by Harper, (1981) include nausea, vomiting, a hot and dry body, a dry tongue, loss of co-ordination and a oncentrated urine of small volume. Death usually occurs when the body loses 10 to 20 percent of its water content.

7.1.6 HORMONES AND ELECTROLYTES IN WATER METABOLISM

The brain, the hypophyses, the adrenal glands and the thyroid gland are all involved in the maintenance of water balance in the body. Any attempt to deprive the animal of water therefore results in serious imbalance in the level of circulating hormones and electrolytes. Under normal circumstances, the osmotic pressure of the plasma varies only slightly despite wide variations in the intake of fluid and solutes. The neurohypophysis releases into the blood an antidiuretic hormone (ADH) or vasopressin, the chief action of which is to increase water reabsorption in the distal tubules and collecting ducts.

This eanables the kidney to defend the osmolarity of the plasma. Water starvation therefore leads to elevated ADH secretion and the production of a concentrated urine. This results in an increase in osmotic pressure and an elevation of the sodium ions in the blood due to the fact that the rate of water loss is usually greater than the rate of electrolyte loss.

Experiments by Verney (1958) revealed that osmoreceptors are present in the hypothalamus and stimulation of the hypothalamus stimulates drinking while its destruction abolishes the sense of thirst.

In another experiment, Grossman (1960, 1962a) found that injection of cholingergic stimulants (such as carbachal) into the hypothalamus enhanced water intake in both satiated and water-deprived rats. Contrariwise, injection of cholingergic blocking agents reduced water consumption (Grossman, 1962b). Also Leibowitz (1970) reported that blocking of cholinergic system in the rat hypothalamus increases the thirst sensation and decreases hunger.

7.2.1 MATERIALS AND METHODS

Three experiments were carried out. All the animals used for the experiment were boars farrowed and reared in the physiology unit of the University of Ibadan Farm as earlier described.

Experiment 1

Eight Large White boars weighing between 38 and 48 kg and aged between 6 and 8 months were used. They were randomly assigned to two experimental groups of 4 boars each.

One group (ES) eas exposed to direct sunlight without water or shade for one hour everyday between 1200 to 1300 hours for five days. The other group (CS) were used as controls and kept in the pen throughout the experimental period. Both the ES and CS groups were slaughtered immediately after the last and final exposure hour.

Experimental 2

Twelve Large White boars (6-8 months old) divided into 3 groups of four boars each were used. In the first group, the four boars were exposed to direct sunlight everyday for one hour between 1200 and 1300 hours for a

3-day period (ES3). In group two, all the boars were challenged with the same exposure treatment but this time for an extended period of six days (ES6). In group three (CS) all the four boars were used as controls and kept indoors throughout the duration of the experiment.

In groups one and two, the animals were slaughtered immediately after the last and final exposure hour. The controls were slaughted at the end of the exposure of the ES6 animals.

Experiment 3

Twelve Large White boars made up of four boars for the control and four in each of the two experimental groups were used. In group one, all the four boars were deprived of drinking water for twenty-four hours with free access to food.

In group two, all the four boars were deprived of drinking water for 48 hours also with uninhibited access to food.

The control group was allowed ad lib feeding and drinking.

Immediately after the water deprivation period, experimental group of animals were slaughtered. The controls were slaughtered at the end of the 48 hour water-deprivation of the group two animals.

Immediately after slaughter, the individual brain samples and hypophyses were dissected out and processed as described earlier.

7.2.2 PHYSIOLOGICAL AND CLIMATIC MEASUREMENTS

During exposure, the rectal temperature and respiratory rate of all boars were recorded every fifteen minutes. Also the dry- and wet-bulb temperatures in the open and in the pen were recorded every fifteen minutes using a zeal (Z.H. Zeal, London), Mason's type wet - and dry-bulb

hygrometer and the relative humidy calculated therefrom. The physiologically effective temperature (PET) was calculated by respectively weighting the dry- and wet-bulb temperatures by 0.6 and 0.4 (Ingram, 1965; Steinbach, 1971).

7.2.3. STATISTICAL ANALYSES

The data were subjected to statistical analyses as described in preceeding chapters.

RESULTS

TABLE 7.1.1

CHANGES IN MEAN TEMPERATURE, RELATIVE HUMIDITY, RESPIRTORY RATE, RECTAL TEMPERATURE AND PHYSIOLOGICALLY-EFFECTIVE TEMPERATURE (PET) DURING THE EXPOSURE OF BOARS TO DIRECT SUNSHINE
(Means ± S.E.M.)

	IN THE OPEN (a)	INSIDE THE PEN (b)	LEVEL OF DIFFERENCE BETWEEN (a) and (b)
Air Temperature (°C)	35.255 ± 0.545	30.175 ± 0.476	P<0.001
Relative Humidity %	50.325 ± 0.131	67.850 ± 0.476	P<0.001
P.E.T. (°C)	32.00 ± 0.245	29.3 ± 0.114	P<0.0001
Respiratory Rates (breaths per minute)	122.2 ± 3.419	48.1 ± 2.084	P<0.001
Rectal Temperature (°C)	41.637 ± 0.056	39.475 ± 0.035	P<0.0001

Table 7.1.1 shows the mean temperatures, relative humidities, physiologically-effective temperature (PET), respiratory rates and rectal temperatures in the open and inside the pen.

These results indicate that the above indices were significantly different with respect to the location of the animals at the time the readings were taken. animals in the open displayed considerable signs of hyperthermia exhibited by very erratic movement, profuse salivation and flushing of the face.

7.3.1 INFLUENCE OF SHORT-TERM EXPOSURE TO TROPICAL SUNLIGHT ON AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY IN THE PORCINE BRAIN AND HYPOPHYSES

The results are displayed in Table 7.1.2. Heat stress sharply elivated AChE activity ($P<0.05$) in the pons, cerebellum, amygdala, hippocampus, midbrain, and medulla oblongata while AChE activity in the hypothalamus of heat-stressed animals was inferior to the control.

However, no significant changes were observed in the cerebral cortex, adenohypophysis and neurohypophysis ($P>0.05$). Total protein levels in heat stressed animals were generally inferior to the control group in the pons, cerebellum, hippocampus, hypothalamus, midbrain, and medulla oblongata ($P<0.05$). No significant changes were observed in the amygdala, cerebral cortex, adenohypophysis and the neurohypophysis ($P>0.05$).

The results also indicate that heat stress significantly elevated SAChE activities in all regions except in the cereberal cortex where no significant effect was observed.

7.3.2 INFLUENCE OF SHORT-TERM EXPOSURE TO TROPICAL SUNLIGHT ON THE CARTIONS CONCENTRATION IN THE PORCINE BRAIN AND HYPOPHYSES

The results are summarized in Tables 7.1.3 and 7.1.4.

CALCIUM

Calcium levels were higher in the pons, hippocampus, hypothalamus, midbrain, adenohypophysis and neurohypophysis ($P<0.05$) of the heat stressed animals than the control. Heat stress however depressed calcium level in the amygdala and cerebral cortex ($P<0.05$ while no significant change was observed in the medulla oblongata ($P>0.05$).

TABLE 7.1.2: INFLUENCE OF SHORT-TERM EXPOSURE TO TROPICAL SUNLIGHT ON ACHE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY IN THE PORCINE BRAIN AND HYPOPHYSES.

a) AChE ACTIVITY	ANIMAL GROUPS	
BRAIN REGIONS	Heat stressed	Control
Pons	8.694 ± 0.531 a	4.945 ± 0.642 b
Cerebellum	10.214 ± 1.154 a	6.641 ± 0.444 b
Amygdala	10.365 ± 0.588 a	7.178 ± 0.424 b
Hippocampus	7.156 ± 0.400 a	5.103 ± 0.514 b
Hypothalamus	7.301 ± 0.880 b	9.699 ± 0.616 a
Cerebral cortex	3.050 ± 0.153 a	3.492 ± 0.438 a
Mid brain	14.795 ± 0.866 a	7.956 ± 0.904 b
Medulla oblongata	15.888 ± 1.266 a	10.892 ± 0.766 b
Adenohypophysis	0.664 ± 0.093 a	1.716 ± 0.129 a
Neurohypophysis	0.528 ± 0.028 a	1.578 ± 0.316 a
GRAND MEAN	7.386 ± 2.127	5.920 ± 1.260

b) TOTAL PROTEIN	ANIMAL GROUPS	
BRAIN REGIONS	Heat stressed	Control
Pons	0.990 ± 0.070 b	1.178 ± 0.0 2 a
Cerebellum	0.612 ± 0.066 b	1.264 ± 0.067 a
Amygdala	0.348 ± 0.028 a	0.442 ± 0.016 a
Hippocampus	0.563 ± 0.029 b	1.084 ± 0.121 a
Hypothalamus	0.356 ± 0.023 b	0.573 ± 0.018 a
Cerebral cortex	0.526 ± 0.082 a	0.501 ± 0.019 a
Mid brain	0.207 ± 0.004 b	0.614 ± 0.010 a
Medulla oblongata	0.432 ± 0.015 b	0.580 ± 0.036 a
Adenohypophysis	0.299 ± 0.034 a	0.361 ± 0.029 a
Neurohypophysis	0.126 ± 0.008 a	0.145 ± 0.004 a
GRAND MEAN	0.446 ± 0.098	0.672 ± 0.149

c) SAChE ACTIVITY	ANIMAL GROUPS	
BRAN REGIONS	Heat stressted	Control
Pons	9.020 ± 1.202 a	4.385 ± 0.847 a
Cerebellum	17.038 ± 1.872 a	5.300 ± 0.380 b
Amygdala	29.986 ± 1.033 a	16.017 ± 0.493 b
Hippocampus	12.912 ± 1.416 a	4.734 ± 0.248 b
Hypothalamus	20.963 ± 3.061 a	16.864 ± 1.027 a
Cerebral cortex	6.198 ± 0.938 a	6.952 ± 0.807 a
Mid brain	71.286 ± 6.061 a	12.942 ± 1.416 b
Medulla oblongata	36.654 ± 1.968 a	13.855 ± 1.114 b
Adenohypophysis	2.421 ± 0.446 a	4.793 ± 0.340 a
Neurohypophysis	4.286 ± 0.507 a	10.804 ± 1.911 b
GRAND MEAN	21.077 ± 3.351	10.165 ± 2.265

Values in the same horizontal column bearing different superscripts differ significantly ($P < 0.05$)
*AChE activity μmole/g/min., ** Total protein in g/100 ml
***SAChE activity in μmole/g protein/min.
Values are means ± S.E.M.

TABLE 7.1.3: INFLUENCE OF SHORT-TERM EXPOSURE TO TROPICAL SUNLIGHT ON THE *CALCIUM, MAGNESIUM AND ZINC LEVELS IN THE PROCINE BRAIN AND HYPOPHYSES.

a) CALCIUM	ANIMAL GROUPS	
BRAIN REGIONS	Heat stressed	Control
Pons	1.641 ± 0.054 a	1.500 ± 0.070 b
Cerebellum	1.565 ± 0.012 a	1.476 ± 0.014 a
Amygdala	1.148 ± 0.002 b	1.487 ± 0.024 a
Hipocampus	1.939 ± 0.055 a	1.344 ± 0.016 b
Hypothalamus	2.041 ± 0.040 a	1.681 ± 0.031 b
Cerebral cortex	1.707 ± 0.016 b	1.915 ± 0.013 a
Mid brain	2.014 ± 0.058 a	1.340 ± 0.012 b
Medulla oblongata	1.500 ± 0.020 a	1.580 ± 0.013 a
Adenohypophysis	3.014 ± 0.555 a	2.329 ± 0.002 b
Neurohypophysis	2.171 ± 0.033 a	1.369 ± 0.027 b
GRAND MEAN	1.874 ± 0.201	1.602 ± 0.124

b) MAGNESIUM	ANIMAL GROUPS	
BRAIN REGIONS	Heat stressed	Control
Pons	1.084 ± 0.004 b	1.203 ± 0.003 a
Cerebellum	1.270 ± 0.008 a	1.200 ± 0.017 a
Amygdala	0.936 ± 0.011 b	1.364 ± 0.021 a
Hippocampus	0.689 ± 0.021 a	0.707 ± 0.025 a
Hypothalamus	0.870 ± 0.021 a	0.670 ± 0.025 a
Cerebral cortex	0.815 ± 0.009 b	1.395 ± 0.008 a
Mid brain	1.116 ± 0.113 b	1.308 ± 0.027 a
Medulla oblongata	1.013 ± 0.004 b	1.189 ± 0.001 a
Adenohypophysis	0.939 ± 0.012 a	1.022 ± 0.028 a
Neurohypophysis	1.311 ± 0.009 a	0.450 ± 0.00 b
GRAND MEAN	1.104 ± 0.078	1.051 ± 0.131

c) ZINC	ANIMAL GROUPS	
BRAIN REGIONS	Heat stressed	Control
Pons	0.398 ± 0.009 a	0.366 ± 0.014 a
Cerebellum	0.380 ± 0.016 a	0.382 ± 0.014 a
Amygdala	0.281 ± 0.003 a	0.315 ± 0.010 a
Hippocampus	0.386 ± 0.012 a	0.253 ± 0.013 b
Hypothalamus	0.310 ± 0.005 a	0.289 ± 0.034 a
Cerebral cortex	0.220 ± 0.007 b	0.364 ± 0.005 a
Mid brain	0.308 ± 0.003 a	0.322 ± 0.005 a
Medulla oblongata	0.318 ± 0.003 a	0.308 ± 0.002 a
Adenohypophysis	0.270 ± 0.002 b	0.374 ± 0.015 a
Neurohypophysis	0.264 ± 0.005 b	0.316 ± 0.007 a
GRAND MEAN	0.313 ± 0.023	0.329 ± 0.017

Values in the same horizontal column bearing different superscripts significantly ($P < 0.05$)

Values are in parts per million (ppm).

TABLE 7.1.4: INFLUENCE OF SHORT-TERM EXPOSURE TO TROPICAL SUNLIGHT ON THE *POTASSIUM SODIUM AND COPPER LEVELS IN THE PROCINE BRAIN AND HYPOPHYSES (Mean ± S.E.M).

d) POTASSIUM	ANIMAL GROUPS	
BRAIN REGIONS	Heat stressed	Control
Pons	12.511 ± 0.501 a	13.290 ± 0.464 a
Cerebellum	20.865 ± 0.479 a	21.656 ± 1.135 a
Amygdala	6.262 ± 0.326 b	18.489 ± 0.380 a
Hippocampus	15.512 ± 0.208 a	10.068 ± 0.739 b
Hypothalamus	15.522 ± 0.836 a	11.265 ± 0.479 b
Cerebral cortex	12.964 ± 0.829 a	9.732 ± 0.510 b
Mid brain	15.190 ± 0.103 b	25.314 ± 0.873 a
Medulla oblongata	4.926 ± 0.145 b	17.756 ± 1.052 a
Adenohypophysis	7.542 ± 0.278 a	8.511 ± 0.345 a
Neurohypophysis	8.429 ± 0.504 a	5.337 ± 0.261 b
GRAND MEAN	11.717 ± 1.969	14.142 ± 2.556

e) SODIUM	ANIMAL GROUPS	
BRAIN REGIONS	Heat stressed	Control
Pons	536.312 ± 3.146 a	520.125 ± 8.502 b
Cerebellum	528 ± 1.554 a	536.250 ± 2.529 a
Amygdala	513.720 ± 4.270 b	534.556 ± 2.527 a
Hippocampus	515.000 ± 6.819 b	532.250 ± 2.780 a
Hypothalamus	538.500 ± 6.383 a	539.500 ± 2.954 a
Cerebral cortex	526.000 ± 3.188 a	529.250 ± 4.916 a
Mid brain	525.250 ± 2.689 b	539.125 ± 1.493 a
Medulla oblongata	171.750 ± 7.836 b	537.250 ± 2.287 a
Adenohypophysis	258.000 ± 5.598 b	541.250 ± 2.353 a
Neurohypophysis	540.000 ± 3.535 a	540.00 ± 4.213 a
GRAND MEAN	465.319 ± 53.538	534.956 ± 2.565

f) COPPER	ANIMAL GROUPS	
BRAIN REGIONS	Heat stressed	Control
Pons	0.230 ± 0.003 a	0.088 ± 0.001 a
Cerebellum	0.113 ± 0.004 a	0.091 ± 0.001 a
Amygdala	0.073 ± 0.006 a	0.159 ± 0.004 a
Hippocampus	0.123 ± 0.021 a	0.082 ± 0.003 a
Hypothalamus	0.070 ± 0.002 a	0.104 ± 0.008 a
Cerebral cortex	0.060 ± 0.008 a	0.116 ± 0.020 a
Mid brain	0,070 ± 0.002 a	0.043 ± 0.002 a
Medulla oblongata	0.079 ± 0.003 a	0.054 ± 0.002 a
Adenohypophysis	0.100 ± 0.004 a	0.149 ± 0.001 a
Neurohypophysis	0.140 ± 0.002 a	0.060 ± 0.003 a
GRAND MEAN	0.106 ± 0.020	0.095 ± 0.015

Values in the same horizontal column bearing different superscripts differ significantly ($P < 0.05$)

*Values are in parts per million (ppm).

MAGNESIUM

Heat stress depressed magnesium level in the pons, amygdala, cerebral cortex, mid brain and medulla oblongata and elevated it in the neurohypophysis ($P<0.05$). No significant changes were observed in the cerebellum, hippocampus, hypothalamus and adenohypophysis ($P>0.05$).

ZINC

Heat stress significantly depressed zinc level in both hypophyses and the cerebral cortex and elevated it in the hippocampus ($P<0.05$). No significant changes were observed in the pons, cerebellum, amygdala, hypothalamus, midbrain and medulla oblongata ($P>0.05$).

POTASSIUM

Heat stress significantly elevated potassium level in the hippocampus, hypothalamus, cerebral cortex, neurohypophysis ($P<0.05$) and depressed it in the amygdala, midbrain and medulla oblongata ($P<0.05$). No significant changes were observed in the pons, cerebellum, and adenohypophysis ($P>0.05$).

SODIUM

Sodium level in the amygdala, hippocampus, midbrain, medulla oblongata and adenohyphysis of the heat stressed group were inferior to the control ($P<0.05$). The pons of the heat stressed boars however had a higher sodium level than the control.

No significant differences were observed in the cerebellum, hypothalamus, cerebral cortex and neurohypophysis ($P>0.05$).

COPPER

No significant differences were observed in all the brain regions and the hypophyses (P>0.05).

7.4.1 EFFECT OF ACUTE AND PROLONGED HEAT STRESS ON THE AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY OF THE PORCINE BRAIN AND HYPOPHYSES

The results are summarized in Table 7.1.5. Animals heat stressed for either 3 days or 6 days had similar AChE activities but were significantly higher than the controls in the cerebellum, hypothalamus, cerebral cortex and medulla oblongata. No significant differences (P>0.05) were observed in the amygdala and the hypophyses. However in the pons and hippocampus, the ES3 animals had higher AChE activity levels than the controls while no significant differences were observed between the ES6 group and the control group. Contrariwise, in the midbrain, the ES6 group was superior to the ES3 group which in turn was superior to the control group.

Total protein levels were unaffected by heat stress (P>0.05) in the pons, amygdala, hippocampus, cerebral cortex and adenohypohysis and the neurohypophysis. In the hypothalamus, medulla oblongata and midbrain the ES6 group had higher protein level than the control groups which were similar to the ES3 groups. Surprisingly however, protein level was least in the cerebellum of the ES3 animals, medium in the control group and highest in the ES6 group. SAChE activities were not affected by heat stress (P>0.05) in the pons, cerebral cortex and neurohypophysis.

TABLE 7.1.5: EFFECT OF ACUTE AND PROLONGED HEAT STRESS ON THE AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY OF THE FORCING BRAIN AND HYPOPHYSES (Means ± S.E.M.)

a) *AChE ACTIVITY	ANIMAL GROUPS		
BRAIN REGIONS	HEAT STRESSED		CONTROLS
	3 DAYS	6 DAYS	
Pons	4.418 ± 0.158 a	4.242 ± 0.107 ab	3.746 ± 0.115 b
Cerebellum	3.649 ± 0.165 a	3.851 ± 0.134 a	2.488 ± 0.082 b
Amygdala	7.276 ± 0.478 a	7.025 ± 0.135 a	7.235 ± 0.205 a
Hippocaumpus	5.278 ± 0.077 a	4.166 ± 0.174 b	3.443 ± 0.093 b
Hypothalamus	4.043 ± 0.088 a	4.067 ± 0.056 a	2.542 ± 0.028 b
Cerebral cortex	6.457 ± 0.279 b	8.375 ± 0.385 a	4.632 ± 0.218 c
Mid brain	6.457 ± 0.279 b	8.375 ± 0.385 a	4.632 ± 0.218 c
Medulla oblongata	5.162 ± 0.171 a	5.764 ± 0.086 a	3.546 ± 0.118 b
Adenohypophysis	0.651 ± 0.032 a	0.826 ± 0.044 a	0.865 ± 0.009 a
Neurohypophysis	0.892 ± 0.012 a	1.117 ± 0.036 a	0.949 ± 0.044 a
GRAND MEAN	3.960 ± 1.130	4.096 ± 1.246	3.170 ± 0.930

b) **TOTAL PROTEIN	ANIMAL GROUPS		
BRAIN REGIONS	HEAT STRESSED		CONTROLS
	3 DAYS	6 DAYS	
Pons	0.611 ± 0.024 a	0.698 ± 0.012 a	0.568 ± 0.019 a
Cerebellum	0.096 ± 0.005 c	0.370 ± 0.017 a	0.257 ± 0.018 b
Amygdala	0.177 ± 0.006 a	0.257 ± 0.004 a	0.249 ± 0.008 a
Hippocampus	0.202 ± 0.014 a	0.258 ± 0.006 a	0.154 ± 0.008 a
Hypothalamus	0.131 ± 0.004 b	0.283 ± 0.010 a	0.194 ± 0.009 ab
Cerebral cortex	0.212 ± 0.022 a	0.230 ± 0.006 a	0.298 ± 0.009 ab
Mid brain	0.294 ± 0.006 ab	0.400 ± 0.014 a	0.191 ± 0.005 b
Medulla oblongata	0.458 ± 0.022 b	0.707 ± 0.009 a	0.462 ± 0.032 b
Adenohypophysis	0.182 ± 0.004 a	0.116 ± 0.003 a	0.170 ± 0.007 a
Neurohypophysis	0.176 ± 0.005 a	0.191 ± 0.005 a	0.202 ± 0.003 a
GRAND MEAN	0.254 ± 0.081	0.351 ± 0.101	0.274 ± 0.067

c) ***SAChE ACTIVITY	ANIMAL GROUPS		
BRAIN REGIONS	HEAT STRESSED		CONTROLS
	3 DAYS	6 DAYS	
Pons	7.293 ± 0.544 a	6.085 ± 0.225 a	6.607 ± 0.254 a
Cerebellum	38.596 ± 2.064 a	10.474 ± 0.687 b	9.827 ± 0.772 b
Amygdala	41.060 ± 1.408 a	27.338 ± 0.416 b	29.180 ± 1.310 b
Hippocampus	20.217 ± 1.402 a	16.150 ± 0.547 b	22.623 ± 1.911 a
Hypothalamus	30.936 ± 1.467 a	14.419 ± 0.682 b	13.095 ± 0.564 b
Cerebral cortex	8.486 ± 1.143 a	6.678 ± 0.260 a	7.573 ± 0.154 a
Mid brain	22.074 ± 0.889 ab	20.395 ± 0.220 a	24.185 ± 0.869 b
Medulla oblongata	11.388 ± 0.894 a	8.161 ± 0.220 b	7.829 ± 0.778 b
Adenohypophysis	3.598 ± 0.271 b	7.155 ± 0.517 a	5.119 ± 0.190 b
Neurohypohysis	5.061 ± 0.102 a	5.857 ± 0.119 a	4.710 ± 0.163 a
GRAND MEAN	18.865 ± 6.988	12.271 ± 3.611	13.075 ± 4.464

Values in the same horizontal line differently superscripted differ significantly ($P < 0.05$)
*AChE activity in umole/g/min; ** Total protein in g/100 ml
***SAChE Activity in umole/g protein/min.

SAChE activities in the CS and ES6 groups were similar and inferior to the ES3 group in the cerebellum, amygdala, hypothalamus and midbrain while in the hippocampus, heat stress for six days significantly depressed SAChE activity and elevated it in the adenohypophysis. In the midbrain, the ES6 group was similar to the ES3 but superior to the CS.

7.4.2 EFFECT OF ACUTE AND PROLONGED HEAT STRESS ON THE MINERAL PROFILE IN THE BRAIN AND HYPOPHYSES

CALCIUM

The results are summarized in Tables 7.1.6 and 7.1.7. Animals heat stressed for 6 days had significantly higher calcium levels than the animals heat stressed for 3 days which were in turn superior to the control in the hippocampus, hypothalamus, medulla oblongata and the adenohypophysis ($P<0.05$).

In the cerebellum and midbrain, the ES3 and CS groups were similar and inferior to the ES6 group. In the amygdala and the cerebral cortex, calcium level was highest in the ES group, medium in the CS group and least in the ES3 group ($P<0.05$).

Heat stress for 3 days depressed calcium level in the pons while the ES6 animals were unaffected. In the neurohypophysis however, both the ES3 and ES6 animals were similar and superior to the control group.

MAGNESIUM

The three-day heat stress period resulted in a decline in magnesium level in the pons, midbrain and neurohypophysis ($P<0.05$) while the six-day heat stress period had no effect.

TABLE 7.1.6: EFFECT OF ACUTE AND PROLONGED HEAT STRESS ON THE *CALCIUM, MAGNESSIUM AND ZINC LEVELS IN THE PROCINE BRAIN AND HYPOPHYSES (Means ± S.E.M.)

a) CALCIUM	ANIMAL GROUPS		
BRAIN REGIONS	HEAT STRESSED		CONTROLS
	3 DAYS	6 DAYS	
Pons	0.954 ± 0.031 b	1.407 ± 0.014 a	1.464 ± 0.076 a
Cerebellum	0.977 ± 0.017 b	2.470 ± 0.031 a	1.001 ± 0.018 b
Amygdala	0.745 ± 0.045 c	1.380 ± 0.006 a	1.110 ± 0.031 b
Hippocampus	1.205 ± 0.082 b	2.041 ± 0.047 a	0.877 ± 0.035 c
Hypothalalmus	1.538 ± 0.086 b	1.860 ± 0.055 a	1.134 ± 0.070 c
Cerebral cortex	0.720 ± 0.008 c	2.019 ± 0.022 a	1.171 ± 0.085 b
Mid brain	1.129 ± 0.010 b	1.905 ± 0.047 a	1.190 ± 0.063 b
Medulla oblongata	1.275 ± 0.019 b	2.162 ± 0.088 a	0.867 ± 0.040 c
Adenohypophysis	1.742 ± 0.019 b	2.032 ± 0.023 a	1.247 ± 0.051 c
Neurohypophysis	1.903 ± 0.031 a	1.932 ± 0.038 a	1.306 ± 0.072 b
GRAND MEAN	1.219 ± 0.201	1.921 ± 0.162	1.137 ± 0.092

b) MAGNESSIUM	ANIMAL GROUPS		
BRAIN REGIONS	HEAT STRESSED		CONTROLS
	3 DAYS	6 DAY	
Pons	0.881 ± 0.017 a	0.649 ± 0.088 b	0.566 ± 0.062 b
Cerebellum	0.852 ± 0.059 a	0.911 ± 0.105 a	0.720 ± 0.040 a
Amygdala	0.980 ± 0.036 a	1.050 ± 0.099 a	0.562 ± 0.085 b
Hipocampus	0.701 ± 0.022 a	0.911 ± 0.070 a	0.511 ± 0.043 c
Hypothalamus	0.845 ± 0.022 a	0.745 ± 0.086 a	0.234 ± 0.071 b
Cerebral cortex	1.058 ± 0.026 a	0.761 ± 0.096 b	0.501 ± 0.022 c
Mid brain	0.695 ± 0.010 a	0.431 ± 0.065 b	0.341 ± 0.027 b
Medulla oblongata	0.762 ± 0.019 b	1.217 ± 0.036 a	0.269 ± 0.036 c
Adenohypophysis	1.526 ± 0.091 a	1.125 ± 0.111 b	0.617 ± 0.029 c
Neurohypophysis	1.400 ± 0.071 a	1.062 ± 0.124 b	0.961 ± 0.055 b
GRAND MEAN	0.969 ± 0.142	0.886 ± 0.121	0.528 ± 0.108

c) ZINC	ANIMAL GROUPS		
BRAIN REGIONS	HEAT STRESSED		CONTROLS
	3 DAYS	6 DAYS	
Pons	0.026 ± 0.002 b	0.032 ±+0.002 b	0.201 ± 0.005 a
Cerebellum	0.047 ± 0.003 b	0.019 ± 0.002 b	0.106 ± 0.002 a
Amygdala	0.401 ± 0.032 a	0.259 ± 0.024 b	0.151 ± 0.001 c
Hippocampus	0.201 ± 0.010 a	0.067 ± 0.008 c	0.113 ± 0.001 b
Hypothalamus	0.073 ± 0.004 a	0.020 ± 0.002 b	0.070 ± 0.004 a
Cerebral cortex	0.326 ± 0.019 a	0.049 ± 0.002 c	0.109 ± 0.003 b
Mid brain	0,074 ± 0.004 a	0.016 ± 0.002 b	0.074 ± 0.002 a
Medulla oblongata	0.021 ± 0.004 b	0.064 ± 0.004 a	0.078 ± 0.001 a
Adenohypophysis	0.494 ± 0.025 b	0.706 ± 0.030 a	0.377 ± 0.020 c
Neurohypophysis	0.502 ± 0.021 a	0.480 ± 0.024 a	0.189 ± 0.008 b
GRAND MEAN	0.216 ± 0.099	0.171 ± 0.124	0.147 ± 0.046

Values in the same horizontal line bearing different superscripts differ significantly ($P < 0.05$)

*Values are in parts per million (ppm)

Table 7.1.7 EFFECT OF ACUTE, AND PROLONGED HEAT STRESS ON THE POTASSIUM, SODIUM AND COPPER LEVELS IN THE PORCINE BRAIN AND HYPOPHYSES (Means = S.E.M.)

POTASSIUM BRAIN REGIONS	ANIMAL GROUPS HEAT STRESSED 3 days	6 days	CONTROL
Pons	16.050 ± 1.415a	12.875 ± 0.314b	12.500 ± 1.085b
Cerebellum	15.500 ± 0.612a	14.875 ± 0.125a	11.200 ± 0.515b
Amygdala	16.875 ± 0.875a	17.375 ± 0.375a	10.850 ± 0.656b
Hippocampus	16.000 ± 0.718a	13.750 ± 0.595b	10.000 ± 0.722c
Hypothalamus	15.625 ± 0.289a	14.625 ± 0.688a	6.725 ± 0.395b
Cerebra Cortex	16.375 ± 0.554a	14.625 ± 0.554ab	12.837 ± 0.827b
Mid Brain	11.125 ± 0.826a	10.500 ± 0.645a	8.300 ± 0.212b
Medulla Oblongata	12.750 ± 0.520a	16.000 ± 0.473b	6.680 ± 0.289c
Adenohypophysis	11.750 ± 0.433b	11.375 ± 0.746b	15.250 ± 0.898a
Neurohypophysis	19.125 ± 0.427a	19.500 ± 0.645a	13.212 ± 0.635b
GRAND MEAN	15.117 ± 1.24	14.550 ± 1.340	10.755 ± 1.427

SODIUM BRAIN REGIONS	3 days	6 days	CONTROL
Pons	290.000 ± 0.607a	270.025 ± 4.516a	292.000 ± 9.120a
Cerebellum	269.750 ± 8.370b	271.500 ± 7.963b	301.500 ± 8.694a
Amygdala	288.500 ± 10.905a	296.000 ± 3.674a	283.250 ± 15.146a
Hippocampus	294.350 ± 8.602a	252.250 ± 8.066b	300.750 ± 8.835a
Hypothalamus	292.000 ± 8.042ab	275.750 ± 5.138b	304.500 ± 10.380a
Cerebral Cortex	275.750 ± 4.564b	265.000 ± 6.455b	300.000 ± 5.401a
Mid Brain	280.500 ± 4.113b	314.750 ± 5.252a	316.750 ± 11.346a
Medulla Oblongata	293.000 ± 5.972a	297.500 ± 10.491a	307.500 ± 5.010a
Adenohypophysis	312.500 ± 8.540a	285.000 ± 6.455b	296.970 ± 2.926ab
Neurohypophysis	322.500 ± 4.790a	322.500 ± 2.500a	296.500 ± 5.694a
GRAND MEAN	291.885 ± 7.931	285.027 ± 11.221	299.972 ± 4.490

COPPER BRAIN REGIONS	3 days	6 days	CONTROL
Pons	0.134 ± 0.004a	0.171 ± 0.002a	0.167 ± 0.003a
Cerebellum	0.137 ± 0.003a	0.159 ± 0.003a	0.167 ± 0.002a
Amygdala	0.125 ± 0.003a	0.151 ± 0.002a	0.129 ± 0.006a
Hippocampus	0.166 ± 0.005a	0.125 ± 0.003b	0.169 ± 0.005a
Hypothalamus	0.182 ± 0.006a	0.166 ± 0.008a	0.167 ± 0.009a
Cerebral Cortex	0.142 ± 0.010a	0.159 ± 0.004a	0.175 ± 0.008a
Mid Brain	0.144 ± 0.005a	0.112 ± 0.001a	0.126 ± 0.009a
Medulla Oblongata	0.162 ± 0.005a	0.106 ± 0.001b	0.150 ± 0.017a
Adenohypophysis	0.150 ± 0.002a	0.162 ± 0.006a	0.147 ± 0.007a
Neurohypophysis	0.161 ± 0.001a	0.150 ± 0.003a	0.166 ± 0.004a
GRAND MEAN	0.150 ± 0.009	0.146 ± 0.012	0.156 ± 0.009

Values in the same horizontal line bearing different superscripts significantly differ ($P<0.05$).

Values are [illegible] per million (ppm).

Magnesium level in the amygdala and hypothalamus of the heat stressed groups were similar and superior to the control ($P<0.05$).

In the cerebral cortex and adenohypophysis, magnesium level was highest in the ES3 group, medium in the ES6 group and least in the CS group.

In the hippocampus and medulla oblongata, the ES6 group were superior to the ES3 group whch in turn were superior to the CS group. No significant effects were observed in the cerebellum ($P>0.05$).

ZINC

Heat stress reulted in a significant delcine in zinc level in the pons and cerebellum while the reverse occured in the neurohypophysis. Results in other brain regions indicate that heat stress for 3 days was more potent in elevating zinc level in the amygdala, hypothalamus and midbrain than heat stress for a 6-day period. However, in the adenohypophysis the six-day heat stress period had a more potent effect in elevating zinc level than the 3-day stress period.

In the cerebral cortex and hippocampus, zinc was highest in the ES6 group while in the medulla oblongata, zinc level in both the ES6 and CS animals were similar and inferior to the ES3 group ($P<0.05$).

POTASSIUM

The results indicate that heat stress significantly elevated potassium levels in the cerebellum, amygdala, hypothalamus, midbrain and neurohypophysis ($P<0.05$) whereas in the cerebral cortex and pons, only the ES3 animals had significantly higher level than the other two groups.

In the medulla oblongata, and hippocampus, potassium was highest in the ES3 group, medium in the ES6 and least in the CS groups. In the adenohypophysis, heat stress significantly depressed potassium level ($P<0.05$).

SODIUM

While no significant treatment effects were observed in the pons, amygdala, medulla oblongata, neurohypophysis ($P>0.05$), other brain regions were appreciably affected.

Thus in the cerebellum and cerebral cortex, the heat stressed groups had significantly depressed sodium levels than the control ($P<0.05$). In the hippocampus and adenohypophysis, sodium levels in the ES3 and the CS goups were similar and superior to the ES6 groups. Heat stress for 3 days significantly depressed sodium levels in the midbrain ($P<0.05$) while the 6-day stress period had no significant effect ($P>0.05$). In the hypothalamus, the six-day stress period was more potent in depressing sodium levels than the 3-day stress period.

COPPER

Only the six-day heat stress period significantly depressed copper levels in the medulla oblongata and hippocampus. ($P<0.05$). No significant effects were observed in the other regions ($P>0.05$).

7.5.1 EFFECT OF ACUTE WATER DEPRIVATION ON THE AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY IN THE PORCINE BRAIN AND HYPOPHYSES

The results are summarized in Table 7.1.8. While no treatment effects were observed in the cerebral cortex, adenohypophysis and the neurohypophysis

($P>0.05$), appreciable results were obtained in other brain regions. Thus in the amygdala, medulla oblongata and hypothalamus, water deprivation significantly depressed AChE actitivy but to varying extents depending on the duration of water deprivation. For instance, in the amygdala, water deprivation for 24 hours significantly depressed AChE activity ($P<0.05$) while it was partially restored after 48 hours of water deprivation. In the hippocampus, water deprivation for 24 hours had no significant effect ($P>0.05$) while the 48-hour deprivation depressed AChE activity ($P<0.05$). In the medulla oblongata and pons, water deprivation for 24 hours depressed AChE activity and was restored after 48 hours.

In the midbrain, animals water-deprived for 24 hours had significantly depressed AChE activity ($P<0.05$) much more than the 48 hour water deprivation did.

In the cerebellum, water deprivation for 24 hours elevated SAChE activity ($P<0.05$) but was depressed after water deprivation for 48 hours.

Generally, water deprivation caused a delcine in protein levels. More specifically, water deprivation for 24 hours was more potent in depressing total protein in the pons and hypothalamus than the 48-hour period ($P<0.05$). In the cerebellum, total protein was only depressed in the group deprived of water for 24 hours. The other group was unaffected ($P>0.05$)> In the amygdala, water deprivation caused a rise in total protein level but only the 24-hour water-deprived group displayed a significant effect. No particularly consistent trend was observed in the cerebral cortex where as shown, the animals water-deprived for 24 hours were superior to the control whereas the 48-hour-water-deprived group were inferior to the control.

TABLE 7.1.8 EFFECT OF ACUTE WATER DEPRIVATION ON THE AChE ACTIVITY, TOTAL PROTEIN AND SAChE ACTIVITY IN THE PORCINE BRAIN AND HYPOPHYSES (Mean ± S.E.M.)

AChE ACTIVITY BRAIN REGIONS	ANIMAL GROUPS WATER DEPRIVED 24 hours	48 hours	CONTROL
Pons	3.436 ± 0.923c	6.058 ± 0.037a	4.374 ± 0.224b
Cerebellum	4.601 ± 0.297a	2.610 ± 0.267c	3.386 ± 0.196b
Amygdala	4.189 ± 0.340c	6.833 ± 0.446b	8.081 ± 0.044a
Hippocampus	4.010 ± 0.230a	3.332 ± 0.489b	4.252 ± 0.256a
Hypothalamus	4.924 ± 0.150b	5.073 ± 0.134b	6.666 ± 0.064a
Cerebral Cortex	1.489 ± 0.110a	1.748 ± 0.055a	1.893 ± 0.051a
Mid Brain	7.006 ± 0.073a	6.339 ± 0.424b	5.614 ± 0.286c
Medulla Oblongata	3.866 ± 0.199b	5.317 ± 0.072a	4.847 ± 211a
Adenohypophysis	0.511 ± 0.025a	0.384 ± 0.016a	0.865 ± 0.009a
Neurohypophysis	0.770 ± 0.030a	1.018 ± 0.232a	0.949 ± 0.044a
GRAND MEAN	3.480 ± 1.012	3.871 ± 1.177	4.093 ± 1.192
TOTAL PROTEIN BRAIN REGIONS	**24 hours**	**48 hours**	**CONTROL**
Pons	0.372 ± 0.021c	0.522 ± 0.020b	0.670 ± 0.019a
Cerebellum	0.149 ± 0.010b	0.219 ± 0.026a	0.239 ± 0.010a
Amygdala	0.257 ± 0.008a	0.225 ± 0.010ab	0.198 ± 0.007b
Hippocampus	0.255 ± 0.013b	0.153 ± 0.008c	0.398 ± 0.022a
Hypothalamus	0.121 ± 0.009c	0.179 ± 0.017b	0.258 ± 0.014a
Cerebral Cortex	0.331 ± 0.015a	0.192 ± 0.002c	0.256 ± 0.008b
Mid Brain	0.325 ± 0.017b	0.213 ± 0.082c	0.490 ± 0.025a
Medulla Oblongata	0.625 ± 0.040b	0.524 ± 0.036c	0.679 ± 0.020a
Adenohypophysis	0.151 ± 0.008b	0.468 ± 0.026a	0.170 ± 0.007b
Neurohypophysis	0.175 ± 0.009b	0.326 ± 0.043a	0.202 ± 0.003b
GRAND MEAN	0.276 ± 0.075	0.302 ± 0.074	0.356 ± 0.097
SAChE ACTIVITY BRAIN REGIONS	**24 hours**	**48 hours**	**CONTROL**
Pons	9.165 ± 0.359ab	11.647 ± 0.551a	6.571 ± 0.493b
Cerebellum	31.578 ± 4.455a	11.971 ± 0.197b	14.240 ± 1.146b
Amygdala	16.247 ± 0.887c	30.252 ± 0.825b	40.766 ± 1.535a
Hippocampus	15.732 ± 0.142b	22.274 ± 4.500a	13.354 ± 0.426c
Hypothalamus	40.861 ± 1.753a	29.114 ± 3.725b	25.980 ± 1.067b
Cerebral Cortex	4.486 ± 0.12b	9.080 ± 0.195a	7.413 ± 0.207ab
Mid Brain	21.699 ± 1.390b	31.601 ± 8.282a	16.771 ± 0.532c
Medulla Oblongata	6.193 ± 0.078b	10.242 ± 0.813a	7.174 ± 0.451ab
Adenohypophysis	3.423 ± 0.316ab	0.821 ± 0.010b	5.119 ± 0.190a
Neurohypophysis	4.432 ± 0.386a	3.242 ± 1.012a	4.642 ± 0.163a
GRAND MEAN	15.382 ± 6.362	16.024 ± 5.694	14.203 ± 5.723

Values in the same horizontal line differently superscripted differ significantly ($P<0.05$).

*AChE activity in N mole/g/min

**Total protein in g/100ml

***SAChE activity in N mole/g protein/min

In the hypophyses however, water-deprivation for 48 hours significantly raised protein level ($P<0.05$) while the other two groups were unaffected ($P>0.05$).

Water deprivation generally elevated SAChE activities in the various brain regions to varying extents ($P<0.05$) except in the neurohypophysis where no significant changes were observed ($P>0.05$), and in the amygdala where the control group recorded the highest SAChE activity levels. The 24-hour water deprivation period was more potent in elevating SAChE activity above normal than the 48-hour water deprivation period in the cerebellum, hypothalamus, and adenohypophysis ($P<0.05$), while the reverse was observed in the pons, hippocampus, medulla oblongata and the midbrain. No particularly consistent trend was observed in the cerebral cortex.

7.5.2 EFFECT OF ACUTE WATER DEPRIVATION ON THE MINERAL PROFILE IN THE PORCINE BRAIN AND HYPOPHYSES

The results are summarized in Tables 7.1.9. and 7.2.0.

CALCIUM

While water deprivation for 24-hours did not significantly affect the cerebellum and cerebral cortex ($P>0.05$), the 48-hour water deprivation significatly lowered calcium levels below normal ($P<0.05$).

In the amygdala, midbrain and hippocampus, calcium levels were highest in the 24-hours water-deprived group, medium in the controls and least in the 48-hours water-deprived group.

TABLE 7.1.9 EFFECT OF ACUTE WATER DEPRIVATION ON THE CALCIUM, MAGNESIUM AND ZINC LEVELS IN THE PORCINE BRAIN AND HYPOPHYSES (Mean ± S.E.M).

CALCIUM BRAIN REGIONS	ANIMAL GROUPS WATER DEPRIVED 24 hours	48 hours	CONTROL
Pons	1.301 ± 0.022b	1.194 ± 0.008c	1.464 ± 0.076a
Cerebellum	1.047 ± 0.014a	0.801 ± 0.027b	1.011 ± 0.018a
Amygdala	1.267 ± 0.048a	0.809 ± 0.033c	1.110 ± 0.031b
Hippocampus	1.491 ± 0.094a	0.752 ± 0.033c	0.877 ± 0.035b
Hypothalamus	1.485 ± 0.341a	1.245 ± 0.047b	1.134 ± 0.070b
Cerebral Cortex	1.158 ± 0.042a	0.849 ± 0.035b	1.171 ± 0.085a
Mid Brain	1.378 ± 0.114a	0.921 ± 0.081c	1.190 ± 0.063b
Medulla Oblongata	1.980 ± 0.088a	0.761 ± 0.030b	0.867 ± 0.040b
Adenohypophysis	1.797 ± 0.017a	1.296 ± 0.030b	1.247 ± 0.051b
Neurohypophysis	1.850 ± 0.048a	1.474 ± 0.010b	1.306 ± 0.072c
GRAND MEAN	1.475 ± 0.155	1.010 ± 0.133	1.138 ± 0.092
MAGNESIUM BRAIN REGIONS	24 hours	48 hours	CONTROL
Pons	1.252 ± 0.130a	0.789 ± 0.014c	0.964 ± 0.051b
Cerebellum	0.992 ± 0.095a	0.746 ± 0.078b	1.032 ± 0.071a
Amygdala	1.015 ± 0.028a	0.917 ± 0.082a	0.579 ± 0.100b
Hippocampus	1.059 ± 0.135a	0.752 ± 0.056b	0.697 ± 0.096b
Hypothalamus	1.113 ± 0.068a	0.797 ± 0.032b	0.800 ± 0.064b
Cerebral Cortex	1.255 ± 0.023a	0.529 ± 0.071c	0.761 ± 0.053b
Mid Brain	1.255 ± 0.041a	0.866 ± 0.041b	0.750 ± 0.046b
Medulla Oblongata	1.484 ± 0.084a	0.750 ± 0.046b	0.550 ± 0.068c
Adenohypophysis	1.322 ± 0.032a	0.862 ± 0.012b	0.617 ± 0.029c
Neurohypophysis	1.254 ± 0.013a	1.104 ± 0.085ab	0.961 ± 0.055b
GRAND MEAN	1.200 ± 0.077	0.811 ± 0.073	0.771 ± 0.085
ZINC BRAIN REGIONS	24 hours	48 hours	CONTROL
Pons	0.377 ± 0.015a	0.085 ± 0.007b	0.126 ± 0.014b
Cerebellum	0.245 ± 0.009a	0.106 ± 0.004b	0.115 ± 0.005b
Amygdala	0.189 ± 0.007a	0.103 ± 0.002b	0.167 ± 0.009a
Hippocampus	0.427 ± 0.010a	0.106 ± 0.006b	0.147 ± 0.008b
Hypothalamus	0.449 ± 0.012a	0.181 ± 0.019c	0.339 ± 0.028b
Cerebral Cortex	0.894 ± 0.009a	0.104 ± 0.004b	0.124 ± 0.006b
Mid Brain	0.846 ± 0.028a	0.100 ± 0.002b	0.108 ± 0.007b
Medulla Oblongata	0.467 ± 0.031a	0.077 ± 0.007c	0.139 ± 0.005b
Adenohypophysis	0.445 ± 0.015b	0.526 ± 0.035a	0.088 ± 0.006c
Neurohypophysis	0.592 ± 0.089b	0.673 ± 0.013a	0.432 ± 0.008c
GRAND MEAN	0.493 ± 0.114	0.206 ± 0.106	0.178 ± 0.057

Values in the same horizontal line differently superscripted differ significantly ($P<0.05$).

*Values are in parts per million (ppm).

TABLE 7.2.0 EFFECT OF ACUTE WATER DEPRIVATION ON THE POTASSIUM, SODIUM AND COPPER LEVELS IN THE PORCINE BRAIN AND HYPOPHYSES (Mean ± S.E.M.)

POTASSIUM BRAIN REGIONS	ANIMAL GROUPS WATER DEPRIVED 24 hours	48 hours	CONTROL
Pons	14.300 ± 1.328a	15.775 ± 1.212a	15.625 ± 0.796a
Cerebellum	17.000 ± 0.115b	15.000 ± 0.393b	21.225 ± 1.314a
Amygdala	17.012 ± 3.165a	17.625 ± 2.048a	10.550 ± 0.368b
Hippocampus	18.112 ± 0.517a	15.125 ± 1.167b	16.775 ± 0.639ab
Hypothalamus	15.912 ± 1.444a	14.775 ± 1.241a	11.500 ± 0.408b
Cerebral Cortex	19.750 ± 0.882a	14.200 ± 0.536b	14.425 ± 0.285b
Mid Brain	12.300 ± 0.892b	13.750 ± 1.453b	17.125 ± 1.083a
Medulla Oblongata	14.450 ± 1.097a	12.450 ± 1.312a	14.275 ± 0.714a
Adenohypophysis	25.175 ± 0.788a	11.750 ± 1.302b	12.302 ± 1.045b
Neurohypophysis	26.750 ± 1.202a	13.875 ± 0.607b	13.212 ± 0.635b
GRAND MEAN	18.076 ± 2.334	14.432 ± 0.831	14.701 ± 1.575

SODIUM BRAIN REGIONS	24 hours	48 hours	CONTROL
Pons	292.500 ± 15.595a	295.000 ± 11.547a	279.750 ± 6,129a
Cerebellum	290.500 ± 4.333a	293.750 ± 9.280a	301.250 ± 5.154a
Amygdala	302.500 ± 14.530a	292.500 ± 14.530a	305.500 ± 11.449a
Hippocampus	297.750 ± 21.657a	267.500 ± 7.638b	291.750 ± 8.929ab
Hypothalamus	308.250 ± 11.667a	301.750 ± 14.450a	305.750 ± 6.169a
Cerebral Cortex	314.750 ± 10.171a	308.000 ± 8.718a	308.000 ± 7.681a
Mid Brain	277.250 ± 4.702b	300.500 ± 14.622ab	306.250 ± 11.614a
Medulla Oblongata	254.500 ± 9.939b	280.250 ± 3.480ab	300.250 ± 3.391a
Adenohypophysis	281.500 ± 21.940a	273.750 ± 7.638a	296.972 ± 2.926a
Neurohypophysis	285.750 ± 11.591a	292.500 ± 8.570a	296.500 ± 5.694a
GRAND MEAN	290.525 ± 8.626	290.550 ± 6.425	299.197 ± 4.285

COPPER BRAIN REGIONS	24 hours	48 hours	CONTROL
Pons	0.149 ± 0.008a	0.086 ± 0.012b	0.167 ± 0.003a
Cerebellum	0.160 ± 0.006a	0.118 ± 0.008b	0.168 ± 0.002a
Amygdala	0.146 ± 0.002a	0.075 ± 0.005b	0.129 ± 0.006a
Hippocampus	0.162 ± 0.003a	0.070 ± 0.005b	0.169 ± 0.005a
Hypothalamus	0.159 ± 0.007a	0.058 ± 0.002b	0.167 ± 0.009a
Cerebral Cortex	0.115 ± 0.004b	0.106 ± 0.010b	0.175 ± 0.008a
Mid Brain	0.114 ± 0.006a	0.078 ± 0.003b	0.126 ± 0.009a
Medulla Oblongata	0.220 ± 0.013a	0.060 ± 0.004c	0.150 ± 0.017b
Adenohypophysis	0.322 ± 0.023a	0.181 ± 0.011c	0.147 ± 0.007b
Neurohypophysis	0.286 ± 0.011a	0.199 ± 0.002b	0.166 ± 0.004c
GRAND MEAN	0.183 ± 0.035	0.103 ± 0.025	0.156 ± 0.009

Values in the same horizontal line differently superscripted differ significantly ($P<0.05$).

*Values are in parts per million (ppm).

In the hypothalamus, medulla oblongataland adenohypophysis, the 48-hours lwater deprived gorup and the controls were similar and inferior to the 24-hours water-deprived group ($P<0.05$).

In the ons, calcium was highest in the controls, medium and least in the 24-hours and 48-hours water-deprived groups respectively ($P<0.05$), while in the neurohypophysis, calcium was highest in the 24-hours water-deprived group, medium in the 48-hours water-deprived group and least in the controls.

MAGNESIUM

The forty-eight hours water deprived and control groups were similar and inferior to the twenty-four hour water deprived group in the hippocampus, hypothalamus and midbrain ($P<0.05$).

In the medulla oblongata and adenohypophysis, magnesium was highest in the 24-hours water deprived group, medium and least in the 48 hours water-deprived and control groups respectively ($P<0.05$).

Water deprivation also elevated magnesium level in the amygdala ($P<0.05$) while in the cerebellum the 48-hours water deprived group was inferior to the other two groups, which were similar. In the pons and cerebral cortex, water deprivation for 24-hours significantly elevated magnesium level above normal ($P<0.05$) while the 48-hours water deprived group had significantly depressed magnesium level. The 24 hours water deprived group was also superior to the control in the neurohypophysis ($P<0.05$).

ZINC

Zinc levels in the 48-hours water-deprived group and the control group were similar and inferior to the 24-hours water deprived group in the pons, cerebellum, cerebral cortex, midbrain and hippocampus ($P<0.05$). In the hypothalamus medulla oblongata however, water deprivation for 24-hours significantly elevated zinc levels while the 48-hours water-deprivation period depressed it ($P<0.05$). Water deprivation for 48 hours also significantly depressed zinc level in the amygdala ($P<0.05$).

In the hypophyses, zinc was highest in the 48-hours water-deprived group, medium and least in the 24-hours water-deprived and the control groups respectively ($P<0.05$).

POTASSIUM

Water deprivation significantly elevated potassium levels in the amygdala and hypothalamus ($P<0.05$) while the reverse ocurred in the cerebellum and midbrain. In the cerebral cortex, hippocampus and the hypophyses, the 24-hour deprivation period caused a significant increase in potassium level over the other two treatments while no significant effects were observed in the pons and medulla oblongata ($P>0.05$).

SODIUM

In the midbrain and medulla oblongata, water-deprivation for the 24-hour period caused a significant decline ($P<0.05$), in sodium levels while the other group was unaffected ($P>0.05$). In the hippocampus, the decline in sodium level was noticed only in the group water-deprived for 48 hours.

The other regions were not significantly affected ($P>0.05$).

COPPER

In the pons, cerebellum, amygdala, hippocampus, hypothalamus and midbrain, copper levels were significantly depresed by the 48-hour water-deprivation treatment ($P<0.05$) whereas the 24-hour water-deprivation treatment had no significant effect ($P>0.05$).

In the medulla oblongata and adenohypophysis, water-deprivation for 24 hours resulted in an increase in coper levels ($P<0.05$), while the 48 hours water deprivation treatment caused a significant decline ($P<0.05$).

Water deprivation aso resulted in significant lowering of copper levels ($P<0.05$) in the cerebral cortex and in the neurohypophysis, copper was highest in the 24-hour water-deprived group, medium and least in the 48-hour water-deprived and control groups respectively ($P<0.05$).

DISCUSSION

7.6.1 Heat Stress

Heat stress is perhaps the most important environmental factor affecting livestock production in the tropics. Heat stress like exogenous hormonal treatment disturbs endocrine dynamics and elicits very active responses in the animal.

The elevation of AChE activity in the pons, cerebellum, amygdala, hippocampus, midbrain and medulla oblongata of heat stressed animals is indicative of increased activity of the neural cells which may induce the marked neuromuscular activity and muscular movements characteristic of heat-stressed animals. It should be mentioned that during heat stress, the animals were very restless, agitated, very aggressive and many of them even attempted to jump the fence restraining them. Such gross muscular activity is indicative of nervous reaction to heat stress and is in line with

reports of early researchers that heat stress induces convulsions, coma and affects the floor of the third ventricle and pons. The increase in AChE activity may also be linked with the increase in blood volume, respiratory and metabolic activities accompanying hyperthermy.

As a rise in AChE activity directly reflects increased secretion of ACh at synaptic junctions, the rise in ACh would need to be quickly removed to prevent the animal from being in a state of continous excitation and convulsive muscular activity which would utlimately lead to exhaustion and collapse.

The rapid removal of ACh by AChE also has a heat regulatory action becuase ACh has been known to cause excitation of the heat production pathway (Findlay and Thompson, 1968). Bedrak et al., (1980) also observed an increase in the activity of enzymes associated with steroid metabolism (in vitro) with increasing temperature of incubation and a concomitant decline in serum testosterone in heat acclimatized rats.

Thus the increased AChE activity not withstanding, heat stress also brings about increased androgen catabolism by the liver and kidney (Bedrak et al 1980) and impaired integrity of sertoli cells. This may explain the lowered AChE activity observed in the hypothalamus of heat stressed pigs. This result is particularly striking in view of the multiple role of the hypothalamus in both androgen production and heat regulation.

The rise in AChE acitivity in the hippocampus concomitant with a decrease in the hypothalamus also lends support to the inhibitory role of the hippocampus on the hypothalamic steroid-metabolic dynamics. The hippocampus is also known to inhibit the pituitary-adrenocortical system which responds directly to hypothalamic stimulation (Kawakami et al, 1968).

Heat stress directly lowers thyroxine and TSH release which may also indirectly lower the release of Thyrotropin releasing hormone (TRH) by the hypothalamus of heat stressed animals and may therefore explain the sharp drop in testicular and reproductive capacity of boars exposed to heat stress.

The relative tolerance of the cerebral cortex to heat stress implies that learning and memory functions are not impaired by heat stress and lends support to the view that the cortex seems to possess special metabolic mechanisms protecting it from abuse by stressors.

From the second experiment, it appears that the 3-day heat stress period did not evoke as drastic a response as the 5 and 6-day heat stress did. This may be due to the fact that the animals were still able to recover fairly quickly after the heat stress period.

The inhibitory effect of thermal stress on Basal metabolic rate (BMR) and a lowered concentration of thyroxine and thyrotropin (Tal and Sulman, 1973) coupled with decreased serum testosterone level suggests an impairment of adenohypophyseal functions. This is evidenced by the observed decline in AChE activity and protein concentrations of the hypophyses. The decreased protein level is reflective of the increased steroid inactivation induced by thermal stress. The vasodilatory effect of thermal stress also implies decreased secretion of vasopressin by the neurohypophysis.

The depression of protein concentraions of the pons, cerebellum, hippocampus, hypothalamus, midbrain and medulla oblongata of heat stressed animals confirms reports that the increase inblood volume arising from vasodilation leads to a drop in total protein concentration as a result of haemodilution (Yanga, 1972). In addition, the lowered basal metabolic rate

and thyroid gland functions are reflective of decreased protein synthesis. The cortex, through a not very clear mechanism was able to maintain normal protein synthesis which may still be resolved by its role in higher mental functions.

It is therefore clear that heat stress when prolonged would eventually lead to brain injury. Although many of the exotic breeds of pigs reared in the tropics are heat-acclimatized, studies by Bedrak et al (1980), Egbunike and Dede (1980) reveal that such animals, when exposed to thermal stress even of short duration evoked changes in enzyme metabolism and reproductive behaviour.

The sharp rise in the SAChE activity of the brain regions of heat stressed boars over the control also simulates the rise observed in AChE activity and attracts the same reasons advanced for the increase.

7.6.2 MINERAL PROFILE IN THE BRAIN AND HYPOPHYSES

The rise in calcium concentration of several brain regions of heat stressed boars suggest increased calcium absorption at the neural centres in response to increased ACh and AChE activity. This supports the report that calcium ions facilitate the release of ACh at neuromuscular junction and neurotransmitter release. The same trend was observed for potassium and indicates increased potassium retention in response to the increase in membrane excitability. The increase in membrane excitability would depolarise the membranes and trigger of the sodium-pump mechanisms resulting in more potassium ions in the extracellular fluid and sodium ions replacing the potassium ions. Although the concentrations of the minerals were not determined at the cellular level, it is presumed that this action may in part explain the decreased sodium concentrations induced by heat

stress. Zinc levels were also similarly reduced by heat stress especially after 6 days in some of the regions with the amygdala and hippocampus being exceptions. This reduction in zinc levels may have a link with the concomitant decline of AChE activity in the hypothalamus and the presumed

stress. Zinc levels were also similarly reduced by heat stress especially after 6 days in some of the regions with the amygdala and hippocampus being exceptions. This reduction in zinc levels may have a link with the concomitant decline of AChE activity in the hypothalamus and the presumed impairement of reproductive function.

The similarities of copper content of the brain regions of both heat stressed and control boars may result from the fact that the increased blood volume by vasodilation induced by heat stress is accompanied by fluid loss and the resultant decrease in red blood cells is offset by the haemoconcentration of the blood.

The lack of a consistent trend in magnesium concentrations in the brain regions of both heat stressed and control boars relate to earlier observation in preceeding chapters that magnesium seems to be antagonistic to calcium and its mode of action is not very clear. In addition, it appears as if the mineral does not play a very clear role in adaptation to environmental and hormonal stress.

While heat stress did not affect copper level in the hypophyses, calcium and magnesium were elevated which is indicative of a shift in the electrolyte balance of the gland. The some argument can be advanced for the lowering of potassium and sodium levels on the adenohypophysis of heat stressed animals. The mechanism by which this shift is brought about is not yet determied.

7.6.3 WATER DEPRIVATION

The importance of the brain in osmotic balance of body fluids and regulation of water intake has been established for quite some time but reports have been inconsistent.

Grossman (1960, 1962a) discovered that the cholinergic system of the hypothalamus enhances water intake in both satiated and water-deprived rats while Leibowitz (1970) reported that blocking of the cholinergic system in the rat hypothalamus increased the thirst sensation and decreased hunger.

The present study showed a decrease in AChE activity of water deprived pigs in the pons, amygdala, hippocampus, hypothalamus and medulla oblongata. The study further indicated that water deprivation for 24 hours caused a higher decrease in the affected regions than water deprivation for 48 hours. Contrariwise, AChE activity was elevated in the midbrain of the same pigs over the control.

The decrease in AChE activity of some regions particularly the amygdala, hippocampus and hypothalamus is suggestive of the fact that these regions are involved in the thirst sensation.

The classical experiments of Verney (1958) revealed that osmoreceptors are present in the hypothalamus and stimulation of the hypothalamus stimulates drinking while its destruction abolishes the thirst sensation.

The thirst mechanism is presumably due to a stimulation of nervous elements specifically sensitive to an elevated sodium chloride concentration of the internal environment. Thus a lowering of AChE activity in the hypothalamus of water-deprived boars may be a direct response to acute sensation of thirst. This response may bring about a suppression of the thirst mechanism.

The rise in AChE activities in the pons (after 48 hours of water deprivation), midbrain and the restoration of activity in the medulla oblongata after the 48-hour water deprivation period is indicative of an

attempt to reduce the stress of thirst sensation through a concomitant decrease of the hunger sensation.

As the tropical environment is essentially stressful, a combination of thermal and water-deprivation stress have very serious concsequences for the animal. Stimulation of the midbrain has been known to suppress the hunger sensation (Adamek, 1976) and the pons co-ordinates reflexes concerned with swallowing, vomitting and cardiovascular controls such as blood pressure and salivation.

It is thus possible that the increase in AChE activity in the pons may enhance the water-conservation mechanism of the brain. A possible role may also be a regulation and perhaps stimulation of increased synthesis of ADH by the neurohyphysis in a bid to concerve water loss.

The slightly more pronounced effects of water deprivation for 24 hours compared to 48 hours indicates that the reaction of the animal to water deprivation during the first 24 hours involves more physiologic and metabolic activities than in the second day. It is also possible that during the second day of water-deprivation, the animal had achieved its optimal water conservation mechanism or status. This suggestion agrees with observation by earlier workers that the onset of thirst creates a dry sensation of the mouth and craving for fluid rapidly becomes compelling but as time goes on, the dryness of the mouth increases, production of saliva decreases, finally ceases and food intake stopped. The cessation of good intake would therefore minimise metabolic activities and put the animal in another physiologic status.

Although the physiological interactions elicited by water-deprivation have not been clearly unravelled, the hypothalamic-hypophyseal adrenocortical axis is presumed to play a major role. This is borne out by

the fact that workers have been able to produce experimental diabetes insipidus and inhibition of ACTH release by destroying the hypothalamic-hypophyseal connections (de Wied et al, 1958). It is therefore presumed that water-starvation causes increased release of the vasopressor ADH which is also thought to facilitate ACTH release by the adenohypophysis. The stimulation of ACTH exerts an inhibitory effect on TSH release (Fortier, 1973), thereby diminishing the activity of the thyroid hormone.

The present study confirms these views. The failure of water deprivation to influence AChE activity of the hypophyses eliminates the possible impairment of hypophyseal function by the treatment. If further enhances the view that both the adenohypophysis and the neurohypophysis possess mechanisms of ensuring osmoneutrality in the animal.

The decrease in the protein concentration of the hypothalamus, hippocampus, medulla oblongata, midbrain and pons of the water-deprived animals implies decreased protein synthesis presumably due to a decrease in feed intake and the utilization of ingested amino acids. It may also be a result of a decrease in the metabolic activity of the body.

The increased protein levels in the hypophyses of the water-deprived animals after 48 hours suggests increase in the turn-over rate of enzymatic activities responsible for the increased synthesis of ACTH and ADH. This increase in protein turn-over rate of the adenohypophyses may contribute to the decline in SAChE activity observed in the adenohypophyses of water-deprived boars. The result also indicates that boars water-deprived for 48 hours had adjusted to the effects of thirst sensation more than those deprived of water for 24 hours.

In the same vein, the increase in the SAChE activities of the brain regions of water-deprived boars is reflective of the decreased total protein and AChE activity observed in these regions. This presumably leads to higher SAChE acttivities in an attempt to maintain the enzyme turnover rate.

Of particular interest is the relative stability ofthe cerebral cortex to water-deprivation which is reflected in its role in learning and memory activities which are not immediately impaired by environmental stress.

7.6.4 MINERAL PROFILE IN THE BRAIN AND HYPOPHYSES

The depression of calcium levels in the brain regions of water-deprived boars suggests a reduction of neuromuscular transmission and is in line with the decline in AChE activity of the brain regions concerned with osmoregulation. It also reflects the lack of efficient incorporation of amino acids into proteins evidenced by lowered protein content of the brain regions of these animals.

The elevation of magnesium in the medulla oblongata, midbrain, hypothalamus, hippocampus and amygdala of water-deprived boars may be a compensatory attempt for the depletion of calcium in the affected regions. In addition, the magnesium may be functioning to prevent electrolyte imbalance that may result in more interference with the metabolic activities of the animals. It is also well known that magnesium is a co-enzyme of many metabolic pathways, thus the mineral may be helping in sustaining the vital physiologic processes of the water-deprived boars. The decline in magnesium content of the pons, cerebellum and cerebral cortex may be an evidence of differential metabolic rates of differnt brain

regions whereby the highly active areas during water-deprivation retain magnesium more than the less active regions.

Although no substantial changes were observed in the potassium content of the hippocampus and medulla oblongata of water-deprived boars, the rise observed in the concentrations of the mineral in the cerebral cortex, amygdala and hypothalamus of the same group of animals coupled with a decline in potassium content of the cerebellum and midbrain also reflects differences in the metbolic rates of the mineral in the brain regions during water-deprivation. Thus the brain areas that exhibited an increase in potassium concentrations over the controls may be more involved in osmoregulation and may be more osmoreceptive than other groups.

The failure of water-deprivation to significantly influence sodium levels in the brain regions save for a decline in the midbrain and medulla oblongata suggests that the brain possessed some mechnanism for maintaining osmoneutrality and homeostasis.

The elevation of zinc in several brain regions may enhance the stimulation of enzyme-metabolic pathways it activates to compensate for the decline in the protein turnover rate of the brain regions.

The elevation of hypophyseal levels of magnesium and zinc by water deprivation may be due to the increased secretion of ACTH and the concomitant release of the Adrenal cortex hormones namely the mineralocorticoids (e.g. aldosterone) and the gluccerticcids. The release of the adrenocorticoids would therefore enhance the retention of the minerals in a bid to maintain osmotic balance both intracellularly and extra-cellularly.

An interesting trend is displayed by copper, calcium, potassium and magnesium where water-deprivation for 24 hours resulted in a rise in the levels of the minerals in the hypophyses followed by a reduction after 48 hours.

A possible explanation for this is that the surge of ACTH released in direct response to the onset of thirst is gradually diminished as the animal adapts to the new condition.

This further confirms the hypothesis that the animals that were water-deprived for 48 hours showed higher adaptability to water-deprivation than those water-deprived for 24 hours.

As suggested earlier, the manifestations of such adaptation would include decreased urine production, decreased salivation, decreased or cessation of feed intake and decreased locomotory activities.

7.6.5 SUMMARY

These results have conclusively shown that the pig suffers from serious brain impairment and possible interference with endocrine dynamics during thermal stress. They also indicate that the present design of the pig pens in use at the university farm protects the animals from the thermal stress of the humid tropical climate and provide optimum temperature and relative humidity conducive to normal development of the animal.

These studies further reveal that water deprivation even for a short period like 24 hours results impairs nerous transmission in the brain with possible interference with normal brain activity. They also show the relative ability of the hypophyses to withstand water-deprivation stress presumably through feed-back mechanisms.

CHAPTER EIGHT

CONCLUSIONS

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CONCLUSIONS

8.1.0 The studies reported herein have not only provided adequate details about the role of sex steroids and enzymes in the maintenance of the physiological integrity of the animal particularly at the central nervous system level but have also elucidated to a large extent, the importance of a physiological balance between the circulating hormones inside the animal and the external environmental factors.

The studies also highlight the critical role of acetylcholinestrease enzyme (AChE) activity as a major link between the endocrine and nervous system.

The ontogenic studies indicate that AChE activity can be used as an index of development in the fetal brain and correlated positively with gestation length. They further suggest that brain development is not necessarily accompanied by a rise in total protein content of the brain. The increase in calcium content of the fetal brain with gestation length is directly related to its vital requirement for bone development while the decline in copper and zinc levels may be responsible for the piglet anaemia normally associated with piglets shortly after birth.

Postnatally, the studies also indicate that the brain regions could be classified into 'high', 'medium' and 'low' activity regions depending on the level of AChE activity obtained in such regions. Thus the 'high activity' areas are the amygdala, midbrain and medulla oblongata which recorded the highest AChE activities. This may be related to the fact that these are areas that are involved in highly active, quick and short-termed

responses such as aggressive sexual behaviour and predatory actions as described by Kang et al., (1970). These are actions characterized by high nervous stimulation and manifested by considerable muscular activities.

The hypothalamus, hippocampus and pons which are concerned with basic reflexes such as respirattion, swallowing and normal hormonal actions and co-ordinated responses thus naturally fall into the 'medium activity' class whereas the constant low activity observed in the cerebral context and cerebellum reflects on the fact that these regions control higher and lower intellectual functions which are acquired by the body over time and retained for a long period of time.

As animals do not posses the ability to regnerate neurons after birth, it is not surprising therefore that a decline in AChE activities after birth with age was observed in the pig brain. Thss further confirms that the pig brain had reached its peak of neuronal development by the time of birth and very little changes in quality of the cellular constituents of the neurons take place. The higher activities observed in some brain regions of the male pigs over the female pigs could be responsible for the more aggressive nature of the male animal over the female.

The increase in total protein concentrations of the pig brain with age relates to the growth requirements of the young animal which include increased rate of RNA Synthesis, protein turnover and tissue deposition.

It is also noteworthy that positive correlations were observed in the calcium, magnesium, potassium, sodium, copper and zinc contents of the pons, cerebellum, medulla oblongata and midbrain which are areas involved in growth and protein synthesis.

The results of the experiments on castration also tend to confirm the notion that castration abolishes sexual behavior. The studies also

reveal that the age at castration has an influence on the effects of castration in the animal. Thus post-pubertally castrated male boars appeared to tolerate the responses due to androgen withdrawal more than pre-pubertal castrates.

The studies further show that trestosterane enhances AChE activity in the central nervous system of the boars while castration interferes with protein synthesis.

The decline in the concentrations of some cations such as calcium, sodium, potassium, copper and zinc in castrated boars is indicative of a possible interference with cation absorption from the diet. This closely agrees with the observation of Kunerth and Pitman (1939) who related calcium ions retention to protein synthesis and according to Fullis (1958), zinc deficiency also brings about testicular atrophy associated with castration.

The striking ability of the cerebral cortex to maintain normal levels of AChE activity in castrated pigs supports the view that the cortex is involved in high mental activities which are not immediately impaired by short-term hormonal inbalance. It is also probable that the failure of castration to significantly influence AChE activity in the hypophyses is presumably due to the feed-back response of the hypophyses to a lowered serum testosterone level brought about by castration.

On the effects of ovariectomy on brain physiology, it is safe to conclude that both estrogens and progesterones are necessary for normal functioning of the nervous system and the nervous system responds to estrogens much more than it does to progresterone.

On the role of testosterone as an anti-estrogen in intact gilts, the results indicate that testosterone administration to intact gilts interfers with AChE activity and mineral profile in the brain and has no distinct advantages at the moment. It is therefore clear that androgens cannot serve as primers in the female animal body.

Apart from endocrine factors, environmental factors also play a part in the physiological well being of the animal. Studies carried out on thermal stress and water deprivation have conclusively shown that the pig suffers from endocrine malfunction during thermal stress. The vulnerability of the pig to high temperatures indicate that the pig cannot be successfully reared on an extensive system of management. However, the results show that the present design of the pig pens at the University farm adequately protects the animals from thermal stress during the day and provides tolerable temperature and relative humidity conducive to the normal functioning of the animal.

The study further indicate that water deprivation even for a short period of 24 hours impairs nervous transmission. It also shows that the pig responds immediately to short-term water deprivation of 24 hours and adjusts progressively as the period of water deprivation increases which is an evidence of adaptability. As acetylcholinesterase is known to influence adaptive behaviour, the ability of the animal to regain fairly normal AChE activity levels in some brain regions after 48 hours of water deprivation may partly explain why water deprivation for 48 hours elicited less reponse than water deprivation for 24 hours. The study further confirms the cerebral cortex as an area that tolerates to a large extent, short-term inbalances in endocrine and environmental factors thereby allowing it to effectively maintain its vital role in learning and high mental activities.

The hypophyses too are presumably able to tolerate the effects of stressors through feed-back mechanisms.

In summary, for optimum productivity of the animal, the basic requirements of energy, protein, vitamins, minerals and managemental procedures should be strictly adhered to. Any attempt to enhance the productivity of the animal by means of hormonal additives, injections or surgical procedures should be carried out with the strictest caution and with due regard to the consequences of such attempts on the nervous and endocrine systems.

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APPENDICES

APPENDIX 1 . ANALYSIS OF VARIANCE TABLES FOR AGE AND SEX EFFECTS ON LIVE WEIGHT AND ORGAN WEIGHT

		Live weight			Brain weight			Adrenal Glands			Thyroid		
SOURCE OF VARIATION	DF	MEAN SQUARES	F. VALUE	P	MEAN SQUARE	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P
Replications	3	2.098	1.277	n.s	11.786	1.017	n.s	0.010	5.755	n.s	0.069	0.751	n.s
Sex	1	9.545	5.812	n.s	57.585	4.929	n.s	0.020	1.115	n.s	0.422×10^{-3}	0.004	n.s
Error A	3	1.642			11.585			0.002			0.092		
Age	4	1339.737	585.357	***	2941.115	354.928	***	0.949	376.641	***	22.265	214.430	***
Interaction	4	2.198	0.960	n.s	13.703	1.653	n.s	0.004	1.818	n.s	0.107	1.033	n.s
Error B	24	2.289			8.286			0.002			0.104		
Total	39	139.525			311.419			0.100			2.371		

ANALYSIS OF VARIANCE TABLES FOR EFFECT OF AGE AND SEX ON AChE ACTIVITIES IN SOME BRAIN REGIONS AND HYPOPHYSIS

		Pons			Cerebellum			Amygdala			Hippocampus			Hypothalamus		
SOURCE OF VARIATION	DF	MEAN SQUARES	F. VALUE	P	MEAN SQUARE	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P
Replications	3	0.176	0.992	n.s	0.160	0.476	n.s	0.327	0.636	n.s	0.410	1.967	n.s	0.145	0.569	n.s
Age	4	20.548	115.668	***	4.804	14.298	**	52.513	102.287	***	1.764	8.461	**	6.652	26.192	***
Error A	12	0.178			0.336			0.513			0.208			0.254		
Sex	1	1.634	10.194	**	0.685	4.735	*	0.428	1.959	n.s	0.575	2.283	n.s	0.943	6.378	*
Interaction	4	0.895	5.582	**	0.126	0.875	n.s	0.437	2.004	n.s	0.361	1.434	n.s	0.765	5.178	**
Error B	15	0.160			0.144			0.218			0.252			0.148		
Total	39	2.371			0.694			5.709			0.452			0.931		
		Cerebral cortex			Mid brain			Medulla oblongata			Adenohypophysis			Neurohypophysis		
Replications	3	0.120	1.226	n.s	0.089	0.199	n.s	0.663	2.580	n.s	0.032	1.655	n.s	0.062	3.288	n.s
Age	4	0.381	39.009	***	2.377	5.302	**	25.512	99.266	***	4.861	247.468	***	1.202	64.106	***
Error A	12	0.010			0.448			0.257			0.020			0.019		
Sex	1	0.009	0.687	n.s	1.981	4.663	**	0.347	1.672	n.s	0.220	17.232	**	0.107	1.904	n.s
Interaction	4	0.301	23.571	***	0.412	0.969	n.s	4.605	22.173	***	0.191	14.986	***	0.542	9.594	**
Error B	15	0.013			0.425			0.208			0.130			0.056		
Total	39	0.079			0.645			3.308			0.537			0.214		

*P<0.05 **P<0.01 ***P<0.001 n.s = not significant (P>0.05)

ANALYSIS OF VARIANCE TABLES (AOV) FOR AGE AND SEX EFFECTS ON BRAIN AND HYPOPHYSES PROTEIN CONTENT

		Pons			Cerebellum			Amygdala			Hippocampus			Hypothalamus		
SOURCE OF VARIATION	DF	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P
Replications	3	0.004	0.602	n.s	0.001	1.357	n.s	0.69×10^{-3}	2.493	n.s	0.002	0.739	n.s	0.006	0.936	n.s
Age	4	0.232	307.904	***	0.071	80.716	***	0.144	519.644	**	0.219	63.98	***	0.068	9.735	***
Error A	12	0.75×10^{-3}			0.88×10^{-3}			0.28×10^{-3}			0.003			0.007		
Sex	1	0.78×10	1.171	n.s	0.001	0.401	n.s	0.008	16.462	***	0.4×10^{-3}	0.263	n.s	0.017	2.719	n.s
Interaction	4	0.003	4.408	**	0.002	0.730	n.s	0.003	7.349	**	0.012	7.786	**	0.023	3.552	*
Error B	15	0.66×10^{-3}			0.002			0.48×10^{-3}			0.001			0.006		
Total	39	0.025			0.009			0.016			0.025			0.015		

		Cerebral cortex			Mid Brain			Medulla oblongata			Adenohypophysis			Neurohypophysis		
Replications	3	0.006	0.936	n.s	0.002	1.114	n.s	0.008	4.180	*	0.006	2.783	n.s	0.42×10^{-3}	1.586	n.s
Age	4	0.068	9.735	**	0.081	36.475	***	0.313	151.123	***	0.526	253.674	***	0.023	87.01	***
Error A	12	0.007			0.002			0.002			0.002			0.26×10^{-3}		
Sex	1	0.017	2.719	n.s	0.005	1.336	n.s	0.0 25	3.027	n.s	0.002	0.187	n.s	0.518×10^{-3}	1.569	n.s
Interaction	4	0.023	3.552	*	0.025	6.152	**	0.033	3.934	*	0.004	0.338	n.s	0.38×10^{-3}	1.157	n.s
Error B	15	0.006			0.004			0.008			0.011			0.330×10^{-3}		
Total	39	0.015			0.013			0.041			0.060					

AOV TABLES FOR SAChE ACTIVITIES

Replications	3	5.321	1.088	n.s	26.311	0.406	n.s	66.789	1.613	n.s	34.160	1.275	n.s	33.355	1.163	n.s
Age	4	6117.708	1251.843	***	793.433	12.249	***	1879.06	45.402	***	1192.399	44.538	***	4878.075	170.217	***
Error A	12	4.887			64.772			41.386			26.772			28.658		
Sex	1	169.057	13.531	**	25.008	0.390	n.s	462.434	17.220	**	30.220	0.918	n.s	749.133	22.424	**
Interaction	4	79.631	6.373	**	3.770	0.058	n.s	338.901	12.620	**	300.959	9.150	**	366.415	10.968	**
Error B	15	12.494			64.024			26.854			32.889			33.406		
Total	39	646.677			128.984			267.538			177.455			581.337		

		Cerebral cortex			Mid Brain			Medulla oblongata			Adenohypophys			Neurohypophysis		
Replications	3	2.759	0.434	n.s	11.807	0.143	n.s	126.472	1.040	n.s	4.525	1.936	n.s	3.236	4.728	*
Age	4	123.746	19.490	***	3809.880	46.242	***	6336.350	52.109	***	901.888	386.007	***	371.633	543.045	***
Error A	12	6.349			82.388			121.596			2.336			0.684		
Sex	1	0.374	0.166	n.s	546.964	8.776	**	269.402	3.454	n.s	25.557	20.177	**	29.177	6.139	*
Interaction	4	46.599	20.721	***	947.690	15.206	***	108.699	1.393	n.s	36.206	28.585	***	71.039	15.022	***
Error B	15	2.248			62.321			77.995			1.266			4.728		
Total	39	20.512			552.208			745.079			98.424			48.425		

AOV FOR CALCIUM

SOURCE OF VARIATION	DF	Pons MEAN SQUARES	F. VALUE	P	Cer MEAN SQUARES	F. VALUE	P	Amygdala MEAN SQUARES	F. VALUE	P	Hippocampus MEAN SQUARES	F. VALUE	P	Hypothalamus MEAN SQUARES	F. VALUE	P
Replications	3	0.002	0.174	n.s	0.048	3.124	n.s	0.002	0.303	n.s	0.070	1.545	n.s	0.044	2.437	n.s
Age	4	0.774	60.981	***	0.440	[illegible]	***	0.451	59.938	***	0.721	15.775	**	0.326	176.433	***
Error A	12	0.013			[illegible]			0.007			0.046			0.002		
Sex	1	0.16 x 10^{-3}	0.013		[illegible]	[illegible]	n.s	0.46 x 10^{-4}	0.002	n.s	0.037	0.927	n.s	0.009	2.218	n.s
Interaction	4	0.031	[illegible]		[illegible]	[illegible]	n.s	0.015	0.900	n.s	0.027	0.688	n.s	0.004	1.139	n.s
Error B	15	0.012			[illegible]			0.017			0.040			0.004		
Total	39	0.091			[illegible]			0.057			0.113			0.004		

SOURCE OF VARIATION	DF	Cerebral cor MEAN SQUARES	F. VALUE	P	Brain MEAN SQUARES	F. VALUE	P	Medulla oblongata MEAN SQUARES	F. VALUE	P	Adenohypophys MEAN SQUARES	F. VALUE	P	Neurohypophysis MEAN SQUARES	F. VALUE	P
Replications	3	0.010	[illegible]		[illegible]	[illegible]	n.s	0.345	1.024	n.s	0.013	0.773	n.s	0.022	5.415	**
Age	4	1.319	20[illegible]		[illegible]	[illegible]	***	1.119	3.318	*	2.569	150.646	***	0.147	36.577	***
Error A	12	0.006			[illegible]			0.337			0.017			0.004		
Sex	1	0.026	9.[illegible]		[illegible]	[illegible]	n.s	0.122	0.332	n.s	0.639 x 10^{-3}	0.084	n.s	0.074	19.395	**
Interaction	4	0.014	5.090		[illegible]	[illegible]	n.s	0.377	1.027	n.s	0.007	1.027	n.s	0.010	2.754	n.s
Error B	15	0.003			0.005			0.367			0.007			0.004		
Total	39	0.141			0.238			0.428			0.273			0.022		

AOV FOR MAGNESIUM

SOURCE OF VARIATION	DF	Pons MEAN SQUARES	F. VALUE	P	Cerebellum MEAN SQUARES	F. VALUE	P	Amygdala MEAN SQUARES	F. VALUE	P	Hippocampus MEAN SQUARES	F. VALUE	P	Hypothalamus MEAN SQUARES	F. VALUE	P
Replications	3	0.010	1.372	n.s	0.002	0.292	n.s	0.031	1.321	n.s	0.001	0.302	ns	0.019	4.331	*
Age	4	0.299	41.726	***	[illegible]	76.014	***	0.037	1.592	n.s	0.292	80.893	***	0.014	3.201	n.s
Error A	12	0.007			[illegible]			0.023			0.004			0.004		
Sex	1	0.040	6.713	*	[illegible]	4.682	*	[illegible]	1.303	n.s	0.008	1.446	n.s	0.005	1.342	n.s
Interaction	4	0.019	3.236		0.004	[illegible]		[illegible]	1.353	n.s	0.016	2.724	n.s	0.003	0.853	n.s
Error B	15	0.006			[illegible]			[illegible]			0.006			0.004		
Total	39	0.309			[illegible]			0.029			0.035			0.006		

SOURCE OF VARIATION	DF	Cerebral cortex MEAN SQUARES	F. VALUE	P	[illegible]ein MEAN SQUARES	F. VALUE	P	Medulla oblongata MEAN SQUARES	F. VALUE	P	Adenohypophysis MEAN SQUARES	F. VALUE	P	Neurohypophysis MEAN SQUARES	F. VALUE	P
Replications	3	0.005	0.527		[illegible]	0.929	n.s	[illegible]	0.361	n.s	0.026	1.143	n.s	0.003	0.500	n.s
Age	4	0.761	86.60[illegible]		[illegible]	[illegible]	*	[illegible]	8.257	**	0.036	15.250	**	0.290	55.398	***
Error A	12	0.009			[illegible]			[illegible]			0.002			0.005		
Sex	1	0.57x10^{-3}	0.19[illegible]		[illegible]	[illegible]	n.s	0.76 [illegible] 10^{-3}	0.174	n.s	0.028	5.602	*	0.043	18.833	**
Interaction	4	0.002	0.786		[illegible]	[illegible]	n.s	[illegible]	1.672	n.s	0.006	1.272	n.s	0.014	5.920	**
Error B	15				[illegible]			0.002			0.005			0.002		
Total	39	0.082			[illegible]			0.006			0.008			0.035		

AOV FOR POTASSIUM

SOURCE OF VARIATION	DF	Pons MEAN SQUARES	F. VALUE	P	Cerebellum MEAN SQUARES	F. VALUE	P	Amygdala MEAN SQUARES	F. VALUE	P	Hippocampus MEAN SQUARES	F. VALUE	P	Hypothalamus MEAN SQUARES	F. VALUE	P
Replications	3	1.520	1.04	n.s	0.299	0.262	n.s	2.157	2.080	n.s	1.500	0.446	n.s	0.291	0.076	n.s
Age	4	413.695	300.624	***	205.649	180.406	***	254.278	245.180		318.827	94.913	***	243.273	63.897	***
Error A	12	1.376			1.140			1.037			3.339			3.807		
Sex	1	0.056	0.023	n.s	0.236	0.058	n.s	10.321	3.409	n.s	2.853	1.522	n.s	24.477	40.921	***
Interaction	4	2.702	1.151	n.s	2.249	0.557	n.s	2.030	0.670	n.s	4.621	2.465	n.s	1.896	3.169	***
Error B	15	2.347			4.032			3.027	1.874					0.598		
Total	39	44.152			23.253			28.202	35.117					27.197		

SOURCE OF VARIATION	DF	Cerebral cortex MEAN SQUARES	F. VALUE	P	Mid Brain MEAN SQUARES	F. VALUE	P	Medulla oblongata MEAN SQUARES	F. VALUE	P	Adenohypophysis MEAN SQUARES	F. VALUE	P	Neurohypophysis MEAN SQUARES	F. VALUE	P
Replications	3	8.534	4.909	*	0.403	0.320	n.s	8.832	3.627	**	1.965	0.958	n.s	0.147	0.089	n.s
Age	4	306.372	176.267	***	421.245	335.644	***	471.339	193.146	***	247.634	120.811	***	78.073	47.365	***
Error A	12	1.738			1.255			2.440			2.049			1.648		
Sex	1	1.580	0.905	n.s	4.828	3.642	n.s	1.015	0.888	n.s	1.741	1.661	n.s	0.461	0.204	n.s
Interaction	4	7.017	4.020	*	3.684	2.778	n.s	4.195	3.670	*	4.468	4.263	*	0.835	0.371	n.s
Error B	15	1.745			1.326			1.143			1.048			2.248		
Total	39	34.045			44.633			50.670			27.086			9.488		

AOV FOR SODIUM

SOURCE OF VARIATION	DF	Pons MEAN SQUARES	F. VALUE	P	Cerebellum MEAN SQUARES	F. VALUE	P	Amygdala MEAN SQUARES	F. VALUE	P	Hippocampus MEAN SQUARES	F. VALUE	P	Hypothalamus MEAN SQUARES	F. VALUE	P
Replications	3	20.943	0.800	n.s	36.408	2.066	n.s	51.738	4.023	*	44.764	1.587	n.s	5.492	0.309	n.s
Age	4	421.787	16.120	***	1574.834	89.368	***	310.162	24.162	***	358.361	12.709	**	724.412	40.783	***
Error A	12	26.164			17.622			12.862			28.196			17.762		
Sex	1	28.476	2.269	n.s	27.225	2.158	n.s	99.225	2.116	n.s	126.914	4.109	n.s	5.625	0.233	n.s.
Interaction	4	3.719	0.296	n.s	13.209	1.047	n.s	14.537	0.310		79.117	2.561	n.s	78.062	3.237	*
Error B	15	12.545			12.612			46.875			30.885			24.108		
Total	39	58.859			176.648			61.815			72.122			97.609		

SOURCE OF VARIATION	DF	Cerebral cortex MEAN SQUARES	F. VALUE	P	Mid Brain MEAN SQUARES	F. VALUE	P	Medulla oblonga MEAN SQUARES	F. VALUE	P	Adenohypophysis MEAN SQUARES	F. VALUE	P	Neurohypophysis MEAN SQUARES	F. VALUE	P
Replications	3	119.825	1.728	n.s	52.319	1.590	n.s	100.073	1.749	n.s	20.600	0.702	n.s	15.558	1.393	n.s
Age	4	91.787	1.324	n.s	1650.095	50.155	***	221.712	3.876	*	481.212	16.407	***	787.212	70.522	***
Error A	12	69.304			32.899			57.187			29.329			11.162		
Sex	1	65.025	2.941	n.s	97.437	6.391	*	120.756	3.346	n.s	40.000	2.292	n.s	342.225	29.651	***
Interaction	4	51.212	2.316	n.s	66.494	4.361	*	118.287	3.278	*	37.312	2.138	n.s	71.787	6.219	**
Error B	15	22.108			15.245			36.081			17.450			11.542		
Total	39	55.379			198.570			77.140			71.528			105.948		

AOV FOR COPPER

SOURCE OF VARIATION	DF	Pons MEAN SQUARES	Pons F. VALUE	Pons P	Cerebellum MEAN SQUARES	Cerebellum F. VALUE	Cerebellum P	Amygdala MEAN SQUARES	Amygdala F. VALUE	Amygdala P	Hippocampus MEAN SQUARES	Hippocampus F. VALUE	Hippocampus P	Hypothalamus MEAN SQUARES	Hypothalamus F.VALUE	Hypothalamus P
Replications	3	0.578×10^{-4}	1.214	n.s	0.007	1.258	n.s	0.483×10^{-4}	0.988	n.s	0.023	1.016	n.s	0.624×10^{-4}	0.304	n.s
Age	4	0.0057	121.438	***	0.043	7.107	**	0.005	112.941	***	0.163	7.205	**	0.012	59.179	***
Error A	12	0.476×10^{-4}			0.006			0.489×10^{-4}			0.023			0.205×10^{-3}		
Sex	1	0.129×10^{-3}	4.430	n.s	0.007	0.970	n.s	0.210×10^{-4}	0.192	n.s	0.029	0.352	n.s	0.422×10^{-5}	0.076	n.s
Interaction	4	0.661×10^{-4}	2.261	n.s	0.007	1.006	n.s	0.708×10^{-4}	0.648	n.s	0.025	0.312	n.s	0.281×10^{-3}	5.077	**
Error B	15	0.292×10^{-4}			0.007			0.109×10^{-4}	0.648	n.s	0.081			0.554×10^{-4}		
Total	39	0.634×10^{-4}			0.010			0.635×10^{-3}			0.060			0.001		

SOURCE OF VARIATION	DF	Cerebral cortex MEAN SQUARES	Cerebral cortex F. VALUE	Cerebral cortex P	Mid Brain MEAN SQUARES	Mid Brain F. VALUE	Mid Brain P	Medulla oblongata MEAN SQUARES	Medulla oblongata F. VALUE	Medulla oblongata P	Adenohypophysis MEAN SQUARES	Adenohypophysis F. VALUE	Adenohypophysis P	Neurohypophysis MEAN SQUARES	Neurohypophysis F. VALUE	Neurohypophysis P
Replications	3	0.601×10^{-4}	0.416	n.s	0.887×10^{-4}	0.997	n.s	0.020	0.984	n.s	0.583×10^{-3}	0.887	n.s	0.162×10^{-3}	0.831	n.s
Age	4	0.008	57.895	***	0.007	75.482	***	0.022	1.107	n.s	0.016	24.041	***	0.007	37.919	***
Error A	12	0.144×10^{-3}			0.889×10^{-4}			0.020			0.656×10^{-3}			0.195×10^{-3}		
Sex	1	0.110×10^{-3}	0.41	n.s	0.65×10^{-4}	0.576	n.s	0.021	1.026	n.s	0.001	1.637	n.s	0.9×10^{-4}	0.762	n.s
Interaction	4	0.116×10^{-3}	1.483	n.s	0.256×10^{-4}	0.227	n.s	0.021	1.039	n.s	0.0012	1.980	n.s	0.108×10^{-3}	0.921	n.s
Error B	15	0.78×10^{-4}			0.113×10^{-3}			0.021			0.623×10^{-3}			0.118×10^{-3}		
Total	39	0.95×10^{-4}			0.770×10^{-3}			0.021			0.002			0.891×10^{-3}		

AOV FOR ZINC

SOURCE OF VARIATION	DF	Pons MEAN SQUARES	Pons F. VALUE	Pons P	Cerebellum MEAN SQUARES	Cerebellum F. VALUE	Cerebellum P	Amygdala MEAN SQUARES	Amygdala F. VALUE	Amygdala P	Hippocampus MEAN SQUARES	Hippocampus F. VALUE	Hippocampus P	Hypothalamus MEAN SQUARES	Hypothalamus F. VALUE	Hypothalamus P
Replications	3	0.001	3.961	*	0.002	3.793	*	0.276×10^{-3}	0.618		0.28×10^{-3}	1.061	n.s	0.524×10^{-3}	1.138	n.s
Age	4	0.066	179.187	***	0.264	403.890	***	0.023	51.926	***	0.014	51.788	***	0.082	178.612	***
Error A	12	0.372×10^{-3}			0.654×10^{-3}			0.445×10^{-3}			0.27×10^{-3}			0.46×10^{-3}		
Sex	1	0.010	17.388	**	0.002	1.454	n.s	0.005	19.728	**	0.19×10^{-4}	0.104	n.s	0.255×10^{-3}	1.036	n.s
Interaction	4	0.002	3.444	*	0.008	5.726	*	0.004	17.030	**	0.67×10^{-4}			0.179×10^{-3}	0.729	n.s
Error B	15	0.598×10^{-3}			0.001			0.240×10^{-3}			0.19×10^{-3}			0.246×10^{-3}		
Total	39	0.008			0.028			0.003			0.002			0.009		

SOURCE OF VARIATION	DF	Cerebral cortex MEAN SQUARES	Cerebral cortex F. VALUE	Cerebral cortex P	Mid brain MEAN SQUARES	Mid brain F. VALUE	Mid brain P	Medulla oblongata MEAN SQUARES	Medulla oblongata F. VALUE	Medulla oblongata P	Adenohypophysis MEAN SQUARES	Adenohypophysis F. VALUE	Adenohypophysis P	Neurohypophysis MEAN SQUARES	Neurohypophysis F. VALUE	Neurohypophysis P
Replications	3	0.003	2.97	n.s	0.67×10^{-3}	1.104		0.530×10^{-3}	0.910	n.s	0.003	8.344	**	0.001	5.283	*
Age	4	0.118	129.207	***	0.206	338.702	***	0.272	467.161	***	0.124	360.64	***	0.064	291.841	***
Error A	12	0.917×10^{-3}			0.61×10^{-3}			0.582×10^{-3}			0.343×10^{-3}			0.22×10^{-3}		
Sex	1	0.011	19.562	**	0.005	45.787	***	0.739×10^{-3}	0.899	n.s	0.221×10^{-3}	0.377	n.s	0.002	6.377	*
Interaction	4	0.006	10.221	**	0.011	14.312	***	0.001	1.304	n.s	0.001	1.879	n.s	0.36×10^{-3}	1.059	n.s
Error B	15	0.551×10^{-3}			0.109×10^{-3}			0.822×10^{-3}			0.586×10^{-3}			0.35×10^{-3}		
Total	39	0.014			0.022			0.028			0.013			0.007		

PENDIX . ANALYSIS OF VARIANCE (AOV) TABLES FOR EFFECT OF PRE-WEANING CASTRATION WITH AND WITHOUT ANDROGEN THERAPY

URCE OF VARIATION	DF	AChE ACTIVITY MEAN SQUARES	F. VALUE	P	PROTEIN MEAN SQUARES	F. VALUE	P	SAChE ACTIVITY MEAN SQUARES	F. VALUE	P
...ions	4	0.640	4.681	**	0.028	1.061	n.s	2.517	1.072	n.s
...ples	12	87.615	640.741	***	0.330	12.378	**	250.284	106.630	***
...ror A	48	0.137			0.027			2.347		
...eatments	2	10.827	56.039	***	11.317	477.454	***	1138.016	409.571	***
...teraction	24	3.257	16.859	***	0.115	4.851	***	73.975	26.623	***
...ror B	104	0.193			0.024			2.778		
...al	194	6.085			0.171			38.487		

		CALCIUM			MAGNESIUM			POTASSIUM		
...plications	4	0.039	1.079	n.s	0.003	0.555	n.s	3.858	0.296	n.s
...mples	9	0.794	21.973	***	0.257	52.592	***	82.900	6.368	***
...ror A	36	0.036			0.005			13.018		
...eatments	2	0.778	23.870	***	0.056	9.710	**	7.965	0.828	n.s
...teraction	18	0.499	15.319	***	0.061	10.547	***	44.698	4.654	**
...ror B	80	0.033			0.006			9.607		
...TAL	149	0.146			0.028	18.921				

		SODIUM			COPPER			ZINC		
...plications	4	279.606	0.394	n.s	0.421×10^{-3}	2.667		0.258×10^{-3}	1.639	n.s
...mples	9	750.307	1.057	n.s	0.032	201.576	***	0.053	334.786	***
...ror A	36	709.402			0.158×10^{-3}			0.157×10^{-3}		
...eatments	2	1553.119	2.469	n.s	0.017	103.862	***	0.067	371.763	***
...teraction	18	1019.700	1.621	n.s	0.004	25.822	***	0.032	179.578	***
...ror B	80	629.003			0.166×10^{-3}			0.182×10^{-3}		
...tal	149	705.978			0.003			0.008		

* = P<0.05
** = P<0.01
*** = P<0.001
...t significant (P>0.05)

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APPENDIX 3 ANALYSIS OF VARIANCE (AOV) TABLES OF EFFECT OF CASTRATION AT 3-4 MONTHS OF AGE WITH AND WITHOUT ANDROGEN THERAPY

SOURCE OF VARIATION	DF	AChE ACTIVITY MEAN SQUARES	F. VALUE	P	PROTEIN MEAN SQUARES	F. VALUE	P	SAChE ACTIVITY MEAN SQUARES	F. VALUE	P
Replications	3	0.153	0.698	n.s	0.027	1.806	n.s	0.702	0.079	n.s
Samples	12	82.520	378.183	***	0.542	36.493	***	763.887	86.116	***
Error A	36	0.219			0.015			8.870		
Treatments	2	35.805	182.655	***	0.817	52.477	***	168.843	18.247	***
Interaction	24	3.109	15.863	***	0.110	7.092	***	162.533	17.565	***
Error B	78	0.196			0.015			9.253		
Total	155	7.485			0.081			93.215		

		CALCIUM			MAGNESIUM			POTASSIUM		
Replications	3	0.05	1.868	n.s	0.008	0.342	n.s	0.527	0.144	n.s
Samples	9	0.913	31.137	***	1.067	42.093	***	183.110	50.038	***
Error A	27	0.029			0.025			3.659		
Treatments	2	12.733	443.845	***	0.315	21.397	***	2073.799	560.414	***
Interaction	18	0.928	32.351	***	0.612	41.533	***	89.367	24.150	***
Error B	60	0.029			0.015			3.700		
Total	119	0.446			0.192			64.929		

		SODIUM			COPPER			ZINC		
...plications	3	962.680	1.111	n.s	0.020	1.486	n.s	0.006	2.597	n.s
...mples	9	37664.265	43.483	***	0.085	6.188	**	0.092	37.525	***
...ror A	27	866.171			0.014			0.002		
...eatments	2	80960.352	89.460	***	0.085	5.054	*	0.516	240.398	***
...raction	18	36768.398	40.628	***	0.039	2.247	*	0.077	36.137	**
...ror B	60	904.979			0.017			0.002		
...tal	119	10447.930			0.026			0.029		

* = P<0.05
** = P<0.01
*** = P<0.001
n.s = not significant (P>0.05)

ANALYSIS OF VARIANCE (AOV) TABLES OF EFFECT OF CASTRATION AT 5-6 MONTHS OF AGE WITH AND WITHOUT ANDROGEN THERAPY

		AChE ACTIVITY			PROTEIN			SAChE ACTIVITY		
SOURCE OF VARIATION	DF	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P
Replications	3	0.167	1.522	n.s	0.002	0.366	n.s	3.954	0.653	n.s
Samples	12	49.069	445.692	***	0.706	117.532	***	495.382	81.908	***
Error A	36	0.110			0.006			6.048		
Treatments	2	7.751	47.715	***	0.815	129.235	***	486.388	99.339	***
Interaction	24	2.226	13.701	***	0.154	24.339	***	82.912	16.933	***
Error B	78	0.162			0.006			4.896		
Total	155	4.354			0.094			61.411		
		CALCIUM			MAGNESIUM			POTASSIUM		
Replications	3	0.508	1.026	n.s	0.010	0.846	n.s	1.124	0.414	n.s
Samples	9	1.746	3.527	**	0.134	11.055	***	160.083	59.106	***
Error A	27	0.495			0.012			2.708		
Treatments	2	11.486	23.936	***	5.183	779.370	***	1353.965	682.426	***
Interaction	18	0.777	1.620	n.s	0.488	73.403	***	30.631	15.438	***
Error B	60	0.480			0.007			1.984		
Total	119	0.809			0.177			41.139		
		SODIUM			COPPER			ZINC		
Replications	3	69.114	0.922	n.s	0.004	0.744	n.s	0.37 x 10^{-3}	0.843	
Samples	9	25550.263	340.874	***	0.015	2.848	*	0.071	162.072	
Error A	27	74.955			0.005			0.439 x 10^{-3}		
Treatments	2	370084.627	2857.152	***	0.037	4.239	*	0.183	420.263	
Interaction	18	24070.777	185.832	***	0.009	1.113	n.s	0.037	86.369	
Error B	60	219.529			0.009			0.436 x 10^{-3}		
Total	119	11877.298			0.009			0.014		

ANALYSIS OF VARIANCE (AOV) TABLES OF EFFECT OF CASTRATION AT 7-8 MONTHS OF AGE WITH AND WITHOUT ANDROGEN THERAPY

		AChE ACTIVITY			PROTEIN			SAChE ACTIVITY		
SOURCE OF VARIATION	DF	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P
Replications	3	0.110	1.244	n.s	0.001	2.285	n.s	2.490	0.069	n.s
Samples	12	98.951	1114.416	***	0.194	281.845	***	1627.369	45.180	***
Error A	36	0.089			0.689 x 10^{-3}			36.019		
Treatments	2	0.161	2.134	n.s	0.468	541.361	***	2518.862	80.331	***
Interaction	24	1.107	14.632	***	0.053	60.820	***	222.753	7.104	***
Error B	78	0.076			0.865 x 10^{-3}			31.356		
Total	155	7.895			0.030			217.175		
		CALCIUM			MAGNESIUM			POTASSIUM		
Replications	3	0.035	0.693	n.s	0.015	1.152	n.s	14.682	2.317	n.s
Samples	9	2.268	44.510	***	0.531	40.720	***	293.508	46.332	***
Error A	27	0.051			0.013			6.335		
Treatments	2	13.363	334.808	***	1.705	71.313	***	3672.363	340.107	***
Interaction	18	0.706	17.685	***	0.460	19.252	***	168.039	15.562	***
Error B	60	0.040			0.024			10.798		
Total	119	0.535			0.154			116.588		
		SODIUM			COPPER			ZINC		
Replications	3	82.119	0.383	n.s	0.178 x 10^{-3}	1.068	n.s	0.003	1.482	n.s
Samples	9	28520.213	133.074	***	0.534 x 10^{-3}	3.204	**	0.226	114.143	***
Error A	27	214.317			0.166 x 10^{-3}			0.002		
Treatments	2	343806.338	1460.249	***	0.014	69.456	***	0.261	125.484	***
Interaction	18	27403.078	116.389	***	0.002	9.877	***	0.145	69.723	***
Error B	60	235.443			0.205 x 10^{-3}			0.002		
Total	119	12249.660			0.732 x 10^{-3}			0.045		

APPENDIX : ANALYSIS OF VARIANCE (AOV) FOR EFFECT OF OVARIECTOMY AND HORMONAL REPLACEMENT THERAPY

		AChE ACTIVITY			PROTEIN			SAChE ACTIVITY		
SOURCE OF VARIATION	DF	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P
Replications	3	0.007	0.051	n.s	0.575×10^{-3}	0.840	n.s	2.519	0.438	n.s
Samples	12	57.689	392.774	***	0.244	356.670	***	694.414	120.945	***
Error A	36	0.147			0.684×10^{-3}	123.298	***	5.741		
Treatments	2	12.618	155.250	***	0.120	11.166	***	20.704	3.913	*
Interaction	24	2.480	30.519	***	0.011			18.342	3.467	**
Error B	78	0.081			0.978×10^{-3}			5.290		
Total	155	5.088			0.023			60.913		
		CALCIUM			MAGNESIUM			POTASSIUM		
Replications	3	0.039	3.195	*	0.388×10^{-3}	0.407	n.s	1.488	0.449	n.s
Samples	9	1.124	90.271	***	0.192	201.896	***	114.083	34.443	***
Error A	27	0.012			0.952×10^{-3}			3.312		
Treatments	2	0.211	11.216	***	0.353	2291.930	***	387.977	70.332	***
Interaction	18	0.229	12.222	***	0.098	78.634	***	680.403	12.400	***
Error B	60	0.019			0.001			5.515		
Total	119	0.136			0.078			29.066		
		SODIUM			COPPER			ZINC		
Replications	3	874.226	0.845	n.s	$0,878 \times 10^{-3}$	0.692	n.s	0.960×10^{-3}	0.554	n.s
Samples	9	1044.866	1.010	n.s	0.0035	28.302	***	0.184	106.407	***
Error A	27	1034.117			0.127×10^{-3}				0.002	
Treatments	2	1089.986	1.423	n.s	0.017	144.826	***	0.117	56.764	***
Interaction	18	1681.422	2.195	*	0.004	32.238	***	0.217	105.180	***
Error B	60	765.752			0.117×10^{-3}			0.002		
Total	119	994.439			0.001			0.050		

* = P<0.05
** = P<0.01
*** = P<0.001
n.s = not significant (P>0.05)

APPENDIX : ANALYSIS OF VARIANCE TABLES FOR EFFECT OF ANDROGEN ADMINISTRATION ON GILTS

		AChE ACTIVITY			PROTEIN			SAChE ACTIVITY		
SOURCE OF VARIATION	DF	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P
Replications	3	5.356	2.690	n.s	0.029	4.203	*	5.731	0.372	n.s
Samples	9	134.318	67.467	***	0.697	99.491	***	1201.371	78.108	***
Error A	27	1.991			0.007			15.381		
Treatments	1	75.695	49.712	***	1.042	95.103	***	2380.540	183.982	***
Interaction	9	18.495	12.146	***	0.102	9.313	***	670.588	51.827	***
Error B	30	1.523			0.011			12.939		
Total	79	19.829			0.112			253.782		
		CALCIUM			MAGNESIUM			POTASSIUM		
Replications	3	0.005	0.719	n.s	0.0013	0.537	n.s	4.648	3.687	*
Samples	9	1.064	159.868	***	0.281	109.693	***	168.023	133.313	***
Error A	27	0.007			0.002			1.260		
Treatments	1	1.478	201.745	***	0.043	9.125	***	94.124	72.831	***
Interaction	9	0.334	45.592	***	0.306	64.525	***	97.664	75.570	***
Error B	30	0.007			0.005			1.292		
Total	79	0.183			0.070			32.558		
		SODIUM			COPPER			ZINC		
Replications	3	134.038	1.737	n.s	0.011	0.767	n.s	0.118×10^{-3}	0.536	n.s
Samples	9	34822.486	451.266	***	0.015	1.050	n.s	0.009	41.135	***
Error A	27	77.166			0.014			0.220×10^{-3}		
Treatments	1	97125.017	1472.267	***	0.019	1.339	n.s	0.005	9.369	**
Interaction	9	37002.862	560.906	***	0.020	1.384	n.s	0.012	23.253	***
Error B	30	65.970			0.014			0.503×10^{-3}		
Total	79	9468.581			0.015			0.003		

* = P<0.05
** = P<0.01
*** = P<0.001
n.s = not significant (P>0.05)

PPENDIX 6 . ANALYSIS OF VARIANCE TABLES FOR EFFECT OF ACUTE HEAT EXPOSURE OF PIGS

		AChE ACTIVITY			PROTEIN			SAChE ACTIVITY		
OURCE OF VARIATION	DF	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P
eplications	4	0.010	0.065	n.s	0.009	2.465	n.s	2.446	0.570	n.s
amples	9	51.873	334.281	***	0.443	117.188	***	128.576	29.963	***
rror A	36	0.155			0.004			4.291		
reatments	1	65.172	485.051	***	0.470	89.916	***	166.619	42.566	***
nteraction	9	4.920	36.618	***	0.116	22.239	***	81.189	20.741	***
rror B	40	0.134			0.005			3.914		
otal	99	5.932			0.059			23.993		
		CALCIUM			MAGNESIUM			POTASSIUM		
eplications	4	0.015	0.308	n.s	0.065	4.366	**	2.047	0.396	n.s
amples	9	1.608	33.190	***	0.038	2.529	*	132.581	25.687	***
rror A	36	0.048			0.015			5.161		
reatments	1	52.201	1256.238	***	3.015	129.002	***	2020.323	226.845	***
nteraction	9	0.624	15.017	***	0.111	4.952	**	38.344	4.305	**
rror B	40	0.041			0.023			8.906		
otal	99	0.765			0.061			41.504		
		SODIUM			COPPER			ZINC		
eplications	4	92.502	1.284	n.s	0.061	0.711	n.s	0.004	0.229	n.s
amples	9	1024.625	14.228	***	0.117	1.354	n.s	0.576	35.294	***
rror A	36	72.014			0.086			0.016		
reatments	1	74.822	1.186	n.s	0.128	1.572	n.s	1.736	123.363	***
nteraction	9	684.867	10.860	***	0.121	1.479	n.s	0.019	1.384	n.s
rror B	40	63.062			0.082			0.014		
otal	99	211.568			0.089			0.083		

* = P<0.05
* = P<0.01
* = P<0.001
s = not significant (P>0.05)

ANALYSIS OF VARIANCE TABLES FOR EFFECT OF ACUTE HEAT EXPOSURE OF PIGS

		AChE ACTIVITY			PROTEIN			SAChE ACTIVITY		
RCE OF VARIATION	DF	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P
plications	3	0.413	1.913	n.s	0.006	0.720	n.s	0.508	0.188	n.s
mples	9	108.861	504.987	***	0.512	56.006	***	1639.431	607.482	***
ror A	27	0.216		*	0.009			2.699		
eatments	5	8.004	41.057	***	0.037	4.037	**	328.207	99.944	***
teractions	45	2.684	13.769	***	0.040	4.408	***	127.413	38.799	***
ror B	150	0.195			0.009			3.284		
tal	239	4.924			0.034	94.964				
		IRON			CALCIUM			MAGNESIUM		
plications	3	0.002	5.755	*	0.006	0.866	n.s	0.052	3.582	*
mples	9	0.165	463.045	***	1.191	154.380	***	0.618	42.173	***
ror A	27	0.356×10^{-3}			0.008			0.015		
eatments	5	0.147	689.253	***	4.411	631.404	***	1.036	45.324	***
teraction	45	0.029	134.438	***	0.415	45.123	***	0.156	6.838	***
ror B	150	0.213×10^{-3}			0.007			0.023		
tal	239	0.015			0.202			0.091		
		POTASSIUM			SODIUM			COPPER		
plications	3	2.030	0.581	n.s	4.546	1.386	n.s	0.001	2.324	n.s
mples	9	25.867	7.409	***	21.066	6.425	***	0.005	7.955	***
ror A	27	3.491			3.278			0.602×10^{-3}		
eatments	5	360.708	165.664	***	27.476	11.465	***	0.019	26.568	***
teraction	45	38.326	17.602	***	11.253	4.693	***	0.003	4.809	***
ror B	150	2.177			2.396			0.715×10^{-3}		
tal	239	17.523			5.418			0.002		
		ZINC								
plications	3	0.869×10^{-3}	1.171	n.s						
mples	9	0.499	673.671	***						
ror A	27	0.742×10^{-3}								
			38.011	***						

APPENDIX 6 ANALYSIS OF VARIANCE TABLES FOR EFFECT OF SHORT AND LONG TERM WATER DEPRIVATION OF PIGS

		AChE ACTIVITY			PROTEIN			SAChE ACTIVITY		
SOURCE OF VARIATION	DF	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P	MEAN SQUARES	F. VALUE	P
Replications	3	1.382	11.574	***	0.005	5.271	**	13.763	2.097	n.s
Samples	9	104.941	878.728	***	0.410	408.207	***	1949.893	297.213	***
Error A	27	0.119			0.001			6.560		
Treatment	5	7.492	70.679	***	0.032	28.554	***	69.345	9.488	***
Interaction	45	3.136	29.582	***	0.031	27.866	***	113.460	15.524	***
Error B	150	0.106			0.001			7.308		
Total	239	4.796			0.023					
		IRON			CALCIUM			MAGNESIUM		
Replications	3	0.0013	0.759	n.s	0.030	3.316	*	0.078	5.482	**
Samples	9	0.417	235.054	***	0.698	76.207	***	0.402	28.068	***
Error A	27	0.002			0.009			0.014		
Treatment	5	0.192	34.003	***	1.643	264.480	***	2.124	158.965	***
Interaction	45	0.034	6.075	**	0.177	28.514	***	0.133	9.968	***
Error B	150	0.006			0.006			0.013		
Total	239	0.029			0.099			0.096		
		POTASSIUM			SODIUM			COPPER		
Replications	3	3.363	1.084	n.s	4.121	1.046	n.s	0.997×10^{-4}	0.335	n.s
Samples	9	100.474	32.398	***	8.892	2.257	*	0.015	51.470	***
Error A	27	3.101			3.938			0.297×10^{-3}		
Treatment	5	189.764	61.248	***	18.430	4.297	**	0.046	176.903	***
Interaction	45	34.750	11.215	***	9.828	2.291	*	0.005	21.011	***
Error B	150	3.098			4.288			0.259×10^{-3}		
Total	239	16.633			5.759			0.003		
		ZINC								
Replications	3	0.003	2.04	n.s						
Samples	9	0.619	448.115	***						
Error A	27	0.001								
Treatment	5	0.957	645.858	***						
Interaction	45	0.112	75.643	***						
Error B	150	0.001								
Total	239	0.065								

Appendix 2(1) Ontogenic development

Intra-uterine Age in weeks		Weight of reproductive tract (kg)	Mean embryo weights (g) Left horn of uterus	Right horn	Mean bran weights (g) Left horn	Right horn	Mean No of embryoes Left horn	Right horn	Mean No of Curpora Lutea Left horn	Right horn
4 weeks	1	0.810	0.042	0.055	-	-	7	6	9	8
	2	0.850	0.059	0.062	-	-	6	4	6	9
	3	0.760	0.062	0.058	-	-	3	5	11	5
6 weeks	1	0.850	26.323±0.120	29.888±2.445	0.812±0.112	0.721±0.001	5	7	9	11
	2	0.831	25.422±2.200	26.422±2.374	0.729±0.102	0.824±0.004	3	6	7	8
	3	0.870	27.415±2.425	29.109±2.423	0.808±0.004	0.814±0.129	6	4	8	9
8 weeks	1	1.939	66.028±2.000	62.425±1.242	9.274±0.142	9.927±0.152	5	5	10	8
	2	1.724	62.422±0.100	60.120±4.222	10.025±0.972	9.024±0.274	4	6	9	11
	3	1.941	68.249±1.200	63.422±2.000	8.245±0.762	9.140±0.183	5	7	8	8
10 weeks	1	8.250	290.472±8.240	248.124±4.438	24.24 ±2.340	25.258±2.422	4	3	5	7
	2	8.470	287.562±6.142	273.144±9.242	21.020±3.122	26.142±2.163	4	2	7	6
	3	8.120	262.142±8.100	270.223±6.145	25.23 ±1.162	22.242±2.401	3	3	5	7
12 weeks	1	7.240	562.450±11.241	510.530±8.211	24.256 1.203	21.126±2.013	3	2	7	4
	2	10.240	534.250± 9.674	578.400±10.244	20.126 2.421	27.142±1.164	4	3	6	5
	3	8.450	544.000±12.122	562.500±8.145	24.20 2.322	24.100±1.003	3	3	8	6

Appendix 2.2 Ontogenic development of AChE, Protein and SAChE

		AChE µmol/g/min.		Protein g/100 mt		SAChE µmol/g protein/min.	
4 weeks		Amniotic fluid	Whole embryo	Amniotic fluid	Whole embryo	Amniotic fluid	Whole embryo
	1	0.517	0.301	0.152	0.243	3.401	1.239
	2	0.457	0.224	0.112	0.209	4.080	1.072
	3	0.501	0.402	0.076	0.232	6.697	7.733
6 weeks		Amniotic fluid	Whole brain	Amniotic fluid	Whole brain	Amniotic fluid	Whole brain
	1	0.201	1.161	0.114	0.244	31.763	4.758
	2	0.197	1.092	0.133	0.197	1.481	5.543
	3	0.185	1.224	0.138	0.240	1.341	5.100
8 weeks	1	0.217	1.245	0.117	0.119	1.855	10.462
	2	0.215	1.342	0.105	0.102	2.048	13.157
	3	0.210	1.427	0.126	0.134	1.667	10.649
10 weeks	1	0.215	1.584	0.136	0.188	1.581	8.426
	2	0.226	1.772	0.140	0.202	1.614	8.772
	3	0.214	1.782	0.140	0.234	1.529	7.615
12 weeks	1	0.322	2.184	0.112	0.102	2.875	21.412
	2	0.215	4.018	0.146	0.166	1.473	24.205
	3	0.236	3.574	0.128	0.188	1.844	19.011