SOME EFFECTS OF HISTAMINE AND SEX HORMONES ON HISTAMINERGIC PATHWAYS IN THE RAT AND CHICK BRAIN

BY

JOSEPH AKPOJO ANUKA

A Dissertation subimitted to the Postgraduate School, Ahmadu Bello University, Zaria, in partial fulfillment of the requirements for the degree of Doctor of Phillosophy

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THESIS APPROVAL

We certify that we have read this thesis and that in our opinion it conforms to acceptable standard of scholarly presentation and is fully adequate in scope and quality as a thesis for the degree of DOCTOR OF PHILOSOPHY (PHARMACOLOGY).

Internal Examiners

O.C. Wambebe, B. Pharm; PhD; MPSN
Professor of Pharmacology and
Director, National Institute for
Pharmaceutical Research and Development
IDU, Abuja.

17/5/93
Date

E.N. Sokomba, B.Sc (Pharm.), PhD, MPSN
Professor of Pharmacology and
Dean, Faculty of Pharmacy, University
of Jos, Jos.

17/5/93
Date

I. Hussaini,
Senior Lecturer in Pharmacology
Department of Pharmacology and
Clinical Pharmacy
Ahmadu Bello University, Zaria.

2/11/92
Date

External Examiner

H.O. Obianwu, B.Sc, PhD
Professor of Pharmacology
Department of Pharmacology
Faculty of Pharmaceutical Sciences
University of Benin.

Date

Dean of Postgraduate School

Prof D. Muhammad, BA, Dip, PhD
Dean, Postgraduate School
Ahmadu Bello University, Zaria

20/09/99
Date

This thesis was submitted to the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria and was accepted as partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY (PHARMACOLOGY).

I. Abdu-Aguye, MBBS, MSC, FMCP
Reader and Head of Department of
Pharmacology and Clinical Pharmarcy,
Ahmadu Bello University, Zaria.

13/4/93
Date

DECLARATION

I hereby certify that the work reported in this thesis was carried out by me in the laboratory of the department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University under the supervision of Prof. O.C. Wambebe, Prof. E.N. Sokomba and Dr. I. Hussaini. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for a degree.

18/5/93

Joseph Akpojo Anuka
Department of Pharmacology & Clinical Pharmacy
Faculty of Pharmaceutical Sciences
Ahmadu bello University
Zaria.

DEDICATION

This thesis is dedicated primarily to my Lord and Saviour, Jesus Christ for his love and wisdom granted me and secondly to my late brother, Mr. Timothy Ntembe Anuka, Madam Comfort Egon and my children

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LIST OF MY PUBLICATIONS

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J.A. ANUKA, C. Wambebe, E. Sokomba and I. Hussaini (1991): The influence of sex hormones on the affinity of histamine H_1 receptors in rat brain. Nigerian Association of Academic Pharmacists. 1:185-194.

J.A. ANUKA, C. Wambebe and E. Sokomba (1991): Effect of sex hormones on histamine induced hypothermia in young chicks. West African Journal of Pharmacology. (In press).

C. Wambebe, J.A. ANUKA, and E. Sokomba. (1991): The role of histamine in reproduction. West African Journal of Pharmacology. (In Press).

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A B S T R A C T

The behavioural effect of histamine in young chicks revealed a triphasic response of initial behavioural sleep which was followed by a longer behavioural stimulation culminating in a very long behavioural depression. HI and H2 receptors are involved in these behavioural changes- The HI receptors were found to be excitatory while **the** H2 receptors were inhibitory. A blockade of the HI receptors eliminates the excitatory component leaving the inhibitory component (H2 receptors) unaffected for endogenous histamine to act. The resulting effect is inhibition. Likewise a blockade of the inhibitory component by H2 receptor antagonist leads to excitation. Chlorpheniramine (an HI receptor antagonist) induced a biphasic response of sedation/excitation.

Both HI and H2 receptors are involved in thermoregulatory effect of histamine in young chicks. Activation of these two receptors leads to hypothermia while a blockage of H2 receptors produced insiginificant increase in temperature. On the other hand, a blockade of HI receptors leads to insignificant fall in temperature. Neither Hi nor H2 receptor antagonists counteracted histamine induced hypothermia but a concurrent administration of HI and

H2 receptor antagonists. Steroid sex hormones (oestradiol 17B, testosterone and progesterone) counteracted hypothermia induced by histamine. They also counteracted hypothermia induced by HI and H2 receptor agonists, 2-thiazoly1-ethylamine and dimaprit respectively- Chicks pretreated with hormone showed fast recovery from hypothermia than the controls. Antioestrogens (clomiphene and tamoxifen) potentiated hypothermia induced by histamine and its selective agonists on H1` and H2 receptors.

The role of histamine on seizure regulation was investigated in chicks. Histamine offered 85% protection against leptazol - induced seizure in young chicks. Both HI and H2 receptor agonists, 2-thiazoly1-ethylamine and dimaprit respectively also offered protection against this seizure. Cimetidine and triprolidine neither protected nor potentiated this seizure. Histamine, its agonists and antagonists were not convulsant at the dose level used in this work but potentiated electroshock seizures.

Intracerebroventricular (ICV) adminstration of histamine was found to induce irregularities and at times persistent oestrous cycle with older rats

exhibiting more cycling irregularities than the younger ones. Cimetidine (H2 receptor antagonist) produced a transient change in the cycle which returned to normal rhythm after a day or two. Triprolidine (HI receptor antagonist) showed no changes in the oestrous rhythm while chlorpheniramine, another HI receptor antagonist produced a non-significant degree of changes in the cycle.

Sex hormones have been found to regulate body weight in rats through their influence on eating and drinking behaviours. Histamine-induced drinking behaviour was enhanced by sex hormones while their influence on histamine — induced eating behaviour was found to be variable. In this case, the eating behaviour was enhanced by testosterone and progesterone but depressed by oestradiol 17B.

Sex hormones particularly oestrogens affected the affinity of histamine HI receptors labelled with mepyramine. The affinity of (^{3}H) mepyramine for histamine HI receptors increased as the binding *capacity* decreased depending on the level of sex hormones. Ovariectomy decreased the affinity of the receptors in all the brain areas examined especially

in the oestrogen concentrated regions (hypothalamus and amygdala). Oestrogen replacement in ovariectomized females showed binding affinity reminiscent of proestrus.

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ABBREVIATIONS

Ach	=	acetylcholine
ACTH	=	adrenocorticotrophic hormone
Ci	=	Curie
cm	=	centimeter
cph	=	count per hour
cpm	=	count per minute
CNS	=	Central nervours system
DA	=	dopamine
dpm	=	disintegration per minute
E	=	Oestradiol
EEG	=	Electroencephalogram
EMG	=	Electromyogram
EO	=	Ethyl oleate
ESH	=	Foliche stimulating hormone
Fig	=	Figure
g	=	gram
hr	=	hour
HS	=	Hyperstriatum
5-HT	=	5-hydroxytryptamine
IC50	=	Concentration producing 50% inhibition
ICV	=	intracerebroventricular
ip	=	intraperitoneal
kg	=	kilogram
kd	=	disociation equilibrium constant

M	=	Molar
Mg	=	Miligram (10^{-3} gram)
min	=	minute
ml	=	millilitre
ul	=	microlitre (10^{-6} litre)
NA	=	Noradrenaline
nm	=	nanomolar (10^{-9} molar)
OT	=	Optic tectum
PH	=	Negative logarithm of the hydrogen ion concentration
PVN	=	Paraventicular nucleus
RF	=	Reticular formation
Sc	=	Subcutaneous
VHM	=	Ventromedial hypothalamus
W/V	=	Weight per volume
W/N	=	Weight per weight
CSF	=	Cerebrospinal fluid

SYMBOLS USED

α	–	Alpha
β	–	Beta
n	–	number of binding sites
♂	–	male
♀	–	female

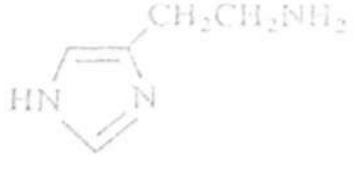
CHAPTER ONE

INTRODUCTION

1.1 HISTAMINE

1.1.1 HISTORICAL

Histamine comprises of an imidazole ring and an amino group connected by a short chain of two carbon atoms. The amino and imidazole groups are both basic and are protonated in acidic medium.



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Fig.1.1 Structure of Histamine

Histamine was first synthesized in 1907 by Windaus and Vogt before its discovery in nature. In 1910 some of its biological activities were established by Barger and Dale. Continuous research into histamine began since 1910 when it was first isolated and some of its biological actions described. Since then much information has accumulated and the involvement of histamine in various pathological phenomena has been demonstrated. However the physiological role of histamine and its biological significance has been less obvious and views on this have been accompanied by considerable scientific controversies. Over the years, research on

histamine has progressed greatly.

In the nineteen forties the, first $H\bar{1}$ receptor antagonists were introduced into medicine to treat the effect of histamine in allergy. The antihistamines therefore provided the pharmacologists with invaluable investigative tools which gave rise to the discovery of $H\bar{1}$ receptors in 1966 by Ash and Schild. This facilitated the identification of many specific biological actions of histamine.

In 1972, the discovery of selective agonists and antagonists led to the discovery of a second type of histamine receptor ($H\bar{2}$) by Black and his coworkers in 1972. The characterization of $H\bar{2}$ receptors established a physiological role for histamine in the control of gastric acid secretion and this led to an immediate clinical application providing a major therapeutic break through for the treatment of peptic ulcers.

Studies have revealed many additional actions of histamine, for example, in the cardiovascular system, on smooth muscles, in immunology and the central nervous system (neurotransmitter) (Rossi, 1977) which can be analysed in terms of its receptors, $H\bar{1}$ and $H\bar{2}$. The value of identifying the receptors involved is to

help in classifying the role of histamine both in physiology and pathology and to indicate the type of drug which may be needed in therapy. Thus, there is great potential for future medical application arising out of the studies in this area of Pharmacology. Since 1972 there has accumulated a large volume of information and considerable new insights into the biological actions and functions of histamine. It is hoped that the present studies will help to unfold new roles for histamine.

1.1.2 Distribution

Histamine occurs widely in both animal and vegetable kingdom including insects. It is widely distributed in the body, being present in almost all the organs and body fluids with the possible exception of cartilage and bone, but the amount varies from organ to organ and from species to species (Felberg 1956). For example, high concentration of histamine is present in the liver of the rabbit, dog, horse as compared to relatively small amounts found in the liver of the rat and guinea pig. In man the concentration of this amine is particularly high in the skin, gastrointestinal mucosa, particularly the acid-secreting parietal cells, lungs and bone marrow. Lesser, but significant

amounts of histamine are also found in peripheral nerves, particularly adrenergic nerves, and the central nervous system where it serves as a neurotransmitter. In the brain, the pattern of distribution is similar to that of 5-hydroxy-tryptamine, for example a relatively high concentration is present in some regions of the brain such as the hypothalamus and thalamus, and low levels in the cerebellum and cerebral cortex (White, 1973).

1.1.3 Biosynthesis and Storage

Almost all the histamine found in animal tissues appears to be formed in the cells by decarboxylation of the amino acid L-histidine. Although the intracellular formation of histamine has not been established conclusively, in-vitro conversion of L-histidine to the corresponding amine has been demonstrated in foetal and adult human tissues (Lindel and Westling, 1966). Mammalian tissues contain at least two enzymes capable of decarboxylating this amino acid. One is the diffusely distributed enzyme, aromatic L-amino acid decarboxylase which has a relatively low affinity for L-histidine, and the other is a selective histidine decarboxylase. It is this specific decarboxylase that is most abundant in histamine-rich mast cells

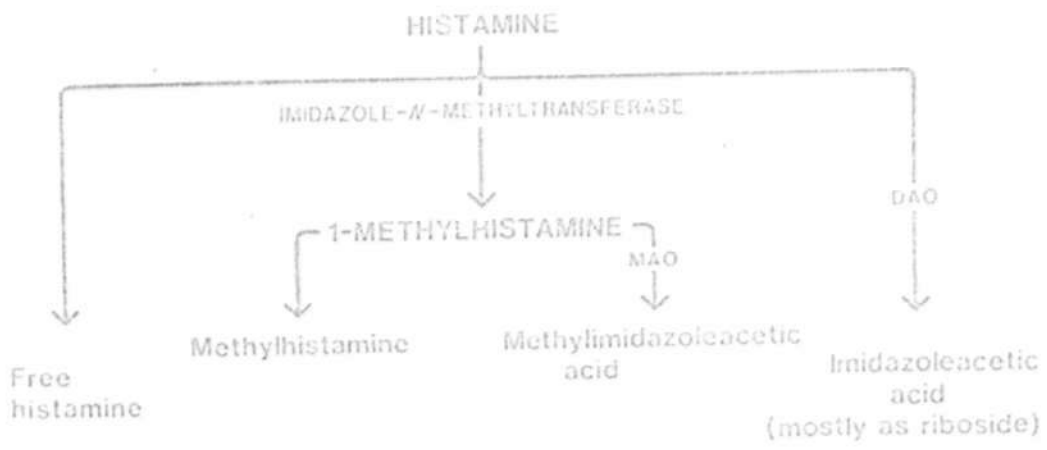
and in certain rapidly growing tissues such as foetal rat liver. Both enzymes require pyridoxal-5 - Phosphate as cofactor and are inhibited by compounds such as semicarbaxide that combine with this cofactor. There is no evidence that tissue histamine is derived from smaller molecules or that it is synthesized by nonenzymatic process. (Rossi, 1977).

A major locus of stored histamine in mammalian tissue is the mast cell (Ehrlich 1977). In man, almost all of the histamine in the blood is found in the basophilic leukeocytes, the cytological counterpart of the mast cell in the tissues. In certain species, the histamine repository function of the basophils is assumed by blood platelets and eosinophils (Rossi 1977). A considerable fraction of the histamine contained in some areas of the body, notably, the epidermis, the brain, and the stomach, is not associated with mast cells (Rossi, 1977).

1.1.4 Metabolism

Metabolic pathways for the biotransformation of histamine have been established in detail, largely by the comprehensive researches of Schayer and his associates (1956, 1959). A major pathway of histamine metabolism in many species, including man, involves ring N-methylation to form 1-methyl-4 (B-aminoethyl)

imidazole through transfer of a methyl group from S-adenosylmethionine in the presence of the enzyme imidazole -N- methyltrasferase. Methylhistamine is, to a large extent, subsequently deaminated by monoamine oxidase to form 1 - methylimidazole-4-acetic acid, the principal urinary metabolite of histamine in man. A secondary catabolic route involves oxidative deamination by diamine oxidase (diaminase) to form imidazole acetic acid, a large fraction of which is conjugated with ribose forming 1 - ribosyl-imidazole - 4 - acetic acid. This reaction appears unique in that histamine is the only endogenous compound to be metabolized by conjugation with ribose (Rossi 1977).



DAO = diamine oxidase, MAO = Monoamine oxidase
Fig.1.2: Metabolism of histamine

1.1.5 Histamine in mammalian Brain

1.1.5.1 A Neurotransmitter role

A considerable amount of evidence from biochemical, anatomical, electrophysiological and neurochemical studies show that histamine is a neurotransmitter in the mammalian central nervous system (CNS) (Schwartz, 1977; Green et al, 1978; Schwartz et al, 1979, Schwartz et al, 1980) and indeed histamine systems in the brain are, in many aspects, quite analogous to the established neurotransmitter systems, in particular, noradrenaline, dopamine and serotonin. However, althoug several lines of evidence suggest a possible neurotransmitter role for histamine in the brain, there is as yet no such evidence that it is a transmitter in the peripheral nervous system (Beaven, 1982). Like other biogenic amines, histamine has been shown to have a nonuniform distribution in the brain and the regional distribution shows that it is present in nearly all areas of the brain with the highest concentration in the hypothalamus followed by amygdala (Hill et al, 1978). In hypothalamus, large amounts of histamine is present in the median eminence and lesser quantities in the premammillary, suprachiasmatic, and arcuate nuclei (Brownstein et al 1974). Histamine and its synthetic enzyme, histidine

decarboxylase, are present in very high levels in hypothalamus with lower levels in cortex, hippocampus, brain stem, thalamus, cerebellum and lower still in the telencephalon (Schwartz et al, 1970; Taylor and Snyder, 1971). The histidine decarboxylase content of hypothalamic nuclei correlates closely with their histamine content except in the median eminence (Pollard et al, 1976).

Histamine is formed by decarboxylation of histidine with the release of CO_2 by the action of enzyme, histidine decarboxylase which uses pyridoxal phosphate (Vit. B6) as cofactor (Beaven, 1982). Histamine appears to be stored in synaptic vesicles and in homogenates of brain tissue; histamine, histidine decarboxylase and histamine - N - methyltransferase are found associated with synaptosomal fractions (Baudry et al, 1973; Snyder et al, 1974). However, specific synaptosomal uptake system typical of other biogenic amine transmitters has not been established for histamine in the brain (Schwartz et al, 1980/82; Beaven, 1982; See Barbin et al, 1980). Thus it is conceivable that in the brain histamine does not appear to be inactivated by the re-uptake mechanism but by methylation to the inactive product, 3-methylhistamine. The enzyme catalysing this reaction, histamine - N-methyltransferase is found in high concentration in

the rat brain. The rate of turnover of histamine in the brain is extremely high where it is metabolised exclusively by methylation, one of the criteria for neurotransmitter function (Pollard et al, 1974, Dismukes and Snyder, 1974a). Histamine-N-methyltransferase is reportedly present in brain of all species investigated (Brown et al, 1959) whereas little or no diaminase (diamine oxidase) activity is found in the brain. However in the periphery histamine is oxidatively deaminated to imidazole acetic acid, a reaction catalysed by the enzyme diamine oxidase.

Histamine has been shown to be released from brain slices by potassium induced depolarization, accompanied by increase in histamine synthesis.

Histamine has been shown to possess a variety of actions in the CNS consistent with neurotransmitter functions. Thus administration of histamine intraventicularly or to specific brain loci has been shown to produce a variety of behavioural and vegetative effects, including central arousal (Sawyer, 1955). Self stimulation (Cohn et al, 1973), hypothermia (Green et al, 1976), enhance water intake (thirst) and hunger (Gerald and Maickel, 1972), increased respiration and vomiting (Bhargava et al, 1968, 1976) and regulation of the adenohypophysis

(Libertum and McCann, 1976). Alteration in histamine sensitivity has been implicated in schizophrenia (Weckonicz and Hall, 1957).

However, the developmental pattern of this amine (histamine) differs markedly from those of other putative transmitters, whereas noradrenaline and serotonin contents in brain increase steadily during ontogenesis (Karki et al, 1962; Bennet and Giarman, 1965), the level of histamine in the rat brain is high at birth then decreases as neural maturation proceeds (Ronnberg and Schwartz, 1969; Pearce and Schanberg, 1969; Schwartz, et al, 1971). A variety of approaches, mainly biochemical but also of morphological nature indicate that histamine is held in at least two classes of cellular stores, neurons and mast cells (Schwartz et al, 1980) and it has been suggested that the high levels of histamine in the newborn brain are contained in the mast cells. This mast cell storage in the neonates changes to a predominantly neural compartmentation as the brain develops (Martres et al, 1975). In adult rat, histamine is found in particulate materials (Baudry et al, 1973) probably in synaptosomes (Stein et al, 1976). However, histamine in mast cells could still function in neural activity. In adult guinea pig, all the histamine of brain appears to be neuronal (Tuomisto, 1977) while in adult rat 20-30% histamine

is present in mast cells and the rest in neuronal elements (Ferrer et al, 1979).

1.1.5.2 Receptors Mediating Histamine Responses

The physiological responses of histamine have been shown to be mediated by at least two distinct types of receptors (H_1 and H_2). The histamine receptors of both H_1 and H_2 distinguished by their differential sensitivities to various agonists and antagonists, have been identified as mediating the cellular actions of histamine (Ash and Schild, 1966; Black et al, 1972) and a variety of biochemical and physiological studies (Schwartz et al, 1980; Taylor and Richelson, 1981) suggest that both types are present in the mammalian brain, depending on the brain area or animal species (Rogers et al, 1975; Kanof et al, 1977). Histamine receptors (H_1 and H_2) in the brain have also been demonstrated, both by competitive binding of labelled ligands (histamine, mepyramine, cimetidine) to neuronal membrane preparations, by histamine induced increases in adenylate cyclase activity in the hypothalamus including other brain areas (Schwartz, 1979; Chang et al, 1978; Tran et al, 1978; Hill et al, 1978) and guineapig ileum (Hill et al, 1977).

1.1.6 Pharmacological characterization of histamine receptors with agonists and antagonists

1.1.6.1 Antagonists

The antihistamines have provided pharmacologists with invaluable investigative tools which have given rise to the concept of Pharmacological receptors for histamine, and also facilitated the identification of many specific biological actions of histamine. Thousands of compounds have been synthesized and tested for antihistamine activity on $H\bar{1}$ and $H\bar{2}$ receptors. The first compounds described by Bovet (1937) as antagonist on $H\bar{1}$ receptors were the benzodioxin derivatives 933F (Unger et al 1937). Others include mepyramine, chlorpheniramine, triprolidine, diphenhydramine, promethazine.

The first compound to be described as $H\bar{2}$ receptor antagonist was burimamide Black et al 1972. Metiamide was next to be synthesized which is a more potent compound (Black et al, 1973).

Burimamide, metiamide, cimetidine, ranitidine, oxymetidine, and no doubt, many more related compounds to come, all belong to a single homogenous pharmacological class, the class of histamine $H\bar{2}$ receptor antagonists (Black et al 1972).

1.1.6.2 Agonists

The respective classes of histamine antagonists provide valuable tools for pharmacological receptor classification of histamine actions but it is often helpful to complement studies by using selective agonists on $H\bar{1}$ receptors and $H\bar{2}$ receptors like 2-thiazolylethylamine and dimaprit respectively that mimic the action of histamine.

1.1.7 Histamine in Central Thermoregulatory Pathways

Neurochemical evidences suggesting that histamine may have a role as a central neurotransmitter have encouraged a search for physiological responses to this amine. That histamine might be involved in neuronal activity in the hypothalamus was suggested by the relatively high concentrations of both histamine and histidine decarboxylase in this part of the diencephalon.

In all species studied, thermoregulatory activity has been established to be by preoptic/anterior hypothalamic nuclei as the major regulating centres. This consideration renders it likely that histamine might be involved in the central thermoregulatory neural network.

Systemic injection of histamine has been shown to induce changes in body temperature in several animal species with direction being a function of environmental temperature. In rat and mice (3mg, s.c) histamine was shown to cause a fall in body temperature at an environmental temperature of 18-20^{o}C, whereas a rise occured at 30^{o}C (Fabinyi and Szebehely, 1949).

1.1.8 Histamine H_1 and H_2 receptors in thermoregulation

Hypothermic effect of intracerebral histamine was inhibited by systemic or central injection of H_1 receptor antagonists. However,the fall in temperature after histidine loading was partially blocked by systemic injection of either pyrilamine or chlorcyclizine (Lomax and Green 1975). These findings confirm the observation of Shaw (1971) that chlorcyclizine was ineffective in antagonizing the hypothermic effect of lateral ventricular injection of histamine.

Injection of the H_2 antagonist into the preoptic/anterior hypothalamic nuclei or into the lateral ventricles did not modify the fall in temperature after histidine loading but injections into the third ventricle abolished the response(Green et al, 1975). These several observations raised the

intriguing possibility that the central thermoregulatory pathways contain both $H\bar{1}$ and $H\bar{2}$ histamine receptors.

1.2 SEX HORMONES

1.2.1 Effects of Steroid hormones in brain development

The relationship between steroid hormones and brain functions are many and varied. They have been shown to possess both long term and short term effects upon the brain, modifying for example, its ultrastructure, the turnover rates of some neurotransmitters and the binding concentrations of high affinity binding sites for some monoamines and biogemic amines (see Thomas and Ani, 1982; Anuka et al, 1986; Biegon et al, 1980).

Regions of the brain that are associated with the sexually stereotypic behaviours include the preoptic-anterior hypothalamus, the ventromedial nucleus, suprachiasmatic nucleus and amygdala. These regions have sexually dimorphic structural characteristics such as cell nuclear size, area volume, and synaptic patterns (Staudt and Dorner, 1976; Gorski et al, 1978, Raisman and Field 1973; Le Bland et al, 1982).

In developing animals, the actions of gonadal steroid hormones permanently alter neural development in certain brain regions and lead to brain sex

differences (Gorski, 1979; Arnold, 1980; Goy and McEwen, 1980). In adult animals, steroid hormones reversibly alter certain behavioural patterns as well as anterior pituitary secretion (McEwen et al, 1979).

1.2.2 Effects of Sex hormones on Gonadotrophin Secretion

Although steroid hormones can cause permanent changes in the brain of developing mammals, they also have some short term effects on the adult animal. The brains of adult male and female rats can be distinguished not only by their morphological nature but also biochemically. This sexual dimorphism is also reflected in differences in the neural mechanisms controlling both sexual behaviour and gonadotrophin release. The differences are expressed essentially by the male exhibiting tonic gonadotrophin release and noncyclic sexual behaviour, while the female exhibits cyclic gonadotrophin release and cyclic sexual behaviour.

The cyclic nature of ovarian changes as a reciprocal interrelationship between ovarian hormones and the anterior puititary has been known since 1932 when Moore and Price suggested that follicle stimulating hormone (FSH) released from the anterior pituitary stimulated ovarian follicular growth and

thence oestrogen secretion. High levels of blood oestrogen were suggested to act directly on the pituitary to inhibit FSH release and enhance the release of luteinizing hormone (LH) leading to ovulation, formation of corpora lutea, and secretion of progesterone which inhibits further LH release.

However, classical experiments by Harris and Jacobsohn (1952) show the involvement of the hypothalamus as the centre which controls the secretory activity of the pituitary. The ovarian hormones, oestradiol and progesterone, therefore appear to act both at the pituitary gland and in the hypothalamus in their feedback regulation of LH and FSH release.

Only females have the ability to respond to high and increasing levels of oestradiol by increasing the release of gonadotrophins. This cyclic release of gonadotrophin is however not mimicked in the adult male even after castration and it is essential for cyclicity. In males, the sex hormones release from the gonads exert only a negative feedback control on the pituitary gland and on the hypothalamus to prevent further release of both gonadotrophins (GTH) and gonadotrophin releasing homrone (GTRH) respectively. In females, however, gonadal hormones not only exert a negative feadback control as in the male but also a positive feedback control.

1.2.3 Effects of Steroid hormones on turnover rate of neurotransmitters

There is increasing evidence that both normally occuring and surgically induced changes in endocrine function can alter hypothalamic monoamines neurotransmitter activity (Beattie et al, 1972; Coppola, 1969; Fuxe et al, 1967; Gudelsky et al, 1977; Jori et al, 1976). During the oestrous cycle of the rat, for example, changes in the circulating levels of ovarian hormones co-vary with the presumptive release of hypothalamic releasing factors, pituitary hormones and brain monoamine Fuxe et al, 1976; Jori et al, 1976; Stefeno and Donoso, 1967). It has been demonstrated that (^{3}H) dopamine uptake, levels of dopamine and noradrenaline and turnover of these catcholamines in the brain or portal blood vary systematically over the oestrous cycle (Ben-Jonathan et al, 1977; Davis et al, 1977; Jori et al, 1976). Exogenous administration of oestradiol has been found to alter dopamine turnover, castration of male rats increased dopamine turnover, tyrosine hydroxylase activity and steady state serotonin levels in certain hypothalamic nuclei. (Coppola et al, 1969; Kiser et al, 1978; Luine and McEwen, 1977). It has been reported that in the ovariectomized rat, the release of dopamine into the hypophysial blood can be inhibited by oestradiol

(Cramer et al, 1979).

In the serotonergic system, serotonin uptake is increased during proestrus, receptor binding decreased and serotonin levels low (Biegon et al, 1980; Keung et al, 1976; Meyer and Quay 1967). In the male rat, it has been shown that castration most consistently results in long term increase in tubero-infundibular dopamine content and turnover (Kiser et al, 1978).

The turnover of whole brain or hypothalamic noradrenaline is accelerated in pro-oestrus (Zschaeck and Wurtman, 1973).

During the oestrous cycle of the female rat adenylate cyclase activity varies, being lower in pro-oestrus than in metoestrus. (Ani et al, 1980; Thomas and ani, 1982).

1.2.4 Effects of Sex hormones on receptor density

Changes in receptor density are known to modify the responsiveness of tissue to neurotransmitter agonists or modify the effects of antagonists including ligand binding. An increase in receptor levels causes supersensitivity, while a reduction in receptor levels produces sub-sensitivity. Thus, changes in receptor levels could be part of the regulation of neurotransmission in the CNS.

A few studies have demonstrated that receptor activity can be regulated as a function of normal physiological changes. Receptor density for some biogenic amines and acetylcholine (Ach) has been shown to fluctuate during oestrous cycle (Biegon et al, 1980, Anuka et al 1986). Biegon et al, 1980 described changes in 5-Hydroxytryptamine (5-HT) receptor density in the basal forebrain during the oestrous cycle, finding 40% reduction in the number of 5-HT binding sites during pro-oestrus and oestrus compared to dioestrus. This reduction could be mimicked by oestradiol administration and was anatomically specific not occuring in hypocampus, caudate or cortex (Biegon and McEwen, 1982). This indicates a role for steroid hormones in the regulation of these receptors. In rat, oestradiol levels are highest at pro-estrus and still elevated during oestrus (Smith et al, 1975; Neguin et al, 1975).

Several studies have also demonstrated changes in B-adrenergic, muscarinic, cholinergic and dopaminergic receptors following oestrogen treatment or ovariectomy (Hruska and Silbergeld, 1980; Wilkinson et al, 1979; Rainbow et al, 1980; Al-Dahan and Thomas, 1981; Vacas and Cardineli, 1982). Some of these changes have been reported to be anatomically

specific (Vacas and Cardineli,1980; Lofstrom, 1977 and Wagner et al, 1979) and for dopamine paralleled by behavioural changes (Hruska and Silbergeld, 1980). Karakiulakis et al (1978), Jalilian-Tehrani et al, 1982) have shown that the number of dopamine binding sites in the cerebral cortex and amygdala of adult male rat is higher than it is in those of adult female and this difference can be mimicked by neonatal androgenization of the female.

A wealth of evidence also show that stereotyped behaviours induced by dopamine agonists are reduced by ovariectomy and potentiated by oestrogens. This potentiation of stereotyped behaviour by oestrogens occurs in males and females and is associated with increased number of striatal dopamine receptors following oestrogen treatment. (Hruska and Silbergeld, 1980).

1.2.5 Steroid hormones and Enzyme Regulation

Gonadal steroid treatment has been shown to result in the alteration of a number of neural enzyme activities (see McEwen and Luine, 1978; McEwen et al, 1979). Reported effects include regulation of hypothalamic tyrosine hydroxylase activity, type A monoamine oxidase activity in hypothalamus and amygdala, choline acetyltransferase activity in

preoptic area and amygdala, and enzyme of intermediary carbohydrate metabolism in hypothalamus as well as pituitary. These effects have been found in brain regions which contain intracellular steroid receptors and therefore reflect steroid acting at the level of the cell nucleus (See McEwen and Luine, 1978; McEwen et al, 1979).

1.2.6 Oestrogen Receptors

Both autoradiography and binding assays have shown that oestrogen receptors are found in various areas of the developing brain, including the cerebral cortex and the limbic structures such as the hypothalamus, preoptic area and amygdala (Barley et al, 1974; Sheridan et al, 1974; Stumpf et al, 1980). Oestradiol has been shown to act by binding to cytoplasmic (cytosol) and nuclear oestrogen receptor system of high affinity and specificity (Barley et al, 1974). The ontogeny of oestrogen receptors in rodent brains reveals that the brains of perinatal mice produce oestrogen receptors as early as embryonic day 13 and both the population of cells with receptors as wells as the density of the receptors within cells increase dramatically over the ensuing perinatal period in the hypothalamus, preoptic area and cortex (Gerlach et al, 1983). However, while neonatal cortices and hypothalami from

both male and female are equally rich in these receptors, in adult these receptors are most concentrated in the hypothalamus and amygdala and relatively sparse in the cortex (Barely et al, 1974).

1.3 Female Reproductive Cycle

The existence of specific sex behavioural patterns in males and females is common knowledge. The most striking sex difference is the ability of the female to ovulate every four days (rodents), monthly (humans) and support oestrous and menstrual cycle. This cyclical nature of the female is in direct contrast with the acyclical nature of the male. Evidence show that these differences in behavioural pattern depends not solely on genetic control but also on the hormonal status of the animal (Doner, 1978; Goy and McEwen, 1980; Gorski, 1979).

The crucial event in reproductive activity of the female is ovulation which is produced by cyclic surge of gonadotrophin (FSH and LH) released from the pituitary. Ovulation, which is the key to the oestrous or menstrual cycle of the female is brought about by the neural activation of the pituitary glands in response to circulating levels of ovarian oestrogens.

Evidence now shows that neuronal control of gonadotrophin is by hypothalamus and ovulation is controlled by a feedback mechanism of sex hormones on the hypothalamus (Flerko, 1966; Everett, 1969). In female, ovulation is regulated by the pre-optic-anterior hypothalamus and studies indicate that this region has specific affinity for labelled oestrogen (Pfaff, 1968; Stumpf, 1970; Zigmond and McEwen, 1970). The pre-optic anterior hypothalamic area is considered to be undifferentiated at birth in rat, or inherently cyclic. In the absence of gonadal steriods, for example, the normal or ovariectomized female or neonatally castrated male, this region develops its full potential for cyclic regulation of gonadotrophin secretion.

1.4 The Oestrous cycle in rat

The structural aspects of the oestrous cycle have been very carefully analysed for a variety of common laboratory and domestic animals. Laboratory rodents, such as mice, rats and hamsters, are polyoestrous species that repeat cycles throughout the year without much variation unless interrupted by pregnancy. Among these, the rat is the most commonly used species in the study of the reproductive function and is used to exemplify species with oestrous cycles. The oestrous cycle in the rat is

four to five days in length, divisible into four stages as exemplified by vaginal cytology. (See Fig.1.3).

1.4.1 Oestrus: The oestrus is a period of heat; copulation is permitted only at this time. It is characterized by rapid maturation of multiple ovarian follicles, uterine enlargement and rapid proliferation of vaginal mucosa resulting in the exfoliation of cornified squamus cells. it is also marked by dry corrugated vaginal wall with fully distended uterus. Oestrus last 10-24 hours and ovulation occurs during this period (Short and Woodnott, 1969; More, 1978).

1.4.2 Metoestrus: Metoestrus is intermediate between oestrus and dioestrus and occurs shortly after ovulation. It normally lasts 10-14 hours. The ovaries are seen to contain corpora lutea and small follicles. The uteri have diminished sizes and vascularity. Many leucocytes appear in the vaginal smears along with some cornified cells. Few spotted epithelial cells may be present. The vaginal wall is moist and the uterus appears fleshy and pink. (Short and Woodnott, 1969; Moore, 1978).

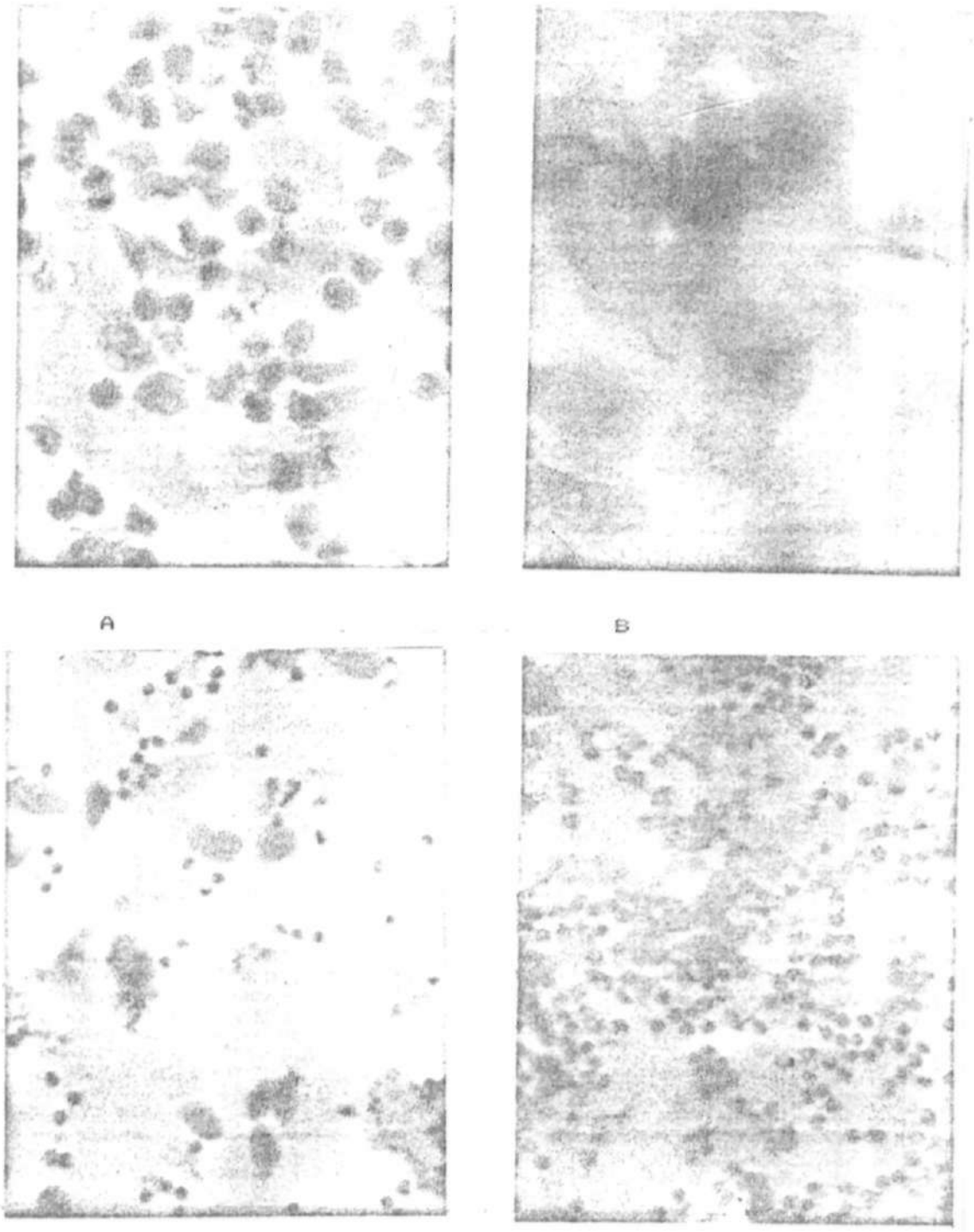


Figure 1.3 A - D: Shows cell types as observed by vaginal smears during the oestrous cycle in adult female rats.

A This is the pro-oestrous stage. In this stage of the oestrous cycle, leucocytes disappear and the smear consists mainly of the epithelial cells with marked nuclei.

B Is the oestrous stage. This stage is the stage at which the female is on heat. The smear consists of cornified cells only and lasts about 10 to 24 hours.

C Metoestrous stage, is the intermediate between oestrus and dioestrus, in this stage the smear consists of leucocytes, cornified and some epithelial cells and lasts about 10 to 14 hours.

D Dioestrous stage, is characterised by vaginal smears consisting almost entirely of leucocytes and lasts longer in duration; about 48 to 70 hours.

1.4.3 Dioestrus: Dioestrus is characterized by vaginal smears almost entirely containing leucocytes, regression of the corpora lutea and uterine regression. However there could be some spotted cornified cells. Dioestrus lasts approximately 48-70 hours. The vaginal wall is moist and the uterus is in resting condition (Short and Woodnott, 1969; Moore, 1978).

1.4.4 Pro-Oestrus: Pro-oestrus is characterized by functional involution of the corpora lutea. Vaginal smears reveals entirely nucleated epithetial cells, and preovulatory swelling of ovarian follicle. Its duration is approximately 12 hours. The vagina becomes dry and uterus starts to become distended. Pro-oestrus immediately proceeds oestrus. (Short and Woodnott, 1969; Moore, 1978).

CHAPTER TWO

EXPERIMENTAL OBJECTIVES

The presence of histamine in the brain as a neurotransmitter and especially its highest concentrations in hypothalamus and amygadala, areas associated with behaviour, temperature regulation, hormone regulation, gonadotrophin regulation, drinking behaviour, feeding etc, suggest that histamine might be involved in neural activity in these regions.

Although systemic administration of histamine has been reported to induce behavioural variables and changes in body temperature in several animal species, so far the exact mechanism(s) of action in bringing about these changes have not been fully elucidated. It is therefore the primary objective of this project to investigate the mechanism whereby histamine induced stimulant effect, depressant effect, eating and drinking behaviours are produced and if possible establish more behavioural variables for this amine.

Histamine and most of its analogues do not easily penetrate the blood-brain barrier thus in order to achieve higher levels of histamine in the brain by systemic administration, young chicks were used in this work. According to Waelsch (1955) and Key and Marley (1962), the young chick (1 day old to five day old) lacks a functional blood-brain barrier

to monoamines. However, although the chicks blood-brain barrier is immature at birth there are reports that the central nervous system (CNS) of the chicks respond to neuropharmacological agents in a similar way to most mammals (Sponer and Winters, 1966). In addition,it is also easy to quantify many behavioural variables in young chicks because of the sensitivity of CNS to chemical agents at this stage in the development of the brain.

Gonadal sex hormones have been shown to interact with a variety of biological substrates in the CNS and in the periphery. They have been shown to possess both long term and short term effects upon the brain. Some of these effects play a crucial role in the modulation of brain structures and functions concerned with behavioural changes and sexual activities in adult life. Although gonadal sex hormones have been reported to affect a number of biological substrates in the brain and periphery, so far little is known about their effects on histaminergic pathways in the brain. This part of the project was carried out (a) To examine the effect of gonadal sex homrones on histamine - induced hypothermia in young chicks. (b) To examine the effect of sex hormones on body weight and histamine - induced eating and drinking behaviour and finally (c) To examine the influence of sex hormones on the

affinity of histamine receptors in rat brain.

In the hypothalamus which contains the highest concentration of histamine in the brain, studies in rat and Rhesus monkey (Brownstein et al 1974) have shown that histamine has its highest intrahypothalamic concentrations in nuclei believed to be directly involved in the regulation of gonadotrophin secretion from anterior pituitary. This suggests that histamine may be involved in the hypothalamo-hypophyseal-gonadal system (Edward et al 1975: Netti et al, 1982). Histamine could therefore play a role in reproduction. This project therefore intends to investigate the role of histamin in reproduction.

The regional distribution of histamine in the brain shows that it is present in nearly all areas of the brain and the physiological responses of this amine in the brain as in the periphery are mediated by at least two distinct types of receptors ($H\bar{1}$ and $H\bar{2}$) (Schwartz et al 1980; Taylor and Richelson 1981). Though histamine has been shown to possess a variety of actions in the central nervous system, its role in regulation of seizures has not been investigated. It is therefore the aim of this project to investigate the role of histamine in seizures and thereby establish its mode of action in seizures regulation. It is hope that these studies will contribute to

better understanding of the physiological role of histamine for histamine and also establish a relationship between histamine and sex hormones in the central nervous system.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Test Animals

Male albino and female wistar rats (bred in Departmental animal House) and white Ranger Cockerels (obtained locally from Arewa Agricultural Enterprises Nigeria Ltd., Zaria) were used for these studies. The chicks were kept under continuous light exposure in order to keep the chicks warm and healthy.

3.2 Chemicals

The chemicals used in this work and their sources of supply are given below:

2-Thiazolyl-ethylamine dihydrochloride, Dimaprit dihydrochloride were donations from SK&F England, Histamine dihydrochloride, Triprolidine hydrochloride, Chlorpheniramine maleate, Cimetidine, Dopamine hydrochloride, Noradrenaline hydrochloride, Cyproheptadine hydrochlorids, Acetylcholine chloride, 5-Hydroxytryptamine, Pentylenetetrazol (leptazol), Apomorphine hydrochloride (Sigma chemical Co.), mepyramine were purchased from May and Baker. (^{3}H) mepyramine (24.1-28.1ci/mmole) was bought from Amercham International.

(a) Histamine dihydrochloride was given both intraperitoneally (ip) and intracerebroventricularly (icv)

(b) Apomorphine hydrochloride was prepared by dissolving the powder in boiled and cooled, air-free normal saline (boiled, closed and allowed to cool) and kept in an amber-coloured air tight bottle. 1% w/w sodium metabisulphite (British Drug Houses Ltd) was added to the weighted quantity of apomorphine hydrochloride before the preparation of each solution to enhance stability, since apomorphine hydrochloride solution decomposes readily when exposed to air or light. The solution was injected subcutaneously.

3.3 STORAGE OF DRUG SULUTIONS

All drugs used in this project were freshly prepared for each day's work to maintain their stability except cimetidine, 2-thiazolyl-ethylamine and dimaprit which were prepared and stored in the refrigerator for use when required. Under this condition their potency could last for more than 30 days. Drugs like dopamine, noradrenaline and apomorphine were stabilized by the addition of 0.1% w/w sodium metabisuphite.

3.4 VOLUME OF THE INJECTED DRUG SOLUTIONS

Drugs were administered as solutions of their respective salts. Their concentrations were adjusted so that the chicks received 0.2ml/10g body weight while each rat received 0.2ml/100g body weight. Hormones were administered at a constant volume such

that each rat/chick receive 0.1ml equivalent to 120ug Destradiol 17B, clomiphene and tamoxifen, 250ug testosterone and 1mg progesterone.

3.5 ROUTES OF ADMINISTRATION

All drugs were administered intraperitoneally (i.p) except hormones and apomorphine that were administered subcutaneously (s.c).In the study of the effect of sex harmones on histamine induced eating and drinking behaviours, histamine was administered intracerebroventricularly (icv).

3.6 CONTROLS

The controls were injected with normal saline (0.9% w/v NaCl). The controls used for the hormones were injected with ethyl-oleate except the controls used for clomiphene that were injected with distilled water since this hormone was dissolved in distilled water. The controls used for cimetidine were injected with 0.1N Hc1 in which the PH had been adjusted to approximately 7 with 0.1N NaoH.

3.7 PRETREATMENT TIMES

A pretreatment time used for behavioural and seizure studies was 30 minutes and 1 hour respectively (based on the onset of the action of the drugs used from previous preliminary studies) before administration of the agonists or antagonists. Both rats and chicks were primed with sex hormones for

four consecutive days before drug treatment. Chicks were also pretreated with reserpine 24 hours prior to administration of 2-thiazolyl-ethylamine.

3.8 STATISTICAL ANALYSIS

All data obtained were compared using the Student's "t" test regarding $P<0.05$ or less as being statistically significant.

3.9 BEHAVIOURAL STUDIES

3.9.1 GROSS BEHAVIOUR

Two-day old chicks (white ranger cockerels obtained locally from Arewa Agricultural Enterprises Nigeria Ltd. Zaria) weighing between 26-41g were used in this work except for an experiment to determine species variation in the action of chlorpheniramine when rats were included in the study. The chicks were kept under continuous light exposure in order to keep the chicks warm and healthy.

Each chick was placed in a separate transparent perspex cage (38.5 cm x 24cm x 15 cm) for one hour to acclimatize to the laboratory conditions before drug administration and had access to both food and water. The experiments were performed in a quiet room free from disturbances with ambient temperature of 27.0 ± 3.0 oC and between 09.00 - 14.00 hours daily to avoid behavioural changes resulting from circadian rhythm. The chicks were scored for the following behavioural

variables; sleep, depression/sedation, activation-locomotion, escape episode, jumping, head and neck jerks and pecking. Vocolization was also noted. Locomotion, pecking, jumping and escape episode were recorded by means of hand tally counter. Locomotion was scored by the ability of the chick to move from one square compartment, drawn on the floor of the cage, to another. Rats were scored for sleep/depression, locomotion, rearing, and climbing .

Chicks were regarded as sedated or depressed when they were quiet and immobile with or without closure of their eyes for over 5 minutes and with or without drooping heads according to Wambebe (1978). The criteria for behavioural sleep in chicks were those described by Fugner and Hoefke (1971). The chick was regarded as asleep when both eyes were closed, the chick was either crouching with the beak on the floor, or standing with the head leaning against the wall of the cage. Either a digital clock or a stop watch was used for timing the onset and duration of sleep.

3.9.2 EATING AND DRINKING BEHAVIOURS

Adult male wistar rats were placed in separate perspex cages (55cm x 33.5 cm x 20cm) and fed daily with tap water and food (Pfizer mouse cubes) until the animals had demonstrated stable water and food intake baselines(less than 5% daily variation). They

were deprived of water and food for 22 hours then fed with 50ml of tap water and 50g of food for 2 hours between 1.00 p.m - 3.00 p.m for 7 days. Intracerebro-ventricular (icv) implantations were carried out on the 7th day followed by hormone administration subcutaneousty (sc) for 3 days starting from the first day. On the 4th day of the surgery (icv. implantation), the test drug (histamine) was administered icv. Both water and food intake were recorded throughout the experiment at 30, 30 and 60 minutes of the test session. Apart from using different rats as controls, the treated rats (with histamine) were also used as their controls.

3.10 BODY WEIGHT

Adult male wistar rats were placed in separate cages (55cm x 33.5cm x 20cm) and fed with Pfizer feeds for 7 days to enable them become used to the feed before starting the experiment. At the beginning of the experiment, rats were primed with sex hormone (oestradiol 17B 120µg, testosterone 250µg and progesterone 1mg total dose) for 6 days while the controls received ethyl oleate. Individual body weights were recorded before and after they were primed with the hormones.

3.11 THERMOREGULATORY STUDIES

Five day old chicks (white ranger cockerels obtained locally from Arewa Agricultural Enterprises

Nigeria Ltd) weighing between 30-51g were used in this study. Chicks treated with sex hormones were six day old and were primed with these hormones for four consecutive days starting from day 2 before they were used for the experiment.

They were allowed 1 hour to acclimatize to the laboratory conditions before commencing temperature measurements and had access to both food and water. Chicks were placed in a big transparent perspex cage (55.0cm x 33.5 cm x 20cm) and supplied with a source of heat from 60W bubs. The experiments were performed in a quiet room at room temperature of about 27.0.c + 3.0 ^{o}C and between 9.00 and 14.00 hours daily to keep uniform circadian oscillations of temperature. Each experiment was repeated five or six times.

The rectal temperature was recorded at regular intervals of 10 minutes with the aid of Edale thermistor thermometer, model K (Edale Instruments, Cambridge, England) before and after drug administration until the temperatures returned to their original or near these values. The thermistor probes were inserted into the birds rectum to a distance of about 2.5cm to give reproducible temperature measurements. The control chicks were injected with the appropriate vehicle. All chicks were injected intraperitoneally(i.p)

3.12 REPRODUCTIVE STUDIES

Young and adult female wistar rats bred locally in our animal house weighing 140g-180g and 200g-250g respectively were used in this experiment. They were kept in separate perspex cages (55.0 cm x 33.5 cm x20.0 cm) and fed daily with water and our locally formulated feed (see appendix). The oestrus cycle of the female rat was determined by vaginal smearing. Rats were subjected to daily vaginal smears between 10.00 a.m and 11.00 a.m and only those rats that exhibited at least four consecutive regular 4 day cycle were included in the study.

A drop of saline was pipetted using a syringe and injected in and out of the vagina, and then transferred to a slide. The smears were examined microscopically in the fresh state and the presence or absence of three kinds of cells: Cornified (C), Epithelial (E) and Leucocytes (L) were noted. The animals were injected intracerebroventicularly (icv) with histamine (80-120µg total dose), intraperitoneally (ip) with triprolidine (50mg/Kg) chlorpheniramine (50mg/Kg) and cimetidine (50mg/Kg) and their oestrous cycle observed for 52 days thereafter.

3.13 SEIZURE STUDIES

3.13.1 ELECTRICALLY - INDUCED SEIZURE

Electroshock was delivered via the upper eye

lids with corneal electrodes using constant current Ugo Basile Electroshock Convulsive Therapy Machine, Model 7800 set to give a shock duration 0.8 seconds, 0.4 pulse width and a frequency of pulse of 100/second. A current giving 20-30% convulsion in the control population was chosen for use on the treated population of chicks. Tonic Convulsion was the parameter used throughout the studies. Protection was defined as prevention of tonic seizure following challenge by a current which produced this pattern in control chicks. Chicks were challenged with electroshock after 1 hour pretreatment. Chicks were then arranged in a group of 5 for each dose of drug and the experiment repeated three or four times using different sets of animals.

3.13.2 CHEMICALLY - INDUCED SEIZURE

In this experiment, chicks were divided in a group of 5 at a time and kept in separate compartments for 1 hour to acclimatize to the environment before drug administration. A dose of leptazol (90mg/Kg) which produced 60% tonic seizure in the control chicks was used. Chicks were pretreated with the drug under test for 1 hour prior to the administration of the convulsant agent and observed for 2 hours later. Lack of tonic extension of the hindlimbs was regarded as no convulsion.

3.14 IMPLANTATION OF ELECTROCORTICAL AND ELECTROMYOGRAPH ELECTRODES

White ranger cockerels, 2 days old and weighing 26-41g were used for this study. The chicks were anaesthetized with halothane (Fluothane, ICI Ltd) and implanted with electrodes made from the pointed ends of stainless steel insect pins NO.00, according to the methods of Spooner (1965)

The optic tectum, hyperstriatum and pontine reticular formation were implanted and secured chronically to the skull with dental cement. The monopolar electrodes implanted onto the hyperstriatum and optic tectum used an electrode implanted onto the comb of the chicks as reference. The electrodes implanted on the pontine reticular formation were bipolar. The sites of the implanted electrodes were located by the aid of stereotaxic instrument using the co-ordinates stated below. Histochemical confirmation of the position of the electrodes at the end of the experiments was carried out on the chicks.

The electrodes for the Electromyograph (EMG) were made with stainless insect pins No. 00 bent at the ends to make hooks. The electrodes were positioned in the proximity of the head and inserted into the semi spinalis capitis muscle of one side of the dorso-lateral aspect of the neck. The electrodes were secured chronically to the neck with dental

cement. The co-ordinates for the chick implantation were HS-antero-posterior + 3.8 mm, lateral - 3.4 mm; OT - at antero-posterior -2.4mm, lateral -3.4mm; RF - antero-posterios - 2.0 mm, lateral + 1.5 mm, vertical - 8.1 mm.

After the implantation process was completed, the chicks were kept warm with an angle poise lamp and allowed 24 hour to recover fully from halothane before EEG recording.

3.14.1 EEG RECORDING

The electrodes of the implanted chick were attached to a male connector and hooked on to the female connector. The chick was then placed inside a screened cage and allowed 1hr to settle down in the new environment before EEG recording. The recordings of both the EEG and EMG were done with Grass Polygraph, model 79D. The chart speed during normal recording was 2.5 mm per second. However, at appropriate times, the speed was changed to 25mm per second for 10 sec to facilitate analysis of voltage and peak frequency.

3.15 INTRACEREBROVENTRICULAR TECHNIQUE

Guide cannulae for intracerebroventricular injection were constructed from 0.61mm diameter intramedic polyethylene tubing. With the aid of flame

from bunsen burner a bulb was blown in the middle of a piece of tubing. The length of the end of the tubing to be inserted into the ventricle was 4mm from the bulb while that from the other end of the bulb on top of the skull through whch injection would be made was 1cm. The dead volume of the cannula was approximately 2ml. Normal saline was passed through the guide cannula before insertion to ensure that it was not blocked.

3.16 SURGERY

Male albino rats weighing between 200-250g were anaesthetized with pentobarbitone sodium solution (45mg/kg,ip). The skin over the skull was cut open with a sterile surgical blade to expose coronal and sagittal sutures. The guide cannula was positioned 1mm posterior to the bregma and 1mm lateral to the midline and with the help of a hand drill, lowered to depth of 4mm from the top of the skull into the left ventricle. In all animals, some CSF appeared in the guide cannulae after insertion. This indicates correct placement into the ventricle as this would not have occurred from an insertion into tissue. A retaining screw was then fixed to the skull opposite the guide cannula to keep it firmly in place. The guide cannula was finally held in position using acrylic fluid and dental cement and closed with

stylets to prevent blocking. Injections were made from 10ul Hamilton syringe over a period of 5 seconds with the needle remaining in position for a further 5 seconds before being withdrawn and immediately replaced by stylets. The total injection volume was 8ul.

3.17 BINDING STUDIES

This work was carried out in Chelsea College, University of London.

(a) Brain Dissection: Adult wistar rats (250 -300g) of either sex were stunned and killed by decapitation between 10.00 and 11.00 hours. The brains were quickly and carefully removed into a beaker containing ice cold assay buffer (Krebs Henseleit buffer) already bubbled with 95% O_2, 5% Co2 and adjusted to PH.7.3. Dissection was performed on an ice-cold petri-dish kept at a temperature 0-4 ^{o}C. Brain regions (hypothalamus, amygdala and cortex) from three animals were pooled together ready for homogenization.

(b) Tissue Preparation: Brain tissues from three rats were homogenized in 15 volumes (w/v) of Krebs Henseleit buffer at PH. 7.3 using 10 strokes of teflon pestle at 300 rph in an MSE homogenizer and suspended in 50 volumes (W/V) of the some buffer. The resulting tissues were then centrifuged in MSE

superspeed 50,at 50,000xg for 20 minutes yielding a pellet and a supernatant fraction. The particulate fractions (pellets) obtained by two successive centrifugations 50x100g x20 minutes and washings were finally suspended in the same buffer (100 volumes) giving a protein amount of 5mg of the original wet weight of tissues.

The series of washings and centrifugations were designed to ensure the removal of endogenous levels of histamine from membrane preparations. The tissue suspensions were immediately frozen with solid CO_2 (dry ice) and stored up to 3 months at -10 ^{o}C ready for use when necessary. There was no evidence of any deterioration of binding properties over this period of storage.

(c) Binding assay: 500ul aliquots of the tissue suspension (5mg original wet weight tissue) were added to the plastic micro-cup-centrifuge tubes (1.5ml capacity) standing on ice containing 25ul Krebs Henseleit buffer and 25ul [3H] mepyramine. Blanks as control for non specific binding contained also 25µl 2nM [3H] mepyramine and $5x10^{-6M}$ unllabelled mepyramine. The resultant tissues were mixed thoroughly using a vortex mixer and incubated at 30 ^{o}C for 15 minutes then cooled in ice to 0-4 ^{o}C for 15 minutes. All the assays were performed in triplicate for Hypothalamus, Amygdala and cortex.

The mixtures were then cooled to 0-4°C, centrifuged at 14x1000g for 2.5 minutes to separate bound radioactivity from free. The pellets were superficially washed three times with ice cold buffer and dissolved in 0.5 N NaoH and neutralized with 0.5N Hc1.

Tubes and contents were transfered to scintilation minivials containing 5ml Dioxon/Naphthalene scintillant, shaken thoroughly and inserted into a polypropylene scintilation tube. These were left in the dark overnight to stabilize and equilibrate before radioactivity was taken.

Radioactivity was measured as cpm using a Beckman L 230 liquid Scintillation counter using [$^{3}_{H}$] - n - hexadecane as a standard. Cpm was converted to dpm for calculations. External standard ratios were constant indicating that the counting efficiency was approximately the same in all the vials.

Incubations containing radiolabelled ligand alone gave a measure of the total 2nM (3HJ mepyramine bound to the tissue preparations while that bound in presence of unlabelled drug, used as control for low affinity binding, gave a measure of nonspecific binding. Specific [$^{3}_{H}$] mepyramine binding was defined as the difference between the total amount of [$^{3}_{H}$] mepyramine bound in the presence and absence of unlabelled mepyramine.

Equilibrium dissociation constant (kd) and saturable binding capacities (Bmax) were estimated by Scatchard analysis (Scatchard, 1949) and protein concentrations were measured by the method of Lowry et al, 1951.

(d) Scatchard Plot: The interaction of radioligand with its receptors in a biological system is best expressed in terms of its affinity (kd) with the receptors and the number of binding sites (n) occupied by the radioligand. These two parameters can be measured by graphical analysis and the Scatchand plot is a popular method of ligand-receptor binding studies in which kd and n can easily be determined.

If we consider a radioligand L, interacting reversibly with a receptor R to form a complex RL as shown below

$$L + R \quad RL \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Then applying the law of mass action at equilibrium

$$kd = \frac{(R)\ (L)}{(RL)} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where kd is the equilibrium dissociation constant of the complex and (R), (L) and (RL) represent the concentrations of free receptors, free ligand and bound ligand respectively. Then the total concentration of the receptors Rt = (R) + (RL)

from equation (2)

$$kd = \frac{(R+ - RL)\ (L)}{RL} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$\therefore \frac{[RL]}{(L)} = \frac{[R+] - [RL]}{(kd)} = \frac{1[RL]}{kd} + \frac{1\ [R+]}{kd} \quad ....(4)$$

which is the form of equation derived by Scatchard (1949). Equation (4) is straightline of the form $y = mx + C$. Thus a plot of the ratio of bound to free ligand (RL)/L, versus the concentration of the bound ligand , (RL) gives a straight line (assuming only one set of binding site with no interaction between sites) with two intercepts. The one on the x-axis giving the total numbers of binding sites (n) per molecule and y- intercept yielding 1/kd

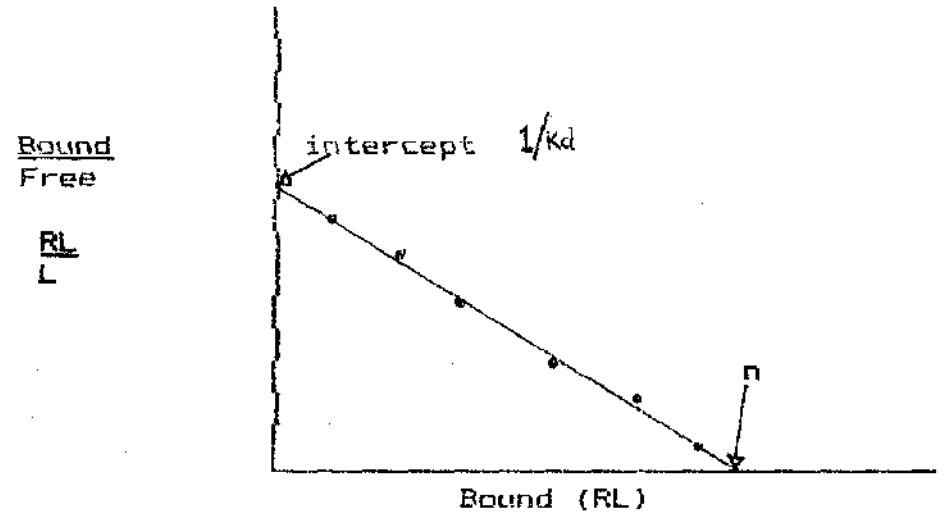


Fig.4.1: Scatchard plot of the binding of a ligand to a receptor.

(e) Oestrous cycle

Animals were treated as early described.

(See reproductive studies 3.12)

CHAPTER FOUR

4.0 BEHAVIOURAL ROLE AND MODE OF ACTION OF BRAIN HISTAMINE IN YOUNG CHICKS

4.1 Summary

4.1.1 The behavioural profile of histamine in young chicks revealed a triphasic response of an initial behavioural sleep, which was followed by a longer behavioural stimulation culminating in a very long behavioural depression.

4.1.2 Two types of receptors ($H\bar{1}$ and $H\bar{2}$) have been implicated in this response. The $H\bar{1}$ receptors were found to be excitatory while the $H\bar{2}$ receptors were inhibitory.

4.1.3 In the central action of histamine the $H\bar{2}$ receptors are first stimulated prior to the $H\bar{1}$ receptors, hence the initial behavioural sleep before stimulation.

4.1.4 The present data also show that the $H\bar{1}$ and $H\bar{2}$ receptors play regulatory roles for endogenous histamine to maintain a balance in the brain during normal physiological process.

4.1.5 A blockade of $H\bar{1}$ receptors (the excitatory component) blocks the endogenous histamine arousal

system leaving the inhibitory component (H_2 receptors) unaffected for endogenous histamine to act. The resulting effect is inhibition. Hence the sedative properties of H_1 receptor antagonists.

4.1.6 Cimetidine (H_2 receptor antagonist) induced behavioural excitation in young chicks, an effect due to a blockade of the inhibitory component, H_2 receptors leaving the endogenous histamine to act on the excitatory component - H_1 receptors.

4.1.7 Chlorpheniramine, H_1 receptor antagonists induced a biphasic response of an initial depression followed by prolonged stimulation in young chicks but only depression in rats and arousal in adult fowls.

4.2 INTRODUCTION

Neurotransmitter role of histamine in the CNS of many mammalian species has been established by many workers (Schwartz, 1977, Greet et al, 1975). The widespread distribution of histaminergic terminals in whole telencephalon as well as its origin in the reticular formation including other brain regions suggests that histamine could play a role in sleep/ arousal behaviour (Schwartz,1975,Verdiere et al,1977) and other behavioural and physiological variables (Muller et al, 1977).

The discovery and characterization of histamine receptors has facilitated the identification of many

specific biological actions of histamine in the peripheral nervous system. A variety of biochemical, physiological, electrophysiological and radio-labelled binding studies suggest that these recepors are also present in mammalian brain (Schwartz et al, 1980, Taylor and Richelson 1981, Hill et al, 1978; Chang at al, 1977, Schwart, 1977) depending on the brain area or mammalian species.

Though these receptors have been found in the brain, the exact role of each of these receptors in the central actions of histamine is not yet clearly understood. The use of selective agonists and antagonists no doubt provides an understanding of the role of histamine recepors in the CNS.

Histamine and most of its analogues do not easily cross the blood brain barrier. Thus, young chicks have been chosen for this work because of their non-functional blood-brain barrier to allow easy penetration of the drugs into the central nervous system. It is also easy to quantify many behavioural variables in young chicks because of the sensitivity of the central nervous system to chemical agents at this stage of brain development.

4.3 Materials and method

Refer to section 3.9.1,3.14,3.14.1

4.4 Results

4.4.1 Behavioural effects of histamine in young chicks

Histamine (5-30mg)kg) was found to induce mixed behavioural changes in young chicks. 80% of the chicks slept immediately after drug administration. The 80% of the chicks that slept and the duration of sleep was dose dependent. This was followed by a longer period of behavioural stimulation (excitation) which was characterized by rapid movements, vocalization, jumping, escape episode, occasional sqation and standing and both head and neck jerks. This period culminated in a very long interval of depression/sleep lasting over 220 minutes at a dose of 30mg/kg before the chicks finally recovered. This resulted in a decrease in locomator activity. There was a decrease in pecking behaviour with increase in dose. The onset of action 1.70 ± 0.67 - 3.2 ± 1.06 min (n=5) was also dose dependent, decreasing with increase in dose (Table 4.1 & Fig 4.1)

4.4.2 Behavioural effects of H_1 and H_2 receptor agonists

It is possible that the mixed behavioural effects elicited by histamine could be due to its action on its receptors or through any other mechanism of action. Using selective H_1 and H_2 receptor agonists it was possible to determine the effects mediated by either H_1 or H_2 receptor stimulation.

TABLE 4.1

Effect of histamine on gross behaviour of two-day old chicks

		Loctomotion, Escape Episode, Jumping and Pecking are expressed in Counts per hour (cph) mean ± SEM									
Dose µg/kg	No. asleep or sedated/ No. used	No.activated/ No. used	No. depressed/ No. used	Duration of sleep/sedated (min)	Duration of activation (min)	Duration of depression (min)	Onset of action (min)	Locomotion	Escape episode	Jump	Pecking	Gross Locomotor activity mean±SEM
Control (Sal.)	0/5	0/5	-	-	-	-	-	66.35±11.75	2.05±0.35	1.3±0.05	59.25±9.5	69.7±4.2
5	2/5	3/5	4/5	9.6±5.3	16.07±7.35	68.3±11.2	3.2±1.06	35.3±11.32	1.95±0.25	13.2±2.26	30.3±8.6	50.4±4.6 *
10	4/5	5/5	5/5	14.0±1.5	21.5 ±5.5	81.3±6.8	2.4±0.9	31.2±6.5	4.2 ±1.2	7.2±2.3	18.2±4.5	42.7±3.5 **
15	4/5	4/5	5/5	15.5±4.5	30.3 ±6.4	134.5±32.5	1.75±0.25	23.5±7.1	3.9 ±2.6	7.6±2.8	12.0±1.3	35.07±4.2 ***
20	5/5	4/5	5/5	16.7±4.4	32.0 ±1.0	137.1±43.3	1.7 ±0.67	20.15±3.6	6.95±1.65	7.3±1.4	8.6±4.1	34.4±2.2 ***
30	5/5	4/5	5/5	37.0±15.2	38.3 ±2.6	205.0±25.0	1.75±0.5	13.14±8.1	9.8 ±2.1	4.5±2.3	0.48±0.02	27.44±4.2 ***

*p<0.02, **p<0.002, ***p<0.001 Compared to saline control: Student's "t" test. n = 5 chicks per experiment.

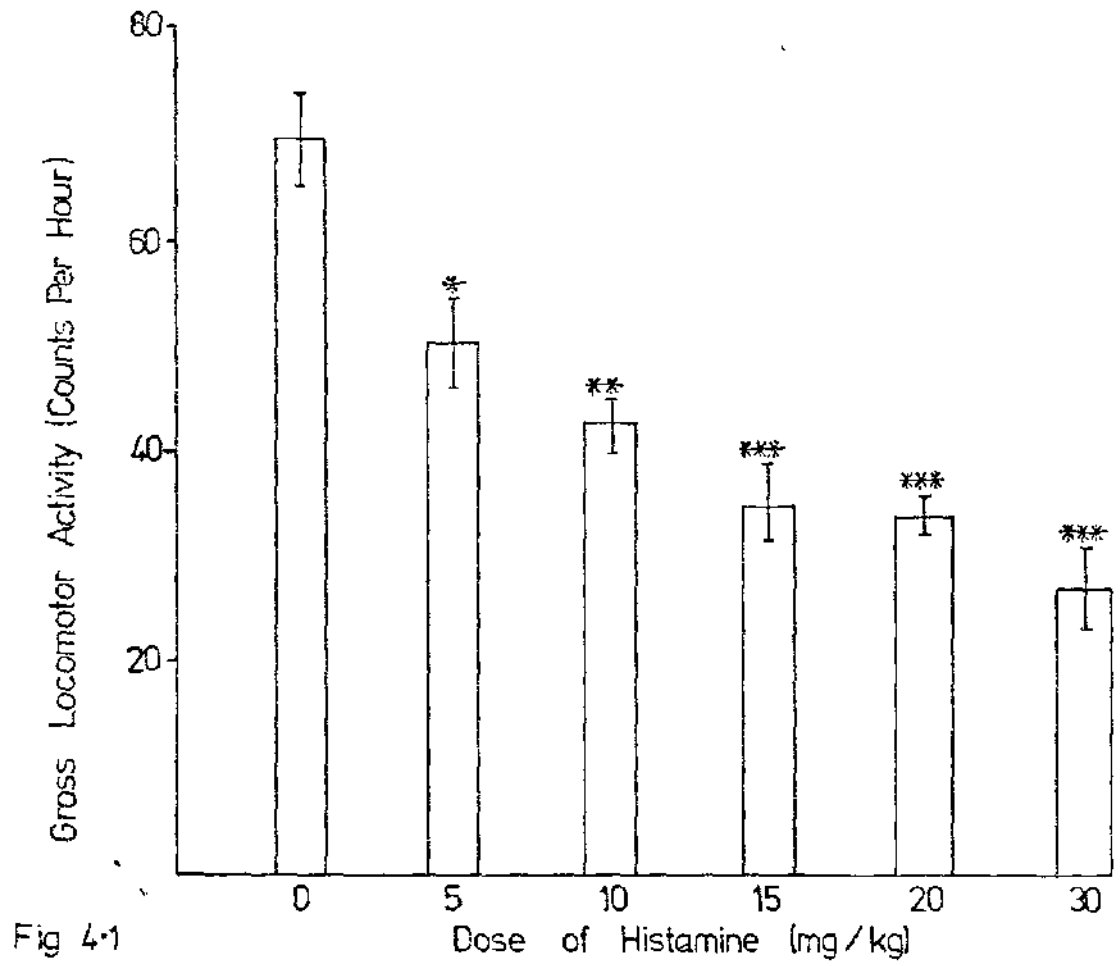


Fig 4·1

Effect of Histamine on gross Locomotor activity in 2-day old chicks.
*P< 0·02 **P< 0·002 ***P< 0·001. Compared to saline control: student's "t" test. Vertical bars represent standard error of the means ± SEM. n = 5.

2 - thiazolyl-ethylamine, a selective H_1 receptor agonist given i p in young chicks induced a dose - dependent behavioural stimulation which was characterized by vocalization (which increased with increase dose) , jumping, cage pecking, rapid movement/running, escape episode, head and neck jerks before the chicks became depressed. This period of activation lasted up to 120 minutes with a dose of 30mg/kg. The onset of action (5.3±2.4 -10.2±3.17 minutes) was dose dependent, decreasing with increase in dose (Table 4.2 & Fig. 4.2). The action of 2-thiazoly-ethylamine was antagonized by mepyramine (15mg/kg) (Table 4.3 & Fig. 4.3) but not by Cimetidine (Table 4.4 & Fig.4.4). To examine if 2-thiazolyl-ethylamine may be acting on dopaminergic receptors to produce behavioural stimulation, the effect of 2-thiazolyl-ethylamine was compared with that of apomorphine. In this study, 2-thiazolyl-ethylamine induced more locomotor activity while apomorphine (0.5mg/Kg) elicited more pecking behaviour especially cage pecking (Table 4.5 & fig 4.5). Pretreatment of reserpine 24 hours before administration of 2-thiazolyl-ethylamine did not effect the action of 2-thiazolyl-ethylamine (Table 4.6 & Fig 4.6).

Dimaprit, a selective H_2 receptor agonist elicited an initial behavioural sleep (5.6 ±1.2 -

TABLE 4.2

Effect of 2-thiazolyl-ethylamine on behavioural activites of two-day old chicks

Dose mg/kg	Behavioural Activity (Counts per hour) mean ± SEM: Locomotion	Excape Episode	Jump	Pecking	Onset (min)	Duration of Activation (min)	Duration of Depression/Sedation (min)	Gross Locomotor Activity(cph) mean ±SEM
Control (Sal.)	73.45±20.6	17.4±6.35	1.1±0.28	78.9±35.4	-	-	91.95±9.1	91.95±9.1
5	+ 120.2 ±11.4	45.8±15.76	72.58±34.6	‡ 230.0±123.6	10.2±3.17	110.0± 12.0	91.0 ±2.3	‡‡ 209.88±20.6
10	+ 109.68±21.6	62.35±10.7	18.54± 4.0	‡ 56.1±10.0	9.26±3.05	83.0± 28.0	106.0 ±4.0	‡ 190.5 ±12.1
15	+ 158.2 ±15.02	68.73±17.89	19.2 ±4.84	‡ 91.3± 37.4	9.03±2.8	79.0± 27.0	105.66±4.4	‡‡‡ 246.06±12.6
20	+ 182.85±27.1	79.5±15.94	23.9 ± 4.3	‡ 116.5±61.6	7.3 ±1.3	85.0 ± 10.0	82.16±8.6	‡‡‡ 286.25±11.8
30	+ 195.0 ±13.0	42.8±5.1	152.0 ±9.5	‡ 151.5±28.6	5.3 ±2.4	98.5± 21.5	62.3 ±6.2	‡‡‡ 389.8 ±9.2

+ Rapid movement/Running, ‡ Cage pecking mainly, ‡P<0.01, ‡‡ P<0.002, ‡‡‡ P<0.001. Compared with saline control: Student's "t" test. n = 5 chicks per experiment.

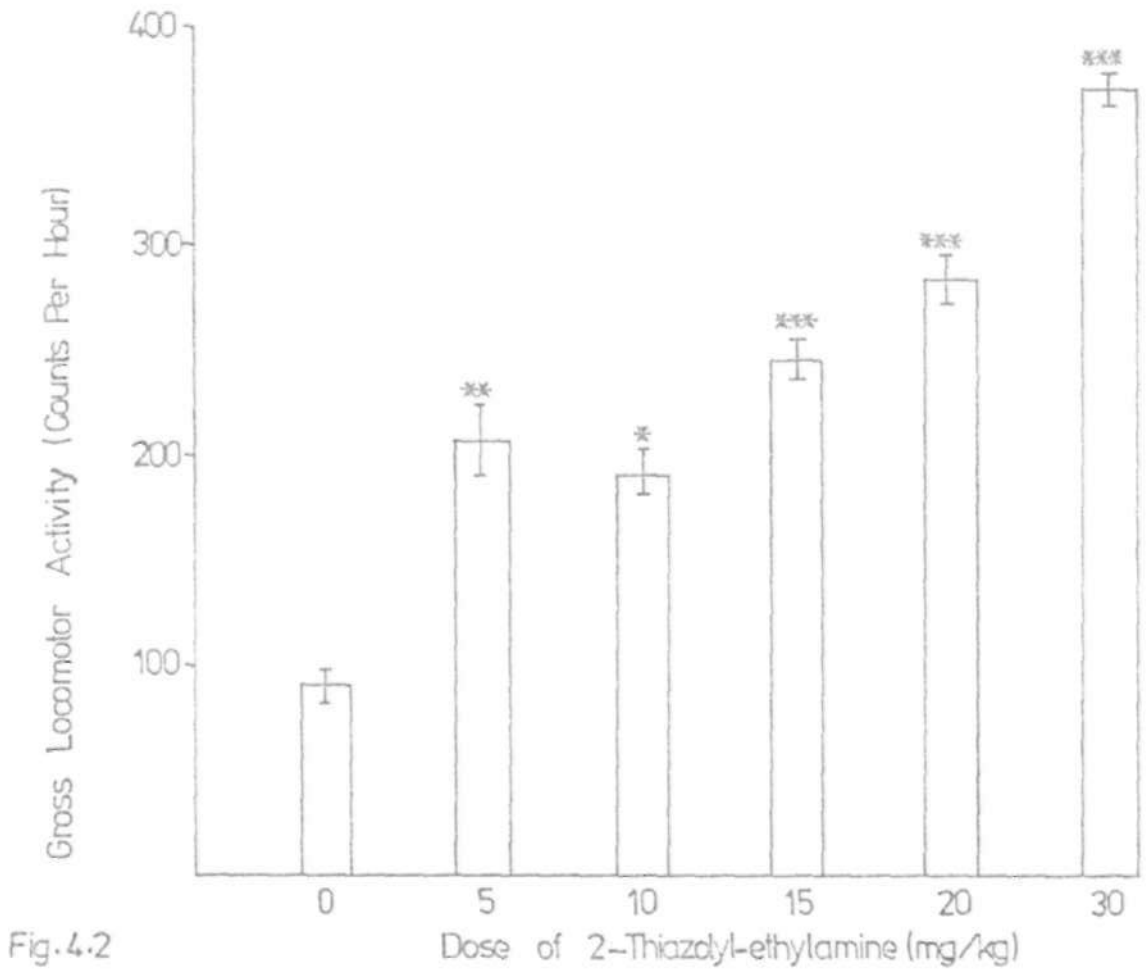


Fig. 4.2

Effect of 2-Thiazolyl-ethylamine on gross locomotor activity in 2-day old chicks. *P<0.01; **P<0.002; ***P<0.001. Compared to saline control; student's "t" test. Vertical bars represent standard error of the means ± SEM. $n = 5$.

TABLE 4.3

Effect of 2-thiazolyl-ethylamine pretreated with 15mg/kg mepyramine on gross behaviour of two-day old chicks

Dose mg/kg	Behavioural activity (counts per hour) Mean ± SEM				
	Locomotion	Excape Episode	Jump	Pecking	Gross locomotor activity mean±SEM
Control (Sal.)	17.2 ±1.2	0.63±0.03	-	9.3 ±0.4	17.83±0.62
5	28.5 ±11.08	1.3 ±0.3	0.8 ± 0.3	11.33±1.6	30.6±3.96
15	47.1 ± 12.3	6.9 ±0.3	4.2 ± 0.6	26.3 ± 10.4	58.2 ±4.4
30	33.8 ± 8.1	2.8 ±1.3	4.3±2.2	17.9 ±12.3	41.1 ±3.9

Pretreatment time prior to administration of the 2-thiazolyl-ethylamine was 30 minutes.

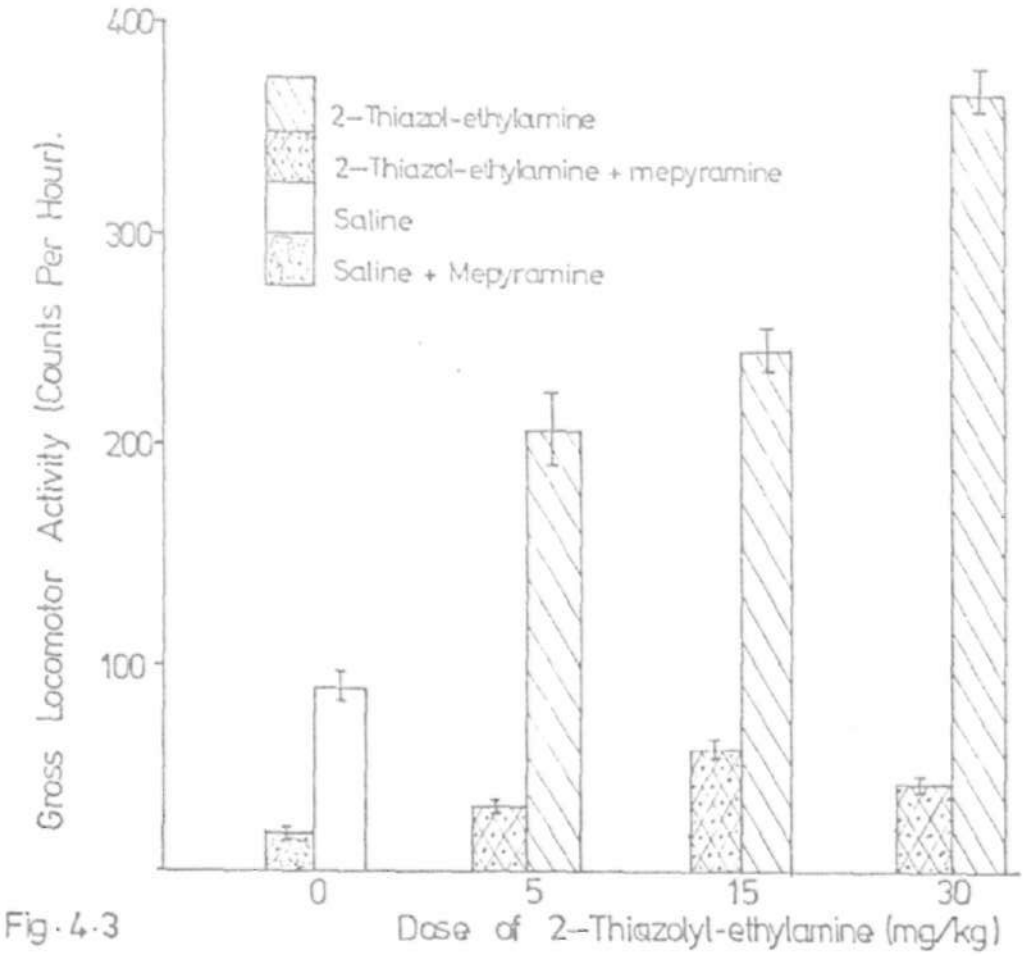


Fig. 4.3 Effect of 2-Thiazolyl-ethylamine pretreated with mepyramine on gross locomotor activity in 2-day old chicks. Vertical bars represent standard error of the means ± SEM. n = 5 chicks per experiment.

TABLE 4.4

Effect of dimaprit and 2-thiazolyl-ethylamine pretreated with 15mg/kg mepyramine and 15mg/kg cimetidine respectively

Locomotion, Escape episode and Pecking are expressed in counts per hour(cph)
Mean ± SEM

A Dimaprit

Dose mg/kg	Locomotion	Excape Episode	Jump	Pecking	Gross locomotor activity mean±SEM
Control (Sal)	46.97 ±2.7	1.05±0.23	0.13±0.01	21.03±0.49	48.15±0.98
30	6.94± 2.16	0.84±0.33	0.00	0.87±0.05	7.78±1.25

B 2-Thiazolyl-ethylamine

Dose mg/kg	Locomotion	Excape Episode	Jump	Pecking	Gross locomotor activity mean±SEM
Control (0.1N NaoH+0.1N Hcl)	31.55 ±9.6	1.55±0.86	0.00	21.38±8.6	33.1 ±5.23
30	78.08±19.2	9.26±2.23	21.5±2.83	66.85±21.35	108.84±8.1

Pretreatment time prior to administration of the agonists was 30 minutes.

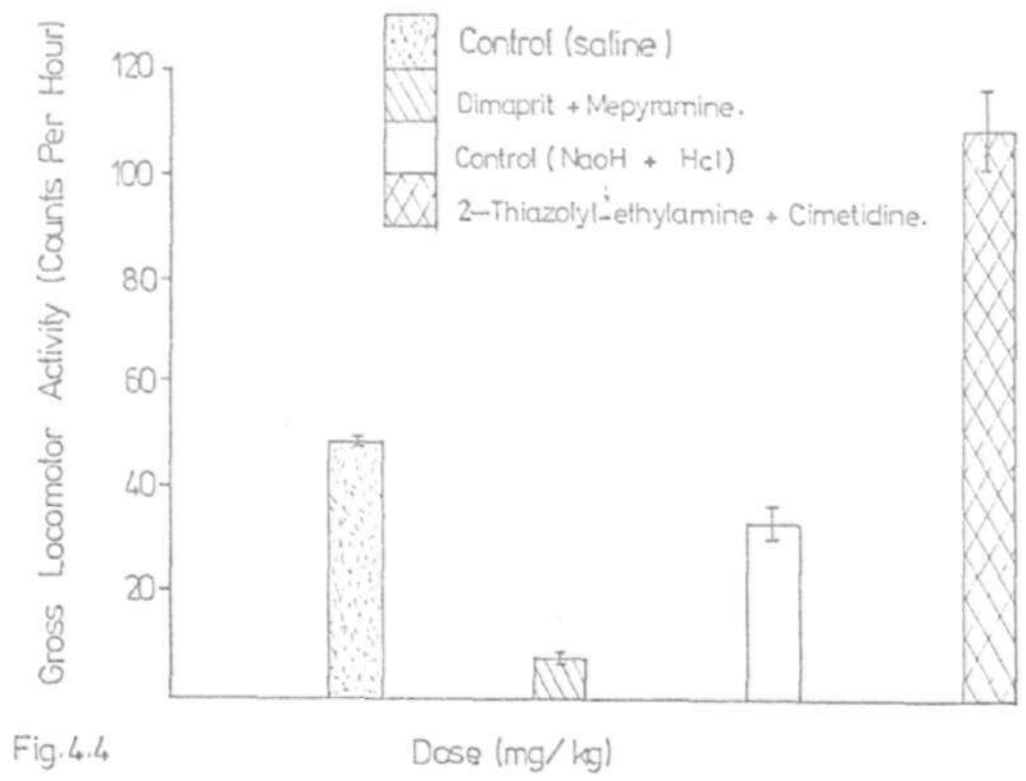


Fig.4.4

Effect of Dimaprit and 2-Thiazolyl-ethylamine pretreated with 15mg/kg Mepyramine and 15mg/kg Cimetidine respectively. Vertical bars represent standard error of the means ± SEM. Pretreatment time was 30 minutes.

TABLE 4.5

Effect of apomorphine and 2-thiazolyl-ethylamine on behavioural activities of two-day old chicks

Dose mg/kg	Behavioural activity (counts per hour) Mean ± SEM				
	Locomotion	Excaupe Episode	Jump	Pecking	Gross locomotor activity mean SEM
Control (Sal.)	72.40± 3.88	7.65±4.56	2.6i ± 0.48	39.3 ±i3.64	82.66±2.83
0.5mg/kg Apomorphine	278.0 ± 17.96	13.16±0.5	35.89±8.55	328.02±97.89	327.05± 9.00
20mg/kg* 2-thiazolyl-ethylamine	305.73±81.24	18.86± 3.49	106.86±22.85	5i.4 ±i3.39	43i.75±35.86

* More Vocalization noted. Apormorphine was administered subcutaneously (SC) while 2-thiazolyl-ethylamine was administered intraperitoneally (ip).

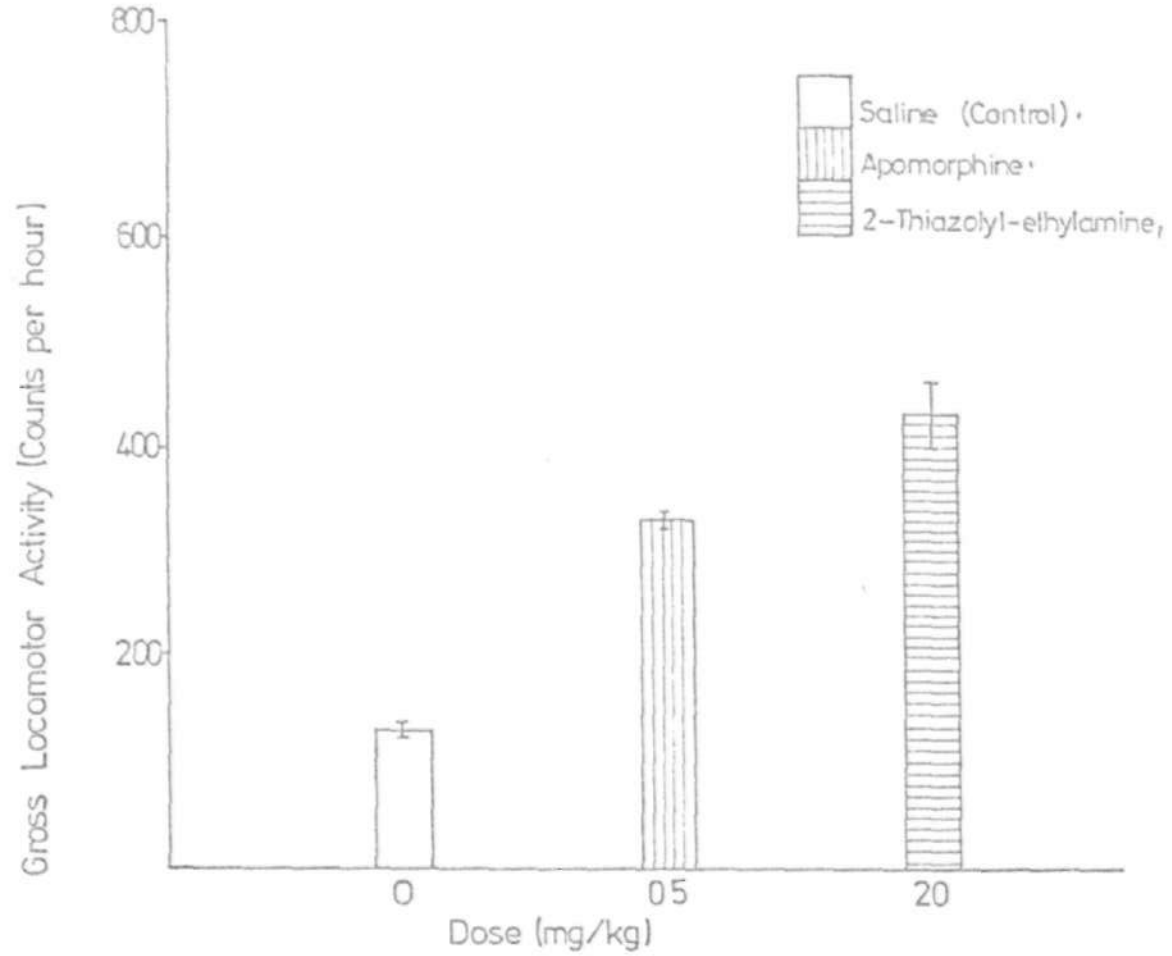


Fig.4.5 Effect of Apomorphine and 2-Thiazolyl-ethlylamine on gross locomotor activity in 2-day old chicks. Vertical bars represent standard error of the means ± SEM. n = 5 chicks per experiment.

TABLE 4.6

Effect of 2-thazolyl-ethylamine pretreated with reserpine on behavioural activities of two-day old chicks

Dose mg/kg	Behavioural activity (counts per hour) Mean ± SEM				
	Locomotion	Excape Episode	Jump	Pecking	Gross locomotor activity mean±SEM
Control	60.49± 2.77	0.86±0.45	1.36 ±0.7	61.6 ±8.9	62.7i±i.3
20mg/kg 2-thiazolyl-ethylamine	193.14± 11.05	14.5 ±3.6	122.86±22.85	51.4 ±15.39	330.57±12.17
20mg/kg 2-thiazolyl-ethylamine + 5mg/kg reserpine	162.09±21.10	96.85±15.65	125.94±29.9	37.8 ±4.03	384.88±22.25

Chicks were pretreated with reserpine 24 hours prior to administration of 2-thiazolyl-ethylamine.

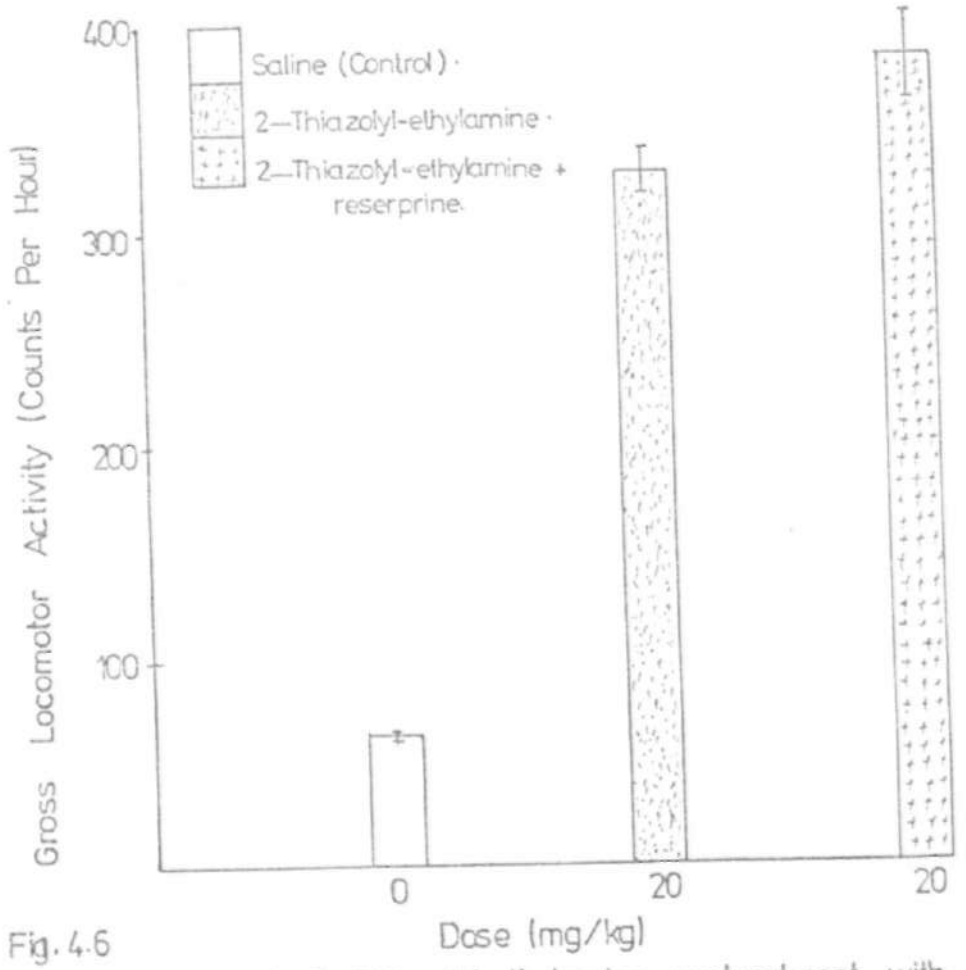


Fig. 4.6

Effect of 2-Thiazolyl-ethylamine pretreatment with reserpine on gross Locomotor activity in 2-day old chicks. Vertical bars represent standard error of the means ± SEM. n = 5.

19.5 ± 3.6 minutes)followed by behavioural sedation/ depression. The chicks showed no escape episode or jump with higher doses. Onset of action (2.2±0.84 - 4.3 ± 2.56) was dose dependent, decreasing with increase in dose (Table 4.7 & Fig. 4.7). The action of dimaprit was counteracted by cimetidine (15mg/kg) Table 4.8 & Fig. 4.8) but not by mepyramine (Table 4.4 & Fig 4.4)

TABLE 4.7

Effect of Dimaprit on Gross Behaviour of Two-Day old chicks

Locomotion, Escape Episode, Jumping and Pecking are expressed in count per hour (cph) mean ± SEM

Dose mg/kg	Locomotion	Excape Episode	Jump	Pecking	Onset of action (min)	Duration of sleep (min)	Duration of Depression/Sedation (min)	No. Sedated/ Depressed No. Used	Gross Locomotor activity Mean ± SEM
Control (Sal.)	77.46±7.57	4.0 ±1.04	8.1±3.2	83.6±12.48	-	-	42.8 ±5.89	2/5 +	89.56±3.9
1	61.83±10.34	8.16±2.09	20.01±1.6	45.06± 8.3	22.9±2.06	-	57.1 ±2.1	2/5	‡ 72.0±4.68
3	37.39± 8.1	2.99±1.6	2.4 ±1.87	8.87 ± 2.8	23.1±2.3	-	77.02±2.7	2/5	‡‡ 42.78±3.86
5	10.32± 3.08	0.00	0.00	9.45 ± 3.8	4.3±2.56	5.8±1.2	150.2 ±10.6	5/5	‡‡ 10.32±3.08
10	4.2 ± 2.3	0.00	0.00	3.8 ± 2.26	3.6±2.16	10.1±2.56	170.0 ±50.1	5/5	‡‡ 4.2 ±2.3
15	3.8 ± 1.56	0.00	0.00	2.0 ± 1.4	3.0±1.6	12.3±1.73	282.0 ±21.86	5/5	‡‡ 3.8 ±1.56
20	3.6 ± 2.1	0.00	0.00	1.8 ± 1.1	2.4±1.67	15.3±2.15	286.8 ±58.6	5/5	‡‡ 3.6 ± 2.1
30	2.1 ± 1.87	0.00	0.00	0.95 ± 0.55	2.2±0.87	19.5±5.6	298.3 ±40.9	5/5	‡‡ 2.1 ±1.87

+ Light sidation ‡ P<0.05, ‡‡ P<0.001 Compared to saline control: Student's 't' test.
n = 5 chicks per experiment.

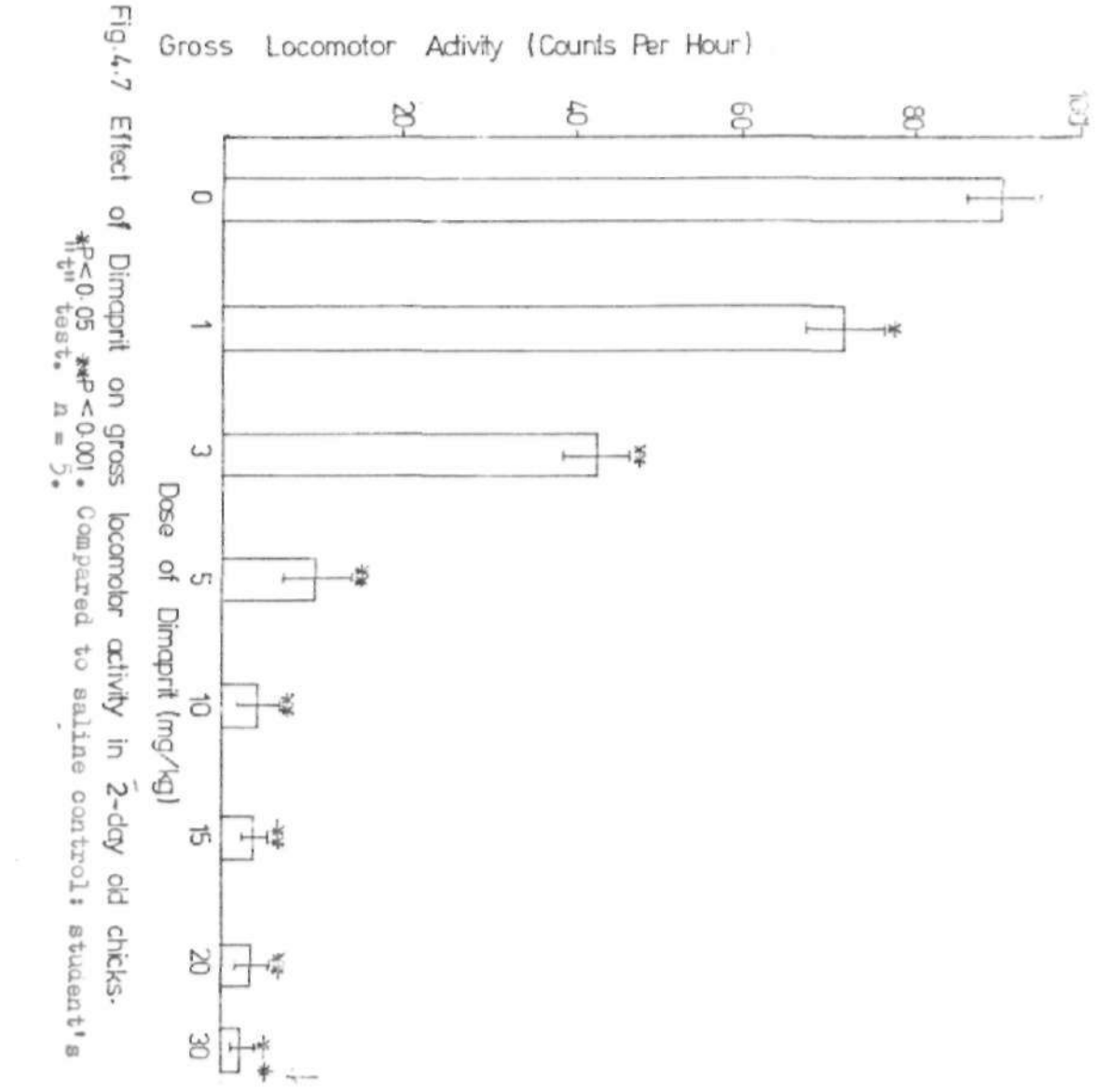


Fig.4.7 Effect of Dimaprit on gross locomotor activity in 2-day old chicks.
*P<0.05 **P<0.001. Compared to saline control: student's "t" test. n = 5.

TABLE 4.8

Effect of dimaprit pretreated with 15mg/kg cimetidine on gross behaviour of two-day old chicks

Dose mg/kg	Behavioural activity (counts per hour) Mean ± SEM Locomotion	Escape Episode	Jump	Pecking	Gross locomotor activity mean±SEM
Control (Sal.+Cimetidine)	61.3 ±11.6	5.5 ±0.5	8.9 ±1.25	51.3 ±10.9	75.7±4.45
5	36.7 ± 8.2	3.6 ±1.3	2.3 ± 0.5	26.9 ±6.1	42.6±3.3
15	19.5 ± 2.3	1.8 ±0.15	-	17.0 ± 3.8	21.3 ±1.3
30	13.5 ± 3.6	1.5 ±0.2	-	13.4 ± 3.2	15.0 ±1.9

Chicks were pretreated with cimetidine 30 minutes prior to administration of dimaprit.

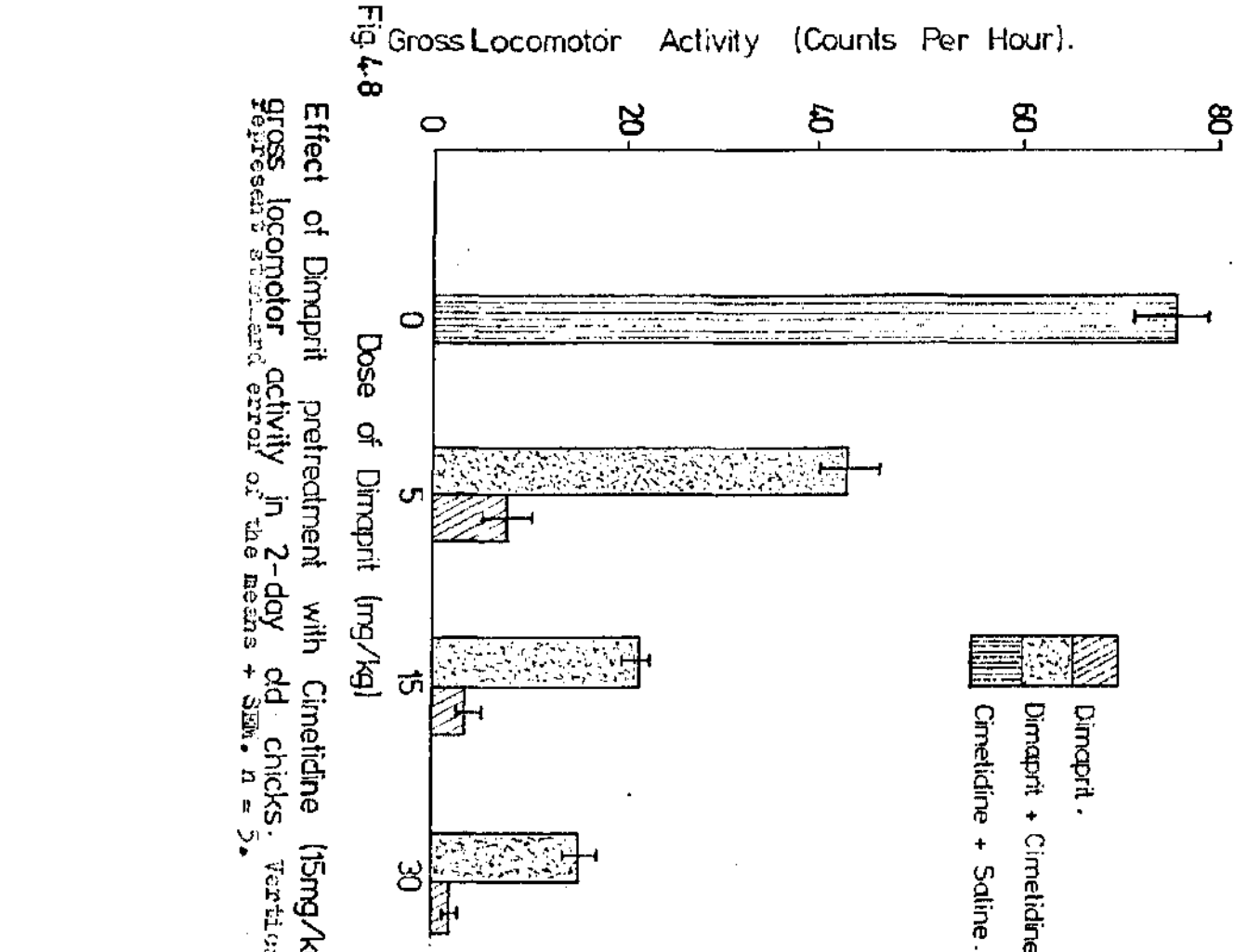


Fig. 4.8 Effect of Dimaprit pretreatment with Cimetidine (15mg/kg) on gross locomotor activity in 2-day old chicks. Vertical bars represent standard error of the means + SEM. n = 5.

4.4.3 Behavioural effects of H_1 and H_2 receptor antagonists.

(i) H_1 antagonists

Both mepyramine and triprolidine induced behavioural sedation/sleep which was dose dependent with a mean value of 53.6±6.2 min for 30mg/Kg mepyramine and 281.0 ± 10.2 min for 30mg/1kg triprolidine. This was also reflected in decrease locomotor activity and decrease in pecking behaviour (Table 4.9 & 4.10 and Fig 4.9 & 4.10).

However ip injection of chlorpheniramine in chicks induced a biphasic response of an initial behavioural sedation/depression lasting more than 50 minutes with 30mg/Kg followed by intense and prolonged activation of more than 200 minutes. This consequently resulted in increased locomotor activity, vocalization, head and neck jerks and cage pecking (Table 4.11 and fig. 4.11)

The initial behavioural sedation/depression is in consonant with the action of classical H_1 receptor antagonists but the behavioural activational phase is a factor which is difficult to correlate with the action of this drug at first sight. It can be speculated that chlorpheniramine might be releasing one of the neurotransmitters in the brain or it is a partial agonist. However it doesn't seem to be any literature up-to-date which points to the speculation that chlorpheniramine is a partial agonist and its

TABLE 4.9

Effect of Mepyramine on gross behaviour of two-day old chicks

Dose mg/kg	Behavioural activity (counts per hour) Mean ± SEM: Locomotion	Excape Episode	Jump	Pecking	Onset of Action	Duration of Depression/Sedation(min)	Gross locomotor activity mean
Control (Sal.)	56.8 ±11.7	15.2 ±7.1	8.3 ±2.3	156.3 ±51.2	-	-	80.3±7.0
10	41.5 ± 8.1	11.2 ±3.11	-	241.3 ±28.3	5.2±2.3	15.8 ± 3.35	* 52.7 ±5.2
20	30.7 ± 4.1	6.3 ±2.4	0.21 ± 0.08	120.6 ±13.5	3.7 ± 1.5	32.2 ± 2.3	** 37.21±2.1
30	21.6 ± 8.3	7.16 ±3.3	2.3 ± 0.2	45.5 ±14.8	3.16± 1.8	53.6 ± 6.2	** 30.86±3.9

*P = < 0.02 Compared to saline control: Student's 't' test.

**P = < 0.001 n = 5

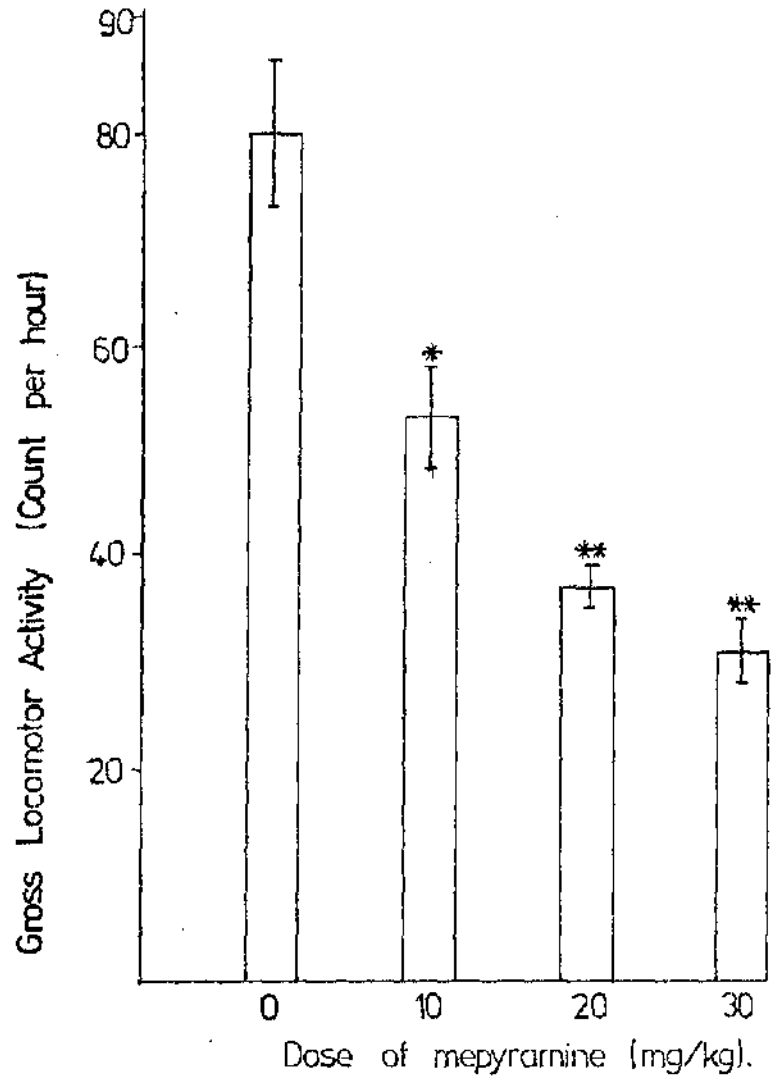


Fig 4.9 Effect of mepyramine on gross locomotor activity in 2-day old chicks. *P =<0.02; **P=<0.001. Compared to saline control: students "t" test. Vertical bars represent standard error of the means ± SEM. n = 5.

TABLE 4.10

Effect of Triprolidine on gross behaviour of two-day old chicks

Locomotion, Escape Episode, Jump and Pecking are expressed in count per hour (cph)
Mean = SEM

Dose mg/kg	Locomotion	Excape Episode	Jump	Pecking	Onset (min)	Duration/Depression/Sedation(min)	Gross locomotor activity mean±SEM
Control (Sal.)	56.86±11.7	5.35±2.3	9.53±3.31	31.16±8.1	-	-	71.74±5.77
15	17.68± 6.45	1.09±0.31	8.1 ±3.25	3.09±0.15	3.67±0.33	214.0 ± 8.02	26.87±3.3
30	18.78± 2.5	0.95±0.36	4.21 ± 1.34	2.93 ± 1.8	2.0 ± 0.58	281.0 ±10.2	25.95±1.4

n = 5

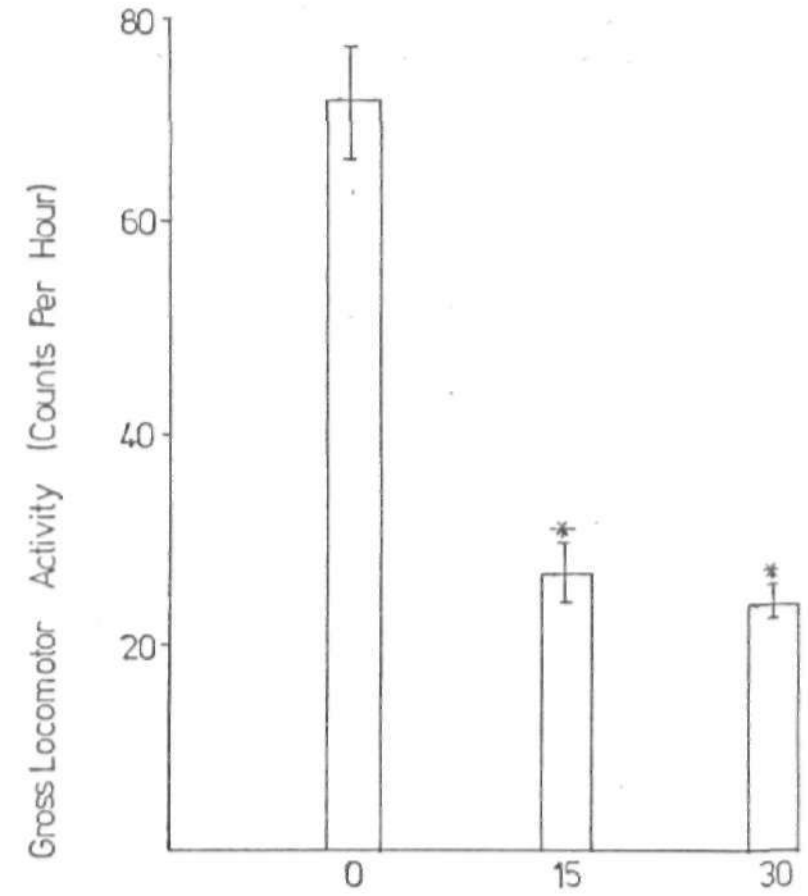


Table- 4.10

Effect of Triprolidine on gross locomotor activity in 2-day old chicks. *P< 0.001. Compared to saline control: student's "t" test. Vertical bars represent standard error of the means ± SEM. n = 5.

Table 4.11

Effect of Chlorpheniramine on Gross Behaviour of Two-Day Old Chicks

Dose mg/kg	Behavioural Activity (Counts per hour) mean ± SEM: Locomotion	Jump	Escape Episode	Pecking	Onset (min)	Duration of Depression/ Sedation (min)	Duration of Activation(min)	Gross Locomotor activity mean±SEM
Control (Sal.)	50.2 ±11.4	0.85±0.425	9.95±9.6	67.55±2.05	-	-	-	61.0 ±5.47
5	+ 171.4 ± 64.3	6.75±2.79	22.37±10.9	‡ 55.9 ±24.5	2.3 ±0.33	30.0±5.0	172.9 ±3.86	‡‡‡ 200.5 ±28.0
10	+ 188.42± 33.5	8.28±2.6	20.2 ± 1.6	‡ 119.4 ± 55.3	2.32±0.88	22.5±0.41	184.3 ± 10.8	‡‡‡ 216.9 ± 29.8
15	+ 180.31±36.25	10.3±2.2	53.95±8.45	‡ 87.75±21.85	1.85±0.2	56.0±20.0	180.86± 11.85	‡‡‡ 244.56± 5.6
20	+ 206.95±13.75	24.9 ±1.14	65.4 ±17.9	‡ 155.8 ±47.7	1.75±0.25	33.4 ± 18.19	183.53±12.76	‡‡‡ 297.25±20.88
30	+ 240.1 ± 29.3	15.0 ±0.01	112.45± 3.4	‡ 137.28±64.05	1.75±0.35	35.5 ± 20.5	182.31±15.22	‡‡‡ 367.55±10.9

‡ Cage Pecking Maint; + Rapid Movement/Running. ‡‡‡P<0.001, Compared with saline control: Student's 't' test. n = 5

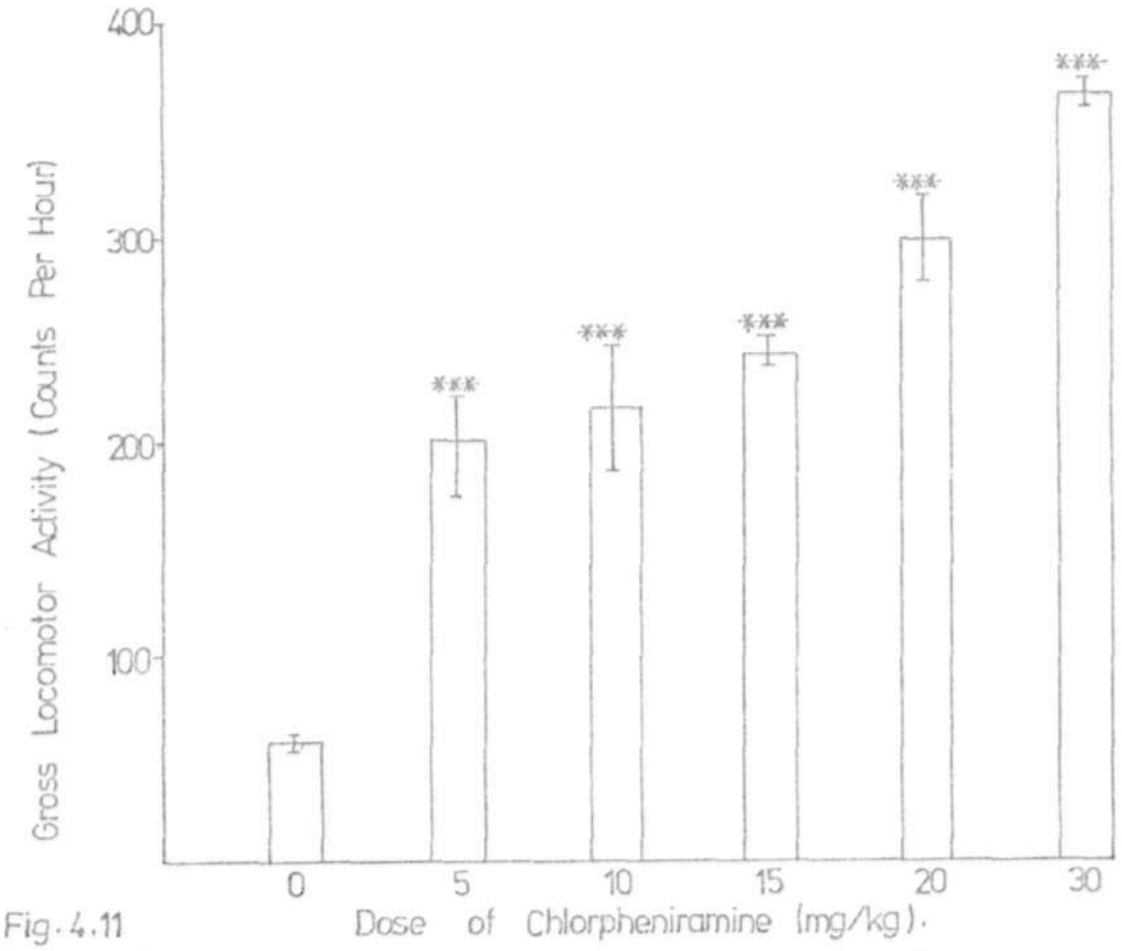


Fig. 4.11 Effect of Chlorpheniramine on gross locomotor activity in 2-day old chicks. ***P<0.001. Compared to saline control: Student's "t" test. Vertical bars represent standard error of the means ± SEM. n = 5.

antagonistic effect on histamine or 2-thiazolyl-ethylamine induced hypothermia in young chicks (Anuka et al, 1991) does not support this view either. We were therefore left with the first speculation that it releases a neurotransmitter which produced the second phase of activation.

Using appropriate agonists and anatagonists to the putative neurotransmitters in the brain like 5 - hydroxytryptamine, dopamine, acetylcholine and noradrenaline, it was possible to show that it does not release any of these nerotransmitters (Table 4.12 and Fig 4.12). It is possible that this effect could be due to species differences or variation typical of this drug. Using adult rats and fowls, only behavioural sleep/depression was observed with rats with no activational phase (Table 4.13 and fig 4.13) but with only activational phase in adult fowls. (Table 4.14 and fig. 4.14)

Intraperitoneal (ip) administration of cimetidine, a $H\bar{2}$ receptor antagonists) elicited an intense behavioural stimulation with onset (1.0 ± 0.58 - 8.63 ± 12 minutes) which was characterized by increase gross locomotor activity, rapid movement/running, increase vocalization which increased with increase dose, head and neck jerks. This effect lasted up to 90 minutes with a dose of 30mg/Kg (Table 4.15 and fig 4.15)

Table 4.12

Effect of chorpheniramine on two day old chicks by comparative studies

Dose mg/kg	%Vocalization Behavioural Activity (Counts per hour) mean ± SEM Locomotion	Jump	Escape Episode	Pecking	Onset (min)	Duration of Depression/ Sedation (min)	Duration of Activation (min)	Gross Locomotor activity Mean±SEM
Control (Sal.)	53.95±11.05	0.43±0.075	8.0±3.5	40.6±29.9	-	-	-	61.99±4.88
30 mg/kg‡ chlorpheniramine	240.1 ± 29.3	15.0±0.01	112.45±3.4	137.26±69.45	1.75±0.35	55.5±20.5	182.5±15.22	387.55±10.9
30mg/kg‡ Chlorpheniramine + 10mg/kg cyprohettadine	271.0 ± 11.2	4.2±1.31	86.4 ± 8.2	26.5 ± 8.36	8.12±3.4	22.8±11.3	178.0 ± 11.2	361.16± 6.9
30mg/kg‡ Chlorpheniramine + 10mg/kg Atropine	128.15±20.85	2.02±0.01	-	13.09± 4.13	1.36±0.26	24.0± 1.85	185.6 ± 18.43	143.26± 10.4
30mg/kg Ach	16.07± 0.11	8.2 ±2.31	0.36± 0.05	6.35± 1.34	2.1 ±0.19	168.23± 19.18	-	24.63± 0.083
30mg/kg Dopamine	24.7 ± 5.2	1.4 ±0.09	3.78± 1.87	16.78± 7.5	5.6 ±0.24	83.0 ± 2.1	-	29.88± 2.38
30mg/kg Noradrenaline	22.2 ± 0.85	-	-	2.3 ± 1.05	1.5 ±0.5	200.3 ± 19.5	-	22.2 ± 0.82
30mg/kg 5-Hydroxy-tryptamine	6.5 ± 2.3	-	1.36± 1.0	0.1 ± 0.01	4.0 ±0.58	303±21.3	-	7.86± 1.8

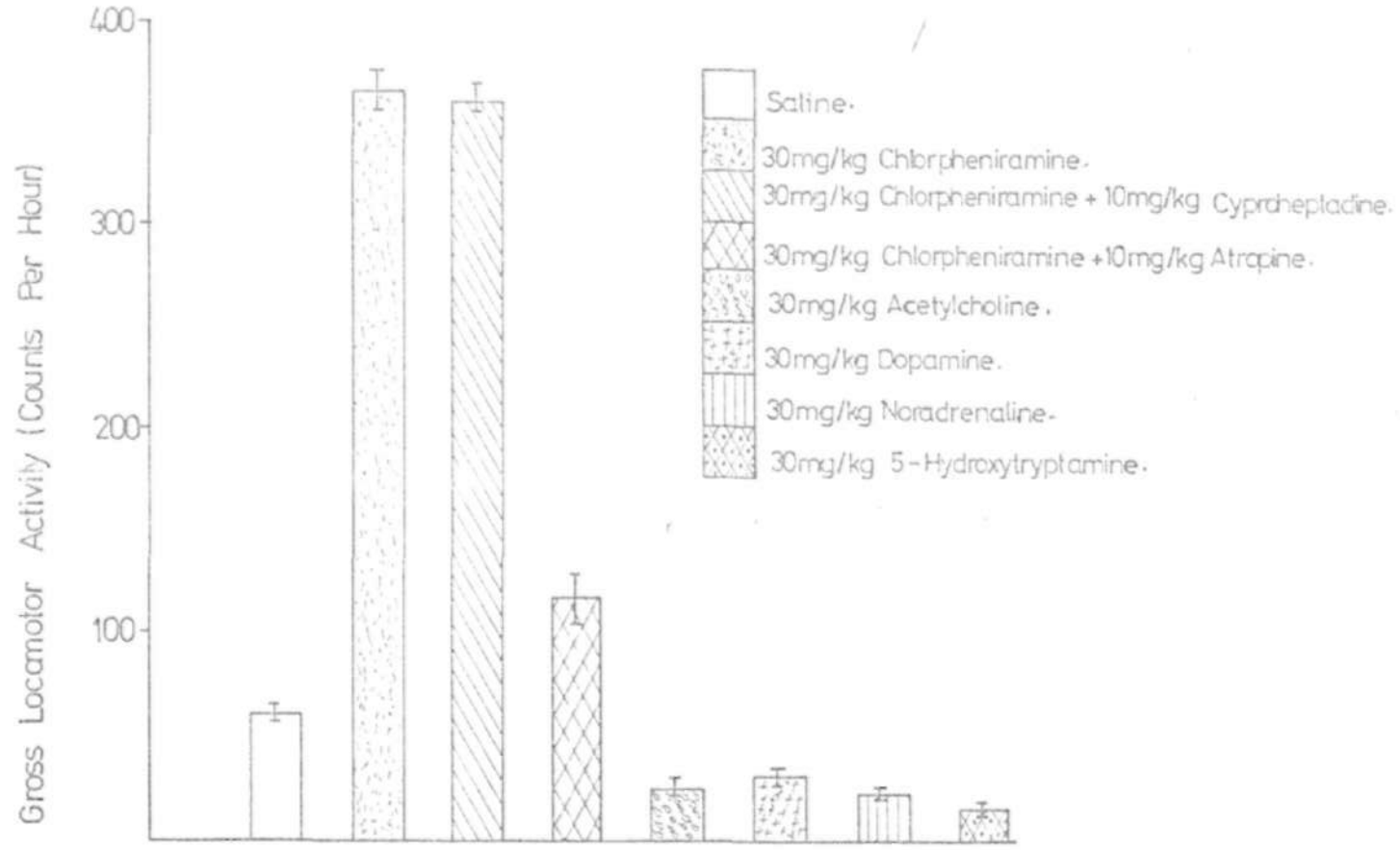


Table 4.12 Assessment of the effect of Chlorpheniramine in 2-day old chicks, by Comparative studies. Vertical bars represent standard error of the Means ± SEM. $n = 5$.

Table 4.13

Effect of chlorpheniramine on gross behaviour in rats

Dose mg/kg	Behavioural Activity (Counts per hour) mean ± SEM				Onset (min)	Duration of sleep/ depression(min)	Duration of Activation (min)	Gross Locomotor netivity mean±SEM
	Locomotion	Rearing	Climbing	Grooming				
Control	24.5 ± 3.0	21.65± 6.9	3.85±0.25	4.35±2.1	-	-	-	28.35±1.6
5	14.85±2.6	6.85±1.2	0.55± 0.02	-	20.0±0.08	52.0± 3.0	-	### 15.4 ± 1.3
15	9.09± 1.5	0.546±0.05	-	-	14.0±1.0	88.0± 1.0	-	### 9.09± 1.5
30	3.9 ± 1.8	0.36 ±0.04	-	-	7.0 ±0.9	102 ± 5.2	-	### 3.9 ± 1.8

P<0.001 Compared to saline control: Student's 't' test. n = 5

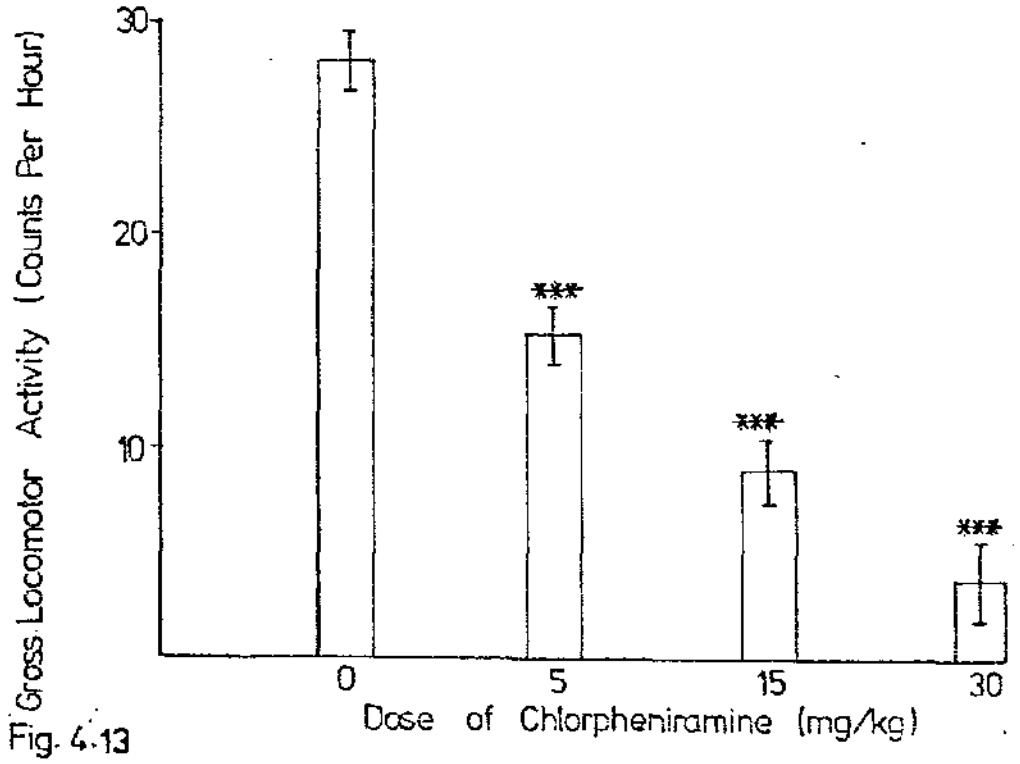


Fig. 4.13 Effect of Chlorpheniramine on gross locomotor activity in adult rat

***P<0.001. Vertical bars represent standard error of the means ± SEM. n = 5.

Table 4.14

Effect of chlorpheniramine on gross behaviour of adult fouls (7½ months)

Dose mg/kg	Behavioural Activity (Counts per hour) mean ± SEM							
	Locomotion	Jump	Escape Episode	Pecking	Onset (min)	Duration of sleep/ depression(min)	Duration of Activation (min)	Gross Locomotor activity mean±SEM
Control (Sal.)	21.2 ± 0.03	-	-	40.6±2.0	-	-	-	21.2 ±0.03
15	40.4 ±3.1	-	20.2±0.5	10.1±4.6	1.0	-	95.33±3.77	♯ 60.6 ± 1.8
30	49.15± 1.6	-	22.7±8.58	35.0±5.3	-	-	102.38±3.93	♯ 7.85±5.09

♯ P<0.001 Compared to saline controls students 't' test. n = 5

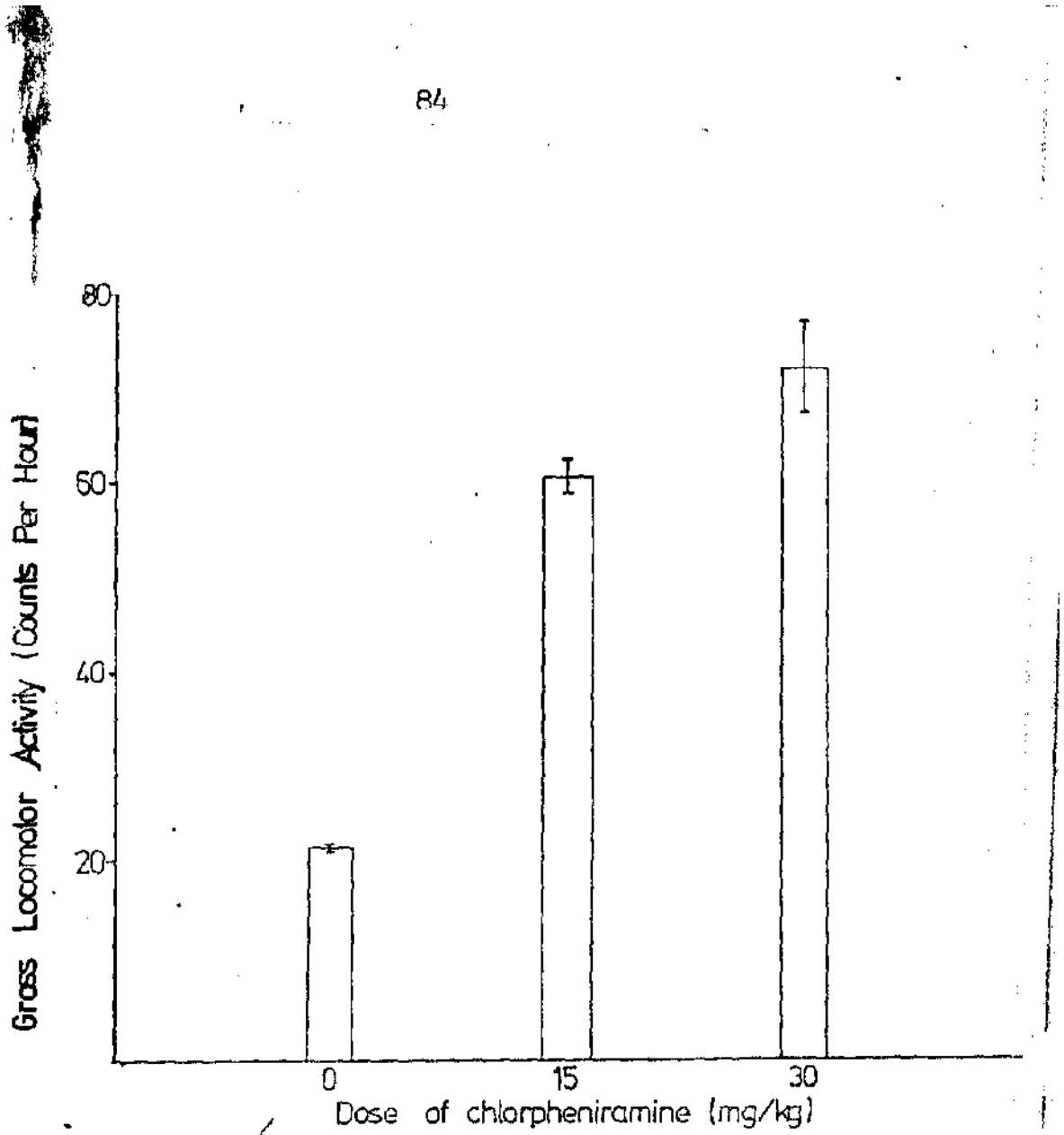


Fig 4.14 Effect of chlorpheniramine on gross behaviour of Adult fouls (3½ months). Vertical bars represent standard error of the means ± SEM. n = 5.

TABLE 4.15

Effect of Cimetidine on two-day old chicks

Dose mg/kg	Behavioural Activity (Counts per hour) mean ± SEM						
	Locomotion	Excape Episode	Jump	Rapid movement/ running	Duration of Activa-tion(min)	Onset of Action(min)	Gross locomo activity mea
Control (Sal.)	40.3 ±9.15	0.03±0.01	-	-	-	-	40.3±9.15
5	45.8 ± 15.2	4.9±1.48	2.05±0.11	-	57.36±10.76	8.63 ± 1.2	N.S 51.75±5.6
10	55.18± 12.1	16.0±3.6	8.33 ± 3.18	7.66 ± 1.2	65.35±10.76	2.33 ± 0.88	** 87.17±5.0
15	58.66±15.3	11.33±2.4	8.0±1.18	6.35± 2.08	60.15±9.87	2.0 ±0.58	* 84.44±5.2
20	57.89± 14.8	16.67±2.34	7.69± 1.86	11.84± 1.77	64.25±3.06	1.0 ± 0.50	*** 94.09.5.1
30	60.8 ± 18.2	35.0 ±2.94	9.58± 1.8	15.7 ± 3.86	80.0 ±11.56	1.0 ± 0.58	*** 119.09±6.5

*P = <0.01. ** P = <0.002; *** P= < 0.001 Compared to saline control: Students't' test.

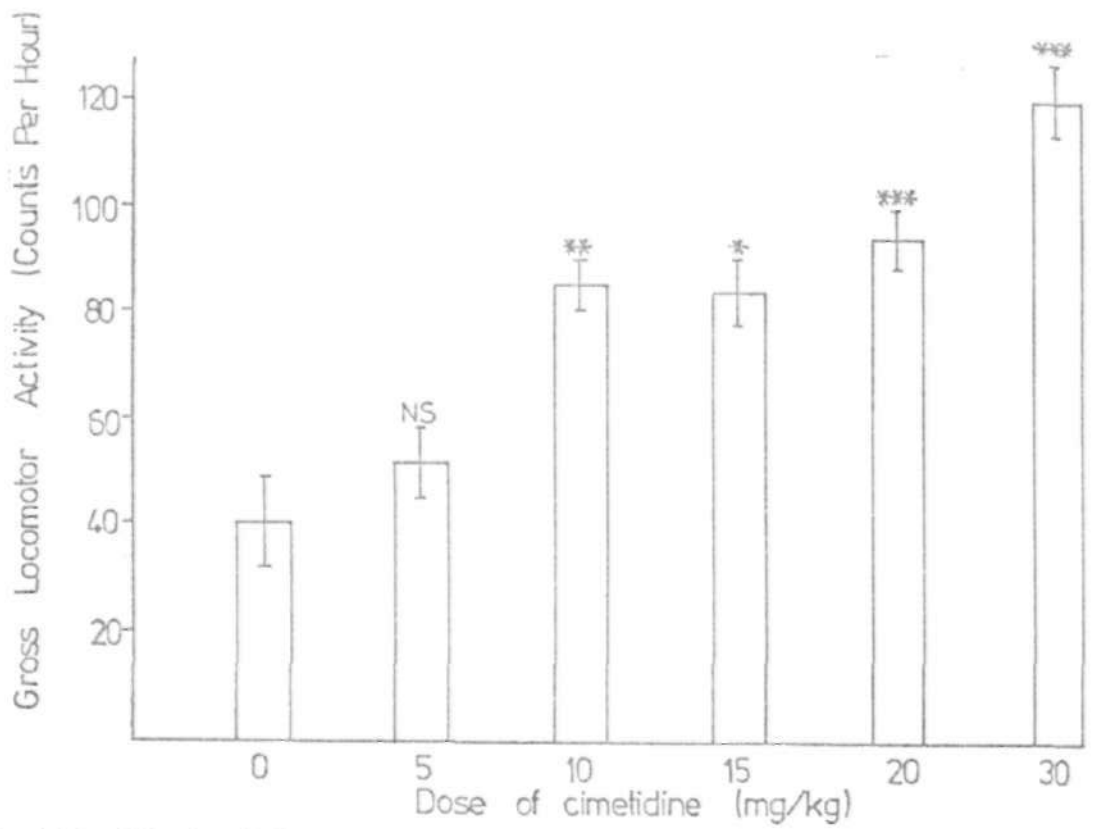


Fig. 4.15 Effect of Cimetidine on gross locomotor activity in 2-day old chicks.

*P =<0.01; **P =<0.002; ***P =<0.001. Compared to saline control: student's "t" test. NS = non-significant. Vertical bars represent standard error of the means ± SEM. n = 5.

4.4.4 Effects of Histamine, 2-thiazolyl-ethylamine and Dimparit on EEG and EMG.

Histamine (20mg/Kg) reduced the EMG activity and sychronized the EEG of the hyperstriatum (HS) while the optic tectum (OT) and pontine reticular formation (RF) were weakly synchronized 5 minutes after drug administration. However, the EEG of HS, OT and RF was desynchronized and EMG activity profundly inceased after 70 minutes. The EEG of the HS. OT and RF was weakly sychronized after 130 minutes with a reduction in muscle activity (EMG). (Fig. 4.16)

2-thiazolyl-ethylamine (20mg/kg) desychronized the EEG of the hyperstiatum, optic tectum and pontine reticular formation while the EMG activity was weakly activated (fig.4.17).

Dimaprit (20mg/kg) synchronized the EEG of the hyperstriatum, optic tectum and pontine reticular formation but the EMG was not affected (fig 4.18)

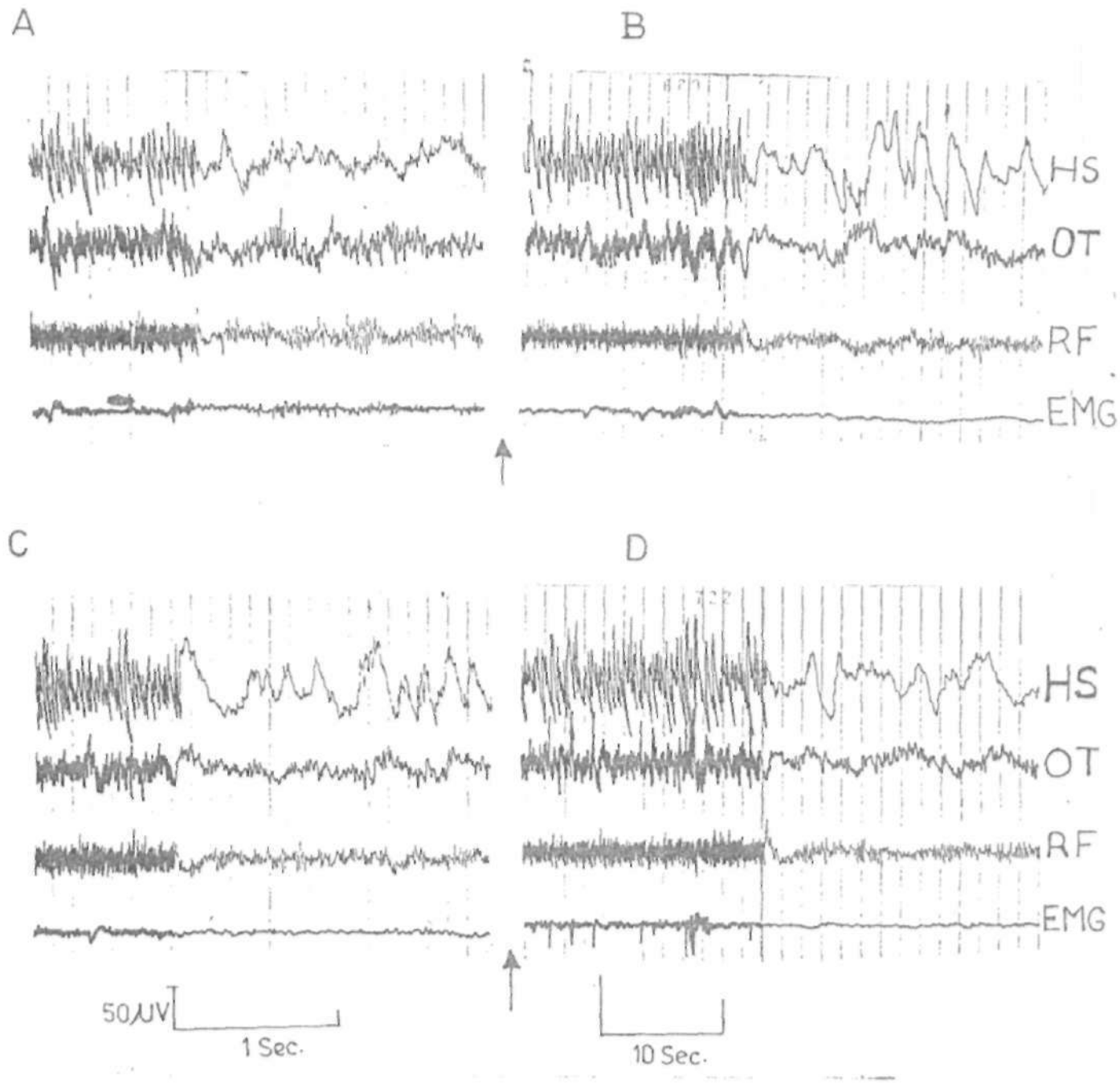


Fig 4.16 The effect of histamine (20mg / kg, ip) on EEG and EMG using 2 day old chicks. Upper pangel (A) is control with saline while pannel (B) is treated with histamine 5minutes after injection, (C) 70 minutes after (D) 130 minutes after.

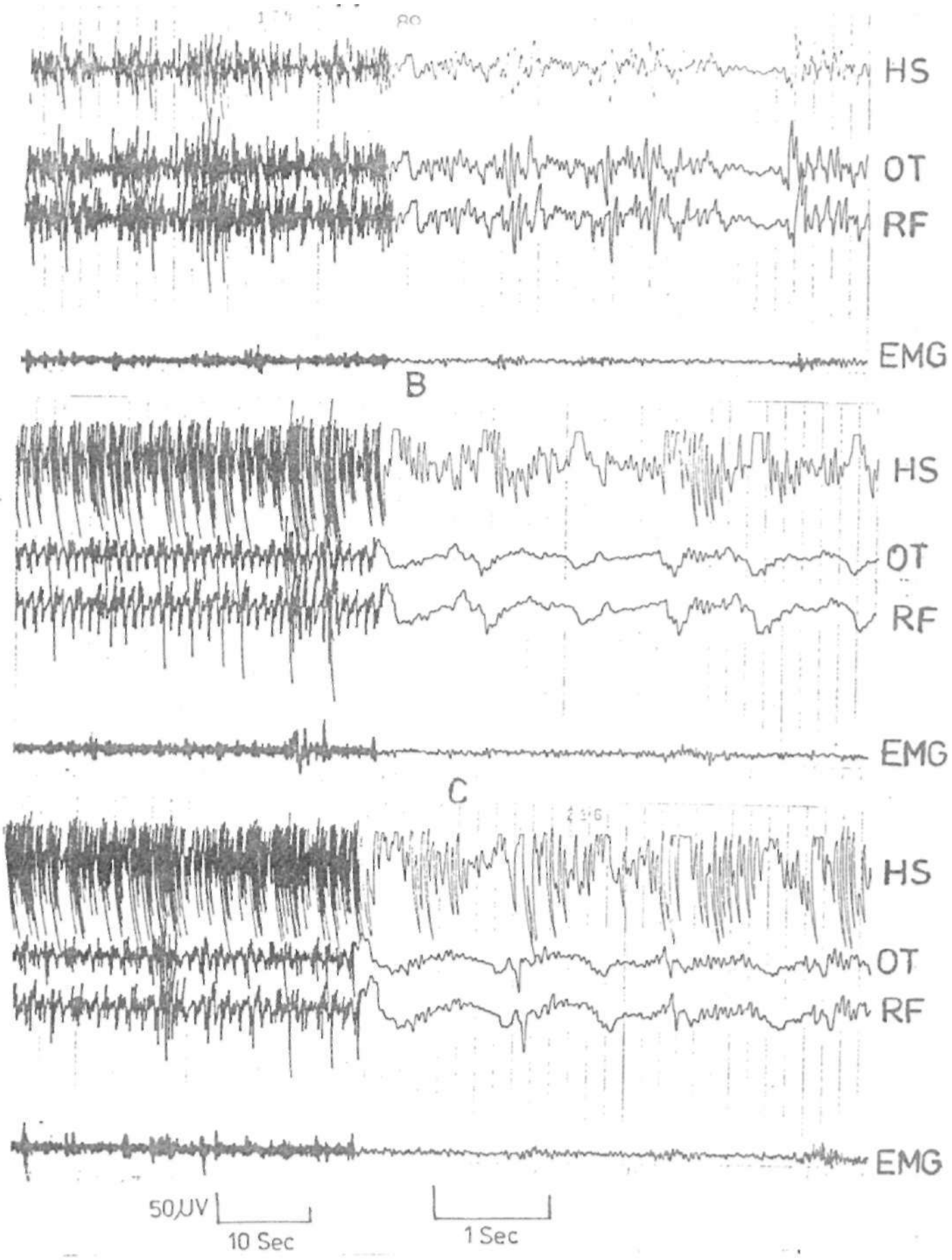


Fig 4.17 The effect of 2- thiazolylethylamine (20mg\kg, ip) on EEG and EMG using 2-day old chicks. Upper pannel (A) is control with saline while (B) is treated with histamine 10 minutes after injection, (C) 30 minutes after

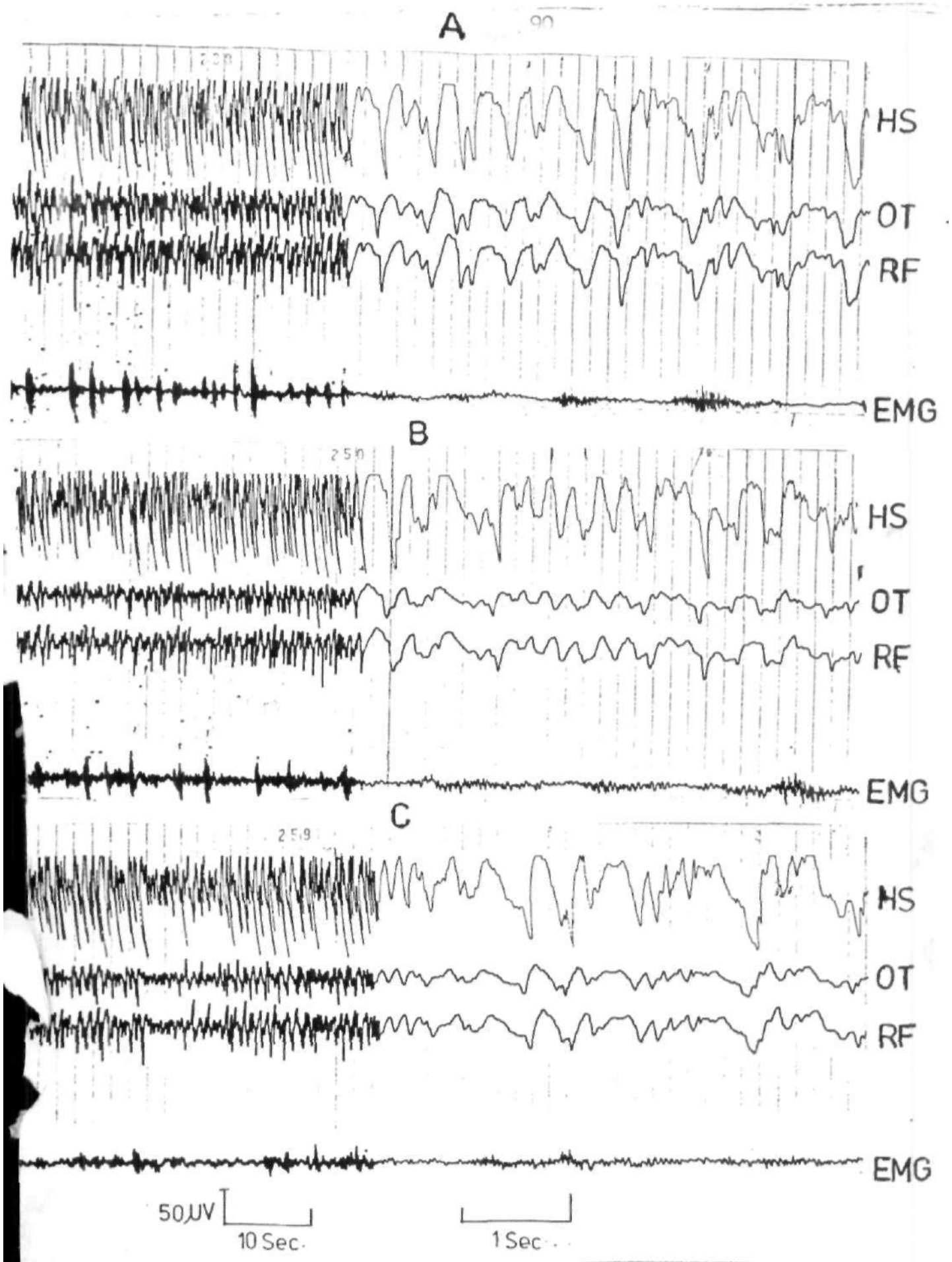


Fig 4.18 The effect of dimaprid (20mg/kg, ip) on EEG and EMG using 2-day old chicks. Upper pannel (A) is control with saline while (B) is treated with dimaprit 5minutes after injection (C) 30minutes after.

4.5 Discussion

The present findings show that histamine induces a mixed behavioural stimulation and sleep/depression in young chicks with the latter lasting for a longer period. Many chicks exhibited triphasic response with an initial behavioural sleep which was followed by a longer behavioural stimulation culminating in a very long behavioural depression of up to 205 ±25.0 minutes at a dose of 30 mg/kg. This consequently resulted in a decrease in locomotor activity and pecking behaviour compared to the controls. The mixed behavioural effects were also shown by the EEG changes of the hyperstriatum, optic tectum, potine reticular formation and muscle activity (EMG). In the rat histamine has been shown to produce a biphasic response consisting of an initial period of hypoactivity which was followed by a prolonged period of hyperactivity following 10ug intracerebroventricular (icv) injection (Kaliva P.W. 1982).

The components of histamine mediating these mixed behavioural effects were revealed by the use of selective $H\bar{1}$ and $H\bar{2}$ receptor agonists and their appropriate antagonists. Thus, activation of $H\bar{1}$ receptors obtained after 2-thiazolyl-ethylamine induced behavioural stimulation (Table 4.2) which was antagonised by mepyramine, an $H\bar{1}$ receptor antagonist

(Table 4.3) but not by cimetidine (Table 4.4) whereas dimaprit, H_2-receptor agonist,elicited behavioural sleep/depression (Table 4.7) an effect which was antagonized by cimetidine, H_2 - receptor antagonist (Table 4.8) but not by mepyramine (Table 4.4). However, it is difficult to know whether the late depressant effect of 2 - thiazolyl -ethylamine after the excitatory phase was due to exhaustion following the stimulatory phase or the action of this drug. In cultures of tuberal hypothalamic neurons, diphenhydramine, a H_1 - receptor antagonist,was found to block specifically the excitant actions of histamine (Geller,1976) suggesting that the latter might be mediated by H_1 receptors.

These observations indicate that the excitatory responses are mediated by H_1 receptors and the inhibitory responses by H_2 recepors.

The present data indicate that 2 -thiazolyl-ethylamine, H_1 receptor agonist,desynchronized the EEG of the hyperstriatum, optic tectum and pontine reticular formation while the EMG activity was weakly activated. Such data indicate electrocortical stimulation. On the other hand, dimaprit, (H_2 receptor agonist) synchronized the EEG of the hyperstriatum, optic tectum and pontine reticular formation but the EMG was not affected. These data indicate electrocortical sleep/depression. Thus, the

EEG results agree with behavioural observation that activation of $H\bar{1}$ receptors induced behavioural stimulation whereas activation of $H\bar{2}$ receptors elicited behavioural sleep/depression.

Dopamine has been shown to cause increased locomotor activity and arousal behaviour in 2- 14 day old chicks (Osuide and Wambebe 1980, Spooner & Winter 1965). Dopamine has also been reported to produce behavioural sleep in 1-28 day old chicks (Key and Marley 1962) Many workers have suggested that dopamine may be a natural inhibitory neurotransmitter in mammals (Bloom et al 1965; York, 1967) while others have suggested inhibition and excitation on different parts of the brain (Hornykicwcz,1966; Cool and Van Rossum, 1976). Results from our study show that dopamine, noradrenaline, 5-hydroxytryptamine and acetylcholine elucited inhibitory effect on 2-day old chicks (Table 4.12). The present data also show that the depletion of catecholamines for 24 hours after treatment with reserpine did not affect the action of 2-thiazolyl-ethylamine and in addition the behavioural profile of apomorphine was found to be different from that of this drug (Table 4.5). Thus neither the excitatory nor the inhibitory action of histamine and its selective agonists can be attributed to their effects on dopaminergic receptors or any other receptors but on specific histamine

receptors since the effects of these agonists which mimick the specific effects of histamine were blocked specifically by their appropriate antagonists, mepyramine and cimetidine.

From the onset of action of the two agonists, it is possible that the H_2 receptors are first stimulated or the effect on H_2 receptors is first established before that on H_1 receptors, hence the initial behavioural sleep which was followed by stimulation.

The action of specific agonists on their receptors seems to suggest a regulatory role of these receptors for endogenous histamine. Hence, in the brain, there seems to be a balance in the action of histamine on the H_1 receptors which are excitatory and the H_2 receptors which are inhibitory.

Cimetidine, (H_2 receptor antagonist) elicited behavioural activation which was characterized by an increased locomotor activity, vocalization and pecking behaviour while H_1 receptor antagonists, mepyramine and triprolidine showed behavioural sedation/depression except chlorpheniramine which elicited an initial behavioural sedation/depression followed by prolonged period of stimulation in young chicks but only sedation/depression in rats and stimulation in adult fowls. The stimulatory effect of cimetidine could be due to a blockade of the

inhibitory component, H2 receptors, leaving the endogenons histamine to act on the excitatory component, $H1^{-}$ receptors. Also a blockade of the excitatory component $H1^{-}$ receptors by $H1^{-}$ receptor antagonists, leaves the inhibitory component, $H2^{-}$ receptors unaffected for the endogenous histamine to act. The resulting effect is inhibitory. The sedative effect of most antihistamines ($H1^{-}$ receptor antogonists) used in therapeutics are consistent with these findings (Carruther et al, 1975; Heinrich 1953).

In the biphasic response of chlorpheniramine, the behavioural sedation/depression is in consonant with the action of classical $H1^{-}$ receptor antagonists but the behavioural activation is a factor which is difficult to correlate with the action of this drug. That the dual role of chlorpheniramine may be due to a release of 5-hydroxytryptamine, dopamine, noradrenaline or acetylcholine was invalidated by the use of specific agonists and some antagonists to these putative neurotransmitters (Table 4.12). It is not a partial agonist either as indicated in the results. It is possible that its stimulatory effect could be due to species variation (differences) as this effect was observed only in young chicks and adult fowls and not in rats. It is also possible

that the biphasic bahavioural effects of 2-thiazolyl-ethylamine involve other brain mechanisms. Further work on this drug may reveal the mechanism underlying this observation.

CHAPTER FIVE

5.0 THERMOREGULATORY EFFECT OF HISTAMINE IN YOUNG CHICKS

5.1 Summary

5.1.1 Thermoregulatory effect of histamine in young chicks has been established in this work and the mechanism involved in this regulation.

5.1.2 Both $H1^-$ and $H2^-$ receptors are involved in this regulation.

5.1.3 Activation of both receptors by their appropriate selective agonists leads to hypothermia.

5.1.4 A blockade of $H2^-$ receptors with appropriate antagonists produced significant increase in temperature ($P<0.001$) while a blockade of $H1^-$ receptors leads to insignficant fall in temperature.

5.1.5 Neither $H1^-$ nor $H2^-$ receptor antagonists could counteract histamine induced hypothermia but a concurrent administration of both $H1^-$ and $H2^-$ receptor antagonists.

5.2 Introduction

The presence of histamine in the brain as neurotransmitter (Schartz, 1977) shows that it has

its highest concentration in the hypothalamus followed by amygdala (Hill et al, 1978). In all species studied, thermoregulatory activity has been established to be by preoptic/anterior hypothalamic nuclei as the major temperature regulating centres. This consideration renders it likely that histamine might well be involved in the central thermoregulatory neural network (Yellin, 1977). Systemic injection of histamine has been shown to induce changes in body temperature in several animal species with direction being a function of environmental temperature (Labinyi and Szebehely, 1947).

Although systemic administration of histamine has been shown to induce changes in body temperature in several species, so far the exact mechanism of action in bringing about these changes in temperature has not been fully elucidated. This study therefore reports on the effect of histamine on temperature regulation in young chicks and the mechanism involved in this regulation. Since histamine does not readily penetrate the blood brain barrier, young chicks (5-day old) were used in this work because of their nonfunctional blood brain barrier.(Spooner and Winter, (1965)

5.3 Materials and Method

Refer to section 3.11

5.4 Results

5.4.1 Effect of Histamine agonists on temperature of Chicks

Intraperitoneal (ip) administration of histamine (5-20mg/Kg elicited immediate and prolonged dose dependent hypothermia in young chicks lasting up to 270 minutes using 15 and 20mg/kg. The highest recorded fall in temperature was 3.8^{o}C with 20mg/kg (Fig.5.1a & b). Selective $H\bar{1}$ and $H\bar{2}$ receptor agonists, 2-thiazolyl-ethylamine and dimaprit respectively also induced dose dependent hypothermia but with less intensity singly when compared with that produced by histamine (Fig.5.2-5.3a&b).

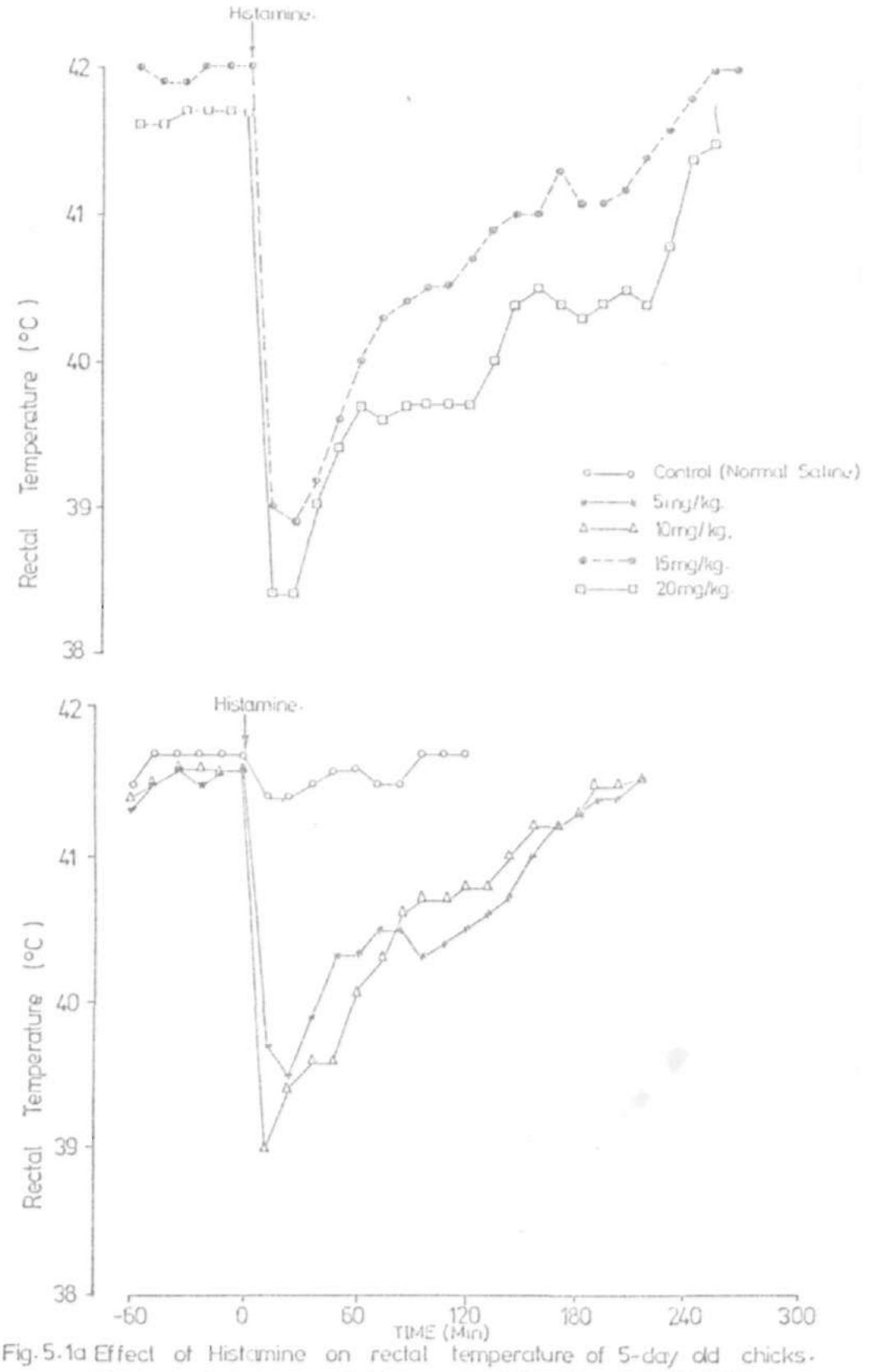


Fig. 5.1a Effect of Histamine on rectal temperature of 5-day old chicks. The points represent the mean from six experiments.

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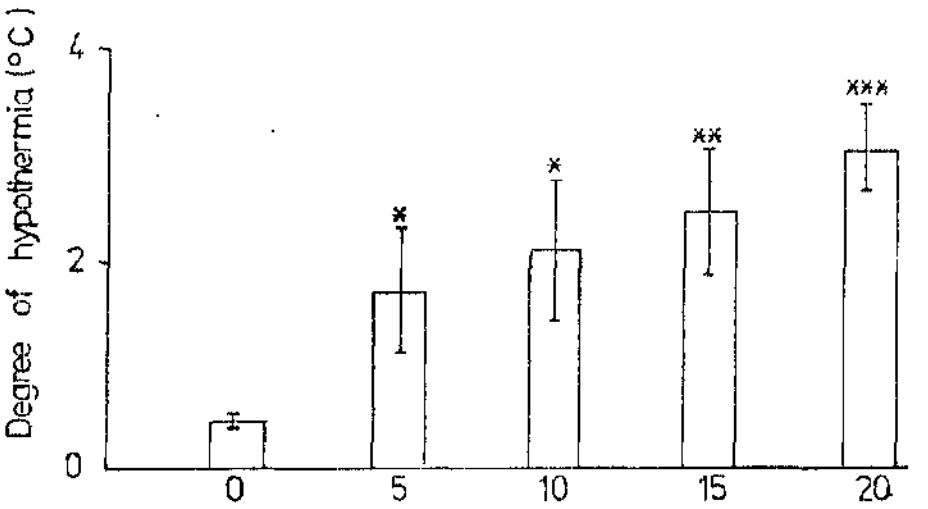


Fig. 5.1b *Dose response relationship of Histamine against rectal* temperature in 5-day old chicks. Vertical bars represent standard error of the means. ± SEM.

* P =< 0.05; **P =< 0.01; ***P =< 0.001. Compared to saline control: student's "t" test. n = 6.

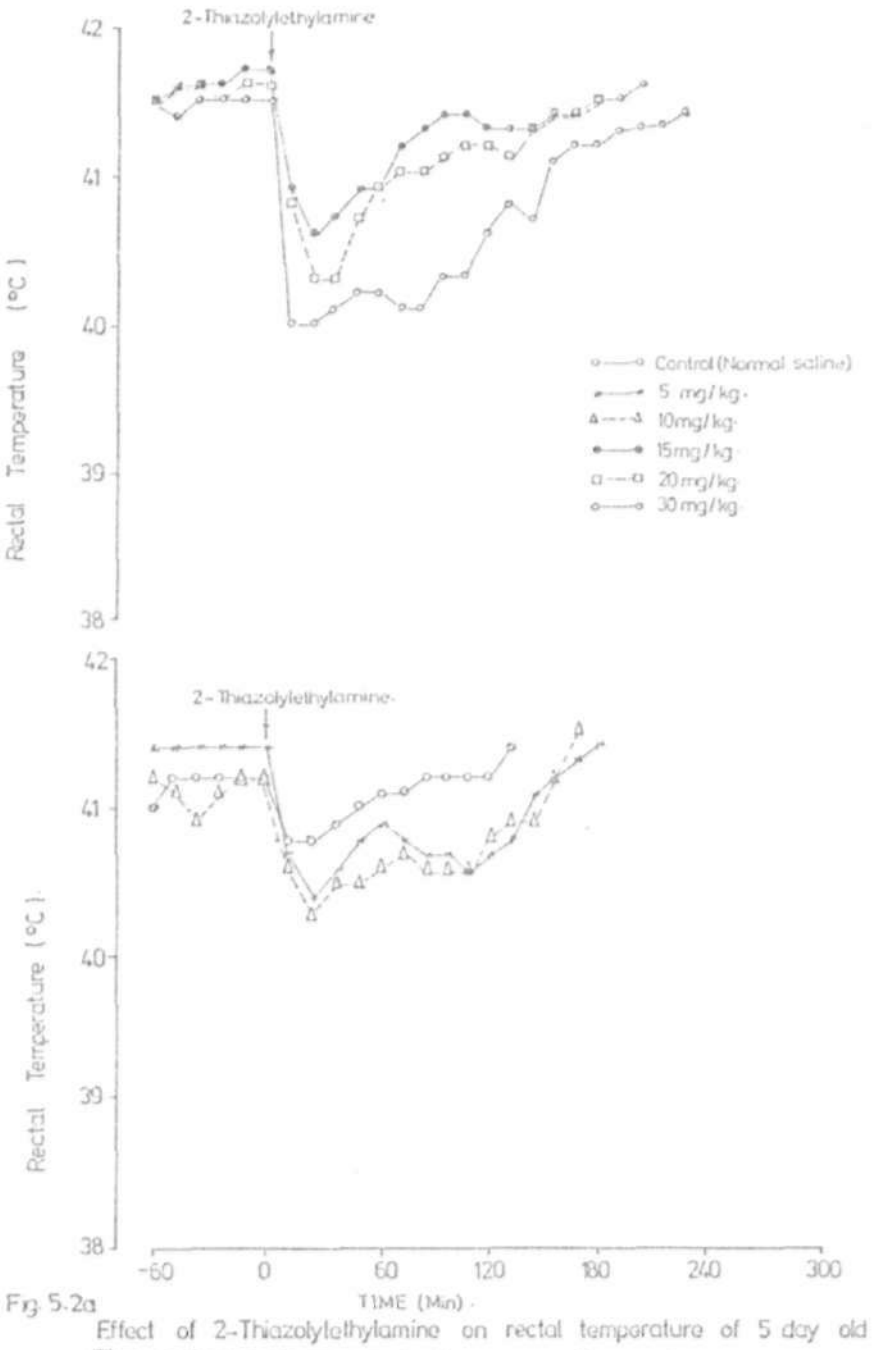


Fig 5.2a Effect of 2-Thiazolylethylamine on rectal temperature of 5 day old chicks. The points represent the mean from six experiments.

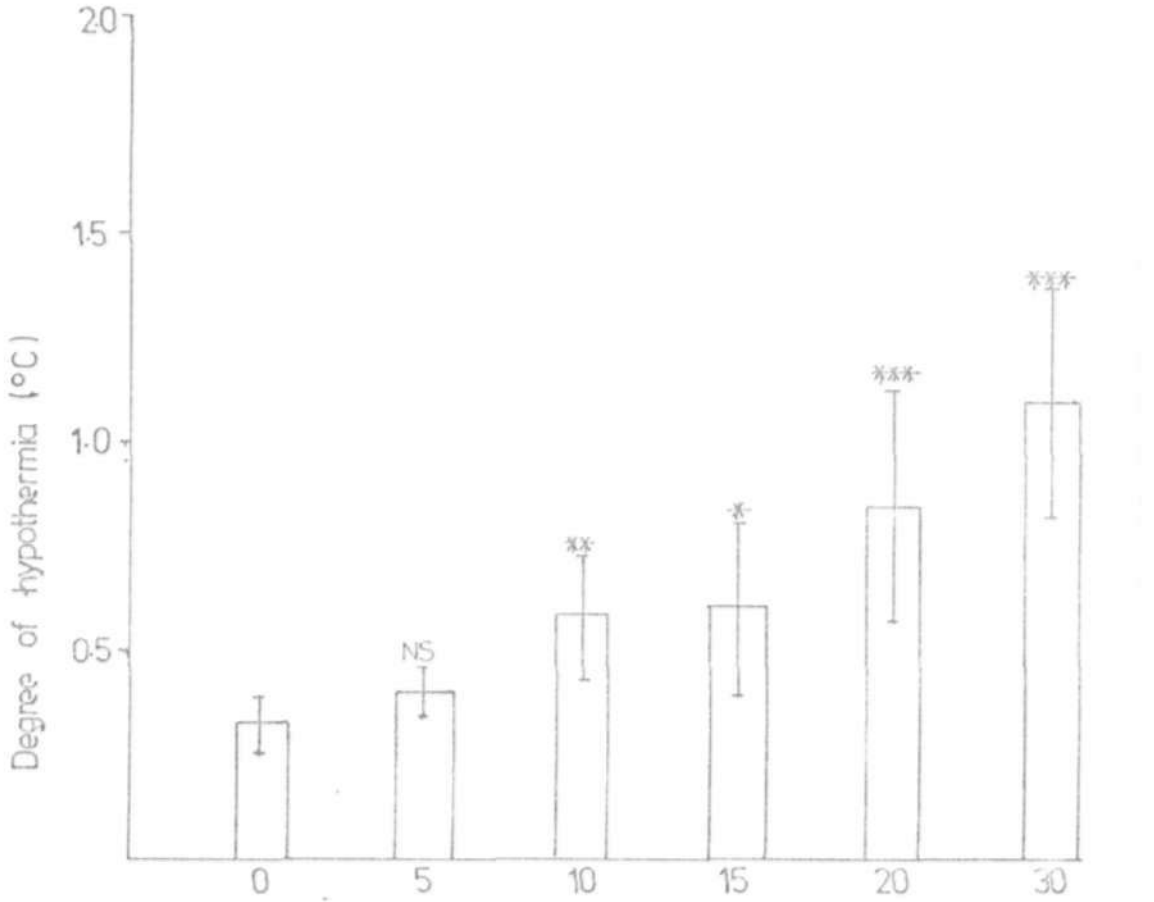


Fig. 5·2b

Dose response relationship of 2–Thiazolyl-ethylamine against rectal temperature in 5 day old chicks.

*P =< 0·05; **P =< 0·01; ***P =<0·001. Compared to saline control: student's "t" test. n = 6. N.S = Non-significant.

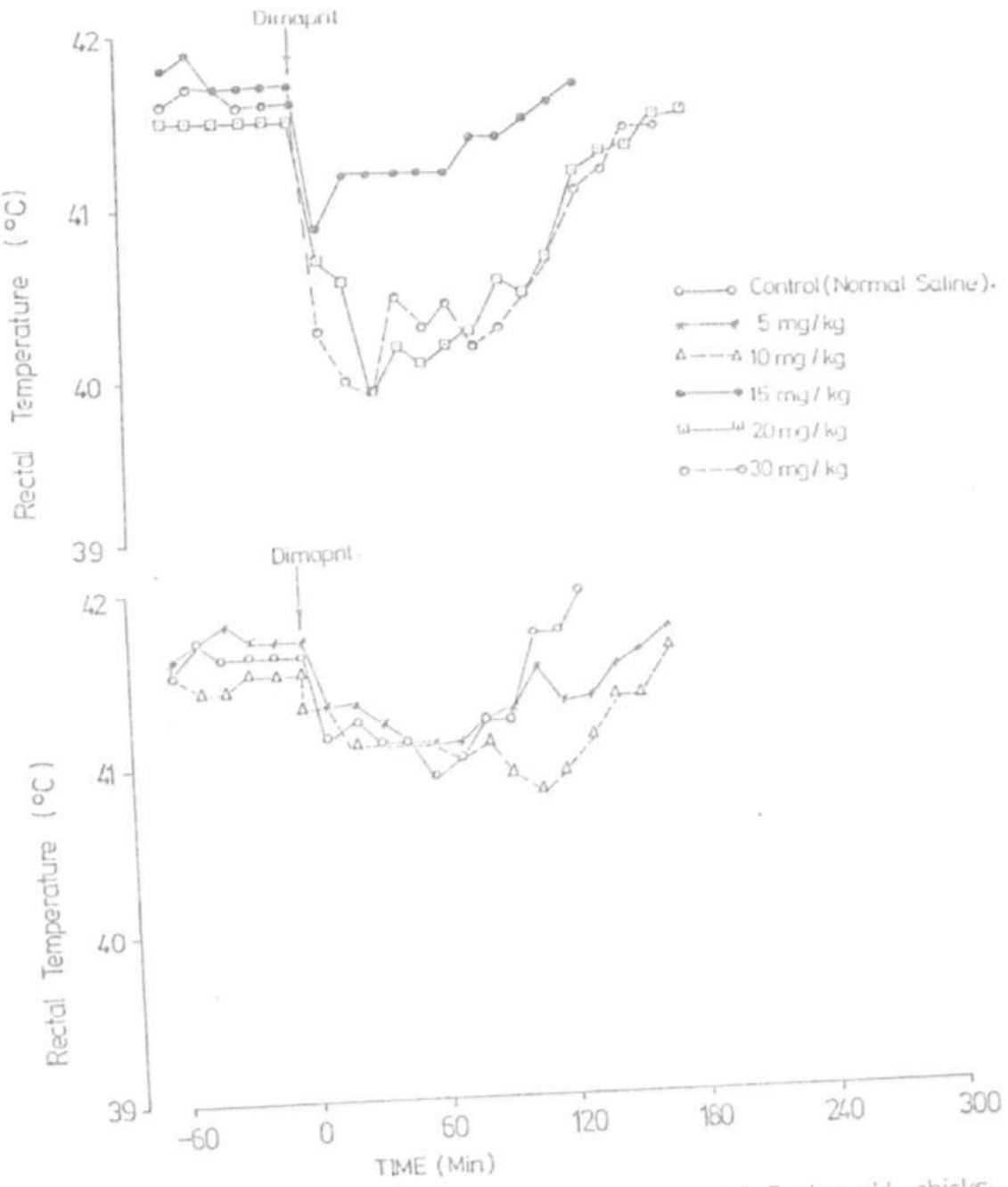


Fig.5.3a Effect of Dimaprit on rectal temperature of 5-day old chicks. The points represent the mean from six experiments.

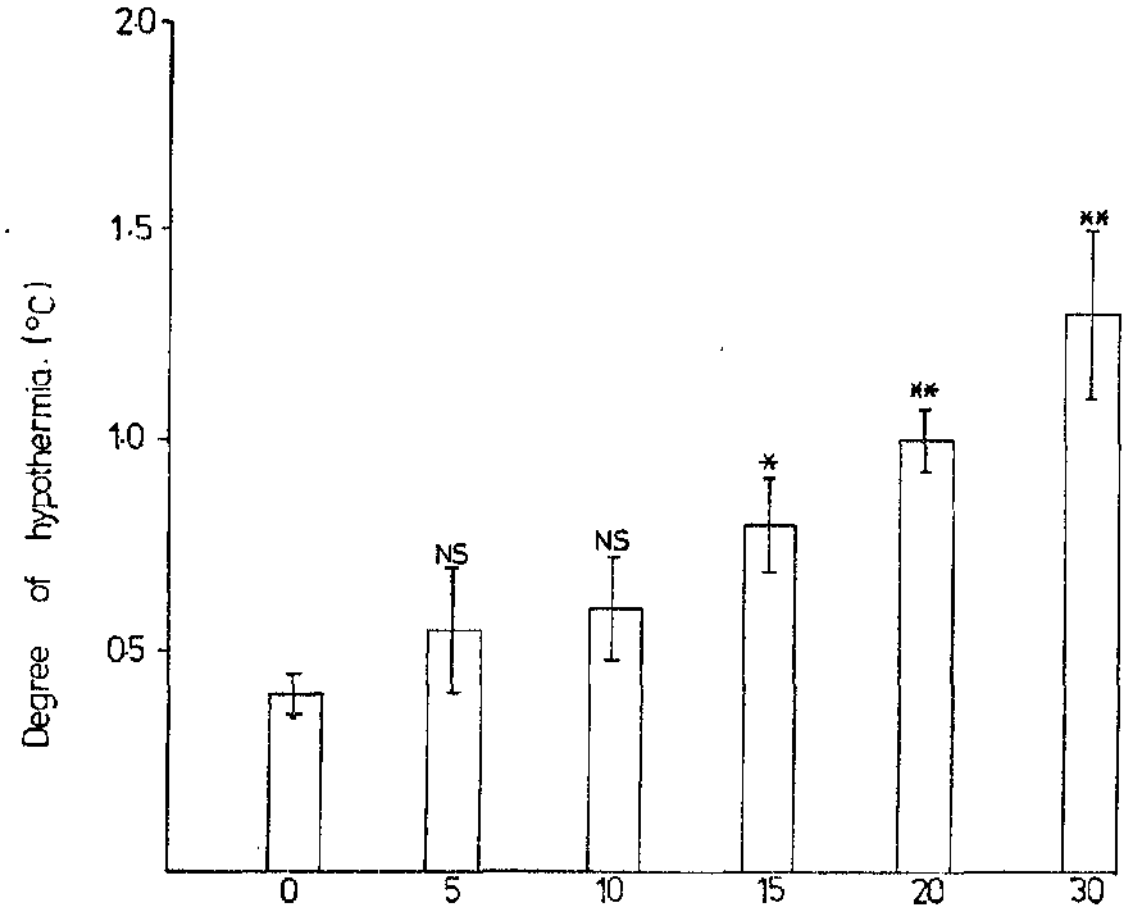


Fig 5.3b

Dose-response relationship of Dimaprit against rectal temperature in 5-day old chicks. * P =< 0.01; * * P =< 0.001. Compared to saline control; student's "t" test. NS = Non-significant. n = 6.

5.4.2 Effect of Histamine antagonists on temperature of chicks.

Chlorpheniramine, mepyramine and triprolidine, all H_1 receptor antagonists produced insignificant fall in temperature (Fig.5.4-5.6) whereas cimetidine, H_2 receptor antagonist produced significant increase in body temperature ($P<0.001$) (Fig.5.7). Hypothermia induced by 2-thiazolyl-ethylamine and dimaprit was completely reversed by chlorpheniramine and cimetidine respectively (Fig.5.8-5.9). Neither H_1 receptor antagonists (Chlorpheniramine and mepyramine, Fig 5.10 - 5.11) nor H_2 recepotr antagonists (cimetidine and metiamide, Fig.5.12 - 5.13) could completely reverse hypothermia induced by histamine. In both cases chlorpheniramine proved to be more active in counteracting this hypothermia than mepyramine while cimetidine proved superiority over metiamide. This might be due to individual effect of antagonist on temperature changes.

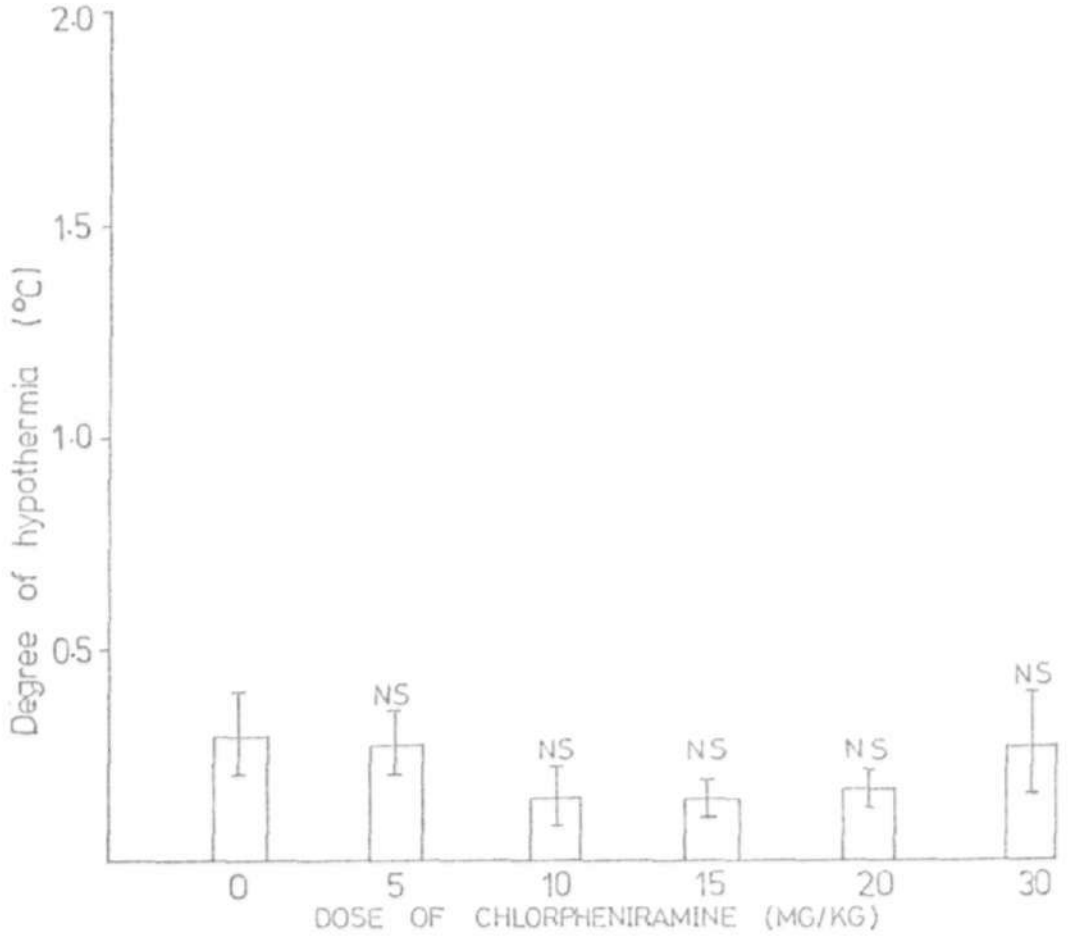


Fig 5.4 Dose response relationship of chlorpheniramine against rectal temperature in 5-day old chicks. Vertical bars represent standard error of mean ± SEM. NS = Non-significant.

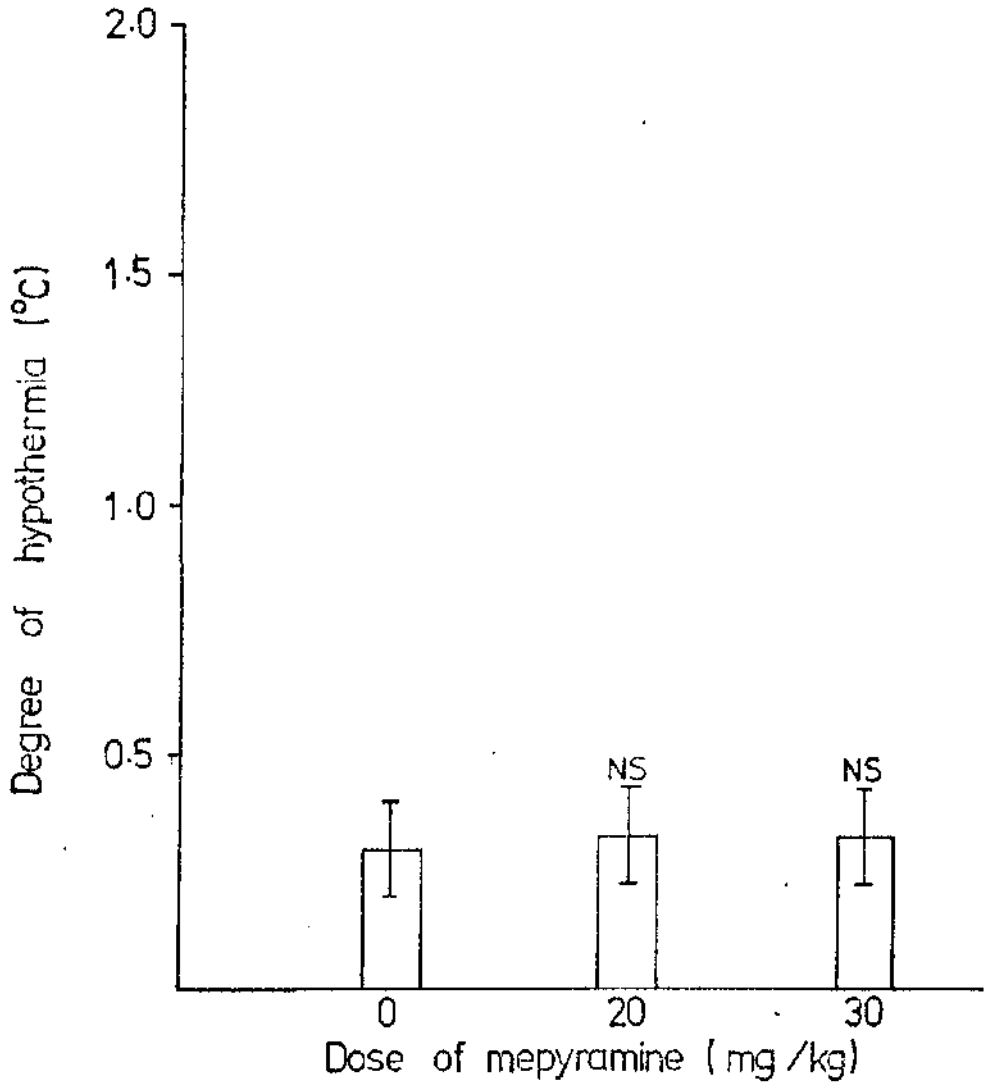


Fig. 5·5 Dose response relationship of mepyramine against rectal temperature in 5-day old chicks. Vertical bars represent standard error of the mean ± SEM. NS = Non-significant.

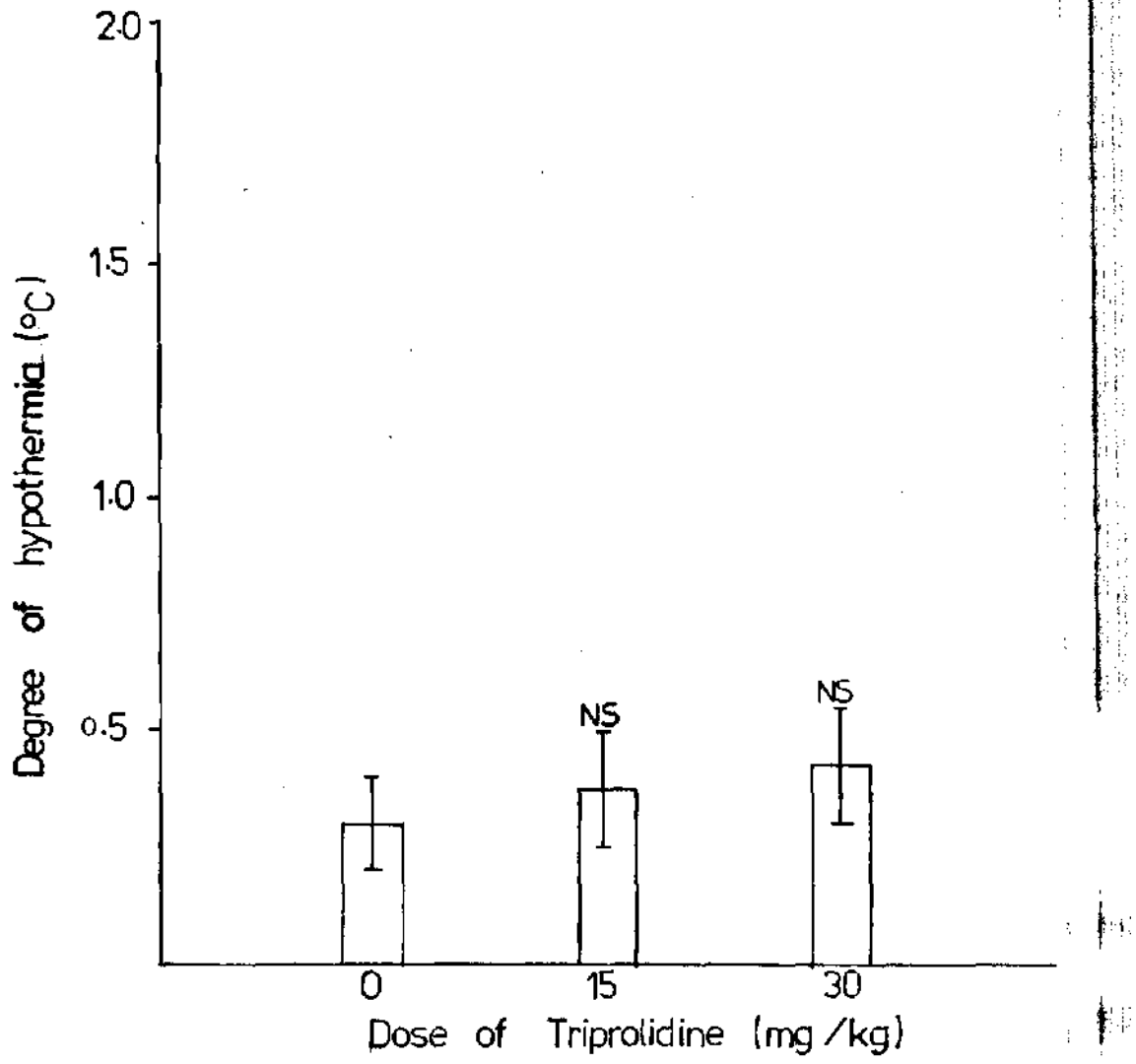


Fig. 5.6 Dose response relationship of Triprolidine against rectal temperature in 5-day old chicks. Vertical bars represent standard error of the mean ± SEM. NS = Non-significant.

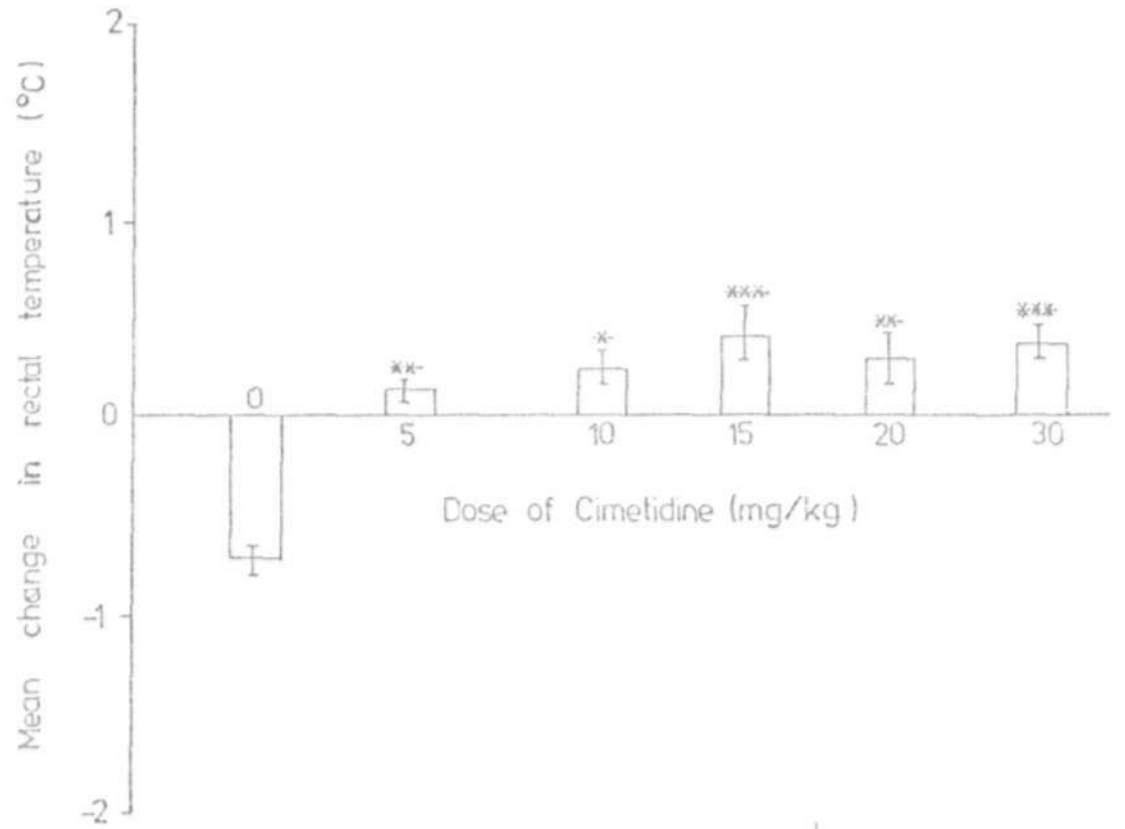


Fig. 5.7

Dose response relationship of cimetidine against rectal temperature in 5-day old chicks. Vertical bars represent standard error from the means. * P = < 0.01; ** P = < 0.002; *** p = < 0.001. Compared to saline control: student's "t" test. Vertical bars represent standard error of the means ± S.E.M. N = 5.

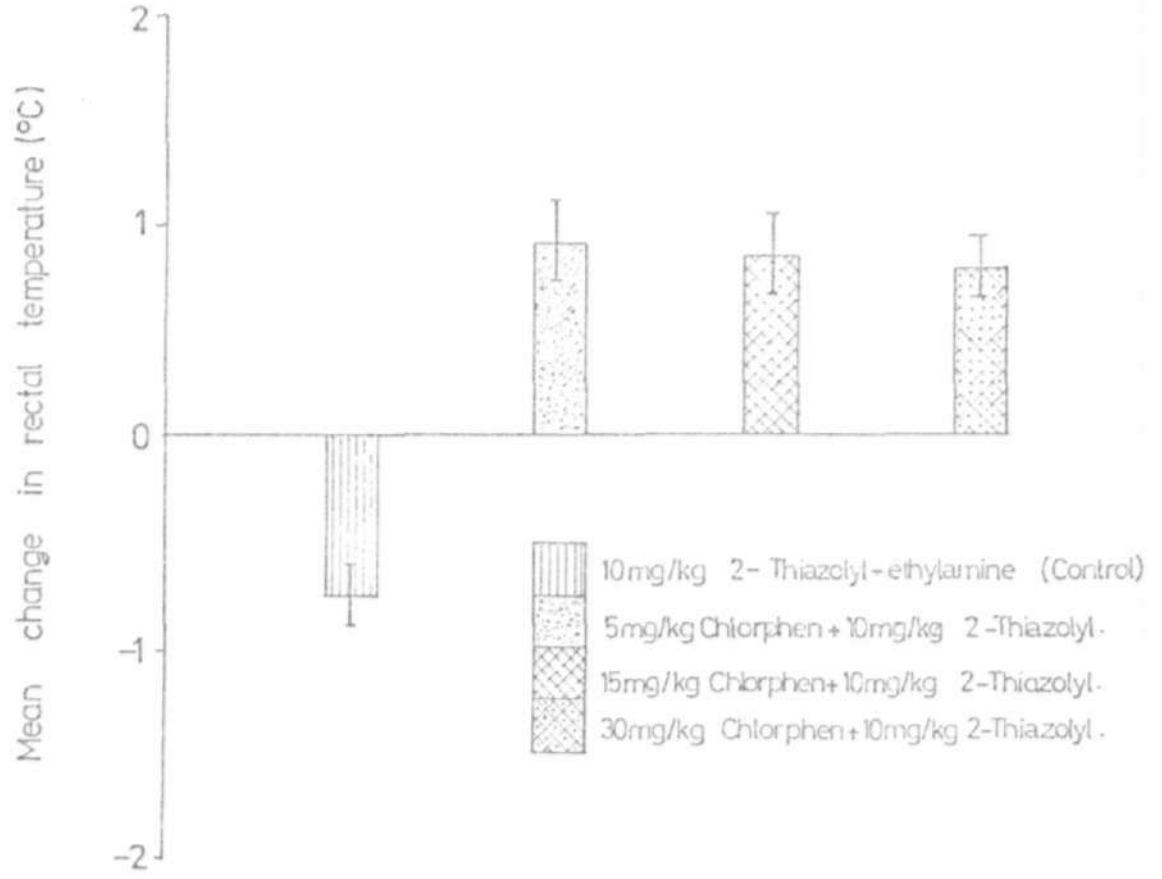


Fig.5.8 Effect of 2-Thiazolyl-ethylamine (10mg/kg) pretreated with chlorpheniramine in 5-day old chicks. Vertical bars represent standard error of the mean ± SEM. n = 6.

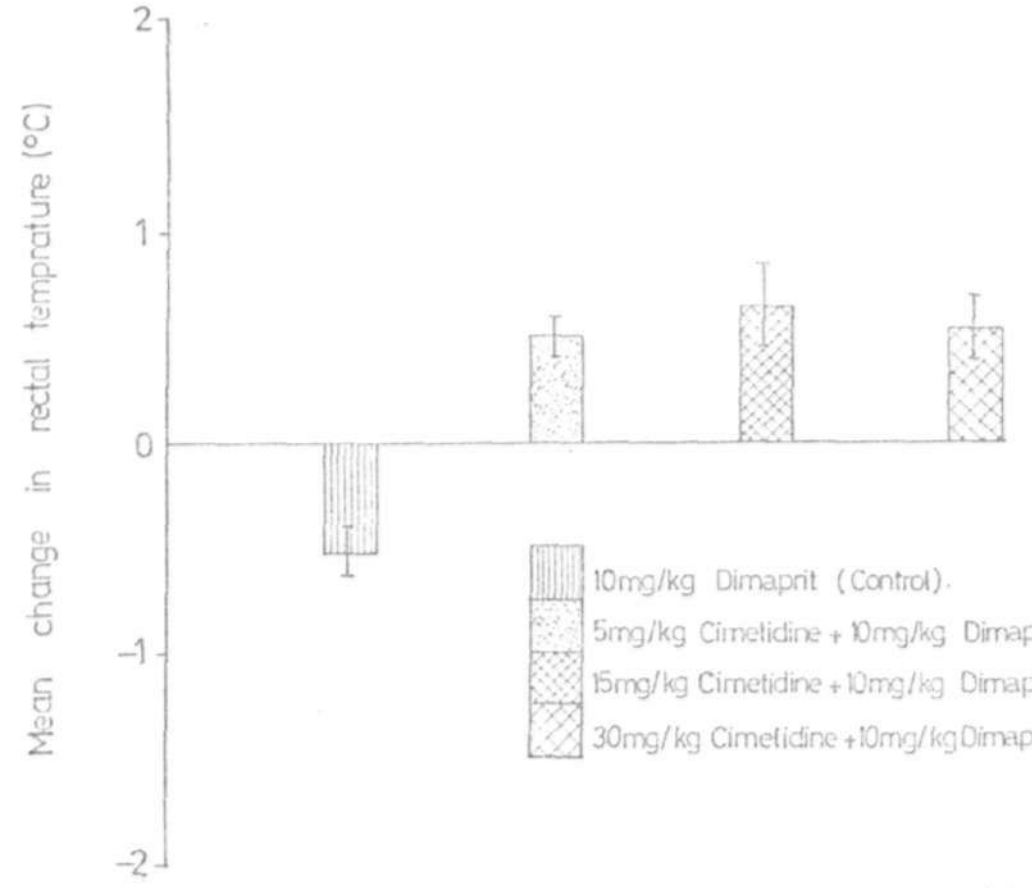


Fig. 5.9 Effect of Dimaprit pretreated with Cimetidine (10mg/kg) in 5-day old chicks. Vertical bars represent standard error of the mean ± S.E. n = 6.

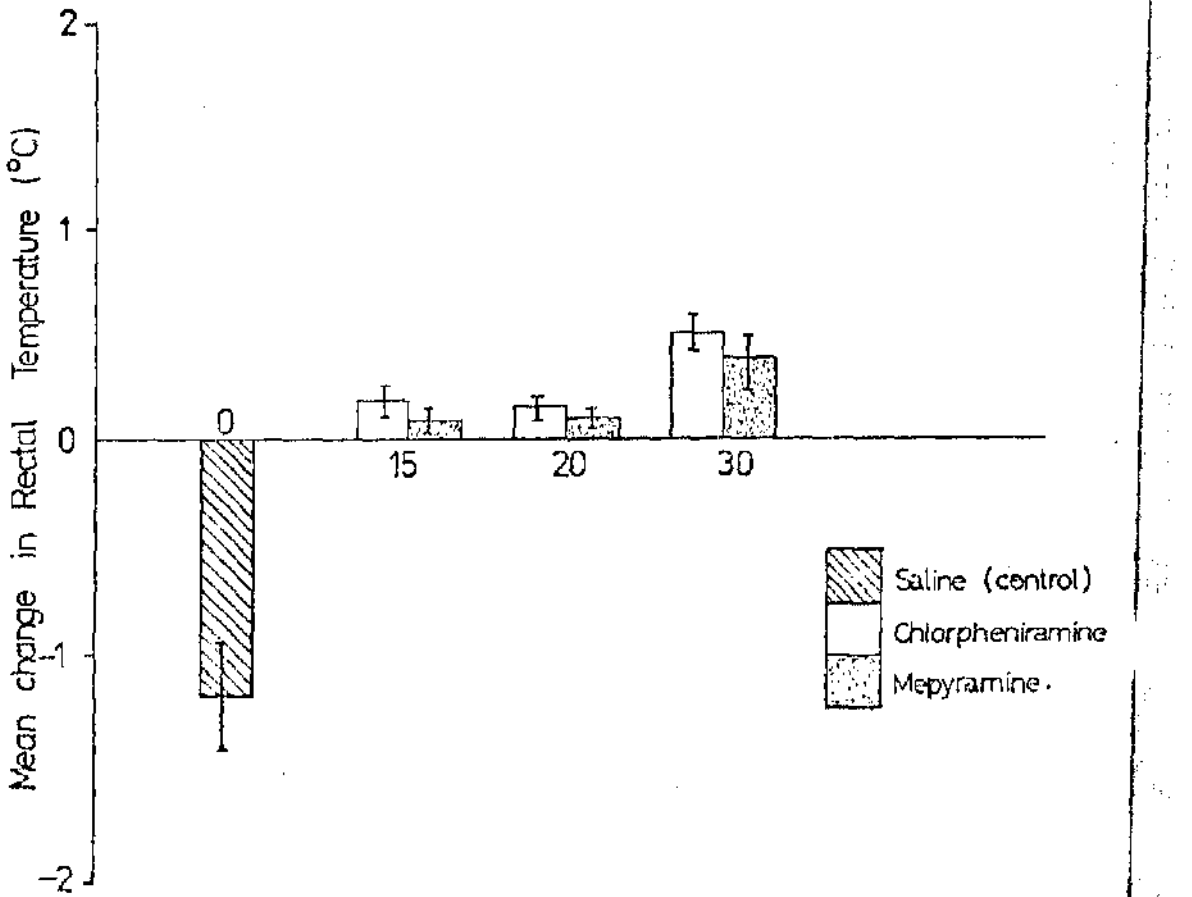


Fig. 5.10 Dose of Chlorpheniramine or Mepyramine (mg/kg)

Effect of pretreatment of chlorpheniramine or mepyramine on hypothermia produced by 5mg/kg Histamine in 5-day old chicks. Vertical bars represent standard error of the mean ± SEM. n = 6.

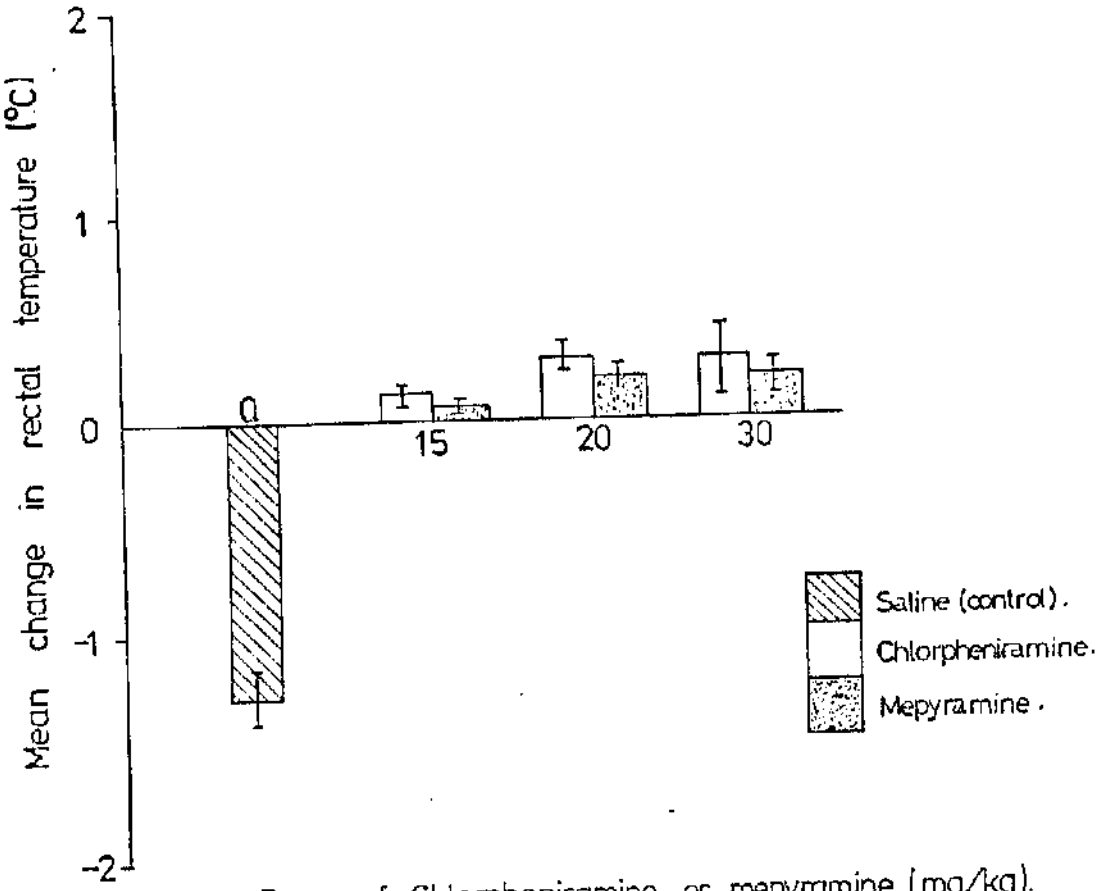


Fig. 5.11 Effect of pretreatment of chlorpheniramine or mepyramine on hypothermia produced by 10 mg/kg histamine in 5-day old chicks.
Vertical bars represent standard error of the mean ± S.E. n = 6.

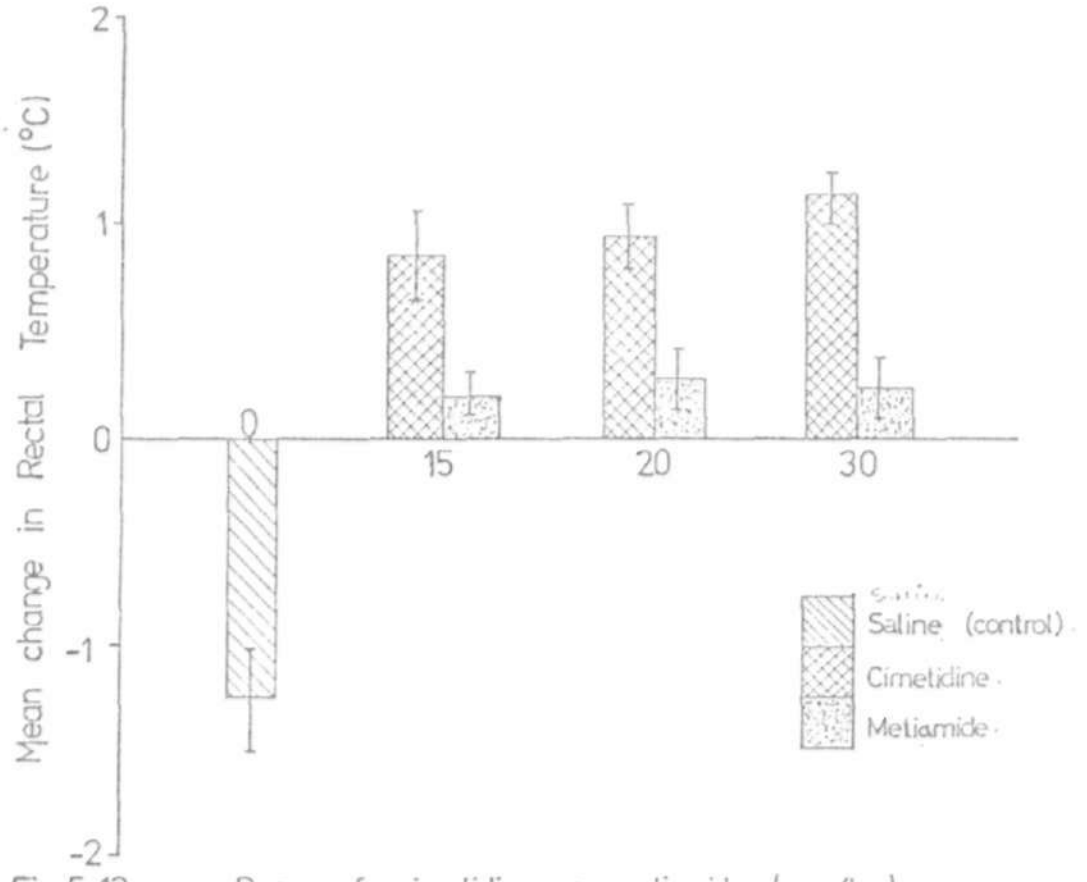


Fig. 5·12 Effect of pretreatment of cimetidine or metiamide on hypothermia produced by 5mg/kg histamine in 5-day old chicks. Vertical bars represent standard error of the mean ± SEM. n = 6.

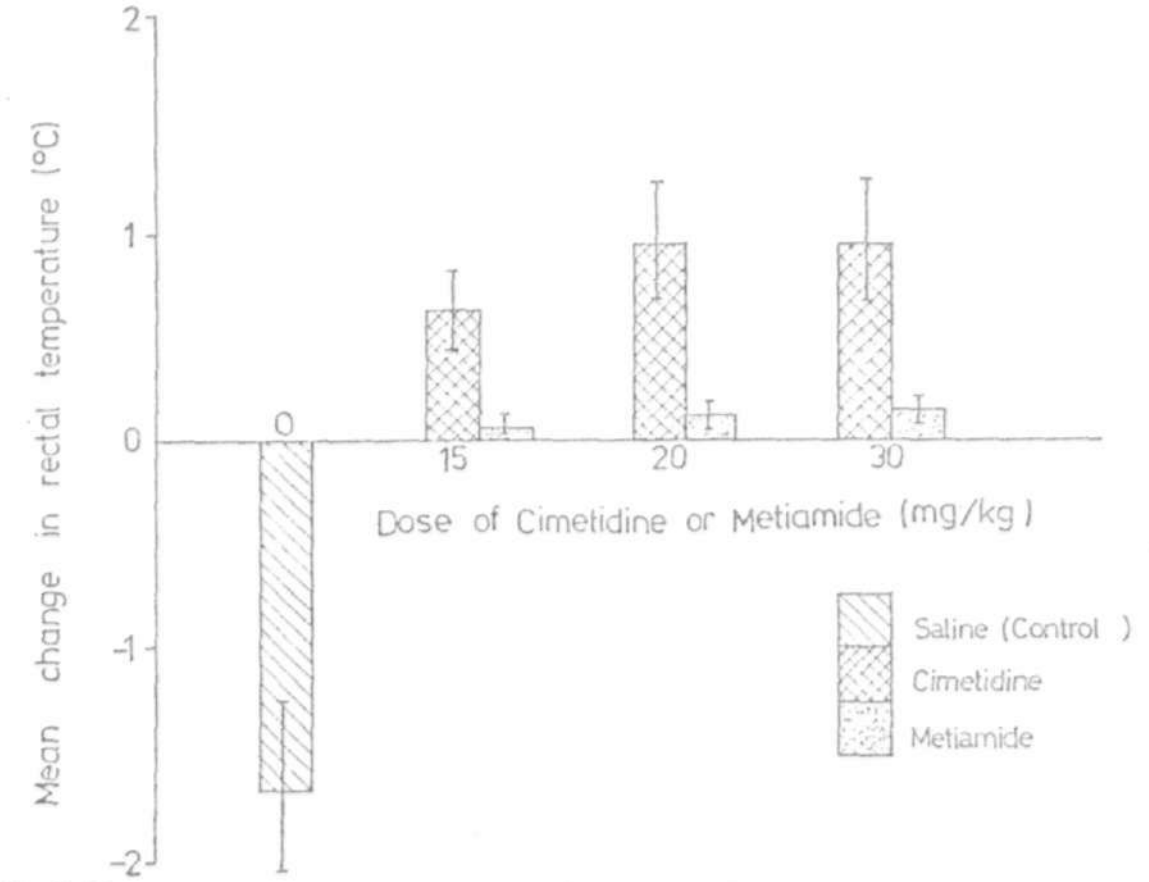


Fig. 5-13

Effect of pretreatment of cimetidine or metiamide on hypothermia induced by 10mg/kg Histamine in 5-day old chicks. Vertical bars represent standard error of the mean ± S.E. n = 6.

5.4.3 Effect of Concurrent pretreatment of H_1 and H_2 antagonists on Histamine-induced hypothermia

A concurrent pretreatment of cholorpheniramine and cimetidine was found to completely counteract histamine-induced hypothermia (Fig.5.14-5.15). However a concurrent administration of mepyramine and metiamide was not as effective as chlorpheniramine and cimetidine in counteracting this hypothermia (Fig.5.16 - 5.17).

5.5 Discussion

Histamine has been shown to be involved in the control of thermoregulatory mechanism in mammalian species (Cox et al, 1975; Green et al, 1976; Brezenoff and Tomax, 1970). Although histamine has been shown to induce changes in body temperature in several animal species, so far the exact mechanism of action in bringing about these changes in temperature has not been fully elucidated. The main emphasis in this study is to establish the thermoregulatory effect of histamine in young chicks and to elucidate its mechanism of action. Selective agonists on H_1 and H_2 receptors and their appropriate antagonists were used to achieve this goal.

In this study histamine-induced hypothermia was rapid, prolonged and dose dependent, lasting over four hours with 15 and 20mg/kg. The highest fall

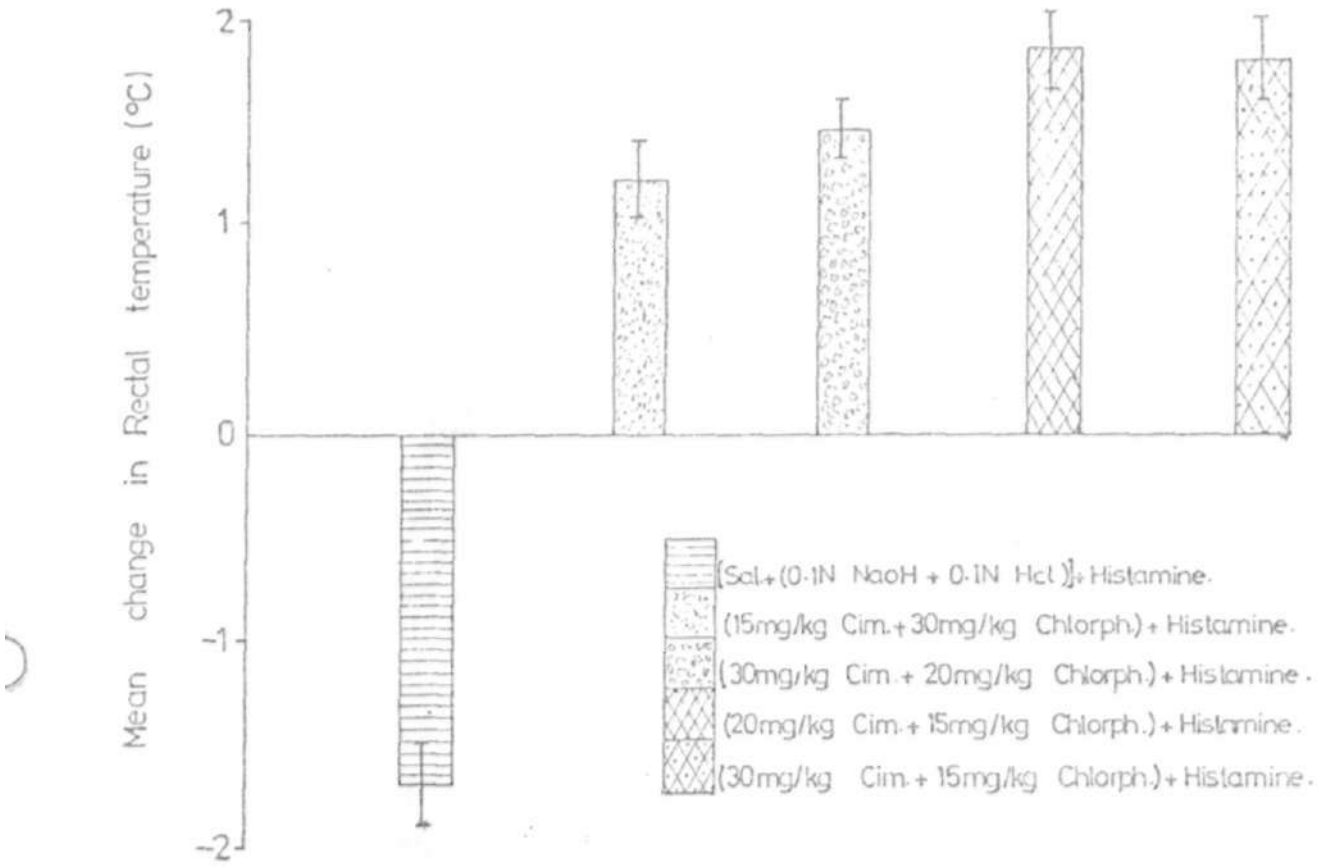


Fig. 5.14 Effect of Concurrent pretreatment of Chlorpheniramine and Cimetidine on hypothermia induced by histamine (5mg/kg) in 5-day old chicks. Vertical bars represent standard error of the mean ± SEM. n = 6.

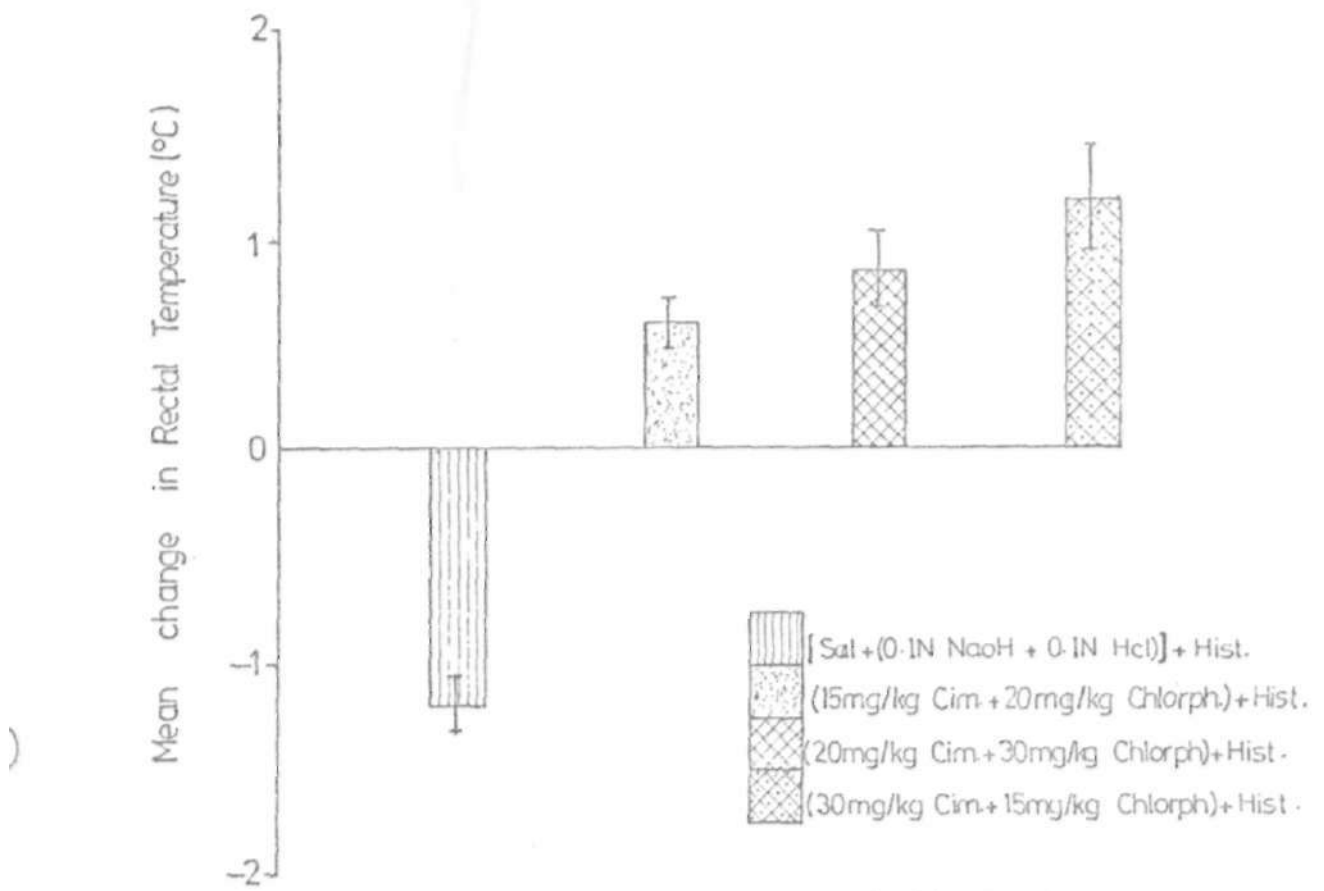
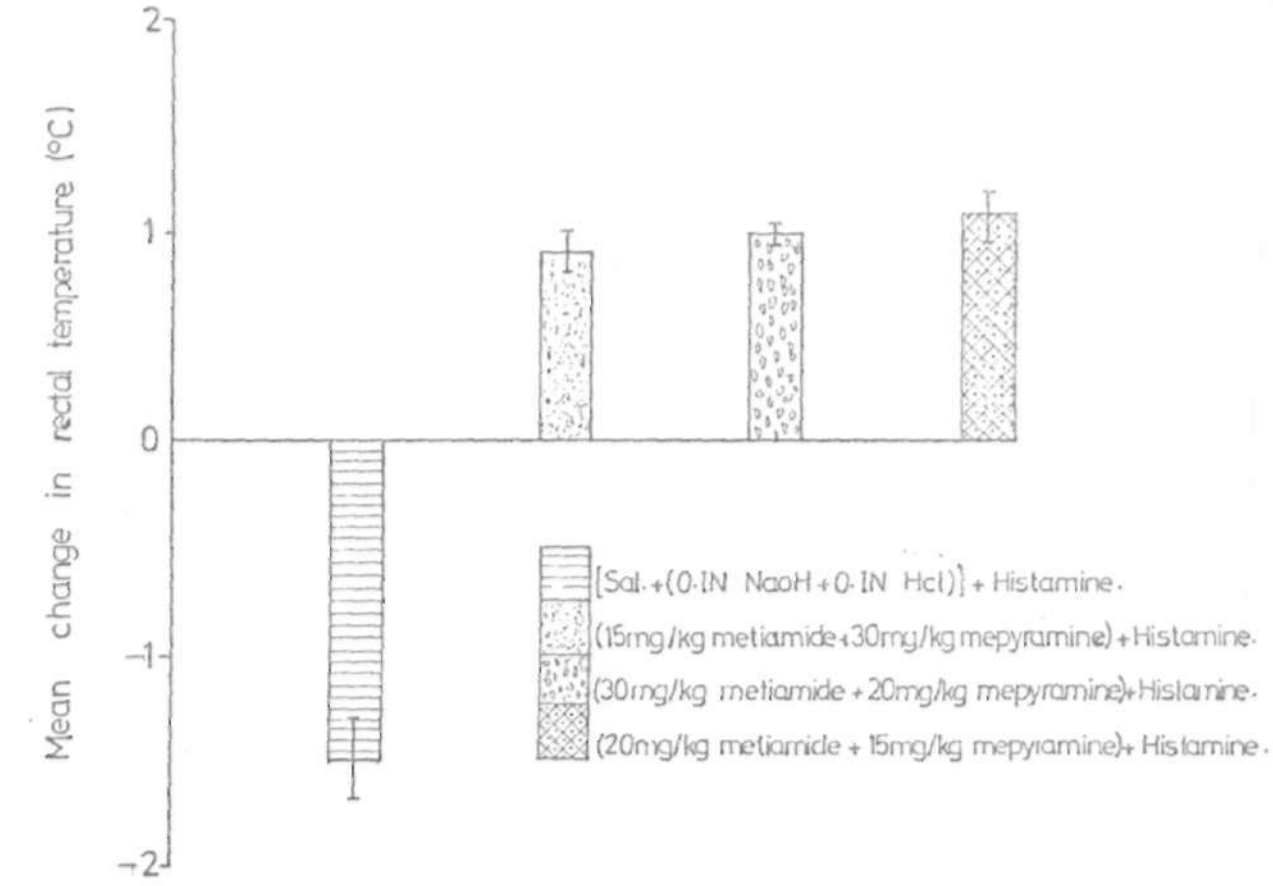


Fig.5·15 Effect of concurrent pretreatment of chlorpheniramine and cimetidine on hypothermia induced by histamine (10mg/kg) in 5-day old chicks. Vertical bars represent standard error of the mean ± SEM. n = 6.



Fig·5·16 Effect of Concurrent pretreatment of Mepyramine and Metiamide on hypothermia induced by histamine (5mg/kg) in 5-day old chicks. Vertical bars represent standard error of the mean ± SEM. n = 6.

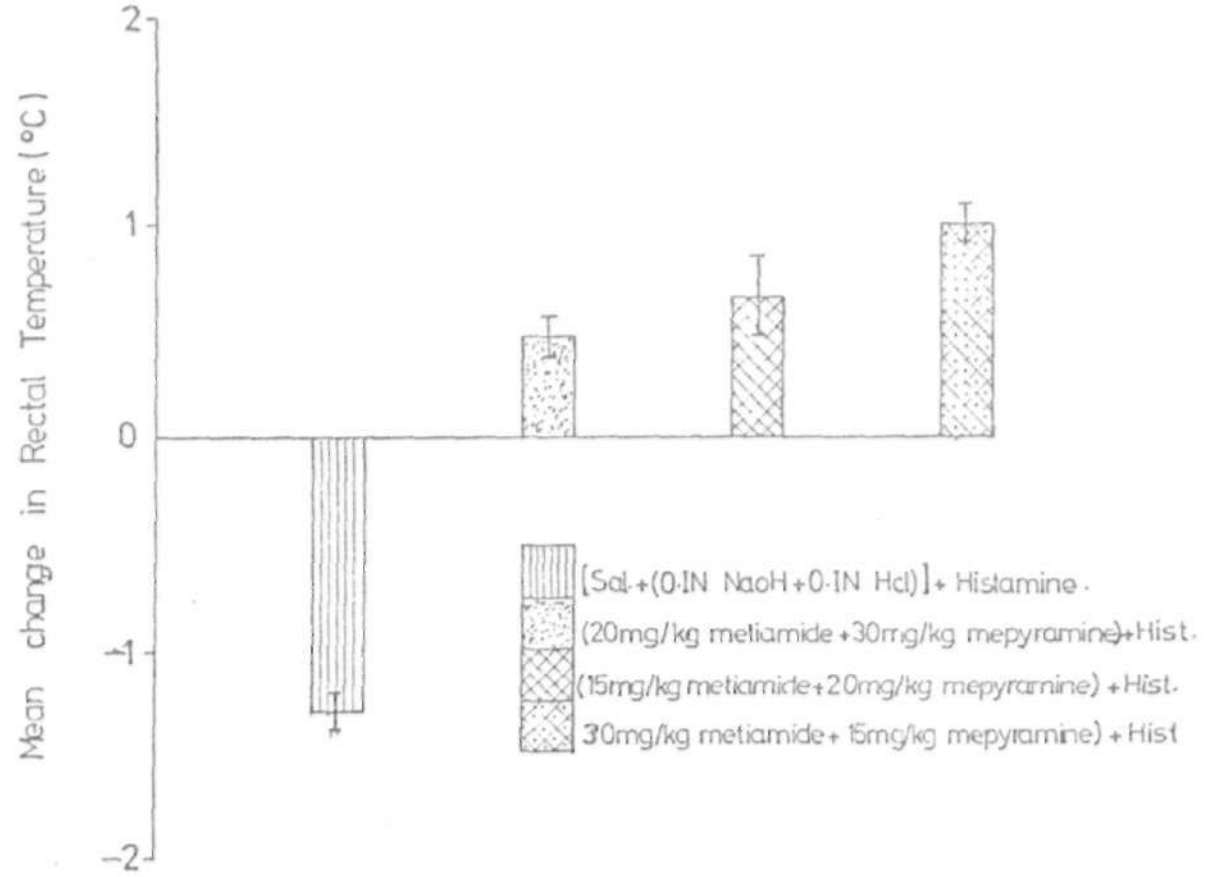


Fig. 5.17 Effect of concurrent pretreatment of mepyramine and metiamide on hypothermia induced by histamine (10mg/kg) in 5-day old chicks.
Vertical bars represent standard error of the mean ± SEM. n = 6.

recorded was 3.8°C which was induced by 20mg/Kg. These results correlate well with the findings of other workers using other species like rats and mice (Brezenoff and Lomax, 1970; Green et al, 1976; Shaw, 1971; Costenin et al, 1972). Like histamine, 2-thiazolyl-ethylamine and dimaprit, selective $H\bar{1}$ and $H\bar{2}$ receptor agonists elicited significant dose dependent hypothermia but with less intensity singly when compared with that induced by histamine. Hypothermia induced by these agonists demonstrates the presence of $H\bar{1}$ and $H\bar{2}$ receptors in the thermo-regulatory centres in young chicks and stimulation of these two components leads to hypothermia.

$H\bar{1}$ receptor antagonists, chlorpheniramine, mepyramine and triprolidine all produced insignificant fall in body temperature in young chicks. On the other hand, cimetidine, $H\bar{2}$ receptor antagonist produced a rise in temperature. This observation shows that a blockade of $H\bar{2}$ receptors can produce increase in temperature. However, the insignificant fall in temperature produced by $H\bar{1}$ receptor antagonists is a complicating factor in interpreting their antagonistic action. This observation is in agreement with that of Shaw (1971) in mice.

Although chlorpheniramine produced insignificant degree of hypothermia on its own, it was able to

completely reverse hypothermia induced by 2-thiazolyl-ethylamine ($H\bar{1}$ receptor agonist). In addition, hypothermia induced by dimaprit was completely counteracted by cimetidine. However, neither $H\bar{1}$ receptor antagonists nor $H\bar{2}$ antagonists could alone counteract histamine - induced hypothermia but a concurrent administration of chlorpheniramine and cimetidine ($H\bar{1}$ and $H\bar{2}$ receptor antagonists). This observation further lend strong support to the suggestion that histamine acts on both $H\bar{1}$ and $H\bar{2}$ receptors to elicite a fall in temperature and both receptors are involved in the central thermoregulatory pathways of histamine in chicks. In counteracting hypothermia induced by histamine, however, both $H\bar{1}$ and $H\bar{2}$ receptor antagonists may be required for effective treatment.

CHAPTER SIX

6.0 EFFECT OF SEX HORMONES ON HISTAMINE-INDUCED HYPOTHERMIA IN YOUNG CHICKS

6.1 Summary

6.1.1 The involvement of sex hormones in thermoregulatory effect of histamine in the brain has been established in this study.

6.1.2 Steriod sex hormones (oestradiol 17β 120ug, testosterone 250ug and progesterone 1mg) counteracted hypothermia induced by histamine, 2-thiazolyl-ethylamine ($H1^{-}$ receptor selective agonist) and dimaprit ($H2^{-}$ receptor selective agonist) in young chicks.

6.1.3 The doses of the hormones used in this study were found to produce no direct effect of their own on the temperature of the chicks.

6.1.4 Chicks pretreated with hormones exhibited fast recovery from hypothermia compared to those without hormone treatment.

6.1.5 Progesterone was found to be more potent on $H2^{-}$ receptors than $H1^{-}$ receptors in counteracting these temperature changes.

6.1.6 Antioestrogens (Clomiphene 120ug and tamoxifen 120ug) (ip) potentiated hypothermia induced by histamine and its selective agonists on H_1 and H_2 receptors.

6.1.7 These results provide evidence that sex hormones can exert a remarkable influence on the thermoregulatory effect of histamine in the brain.

6.2 Introduction

Steroid sex hormones particularly oestrogens have been shown to interact with a variety of biological substrates in the central nervous system (CNS) and in the periphery. They have been shown to possess both long term and short term effects upon the brain, modifying for example, its ultrastructure, the turnover rates of some neurotransmitters, the enzyme systems, and modifying the binding concentrations of high affinity binding sites for some biogenic amines (Thomas and Ani, 1982; Riegon et al, 1980). The present work was therefore carried out to examine the effect of gonadal sex hormones on histamine-induced hypothermia in young chicks.

6.3 Materials and Method

Refer● to section 3.11.

6.4 Results

Histamine-induced hypothermia in young chicks was counteracted almost completely by oestradiol 17B followed by testosterone and progesterone, all of which showed significant reversal ($P<0.001$; Fig.6.1a&b). In order to establish the mechanism of action of sex hormones in counteracting temperature changes induced by histamine, histamine selective agonist on $\bar{H1}$ and $\bar{H2}$ receptors were used. The doses of the hormones used in this study were chosen such that they did not elicite a direct effect of their own on the temperature of the chicks (Fig.6.2). Both oestradiol and testosterone significantly reversed 2-thiazolyl-ethylamine-induced hypothermia ($P<0.002$) while progesterone only reduced this hypothermic effect ($P<0.10$; Fig.6.3). Dimaprit-induced hypothermia was also significantly counteracted by oestradiol ($P<0.002$), testosterone ($P<0.01$ and progesterone ($P<0.001$; Fig.6.4). Results also show that progesterone is more potent on $\bar{H2}$ receptors than on $\bar{H1}$ receptors in counteracting these temperature changes (Fig.6.4). Hypothermia induced by histamine, 2-thiazolyl-ethylamine and dimaprit were potentiated by antioestrogens (Ciomiphena and tamoxifen) (Fig.6.5a,b,c d & 6.6).

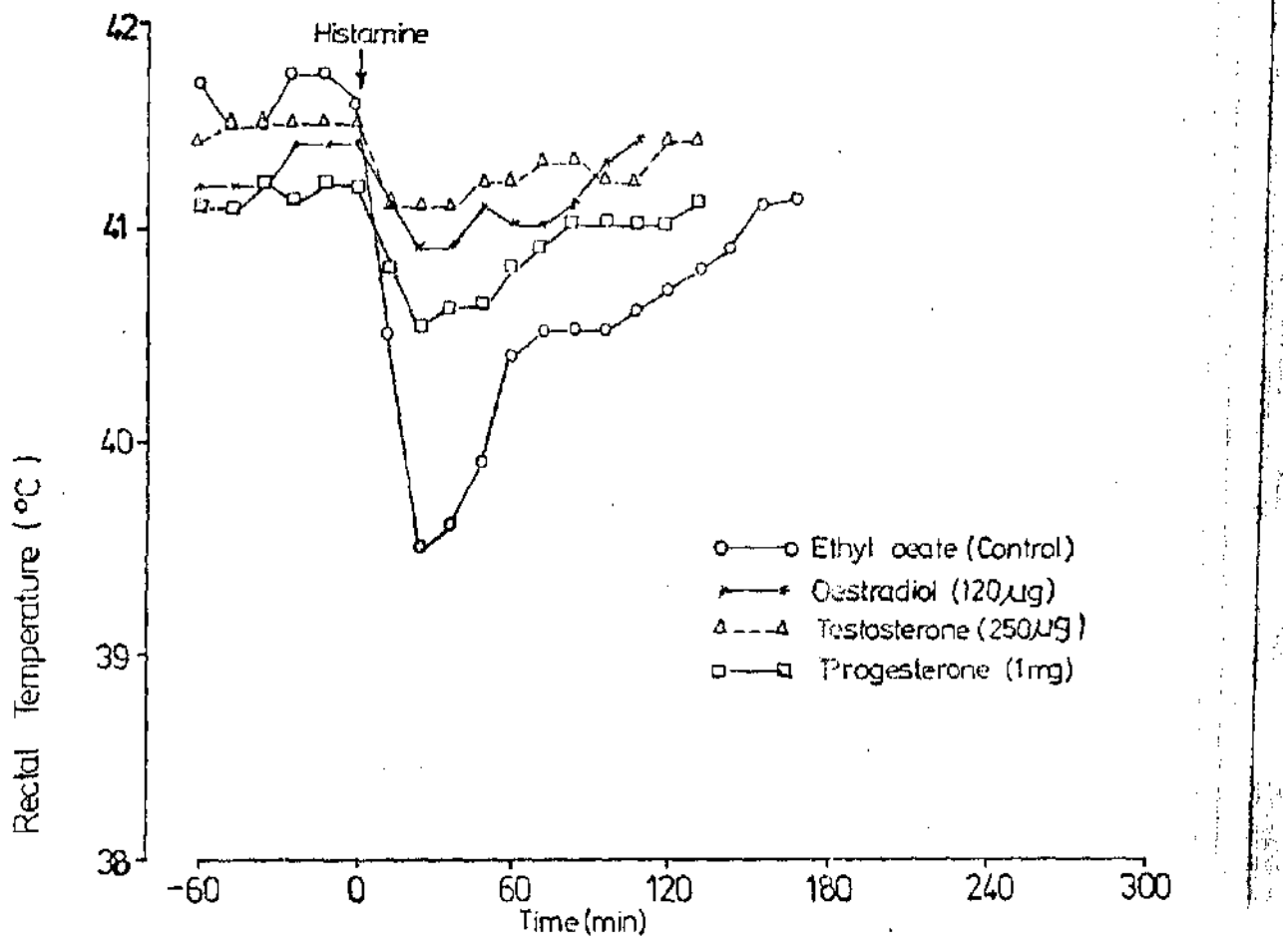


Fig. 6.1a Effect of Histamine (10mg/kg) on rectal temperature of 6-day old chicks pretreated with ethyl oleate, Oestradiol (120 µg), Testosterone (250 µg) and Progesterone (1mg) n= 6. The points represent the mean from six experiments.

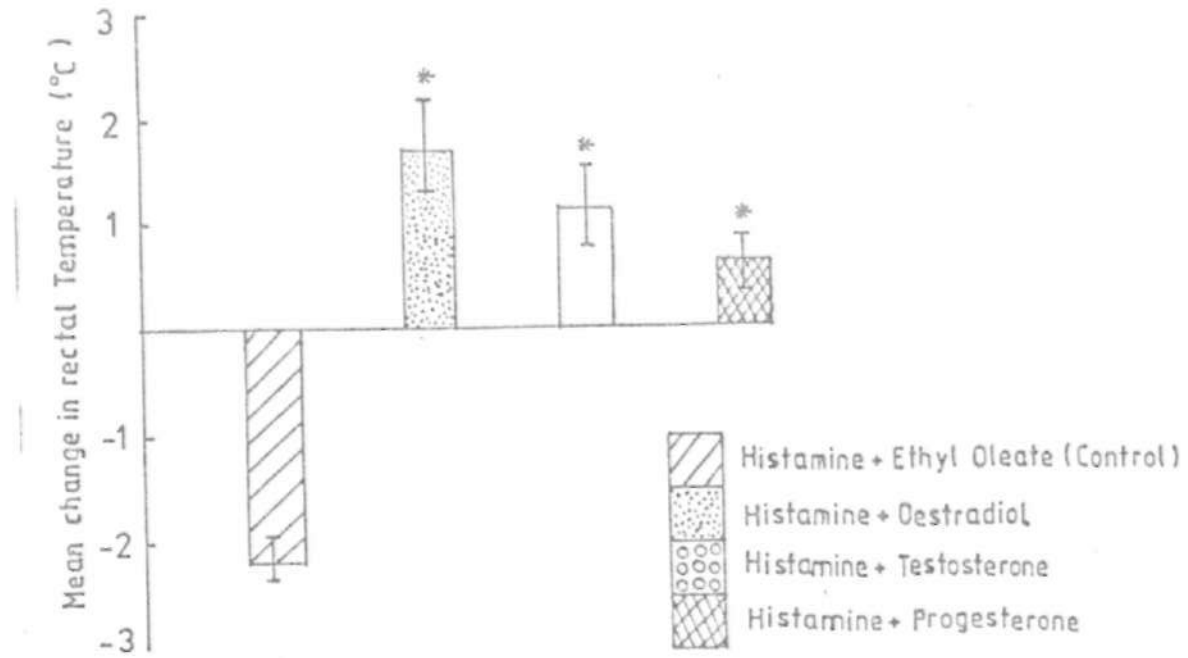


Fig. 6-1b

Effect of sex hormones on histamine induced hypothermia in 6-day old chicks. statistical significance of the difference in temperature (Student's t test) between chicks pretreated with sex hormones and those injected with ethyl oleate at the maximum change in temperature. Vertical bars represent standard error of the mean ± SEM.

* P = <0·001

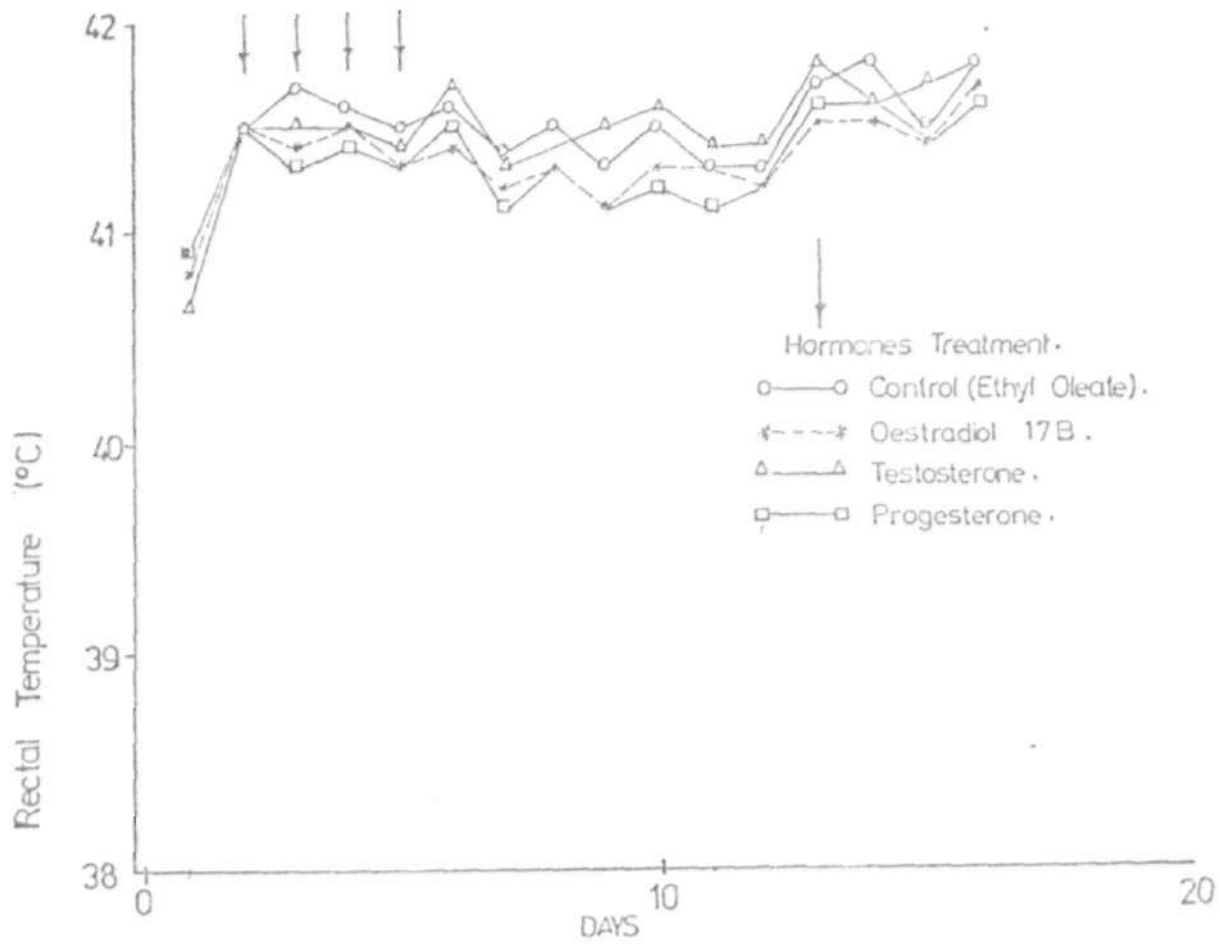


Fig.6.2 Effect of sex hormones on rectal temperature of 1-16days old chicks. The points represent the mean from six experiments.

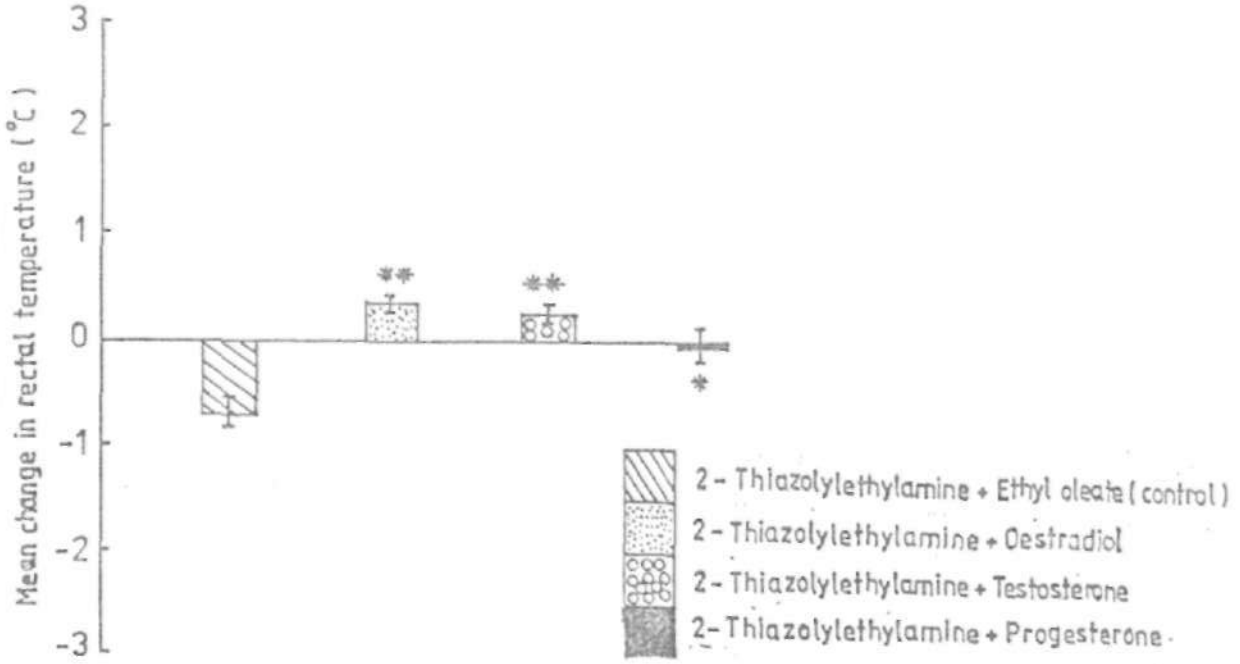


Fig. 6.3

Effect of sex hormones on 2-thiazolylethylamine induced hypothermia in 6-day old chicks. Vertical bars represent ± S E M. Statistical significance of the difference in temperature (student's t-test) between chicks pretreated with sex hormones and those injected with ethyl oleate at the maximum change in temperature: ** $P<0.002$ * $P<0.10$. Vertical bars represent standard error of the ± SEM.

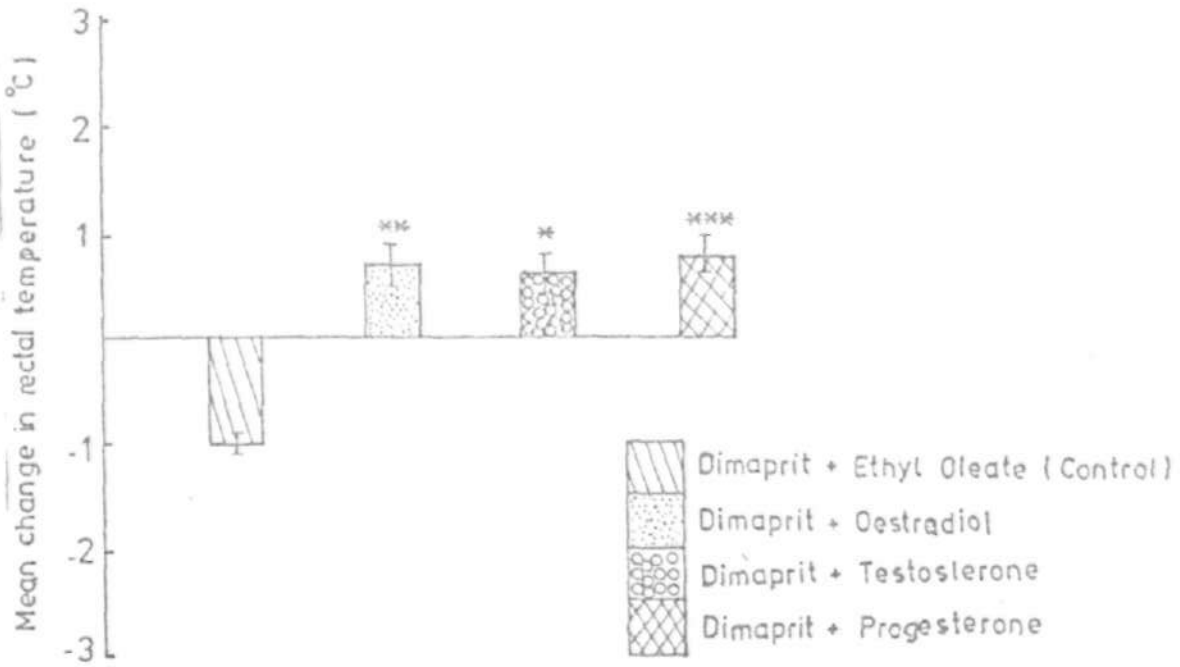


Fig. 6.4.

Effect of sex hormones on Dimaprit-induced hypothermia in 6-day old chicks. Vertical bars represent ±SEM. Statistical significance of the difference in temperature (Student's t-test) between chicks pretreated with sex hormones and those injected with Ethyl Oleate at maximum change in temperature: *** $P < 0.001$, ** $P < 0.002$ * $P < 0.01$. Vertical bars represent standard error of the mean ± SEM.

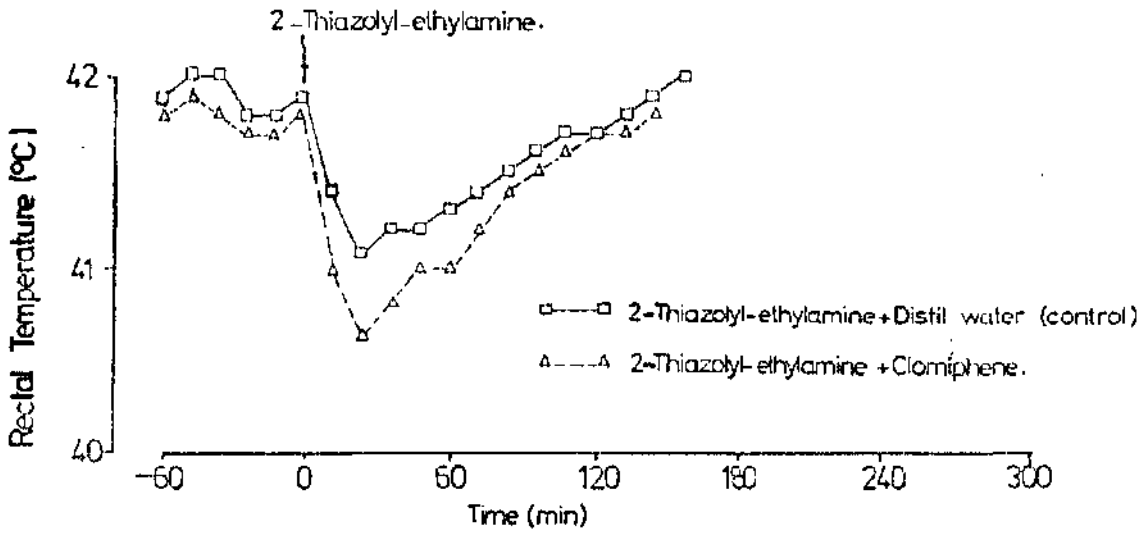


Fig 6.5a Effect of 2-Thiazolyl-ethylamine (20mg) on rectal temperature of 6-day old chicks pretreated with clomiphene (120 µg).

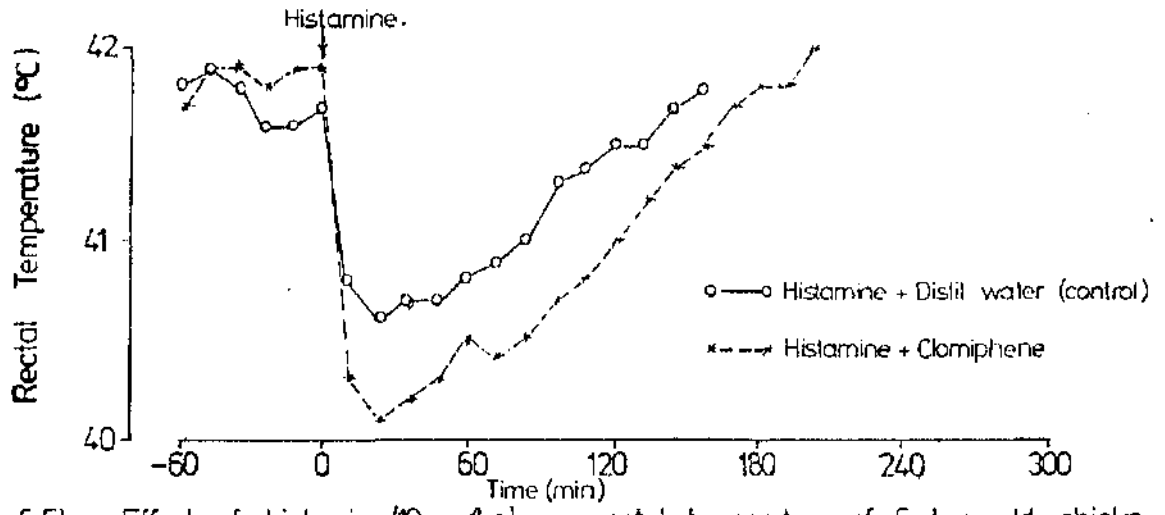


Fig. 6.5b Effect of histamine (10mg/kg) on rectal temperature of 6-day old chicks pretreated with clomiphene (120 µg). The points represent the mean from six experiments.

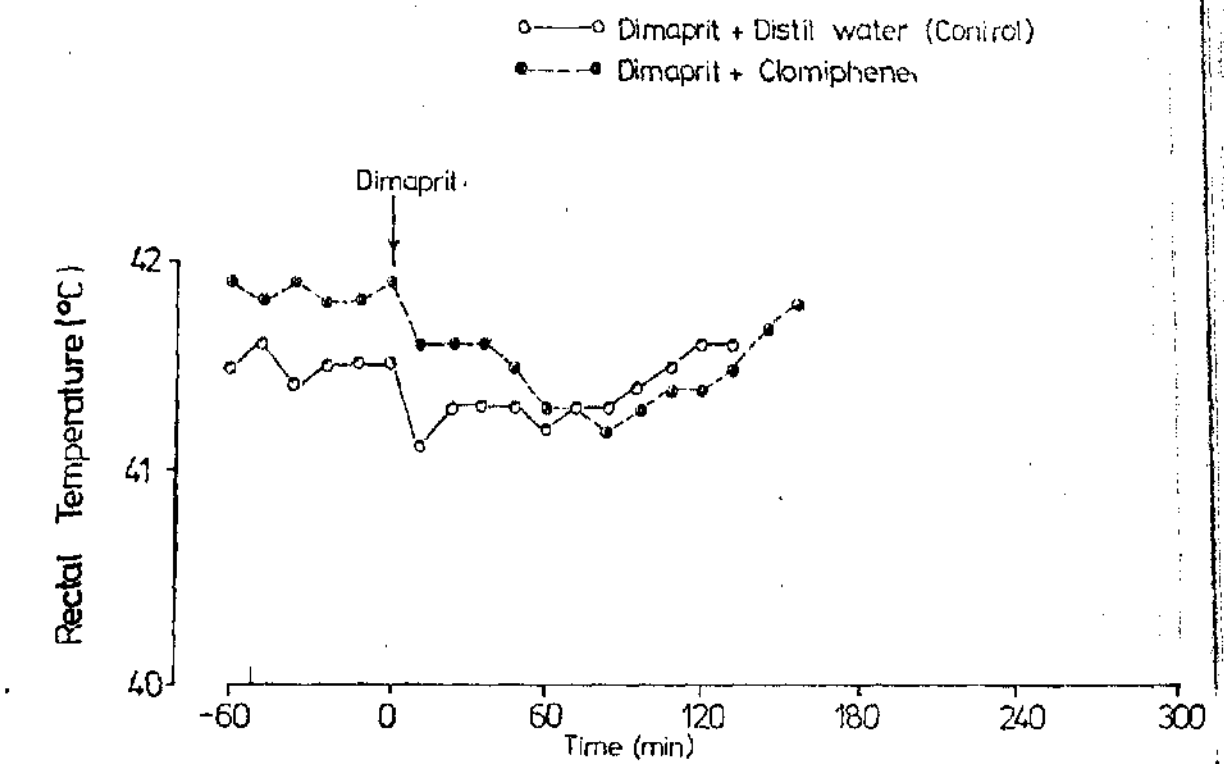


Fig. 6.5c Effect of Dimaprit (20mg/kg) on rectal temperature of 6-day old chicks pretreated with clomiphene (120 wg). The points represent the mean from six experiments.

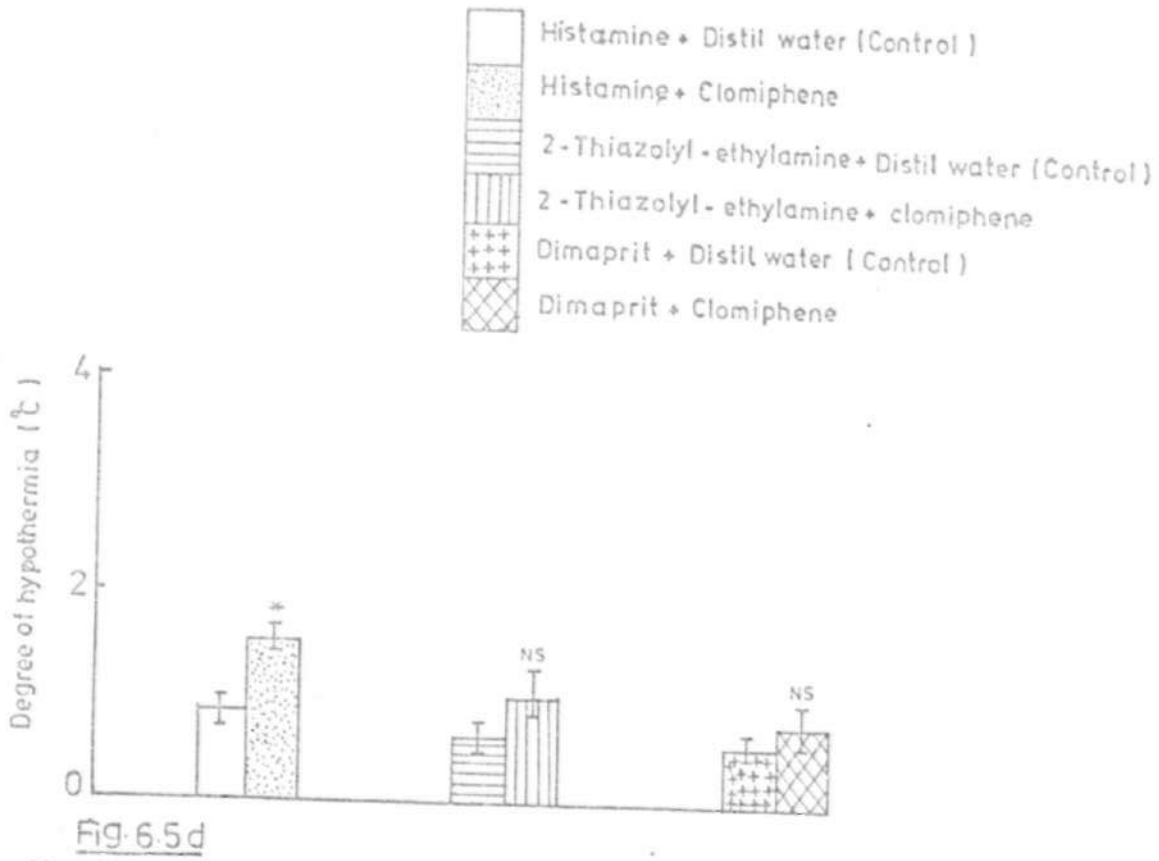


Fig 6.5d

Effect of clomiphene on histamine (10 mg/kg), 2-Thiazolyl ethylamine (20mg/kg) and Dimaprit (20mg/kg) induced hypothermia in 6-day old chicks. * $P < 0.02$. Compared with saline control: student's "t" test. Vertical bars represent standard error of the mean ± SEM. NS = Non-significant.

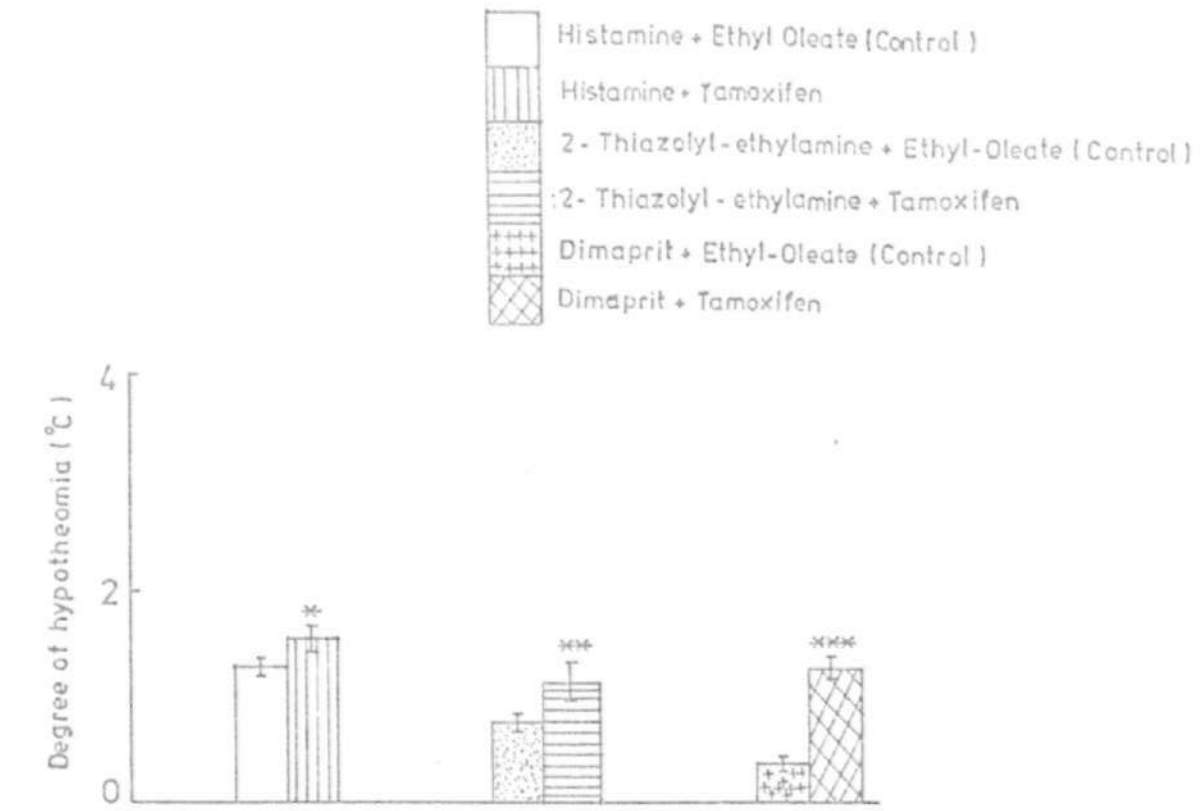


Fig. 6.6.

Effect of Tamoxifen on histamine (10 mg/kg), 2-Thiazolyl-ethylamine (20 mg/kg) and Dimaprit (20 mg/kg) induced hypothermia in 5-day old chicks * $P < 0.10$, ** $P < 0.05$, *** $P < 0.002$ Compared with saline control: student's "t" test. Vertical bars represent standard error of the mean ± SEM.

6.5 Discussion

The present study shows that hypothermia induced by histamine, 2-thiazolyl-ethylamine and dimaprit was counteracted by steroid sex hormones (Oestradiol 17B, testosterone and progesterone). Not only did they affect the degree of hypothermia produced but also the onset of hypothermia and the time of the recovery (fast recovery). The dose of the hormones used in this work did not produce changes in temperature on their own. Hence the effect of the hormones observed in this work was due to their influence on histaminergic pathways. This work therefore provides evidence that sex hormones can exert a remarkable influence on thermoregulatory effect of histamine in the central nervous system and further lend support for a strong mutual correlation between histamine and hormones in the central nervous system (Portaleone et al, 1978). The fact that sex hormones counteracted 2-thiazolyl-ethylamine ($H1^{-}$ receptor agonist) and dimaprit ($H2^{-}$ receptor agonist) is an indication that they may influence both $H1^{-}$ and $H2^{-}$ receptors in the brain.

The overall action of sex hormones in the brain is an inhibitory influence on the termoregulatory effect of histamine. This correlates well with other findings which support the hypothesis that oestrogens play inhibitory role in the development of histamine

sensitive adenylate cyclase; possible interaction between oestrogens and histaminergic receptors in CNS (Portaleone et al, 1978). Steroid sex hormones have been shown to modify many categories of brain cell functions like neurosecretion, turnover rates of neurotransmitters, enzyme regulation and receptor density regulation (see MCEwen, 1980). Histamine receptor availability has been shown to be considerably influenced by oestrogens: binding capacity was reduced and affinity increased (Anuka et al, 1986 and 1991). (Fig 6.3b & 6.4b). The results obtained in this study are largely attributed to the influence of sex hormones on histamine receptor availability and suggest a downward regulatory mechanism of $H1^{-}$ and $H2^{-}$ receptors by sex hormones. With this mechanism, few receptors are available for either histamine or its agonists to act upon. This situation gave rise to the inability of the hormones to completely reverse hyothermia induced by either the agonists or histamine alone. Thus, the mechanism whereby sex hormones counteract hypothermia induced by histamine or its agonist is different from that of histamine antagonists. While histamine antagonists induced this effect by blocking the effect of histamine on the appropriate receptors (Anuka et al, 1991), sex hormones reduced the number of binding sites available for histamine or its agonists to act upon

(genomic mechanism).

The potentiating effect of antioestrogens (Clomiphene and tamoxifen) on either histamine, 2-thiazolyl-ethylamine or dimaprit-induced hypothermia is of particular interest in this work. Antioestrogens have been shown to bind to cytoplasmic oestrogen receptors and to cause translocation to the cell nucleus. Once in the cell nucleus however, the antioestrogenic activity of these compounds is thought to be due to their ability to reduce the number of oestradiol-receptor complex entering the target cell and nucleus (Wade and Blaustein, 1978). From our results (Fig.6.5d&6.6d), it is possible that apart from their antioestrogenic effects, both clomiphene and tamoxifen might be causing an upward regulation of histamine receptors after treatment for a certain length of time. With this penomenon, it seems more receptors will be available for the exogenously administered histamine or its agonists to act upon, thus potentiating hypothermic effects of histamine, dimaprit (H_2 receptor agonist) or 2-thiazolyl-ethylamine (H_1 receptor agonst).

The observation that sex hormones are involved in the regulation of histamine receptors in the CNS may have important implication in understanding female reproductive physiology.

CHAPTER SEVEN

7.0 THE ROLE OF HISTAMINE IN SEIZURES

7.1 Summary

7.1.1 The role of histamine on seizure regulation was investigated in chicks.

7.1.2 Histamine (30mg/kg) was found to offer 85% protection against leptazol-induced seizure in young chicks ($P < 0.001$).

7.1.3 Dimaprit, an $H2^-$ receptor agonist gave a significant degree of protection ($P<0.001$) against leptazol-induced seizure whereas protection offered by 2-thiazolyl-ethylamine, an H1-receptor agonist, was insignificant.

7.1.4 Cimetidine and triprolidine neither protected nor potentiated leptazol-induced seizures. However, chlorpheniramine offered a significant protection ($P<0.01$) against the seizures.

7.1.5 Histamine, its agonists and antagonists on both $H1^-$ and $H2^-$ receptors are not convulsant at the dose levels used in this work but potentiated electroshock seizures.

7.1.6 The potentiating effect of histamine on electroshock seizures was dose-dependent ($P<0.02$ for 10mg/kg and $P<0.002$ for 30mg/kg). Unlike histamine, the potentiating effect of chlorpheniramine was not dose dependent.

7.1.7 These results suggest that histamine in the brain could serve as a protective substance against petit mal seizure, common in children, when the attack is precipitated.

7.1.8 It is also possible that children who are prone to petit mal attack may suffer from lower levels of histamine in the brain during this developmental stage.

7.2 Introduction

A wealth of evidence now shows that histamine is a neurotransmitter in the mammalian central nervous system (CNS) (Schwartz, 1977). The regional distribution of histamine in the brain shows that it is present in nearly all areas of the brain with the highest concentration in the hypothalamus followed by the amygdala (Hill et al, 1978). The physiological responses of this amine in the brain as in the periphery are mediated by at least two distinct types of receptors ($H\bar{1}$ and $H\bar{2}$) (Schwartz et al, 1980; Taylor and Richelson, 1981).

Though histamine has been shown to possess a variety of actions in the central nervous system, its role in the regulation of seizures has not been investigated. This study was therefore designed to examine the role of histamine and its related substances in both electroshock and chemically-induced seizures in young chicks. Both histamine agonists and antagonists were used to determine the mechanism of action of histamine in seizures.

7.3 Materials and Methods

Refer to section 3.13.1; 3.13.2.

7.4 Results

7.4.1 The effect of histamine on electrically induced seizures

Histamine as well as its agonists and antagonists at $H\bar{1}$ and $H\bar{2}$ receptors are not convulsant at the dose levels used in this work (5-30mg/Kg). Neither histamine nor its agonists and antagonists offered protection against electroshock seizures. However, histamine (Fig.7.1),its agonists (Fig 7.2) and antagonists (Fig.7.3 & 7.5) on both $H\bar{1}$ and $H\bar{2}$ receptors potentiated electroshock seizures. The potentiating effect of histamine on electroshock seizure was signidicant and dose dependent ($P < 0.02$ for 10mg/kg and $P < 0.002$ for 30mg/kg; Fig 7.1). Both dimaprit and 2-thialzolyl-ethylamine, $H\bar{2}$ and $H\bar{1}$

receptor agonists respectively gave signidicant degree of seizure potentiation ($P < 0.002$; Fig.7.2). Chlorpheniramine and cimetidine, H_1 and H_2 receptor antagonists also elicited significant ($P < 0.001$) potentiation while triprolidine(another H_1 receptor antagonist) induced insignificant increase in this seizure. Unlike histamine, the potentiating effect of chlorpheniramine was dose-independent (Fig.7.4).

7.4.2 Effect of Histamine on Chemically-induced seizures

Histamine was found to offer 85% protection against leptazol-induced seizures ($P < 0.001$; Fig.7.6). Dimaprit, an H_2 receptor agonist also gave a significant degree of protection ($P < 0.001$) against this seizure whereas protection offered by 2-thiazolyl-ethylamine (H_1 - receptor agonist) was insignificant (Fig.7.6). Cimetidine and triprolidine neither protected nor potentiated leptazol-induced seizures in chicks. However, chlorpheniramine offered a significant protection ($P < 0.01$) against the seizures. (Fig.7.7).

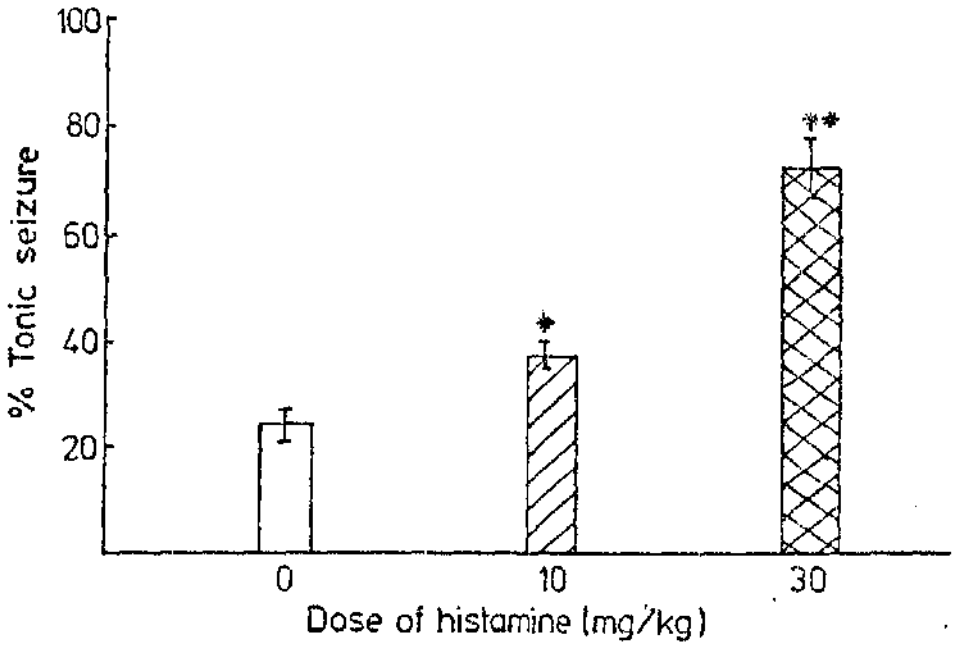


Fig.7.1 The effect of dose on seizure threshold. Vertical bars represent standard error of the means. Current = 47 mA

* $P < 0.02$ ** $P < 0.002$

Compared to saline control : Student's t. test. $n = 15$.

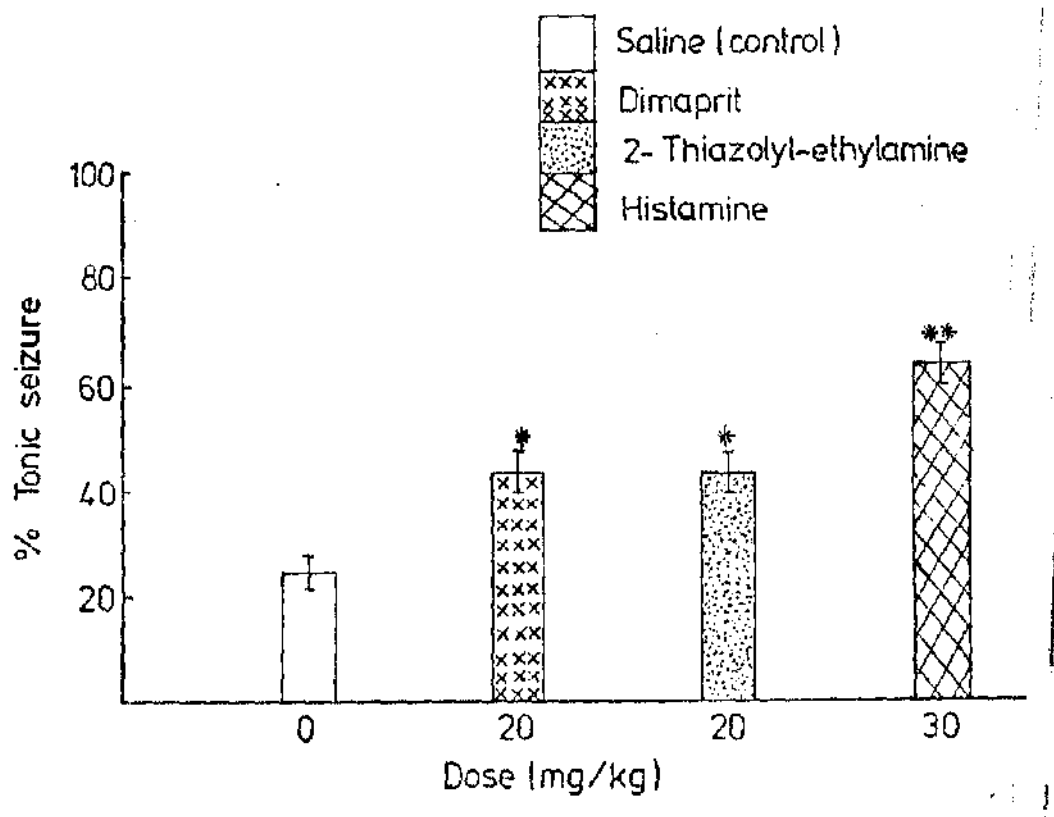


Fig.7-2 The effect of histamine agonists on electroshock seizure

$*p<0.002$ $**p<0.001$

Compared to saline control: student's "t" test. Vertical bars represent standard error of the mean ± SEM.

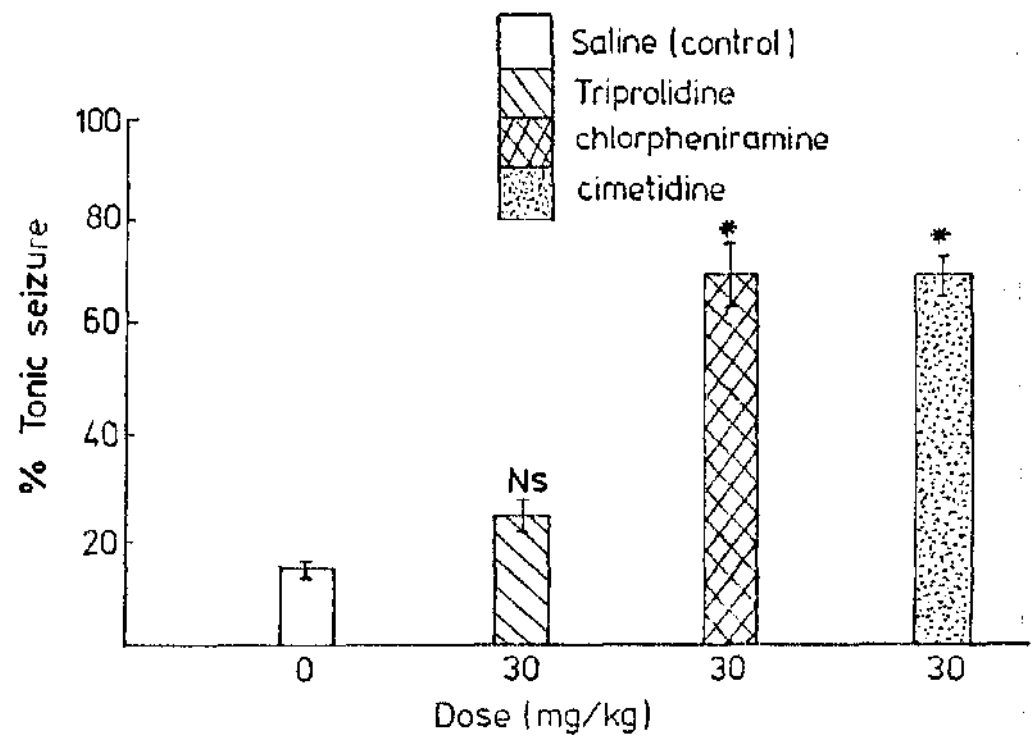


Fig.7.3 The effect of histamine antagonists on electroshock seizure

* $p < 0.001$. Compared to saline control: student's "t" test. Vertical bars represent standard error of the mean ± SEM. NS = Non-significant. n = 15.

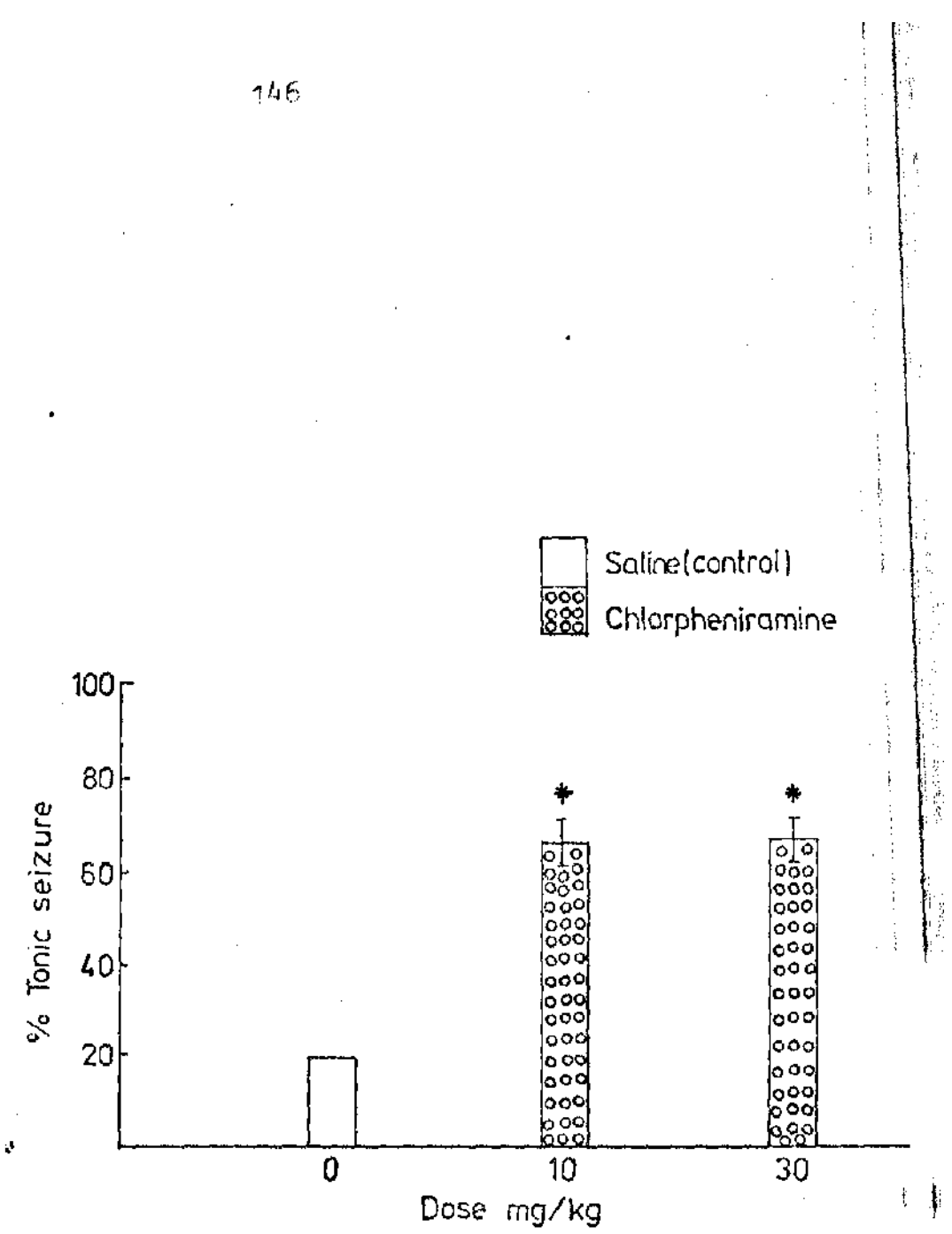


Fig.7.4 The influence of dose on potentiating effect of chlorpheniramine on electroshock seizure

* $P < 0.001$. Compared with the control: student's "t" test. Vertical bars represent standard error of the mean ± SEM. n = 15.

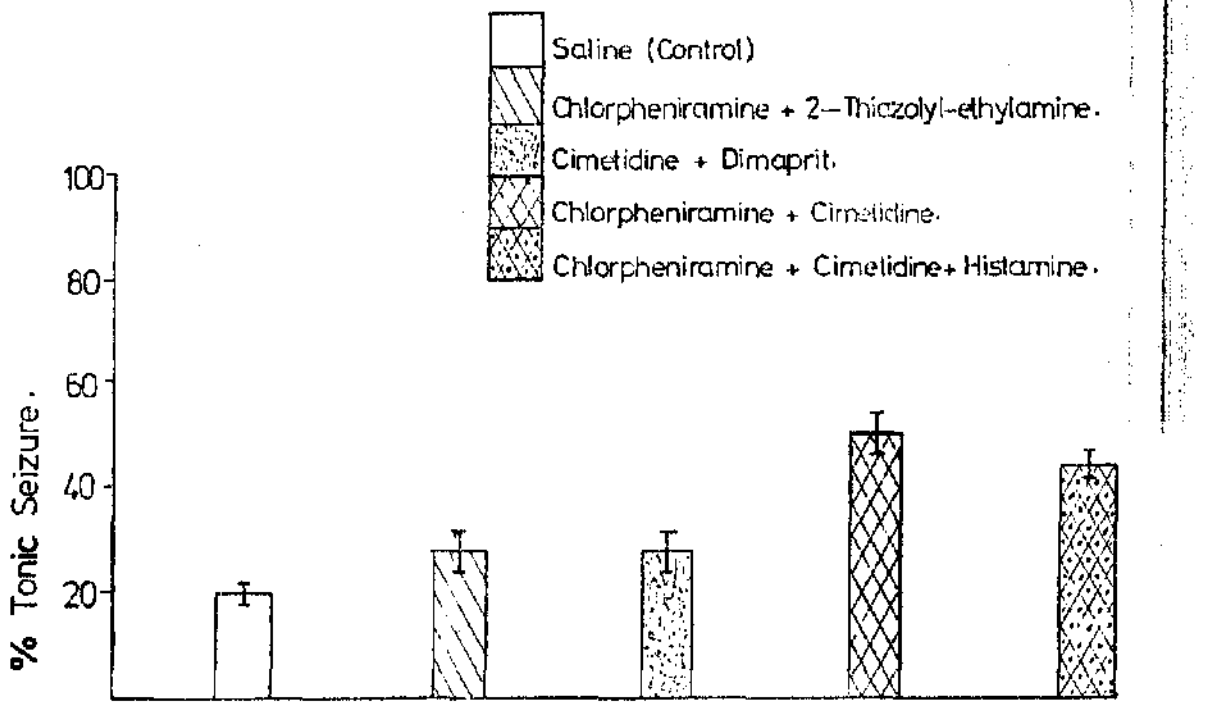


Fig. 7.5 Effect of Histamine agonists pretreated with the antagonists on electroshock seizure. Vertical bars represent standard error of the mean ± SEM.

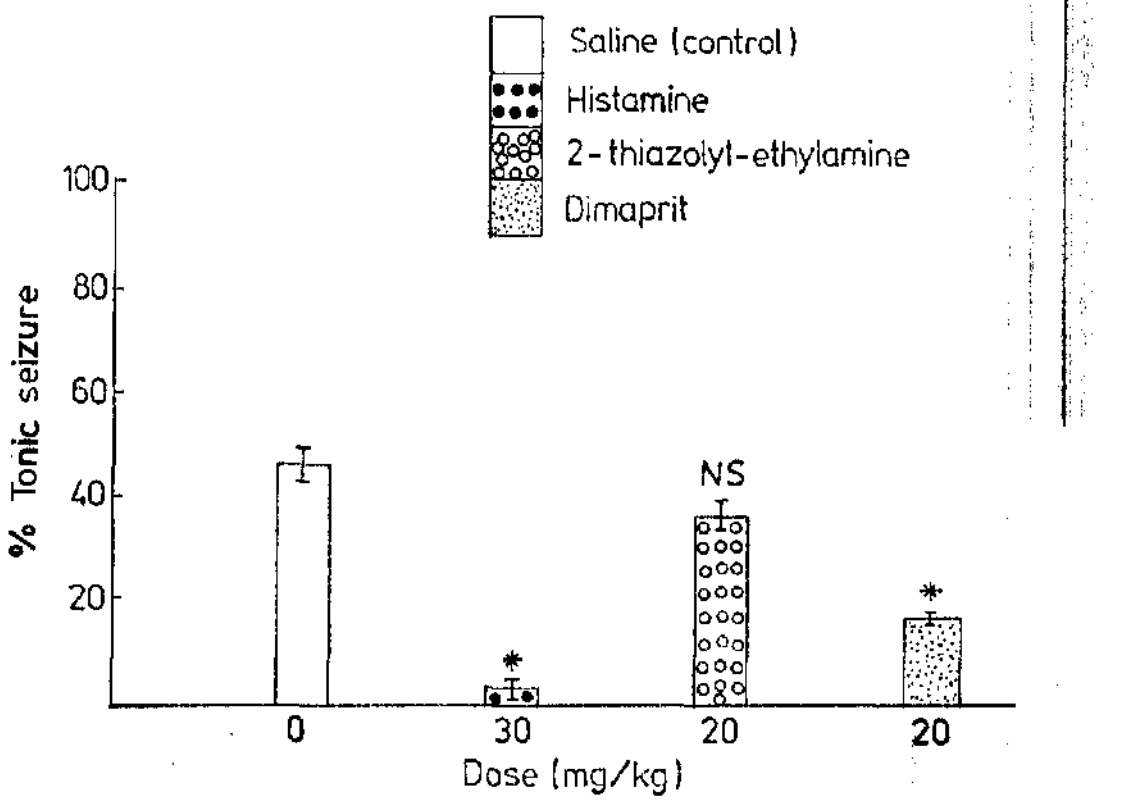


Fig.7.6 The effect of histamine agonists on leptazol - induced seizure

* $P < 0.001$. Compared to saline control: student's "t" test. Vertical bars represent standard error of the mean ± SEM. NS = Non-significant. n = 15.

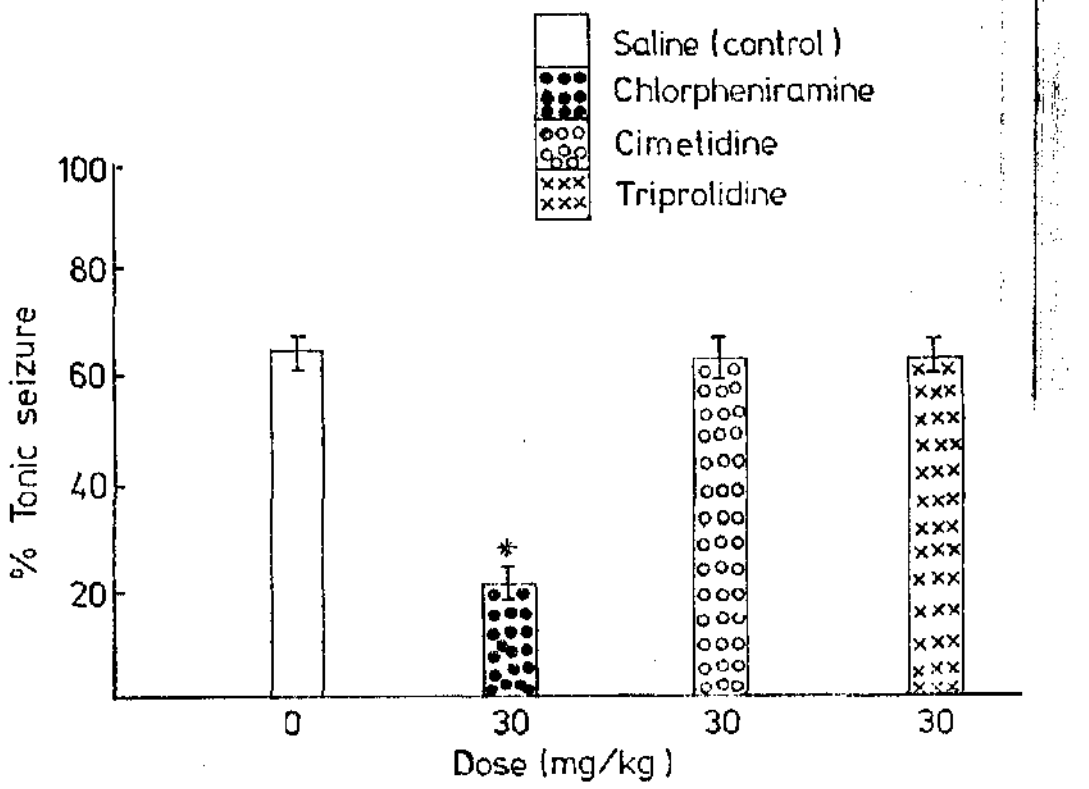


Fig.7.7 The effect of histamine antagonists on leptazol - induced seizure

$*P < 0.01$. Compared to saline control: student's "t" test. Vertical bars represent standard error of the mean $\pm$ SEM. n = 15.

7.5 Discussion

The results of this study show that histamine together with its agonists on H_1 and H_2 receptors offered protection against leptazol-induced seizures (Fig.7.6) but potentiated electroshock seizures (Fig.7.2). The potentiating effect of histamine on electroshock seizure was dose dependent (Fig.7.1). The role of histamine in seizure regulation is not clearly understood but from this study, H_1 and H_2 receptors could play significant roles in this regulation.

Leptazol is a central nervous system stimulant which produces motor activity characterized by forelimb and jaw clonus in its threshold convulsive dose. Studies of this drug on single spinal motor neurons and interneurons have failed to give the mechanism of its stimulant action. However, there is evidence that the excitatory effect of leptazol may be due to a decrease in neural recovery time (Levin and Esplin, 1961).

The role of brain monoamines in the regulation of seizure activity has been reported by numerous investigators (Alexander and Kopeloff, 1976, Stull et al, 1977, Osuide and Wambebe, 1979). Wambebe (1978) showed that dopamine offered protection against both

electroshock and leptazol-induced seizures. In his work, this author showed that the mechanism whereby dopamine offered protection against both chemically and electrically-induced seizures is the same. He suggested that dopamine exerts a central nervous system inhibitory effect which limits the spread of seizure discharge and consequently modulate the intensity of the chemically-induced seizures. The present data also suggest that histamine through its predominantly inhibitory role in the brain like dopamine, may produce its anticonvulsant effect on chicks by a similar mechanism.

Recently, adenosine has been proposed as a possible inhibitory neuromodulator that may be responsible for arresting seizures. The biochemical basis of adenosine anticonvulsant effect is not clear but it has been claimed that adenosine-induced accumulation of c-AMP may be involved (Oragunow, 1967). It has also been demonstrated that experimental seizures alter cyclic nucleotide levels in the brain. Ferrendelli (1983) suggested that the elevation of cyclic AMP may be involved in the mechanisms of inhibiting seizure activity, since he found that animals pretreated with C-AMP lowering agents (e.g. reserpine, propranolol, aminophylline) showed enhanced sensitivity of central nervous system (Gross and Ferrendelli,1979).Cyclic AMP could therefore

play a major role in seizure regulation.

Histamine has been found to be a potent stimulant of c-AMP synthesis in the CNS and elicited its action by interacting with two types of receptors, $H\bar{1}$ and $H\bar{2}$ receptors (Dismukes et al 1976, Hegstrand et al, 1976, Schwartz 1977). The magnitude of the response and relative contribution of $H\bar{1}$ and $H\bar{2}$ receptors to histamine elicited accumulation of c-AMP varies with species. However, while $H\bar{2}$ action seems to occur via receptors directly coupled to adenylate cyclase (Hegstrand et al, 1976, Green et al, 1977), $H\bar{1}$ action is indirect (Dally, 1977) and depends on the presence of a directly acting agonist, such as adenosine or indeed histamine H2 itself; the effect being observed only in the region where $H\bar{2}$-mediated response is found (Palacios et al, 1978). This explains why in this study, dimaprit, an $H\bar{2}$ receptor agonist, offered a better protection compared to 2-thiazolyl-ethylamine, $H\bar{1}$ receptor agonist. It can therefore be suggested that the anticonvulsant effect of histamine against leptazol-induced seizure may be due to its effects on $H\bar{2}$ and $H\bar{1}$ receptors via c-AMP accumulation in addition to its inhibitory effect which limits the spread of seizure discharge and consequently modulates the intensity of the chemically-induced seizures.

However, the mechanism of the anticonvulsant effect of chlorpheniramine is not clearly understood and cannot be fully accounted for by these observations.

Drugs which are effective against petit mal seizure are usually more successful in protecting mice against seizures induced by leptazol than they are in reducing the intensity of maximal electroshock convulsion. However, drugs which prevent grand mal attack usually exhibit opposite pattern of activity. Histamine could therefore offer protection against petit mal epilepsy but potentiates grand mal as shown in this study.

7.6 Conclusion

The level of histamine in the rat brain has been found to be high at birth then decreases as neural maturation proceeds (Schwartz et al, 1971). If the developmental pattern of this amine also applies to humans, it is reasonable to suggest that histamine in the brain could serve as a protective substance against petit-mal seizures, common in children, when the attack is precipitated. It is possible that children who are prone to petit mal attack may suffer from lower level of histamine in the brain during this developmental stage. Antihistamines should be avoided, if possible, on children with petit mal episode.

CHAPTER EIGHT

8.0 THE ROLE OF HISTAMINE IN REPRODUCTION

8.1 Summary

8.1.1 Intracerebroventricular (icv) administration of histamine (80-100ug) to adult rats was found to induce irregularities and at times persistent oestrous cycle when administered at different stages of the cycle.

8.1.2 Cimetidine (50mg/kg), an $H\bar{2}$ receptor antagonist, administered intraperitoneally (ip) produced a transient change in the cycle which returned to normal rhythm after a day or two.

8.1.3 Triprolidine (50 mg/kg; ip), an $H\bar{1}$ receptor antagonist, showed no changes in the oestrous rhythm while chlorpheniramine (50mg/kg;ip), another $H\bar{1}$ receptor antagonist, produced a non-significant degree of changes in the cycle.

8.1.4 Older rats exhibited more cycling irregularities than the younger ones when they were injected with histamine.

8.1.5 These results suggest that histamine could play a role in reproduction.

8.2 Introduction

Several studies have shown that histamine is a neurotransmitter in the mammalian central nervous system with the highest concentrations in hypothalamus followed by amygdala (Schwartz, 1977; Hill et al, 1978). In the hypothalamus where the highest concentrations of histamine are found,studies in rat and rhesus monkey (Brownstein et al, 1974) have shown that histamine has its intrahypothalamic concentrations in nuclei believed to be directly involved in the regulation of gonadotrophin secretion from anterior pituitary. This suggests that histamine may be involved in the hypothalamo-hypophyseal-gonadal system (Edward et al, 1975, Netti et al, 1982). Histamine could therefore play a role in reproduction. This study was carried out to examine the effect of histamine on the reproductive activity in rats.

8.3 Materials and method

Refer to section 3.12; 3.15; 3.16.

8.4 Results

8.4.1 Effect of histamine on the Oestrous cycle of rats

Rats injected with histamine (80-120ug) (icv) were found to show either irregularities in the oestrous cycle or persistent cycle when injected at

different states of the cycle. Some rats exhibited persistent oestrus stage throughout the period of observation ($P < 0.002$; Fig 8.1; Table 8.1). Histamine also produced more cycling irregularities when administered to rats that were not cycling normally. Older rats exhibited more cycling irregularities than the younger ones when injected with histamine.

8.4.2 Effect of histamine $\overline{H1}$ and $\overline{H2}$ receptor antagonists on the oestrous cycle of rats

Rats injected with cimetidine (50mg/kg) showed transient changes in the cycle ($P<0.05$) which returned to the normal rhythm after a day or two (Fig.8.4; Table 8.4). However, no rat showed changes in the cycle when injected with triprolidine 50mg/kg (Fig.8.3; Table 8.3) but rats injected with 50mg/kg chlorpheniramine showed insignificant changes in the cycle (Fig.8.2; Table 8.2).

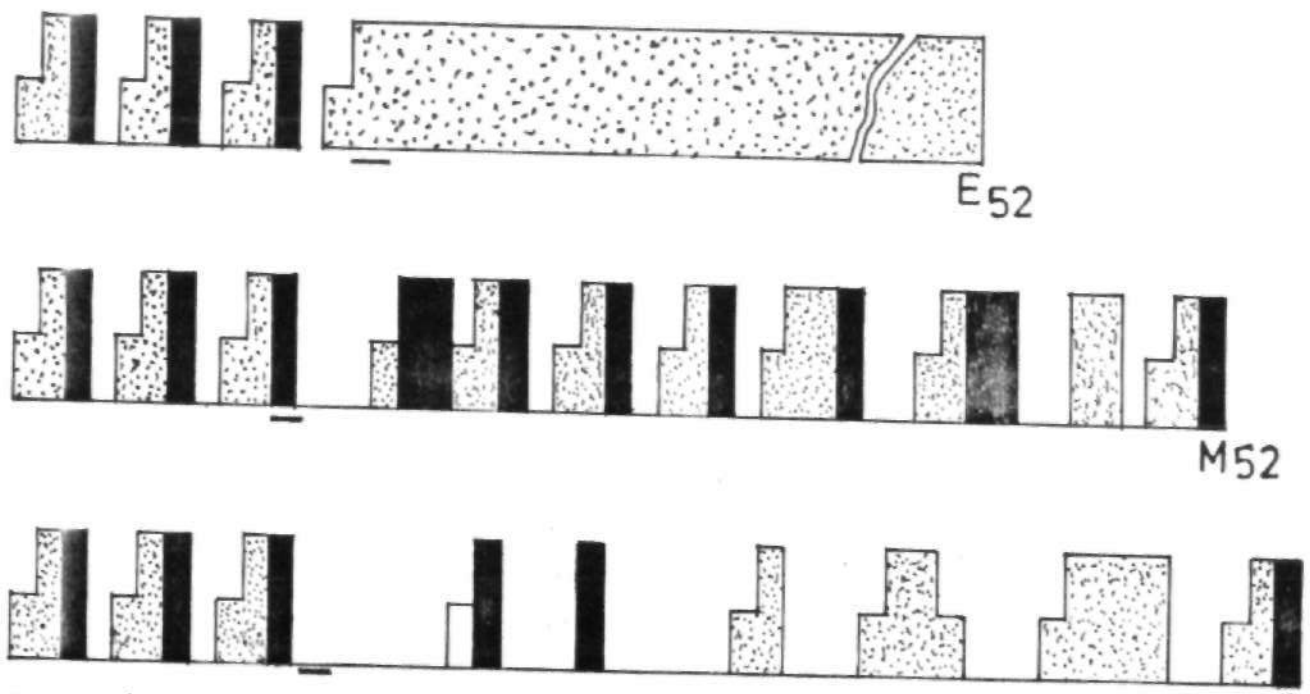


Fig. 8.1

Changes in oestrous cycle before and after intracerebroventricular (icv) administration of histamine (80-120 ug). Proestrus oestrus, metoestrus and dieoestrus are respectively indicated by low column, high column, solid column and Blank A solid bar below each record stands for the period the drug was administered and the number at the end indicates the duration after drug administration.

Table 8.1: Changes in Oestrous cycle before and after intracerebroventricular (icv) administration of histamine.

Dose	No. of rats	Occurrence of Cycles*		
		Before**		After**
		97.2±2.8%	P<0.002	39.58±16.0%
80-120ug	6	6 cycles/24days		13 cycles/52days

* Statistical difference between groups or periods is shown in the middle line

** Mean ± standard error.

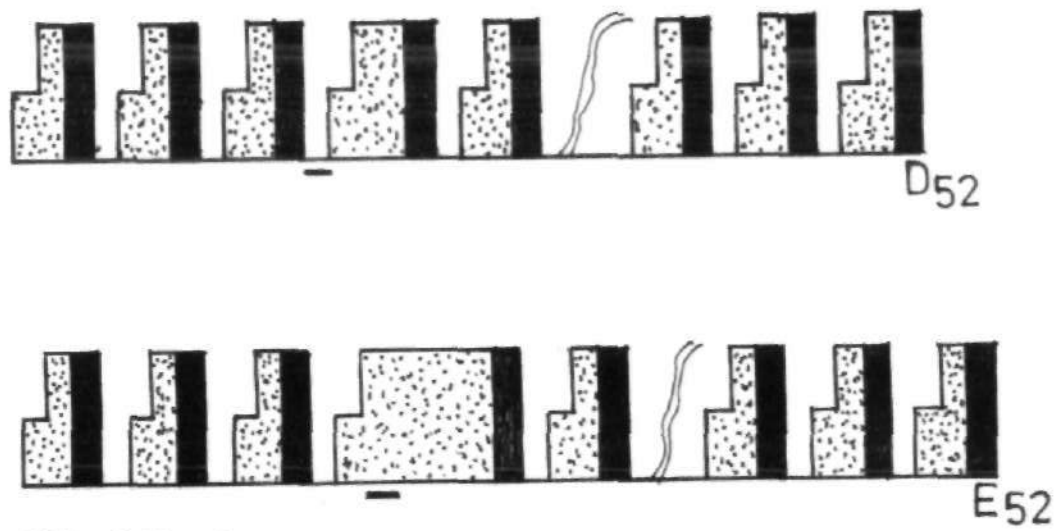


Fig. 8.2

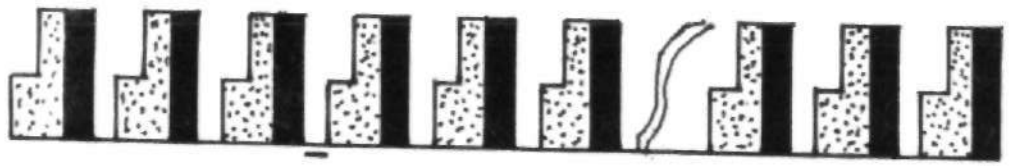
Changes in oestrous cycle before and after intraperitoneal (i.p.) administration of chlorpheniramine (50mg/kg)
Proestrus, oestrus, metoestrus and dioestrus are respectively indicated by low column, high column, solid column and Blank
A solid bar below each record stands for the period the drug was administered and the number at the end indicates the duration after drug administration

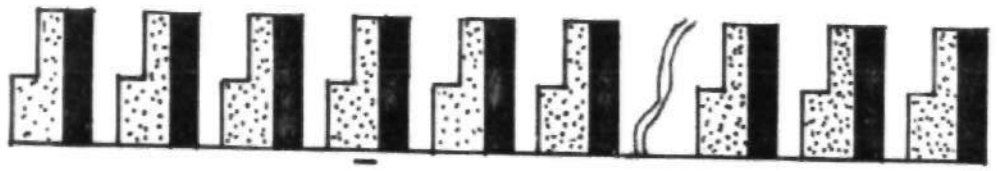
Table 8.2: Changes in Oestrous cycle before and after intraperitoneal (ip) administration of Chlorpheniramine

Dose	No. of rats	Occurrence of Cycles*	
		Before**	After**
		100% N5	87.38±9.4%
50mg/kg	4	6 cycles/24days	13 cycles/52days

* Statistical difference between groups or periods is shown in the middle line

** Mean ± standard error.





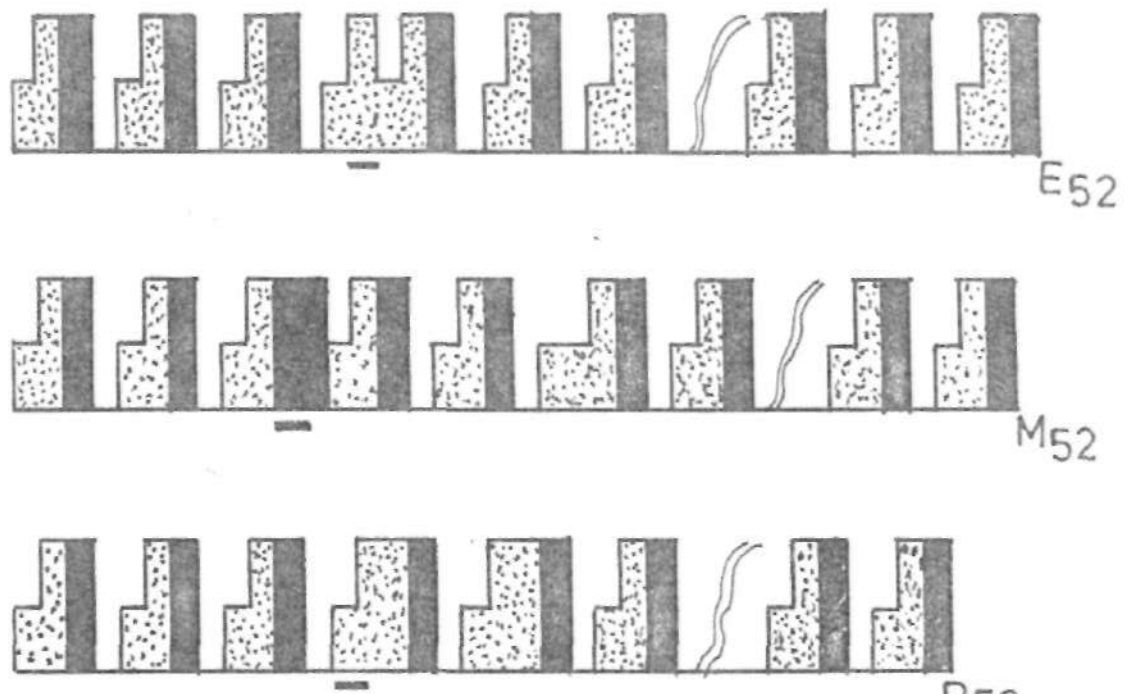
Fig·8·3

Changes in oestrous cycle before and after intraperitoneal (ip) administration of triprolidine (50mg/kg). Proestrus, oestrous, metoestrus and dioestrus are respectively indicated by low column, high column, solid column and Blank - A solid bar below each record stands for the period the drug was administered and the number at the end indicates the duration after drug administration.

Table 8.3: Changes in Oestrous cycle before and after intraperitoneal (ip) administration of triprolidine

Dose	No. of rats	Occurrence of Cycles*	
		Before*	After*
		100%	100%
50mg/kg	4	6 cycles/24days	13 cycles/52days

* Mean



Fig·8·4

Changes in oestrous cycle before and after intraperitoneal (i p) administration of cimetidine (50 mg / kg). Proestrus oestrus, metoeotrus and dioestrus are respectively indicated by low column, high column, solid column and Blank. A solid bar below each record stands for the period the drug was administered and the number at the end indicates the duration after drug administration.

Table 8.4: Changes in Oestrous cycle before and after intraperitoneal (ip) administration of Cimetidine.

Dose	No. of rats	Occurrence of Cycles*		
		Before**		After**
		100%	P<0.05	84.38±5.98%
50mg/kg	4	6 cycles/24days		13 cycles/52days

* Statistical difference between groups or periods is shown in the middle line

** Mean ± standard error.

8.5 Discussion

Results obtained from this study showed that histamine induced irregularities and at times persistent oestrus cycle when administered at different stages of the cycle. The critical event in the oestrous cycle of the rat and similar animals is ovulation. Development of the follicle and the production of oestrogen are promoted by luteinizing hormone (LH) and follicle stimulating hormone (FSH). When the blood oestrogen is high, FSH release is decreased and LH release is enhanced. The mechanism by which LH surge occurs resulting in ovulation is now known to be regulated by hypothalamic neuron system which themselves are controlled by endogenous circadian rhythm - generating mechanism within the suprachiasmatic hypothalamic nucleus (Fitzgerald et al, 1976). It has also been recognized that lesions in the suprachiasmatic region in the rat resulted in constant oestrous activity (Moore et al, 1976; Flerko, 1966). That is, the female rat exhibits continuous vaginal oestrous for prolonged periods without evidence of oestrous cycle. These animals are sterile and will not mate or become pregnant.

In the hypothalamus where the highest concentration of histamine is found, large amounts are present in median eminence and lesser quantities in the premamillary, suprachiasmatic and arcuate nuclei

(Brownstein et al, 1974). From the results of this study, it seems likely that higher levels of histamine may be liable to cause adverse changes or damage to the clock mechanism (suprachiasmatic nucleus) controlling the gonadotrophin secretion and thus affect the reproductive functions. It is also likely that histamine might contribute to the aging process of the hypothalamic-pituitary-ovarian system, more or less inducing irregularities in the oestrous cycle.

Cimetidine, an $H\bar{2}$ receptor antagonist, produced transient changes in the cycle which returned to normal rhythm after a day or two. The transient disturbance in the oestrous cycle during cimetidine administration confirms the observation that both cimetidine and metiamide increase prolactin levels in non-suckling mothers to levels observed during suckling (Arakelian and Libertun, 1977).

The changes in the oestrous cycle during chlorpheniramine administration is consistent with the observation that some $H\bar{1}$ receptor antihistamines have been known to produce changes in the cervical mucus in the human (Billings and Westmore, 1980). However, the mechanism whereby some antihistamines produce these changes while others like triprolidine

(Fig. 8.3) do not, is not fully understood. This needs further investigation and it will be premature to suggest an explanation based on the present results.

CHAPTER NINE

9.0 EFFECT OF SEX HORMONES ON BODY WEIGHT AND THEIR INFLUENCE ON HISTAMINE - INDUCED DRINKING AND EATING BEHAVIOURS IN RATS

9.1 Summary

9.1.1 Regulation of body weight by sex hormones has been established in this study.

9.1.2 Sex hormones may regulate body weight through their influence on eating and drinking behaviours of the animal.

9.1.3 Histamine - induced drinking behaviour was significally enhanced by sex hormones.

9.1.4 The influence of sex hormones on histamine - induced eating behaviour was found to be variable. In this case, the eating behaviour was enhanced by both testosterone and progesterone but depressed by oestradiol 17B. None of these effects showed any significant value.

9.1.5 The difference of sex hormones on histamine induced eating and drinking behaviours may be due to the functional difference of histaminergic pathways through ventromedial hypothalamus (VMH) and paraventricular nucleus (PVN) involved in eating and drinking behaviours respectively.

9.1.6 These results suggest an interaction of sex hormones on histaminergic pathways in the brain.

9.2 Introduction

Behavioural and electrophysiological findings suggest that neuronal histamine in the hypothalamus _modulate feeding and drinking behaviours through H1 receptors (Fukagawa, K. et al, 1989). The ventromedial hypothalamus (VMH) and paraventricular nucleus (PVN) are known to be involved in this control (Palacios et al 1981) and histaminergic pathways through these regions seem to be functionally different. For example,those thorough the histaminergic pathways in the VMH are involved in feeding modulation while those in the PVN mediate drinking behaviours. (Sakata,T.et al 1988). Studies from other workers (Portaleone et al 1980; Dornoso, 1976; Libertun, C, and McCann, S.M. 1976) strongly suggested a mutual correlation between histamine level and hormones in the CNS. In the CNS and periphery, sex hormones particularly oestogens have been shown to interact with a variety of biological substrates. They have been shown to possess both long term and short term effects in the brain (Thomas and Ani, 1982). This work examined the effect of sex hormones on body weight and their influence on histamine -induced drinking and eating behaviours.

9.3 Materials and Method

Refer to Section 3.9.2

9.4 Results

Oestradiol 17B was found to cause a significant decrease in body weight ($p<0.002$) while progesterone and testosterone produced insignificant increase (fig. 9.1). Progesterone and testosterone showed insignificant increase in both water and food intake whereas oestradiol 17B showed a significant decrease ($p<0.02$) (Fig. 9.2 & 9.3). The three hormones produced a significant increase in histamine induced drinking behaviour (oestradiol $p < 0.05$; testosterone and progesterone $p<0.002$) (Fig. 9.4). The influence of sex hormones on histamine induced eating behaviour was found to be variable. In this case, the eating behaviour was enhanced by both testosterone and progesterone but depressed by oestradiol 17B. None of these values were significant (Fig 9.5).

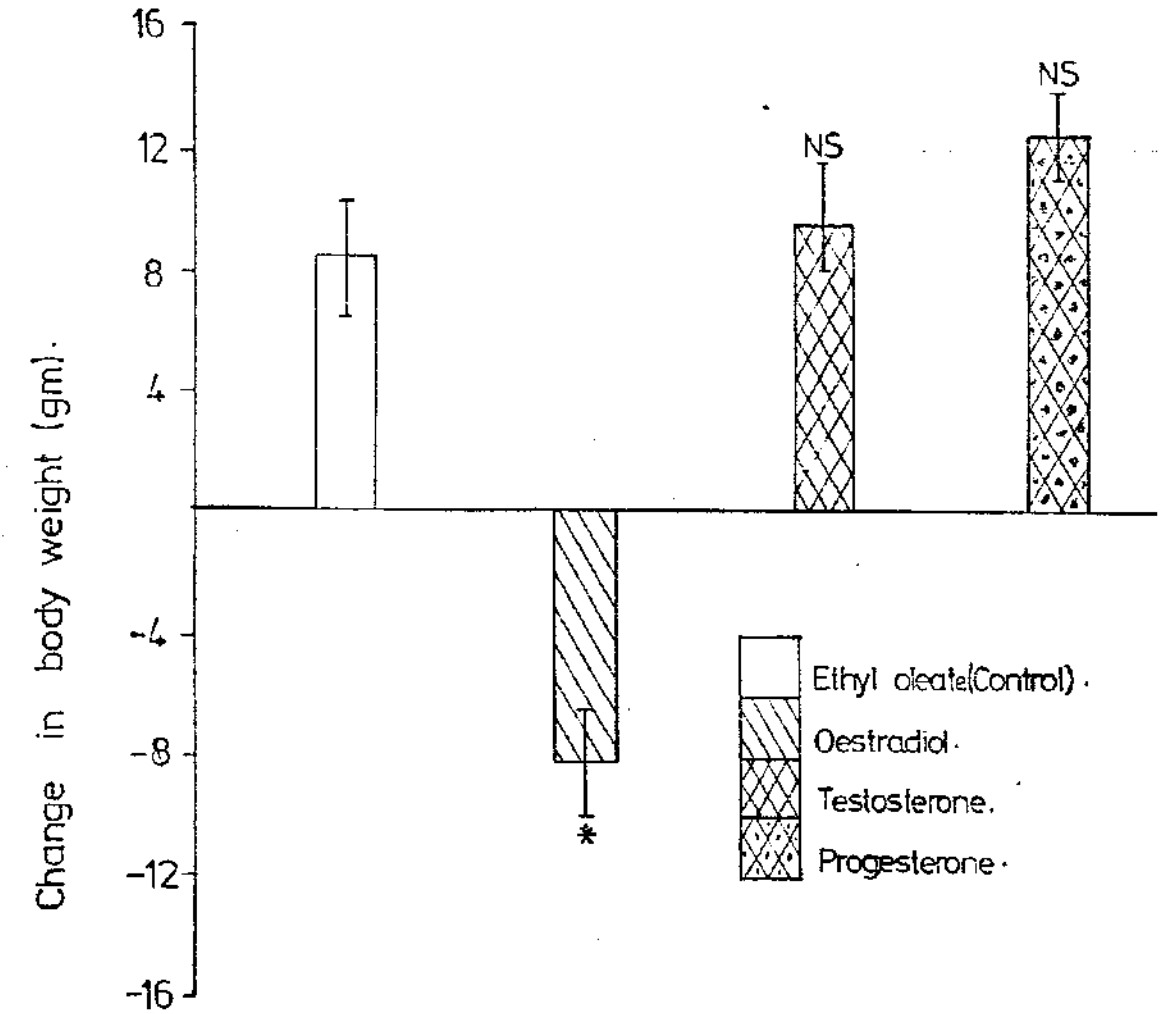


Fig 9.1 Effect of sex hermones on body weight. Rats were treated with the hormones for 6 days while the control recieved ethyl-oleate SC. *P<0.002. Compared to saline control: student's "t" test. Vertical bars represent standard error of the mean ± SEM. NS = Non-significant.

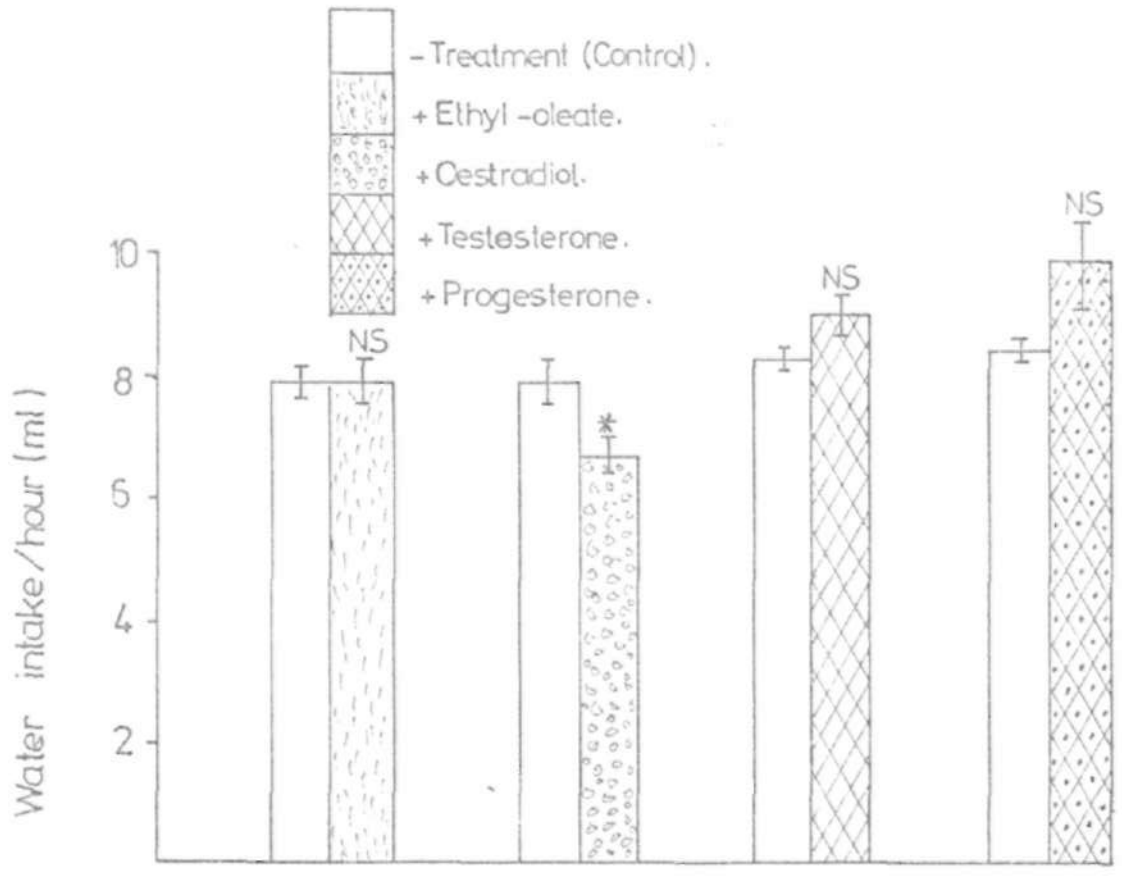


Fig.9.2 Effect of sex hormones on water intake.

*P<0.02. Compared to saline control: student's "t" test. Vertical bars represent standard error of the mean ± SEM. NS = Non-significant.

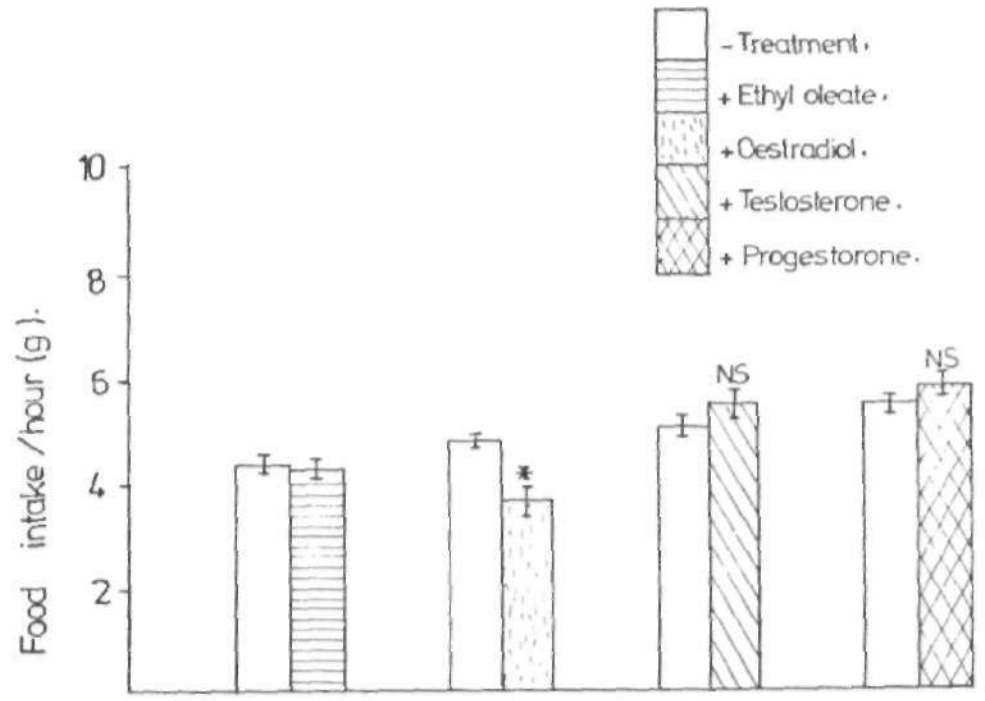


Fig.9·3 Effect of sex harmones on food intake.

*P<0·02. Compared to saline control: student's "t" test. Vertical bars represent standard error of the mean ± SEM. NS = Non-significant.

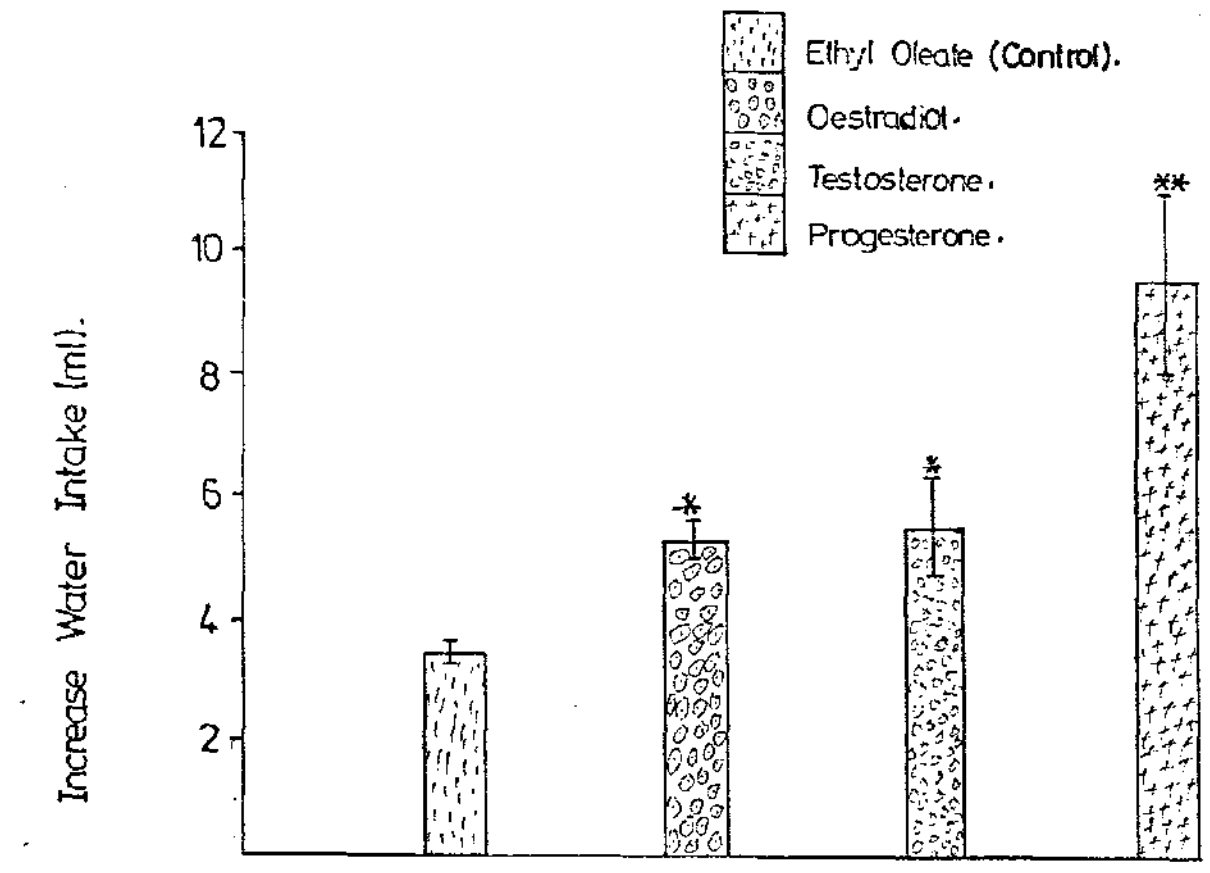


Fig. 9-4 Effect of sex hormones on histamine (80µg) induced drinking behaviour. Histamine was administered intracerebroventricullarly (icv) after the rats had been primed with sex hormones for 7days.

*P<0.05; **P< 0.002. Compared to saline control: student's "t" test. Vertical bars represent standard error of the mean ± SEM.

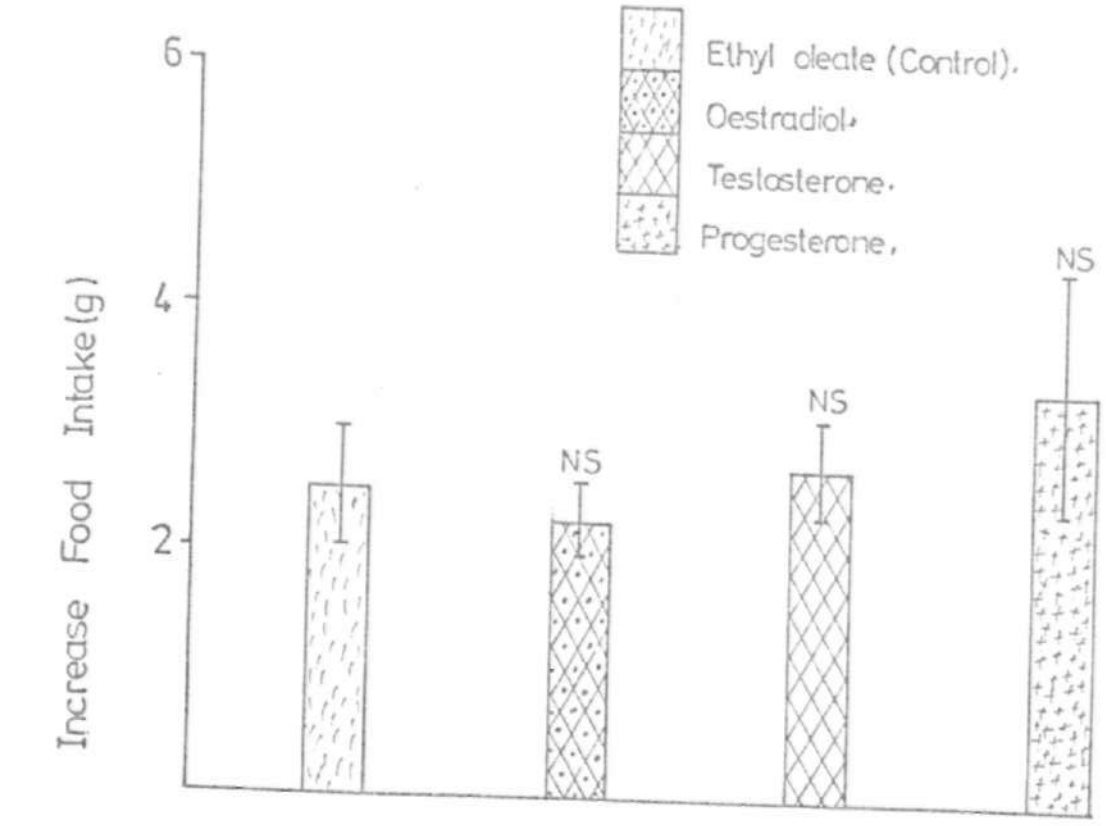


Fig.9.5 Effect of sex hormones on histamine (80µg) induced eating behaviour. Histamine was administered intracerebroventricularly(icv) after the rat had been primed with sex hermones for 7days.
Compared to saline control: student's "t" test. Vertical bars represent standard error of the mean ± SEM. NS = Non-significant.

9.5 Discussion

Results of this study show a striking difference of the effect of sex hormones in regulating body weight. In this study, oestradiol 17B was found to induce a significant decrease in body weight while both progesterone and testosterone produced an increase which was not significant. Suppression of body weight by oestradiol 17B has been noted by Bowman and his Coworkers (1983) who in addition observed a similar change with tamoxifen. Apart from producing androgenic effect of its own, testosterone is metabolized into oestradiol 17B and a more potent androgen, 5 alpha dihydrotestosterone (DHT) which has been shown to stimulate growth. This conversion occurs in both the CNS and the periphery. It is interesting to note that the amount of oestradiol resulting from this conversion did not influence the change in body weight produced by testosterone. It is likely that the effect of oestradiol which might have resulted from the metabolism of testosterone seem to be counteracted by a second metabolite, 5 alpha DHT which has been known to stimulate growth in many tissues or testosterone itself.

In order to examine the mechanism by which sex hormones affected body weight, the effect of the hormones was studied on both water and food intake. In this study, oestradiol 17B produced a significant

decrease (P<0.002) in both water and food intake whereas testosterone and progesterone induced insignificant decrease. These results indicate a possibility that both testosterone and progesterone may stimulate eating and drinking behaviours in animals to increase their body weight whereas the suppresant influence of oestradiol on these two factors results in decrease in weight. Thus, the mechanism by which sex hormones may regulate body weight could largely be attributable to their influence on eating and drinking behavours. However, the mechanism by which oestradiol and possibly testosterone and progesterone affected food and water intake is not fully understood as these activities are unrelated to the high affinity oestrogen receptors (Bowman et al, 1983).

While histamine - induced drinking behaviour was significantly enhanced by the action of sex hormones, the influence of sex hormones on histamine eating behaviour was found to be variable. The eating behaviour was enhanced by both testosterone and progesterone but depressed by oestradiol 17B. None of these effects showed any significant value. It is pertinent to note that the effect of oestradiol 17B on eating and drinking behaviours including histamine eating behaviour was depressant but enhanced histamine-induced drinking behaviour. The difference

of sex hormones on histamine-induced drinking and eating behaviours could be due to the functional difference of histaminergic pathways through PVN and VHM involved in drinking and eating behaviours respectively (Sakata et al 1988).

It is concluded from this study that sex hormones may regulate body weight through their influence on drinking and eating behaviours of the animal but their influence on histamine induced eating and drinking behaviours may be due to the functional difference of histaminergic pathways through VMH and PVM involved in these behaviours.

CHAPTER TEN

10.0 THE INFLUENCE OF SEX HORMONES ON THE AFFINITY OF HISTAMINE H1-RECEPTORS IN RAT BRAIN.

10.1 Summary

10.1.1 The influence of sex hormones on the high affinity of (^{3}H) mepyramine for histamine H1 receptors was investigated in this study.

10.1.2 Sex hormones particularly oestrogens affected the affinity of histamine H_1 receptors labelled with mephyramine.

10.1.3 The affinity of (3H) mepyramine for histamine H_1 receptors increased as the binding capacity decreased depending on the level of sex hormones.

10.1.4 Affinity of (^{3}H) mepyramine for H_1 receptors was found to be higher in proestrus than in males or metoestrus females.

10.1.5 Ovariectomy decreased the affinity of the receptors to the labelled mepyramine in all the brain areas examined especially in the oestrogen concentrated regions (hypothalamus and amygdala).

10.1.6 Oestrogen replacement in ovariectomized females showed binding affinity reminiscent of proestrus.

10.1.7 There was no significant difference in binding between the males and the metoestrus females but there was a statistically significant difference in males and proestrus females.

10.2 Introduction

Sex hormones have been shown to have both short term and long term effects on the binding concentration of high affinity binding sites for some monoamines and biogenic amines in the brain. Several studies have demonstrated changes in B-adrenergic, muscarinic cholinergic, serotonergic and dopaminergic receptors following oestrogen treatment or ovariectomy (Hruska et al, 1980; Wilkinson et al, 1979; Al-Dahan and Thomas, 1982; Rainbow et al, 1980; Vicas et al, 1982). Though sex hormones have been reported to affect a number of substances in the brain and in the periphery, so far little is known about the effect of these hormones on histaminergic pathways. This study was therefore designed to examine the effect of sex hormones on the affinity of histamine H_1 recepors in rat brain.

10.3 Materials and method

Refer to section 3.17

10.4 Results

10.4.1 Conditions necessary for optimal affinity to Histamine H_1-receptors

The binding of a ligand to its binding sites

should be reversible and consequently exhibit equilibrium characteristics. Figures 10.1 and 10.2 show the time course for (^{3}H) mepyramine binding to membrane preparations from adult male rats. Both the total and specific (3H) mepyramine binding increased up to 5 minutes (after which there appeared to be no significant increase) reaching a plateau in 10 minutes. Because of these results, a 15 minute incubation time was selected as suitable for subsequent experiments.

The interaction of hormones with recepors depends to a large extent on a number of factors including PH. Fig 10.3 shows the influence of PH on high affinity (3H) mepyramine binding to $H1^{-}$ recepors. There was a gradual increase from 6.5 to 7.0 after which there was a sharp rise with a peak value between 7.2 and 7.4. After this maximum, there was followed a slow progressive decline and at 8.00 the lowest level was attained. Fig 10.4 shows the effect of tissue concentration on (3H) mepyramine binding to neural membrane preparations. Binding was linear within tissue concentrations in the range of 1.25mg to 10mg wet weight tissue per incubation. A protein concentration of 5 mg wet weight (within the linear range) was chosen for all measurements.

Ligand binding generally consists of two components; low affinity (non-specific) binding which

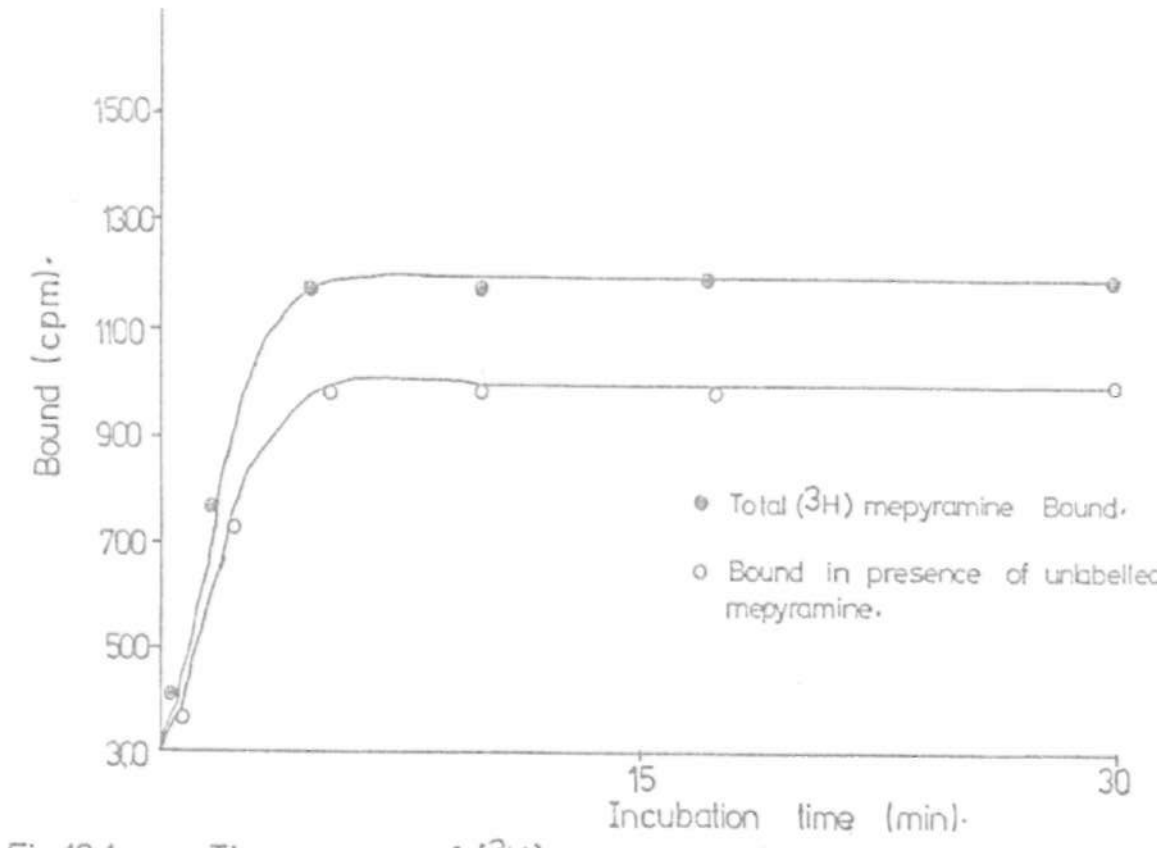


Fig.10.1 Time course of [^{3}H] mepyramine binding to rat brain tissues.
The points represent mean from three experiments.

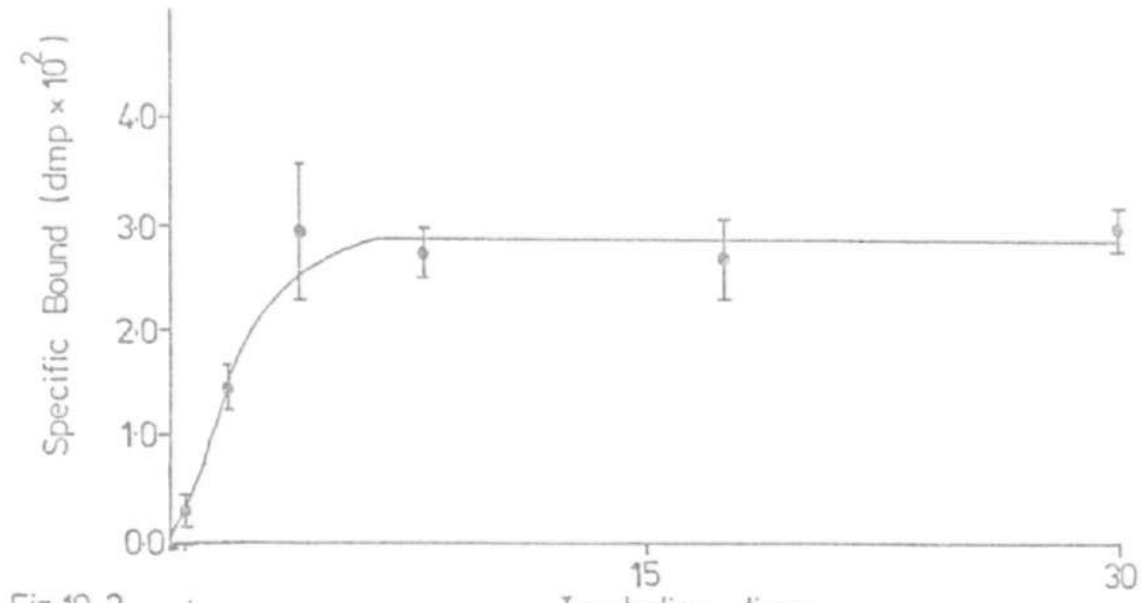


Fig. 10.2

Time course of specific [^{3}H] mepyramine Binding to rat brain tissues
The points represent the mean from three seperate experiments each carried out in triplicate. The bars depict the range of results.

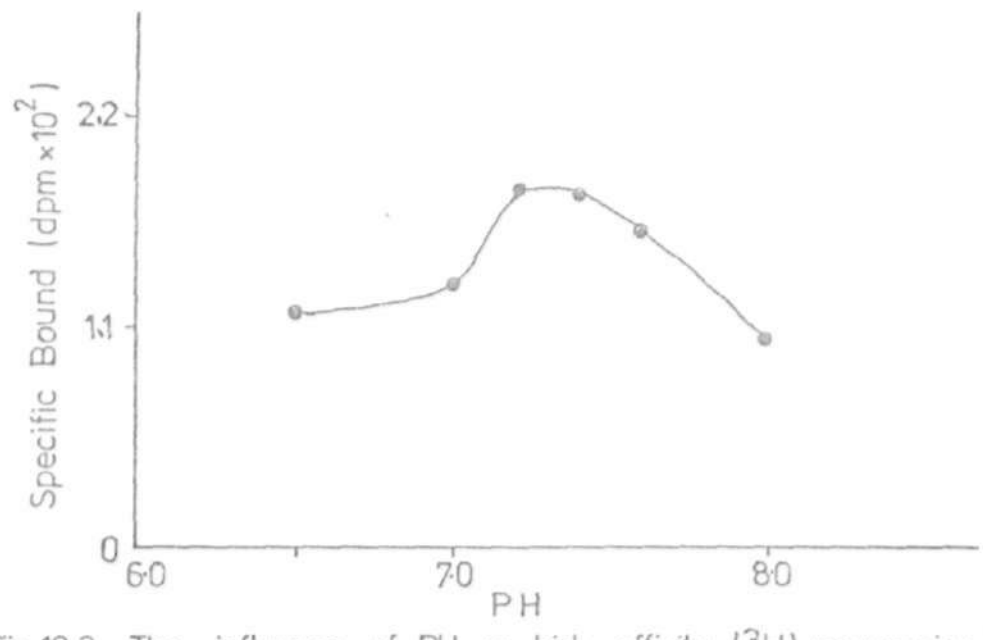


Fig. 10·3 The influence of PH on high affinity (^{3}H) mepyramine Binding. The points represent the mean from three experiments

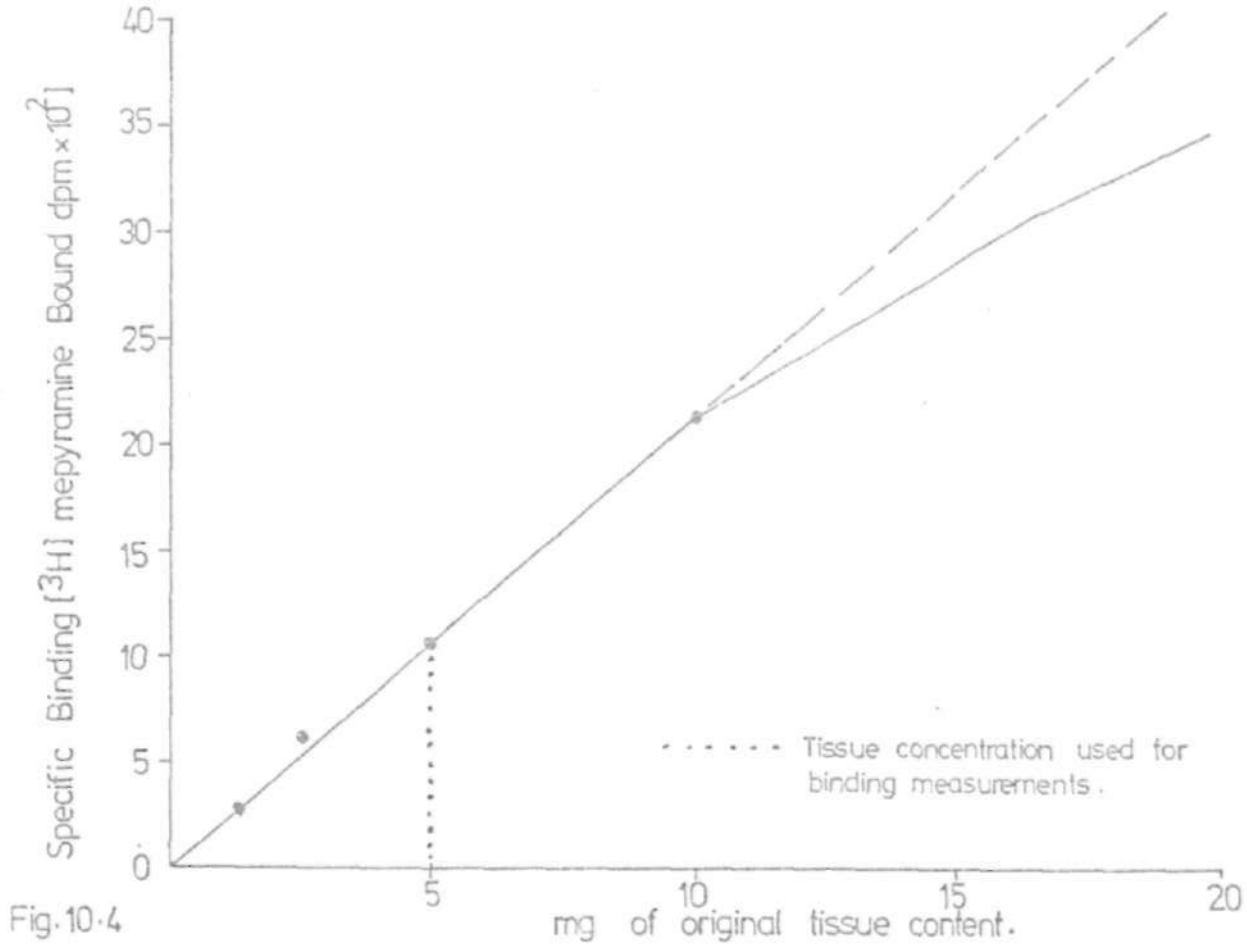


Fig. 10·4

The influence of protein concentration on [3H] mepyramine binding sites in rat brain. The points represent mean from three experiments.

under normal assay condition is non-saturable and exhibits linear characteristics; and high affinity binding (specific) which shows saturable characteristics.

Low affinity (non-specific) binding was measured by incubating 2nM (3H) mepyramine concentration with 5mg wet weight tissue in the presence of increasing concentration of unlabelled mepyramine (fig. 10.5). The total (3H) mepyramine bound to neural membrane preparations decreased with increase of the competing drug thus giving two distinct components. The first component, high affinity (specific) binding became saturable at concentrations between 5×10^{-7}M – 7x10-6 M mepyramine. On the basis of this result, 5x10-6 M mepyramine was selected to control for non-specific (low affinity) binding. The second component probably representing another binding site for (3H) mepyramine is apparently unsaturable even at 10-3M mepyramine which may represent non-specific site.

Low affinity binding may be due to ligand interaction with cell constituents or experimental apparatus or it may be the result of supernatant contamination of the membrane pellet after centrifugation of the incubate. High affinity (3H) mepyramine binding was routinely estimated by deducting low affinity from the total binding occuring in an incubation (fig 10.6)

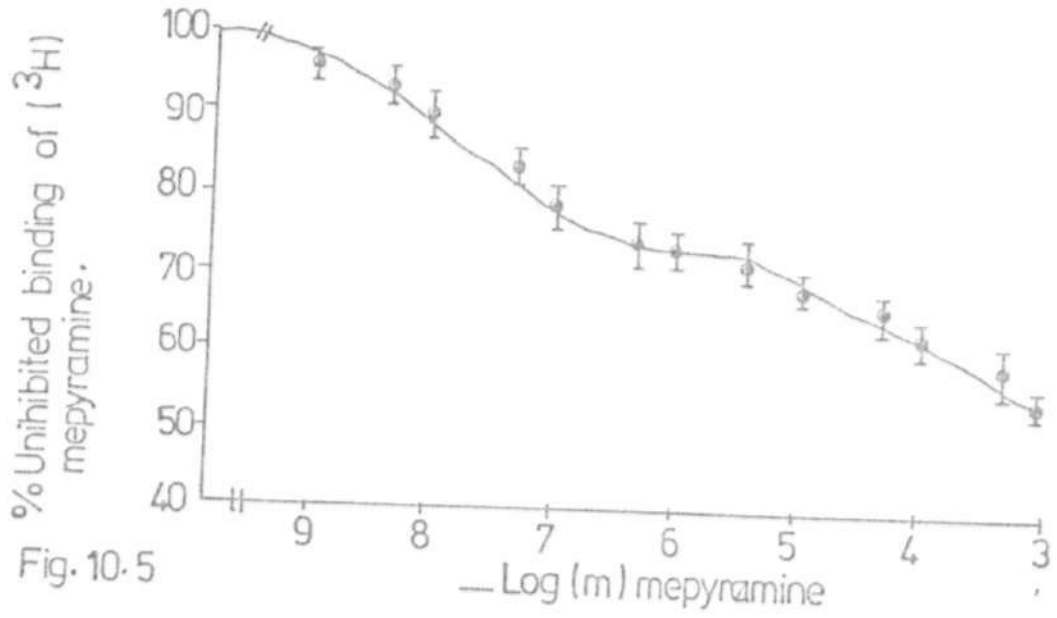


Fig. 10.5

Inhibition of the binding of 2nM (^{3}H) mepyramine.

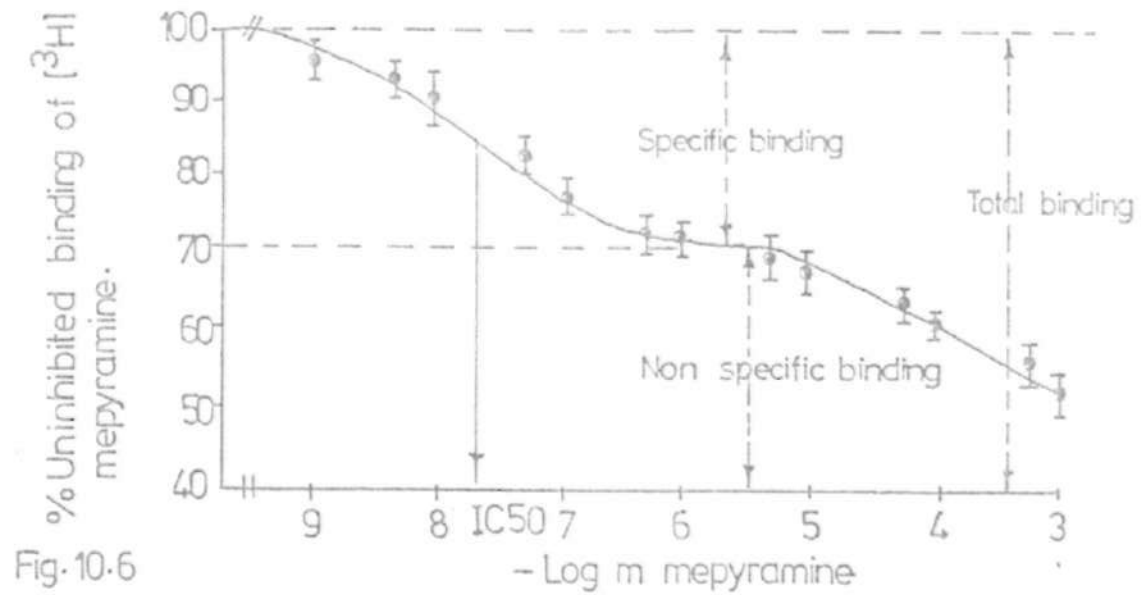


Fig. 10.6 Estimation of specific and non-specific ^{3}H mepyramine binding.

10.4.2 The influence of sex hormones on the high affinity of (^{3}H) mepyramine for Histamine H1-receptors.

The result in fig. 10.7 shows the effect of sex on (3H) mepyramine binding in the rat brain. There was no significant difference in binding between the males and the metoestrus females. In this case, binding was lower in hypothalamus ($P<0.001$), amygdala ($P<0.002$) and cortex ($P<0.001$) during proestrus compared to the males or metoestrus. The regional distribution of (3H) mepyramine binding to $H\bar{1}$ receptors showed a pattern which is similar to that of endogenous histamine itself. Thus, in metoestrus as well as proestrus, higher binding sites were found in hypothalamus and amygdala but least in the cortex. A similar pattern was observed in males. These findings are similar to those reported by other workers (Chang et al, 1978, Chang et al, 1979).

In all the regions examined there was an increase in the affinity of (3H) mepyramine for histamine $H\bar{1}$ receptors in female proestrus compared to the male and metoestrus females (Table 10.1). This high affinity was mimicked by hormone administration of ovariectomized females (Table 10.2). Binding affinity was slightly higher in males than the ovariectomized females especially in hypothalamus and amygdala (Table 10.2). In the male hypothalamus/preoptic area and amygdala testosterone

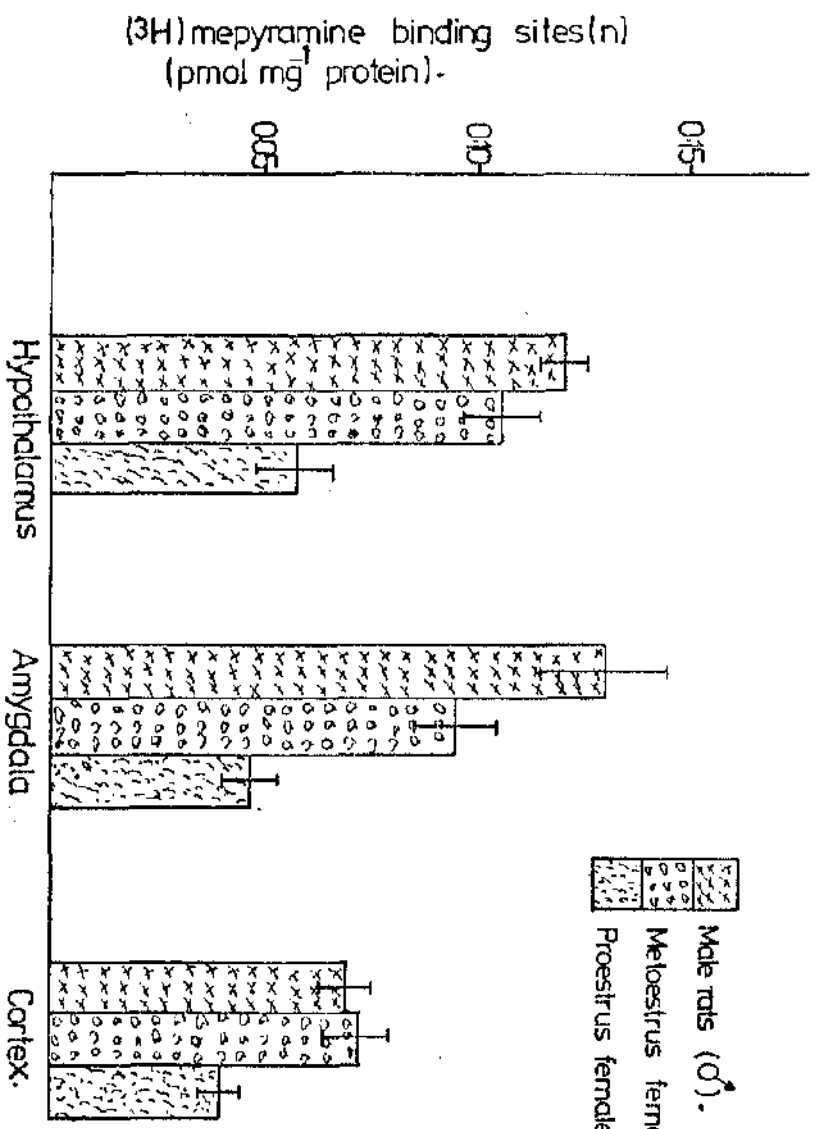


Fig. 10.7 The effect of sex on [3H] mepyramine binding and the distribution of sites.

Table 10.1: The Effect of sex and Oestrous cycle on the affinity of (^{3}H) mepyramine for histamine H1- receptors

Status	Brain Regions	Kd(nM)
Metoestrus females	Hypothalamus	4.3±0.5
	Amygdala	4.3±0.25
	Cortex	4.1±0.76
Proestrus females	Hypothalamus	2.6±0.4
	Amygdala	2.98±1.09
	Cortex	2.5±0.4
Males	Hypothalamus	4.2±1.3
	Amygdala	4.0±1.0
	Cortex	3.1±1.18

Table 10.2: The Effect of sex,Ovariectomy and Oestrogen replacement on the affinity of (^{3}H) mepyramine for histamine Hi- receptors

Status	Brain Regions	Kd(nM)
Male	Hypothalamus	4.2±1.3
	Amygdala	4.0±1.0
	Cortex	3.1±1.18
Ovariectomy	Hypothalamus	5.4±1.0
	Amygdala	5.0 ±0.08
	Cortex	3.6±0.46
Ovariectomy + Oestrogen	Hypothalamus	2.3±0.2
	Amygdala	2.75±0.75
	Cortex	2.7±0.6

can still be aromatized into oestradiol 17B (Reddy et al, 1974) and thus increase the binding affinity for these recepors. However, the proportion of oestradiol 17B resulting from this conversion is comparatively low. This explains why affinity was slightly higher in males than the ovariectomized females where all sources of sex hormones have been removed.

10.5 Discussion

The work described here shows that sex hormones, particularly oestrogens, affect the affinity of histamine H_1 receptors labelled with mepyramine. Changes in binding capacity could be the result of changes in receptor concentration and /or affinity. In this work, the affinity of (3H) mepyramine for histamine H_1 receptors was found to increase as the binding capacity decreased depending on the level of sex hormones. The affinity of (3H) mepyramine for H_1-receptors was found to be higher in proestrus than in males and metoestrus females. In rats, evidence show that oestrogen levels are higher in proestrus than in metoestrus (Thomas and Ani, 1982, McDonald and Doughly, 1974; Butcher et al 1974). The fact that the affinity was higher in proestrus when oestrogen levels are higher but lower in males and metoestrus females suggest that oestrogens increase the affinity of (3H) mepyramine for H_1-receptors in the brain with decrease in binding capacity.

Changes in the affinity of $H\bar{1}$-receptors for (3H) mepyramine binding in the brain throughout the oestrous cycle may not be due to sex hormones. However, in order to establish that sex hormones are really involved,the first step was to ovariectomize the animals (remove their main source of oestrogens and progesterone) and to observe what happens. Ovariectomy decreased the affinity of the receptors to the labelled mepyramine in the oestrogen concentrated brain regions (Pfaff and Keiner, 1973, ie hypothalamus and amygdala) compared to the males. This is consistent but does not prove the hypothesis that oestrogens increase the affinity of $H\bar{1}$ receptors to mepyramine binding. In order to define the effect of oestrogen more precisely in the brain, oestrogen was injected into ovariectomized females. Oestrogen replacement in ovariectomized females showed binding affinity reminiscent of proestrus. Thus, a decrease in affinity during ovariectomy compared to proestrus and even metoestrus and males, coupled with the fact that affinity in proestrus was mimicked by oestrogen replacement of gonadodectomized females lend strong support for the increase in affinity of oestrogen on binding to $H\bar{1}$ receptors. This finding may explain a physiological process in which the binding affinity is regulated during the oestrous cycle. This observation is similar to that of Biegon and McEwen

(1982) who reported a reduction in serotonin binding in both the hypothalamus and non-hypothalamic brain regions with no increase in affinity following oestrogen administration. This was however followed by an increase in receptor level at 72 hours after oestrogen injection with no significant changes in the affinity. In this work, although binding affinities were recorded after 1 hr., 48 hr. and 144 hr of oestrogen treatment, there was no biphasic effect as with serotonin.

The affinity of $H\bar{1}$ receptors for (3H) mepyramine binding in gonadodectomized females was less than that of the males, metoestrus and proestrus females. This observation suggests that not only did oestrogens influenced the affinity of $H\bar{1}$ receptors in this study but also testosterone as well as progesterone. Both testosterone and progesterone including oestrogen have been shown to counteract hypothermia induced by histamine and its agonists on both $H\bar{1}$ and $H\bar{2}$ receptors in young chicks. This observation was attributable to a down-regulation of histamine receptors by sex hormones (Anuka et al, 1991). This finding thus supported our observation in this study that sex hormones can influence the affinity of histamine on $H\bar{1}$ and $H\bar{2}$ receptors in the brain.

CHAPTER ELEVEN

GENERAL DISCUSSION

Histamine induced mixed behavioural and electrocortical changes in young chicks. The behavioural effects of histamine seem to depend largely on the species. In this study, histamine exhibited a triphasic response in chicks consisting of an initial behavioural sleep which was followed by a longer behavioural stimulation culminating in a very long behavioural depression accompanied with sleep before the chicks finally recovered. In rats, only a biphasic response could be recorded consisting of initial period of hypoactivity followed by a prolonged period of hyperactivity (Kaliva, et al 1982).

The components of histamine mediating the mixed behavioural effects were revealed by the use of selective agonists and antagonists on $H\bar{1}$ and $H\bar{2}$ receptors. Thus, two types of receptors are implicated in this behavioural effects of histamine. The effects on $H\bar{1}$ receptors were found to be stimulatory while on $H\bar{2}$ receptors they were inhibitory. These receptors thus provide a regulatory role for the endogenous histamine to maintain a balance in the brain during the normal physiological processes. The sedative properties of most $H\bar{1}$ receptor antagonists is therefore attributed to a

blockade of the excitatory components (H_1 receptors) leaving the inhibitory components (H_2 receptors) unaffected for endogenous histamine to act. This agrees with the findings of the other workers (Carruther et al, 1975; Heinrich, 1953). However, some H_1 receptor antagonist do not conform to this behavioural pattern. The present data show that chlorpheniramine exhibited a biphasic response of depression/activation in young chicks but only depression in rats and arousal in adult fowls. The dual role of this drug was not due to the release of a neurotransmitter or acting as a partial agonist but has been attributed to species difference and age variation.

In the central action of histamine, the inhibitory components (H_2 receptors) seem first to be stimulated prior to the excitatory components, hence the initial behavioural sleep in chicks or hypoactivity in rats (Kaliva, P.W; 1982) prior to stimulation.

The stimulatory effect of cimetidine suggests a blockade of the inhibitory components, H_2 receptors, leaving the endogenous histamine to act on the excitatory components, H_1 receptors.

Although systemic administration of histamine has been shown to induce changes in body temperature

in several animal species,so far the exact mechanism of action in bringing about these changes in temperature has not been fully elucidated. Various views have been expressed about the mechanism of action whereby histamine produces hypothermia in these animal species. Brezenoff and Lomax (1970) have suggested that the hypothermia induced by histamine was due to endogenous norepinerphrine released from site within the hypothalamus. Green et al (1975) suggested that two receptors, $H\bar{1}$ and $H\bar{2}$ are involved in the hypothermic effect of histamine. While he suggested that activation of $H\bar{1}$ receptors lowers body temperature, he concluded that activation of $H\bar{2}$ receptors caused a rise in body temperature in cat but a fall in rat. Certainly,$H\bar{2}$ receptors can not behave differently in different species. Failure to establish the exact mechanism whereby histamine produces hypothermia is clearly due to lack of selective agonists on histamine receptors. Using selective agonists and their antagonists in this work it was possible to establish the exact mechanism of action in which histamine produces hypothermia. It is here shown that thermoregulatory effect of histamine in young chicks is due to its effects on both $H\bar{1}$ and $H\bar{2}$ receptors and activation of these receptors leads to hypothermia. A blockade of $H\bar{2}$ receptors by their

appropriate antagonists produced a significant increase in temperature while a blockade of H_1 receptors leads to insignificant fall in temperature. However, the insignificant fall in body temperature produced by H_1 receptor antagonists is a complicating factor in interpreting their antagonistic tendency. This observation is in agreement with that of Shaw (1971). It is worth noting that, although H_1 receptor antagonists e.g chlorpheniramine, produced insignificant degree of hypothermia on their own, they can reverse hypothermia induced by 2-thiazolylethylamine (H_1 receptor agonist) while hypothermia induced by dimaprit (H_2 receptor agonist) was counteracted by H_2 receptor antagonists e.g. cimetidine. Neither H_1 nor H_2 receptor antagonists could effectively alone counteract histamine induced hypothermia but a concurrent administration of H_1 and H_2 receptor antagonists. This finding reinforces the observation that the hypothermic effect of histamine is due to its action on both H_1 and H_2 receptors through heat loss.

Steroid sex hormones can interact with a variety of biological substrates in the CNS and periphery. They can also produce both long term and short term effects upon the brain (Thomas and Ani, 1982). The involvement of sex hormones in thermoregulatory

effect of histamine has been established in this work. Results here show that hypothermia induced by histamine, 2 - thiazolyl-ethylamine and dimaprit was counteracted by steroidal sex hormones (Oestradiol 17B, testosterone and progesterone). These results thus provide evidence that sex hormones can exert a remarkable influence on thermoregulatory effect of histamine in the CNS and further lend support for a strong mutual correlation between histamine and hormones in the CNS (Portaleone et al, 1978). The fact that sex hormones counteracted 2-thiazolyl-ethylamine ($H\bar{1}$ receptor agonst) and dimaprit ($H\bar{2}$ receptor agonist) is an indication that, to some extent, they influence both $H\bar{1}$ and $H\bar{2}$ receptors in the brain. However, while histamine antagonists can produce this effect by blocking the effect of histamine on the appropriate receptors, sex hormones do so by reducing the binding sites (Bmax) available for histamine or its agonist to act upon (genomic mechanism), an effect which requires days for the effect to be noticed. On the other hand, antioestrogens (clomiphene and tamoxifen) were found to potentiate histamine, 2-thiazolyl-ethylamine and dimaprit induced hypothermia, a phenomenon attributed to an upward regulation of histamine receptors after treatment for a certain length of time.

Sex hormones may also influence histamine receptors by affecting the affinity (kd) of these receptors. The affinity of [3H] mepyramine for H_1 receptors was found to be increased as the binding capacity was decreased depending on the level of sex hormones. The effinity of (3H) mepyramine for these receptors was found to be higher in proestrus than in males or metaestrus, while ovariectomy decreased the affinity of these receptors. Oestrogen replacement in ovariectomized females showed binding affinity reminiscent of proestrus. There was no significant difference in binding between males and the metoestrus but there was a stastistically significant difference in males and proestrus. The observation that sex hormones are involved in the regulation of histamine receptors in the CNS may have important implication in understanding female reproductive physiology.

Data presented here show that sex hormones can influence body weight through eating and drinking behaviours, activities which are unrelated to the high affinity oestrogen receptors (Bowman et al, 1980). However, their effect on histamine eating and drinking behaviours was found to be variable. This difference of sex hormones on histamine induced eating and drinking behaviours was attributed to the functional difference of histaminergic pathways

through VMH and PVN involved in these behaviours.

This work also shows that histamine together with its agonists on $H\bar{1}$ and $H\bar{2}$ receptors offered protection against leptazol induced seizure but potentiated electroshock seizure. Histamine could therefore offer protection against petit mal epilepsy but potentiates grand mal as has been shown in this work.

The role of histamine in seizure regulation is not clearly understood but both $H\bar{1}$ and $H\bar{2}$ receptors seem to play a major role. Histamine has been found to be a potent stimulant of C-AMP synthesis in the CNS and produce its action by interacting with $H\bar{1}$ and $H\bar{2}$ receptors (Dismukes et al 1976); Hegstrand et al, 1976; Schwartz 1977). A number of workers have shown that C-AMP could play a major role in seizure regulation. (Gross and Ferrendelli, 1979 & 1983). It is therefore suggested here that the anticovulsant effect of histamine against leptazol-induced seizure may be due to its effect on $H\bar{2}$ and $H\bar{1}$ receptors via cyclic AMP accummulation in addition to its inhibitory effect which limits the spread of seizure discharge and consequently modulate the intensity of chemically - induced seizures.

Since the level of histamine has been found to be high at birth then decreases as neural maturation proceeds (Schwartz et al, 1971), it is possible that

histamine in the brain could serve as a protective substance against petit-mal, common in children, when the attack is precipitated. It is also possible that children who are prone to petit-mal attack may suffer from lower levels of histamine in the brain during this developmental stage.

In the hypothalamus where the highest concentration of histamine is present, large amounts are present in median eminence and lesser quantities in the premamillary, suprachiasmatic and arcuate nuclei (Brownstein et al, 1974). The critical event in reproductive activity of the female is ovulation which is controlled by gonadotrophin release. The gonadotrophin secretion is controlled by suprachiasmatic nuclei (Fitzgeral et al, 1976). Lesions of the suprachiasmatic nucleus has been shown to result in constant oestrous (Moore et al, 1976; Ferko, 1966) with the female exhibiting continuous vaginal oestrus for prolonged periods without evidence of oestrous cycle. Such females are sterile and will not mate or become pregnant. The present data indicate that histamine induced irregularities and persistent oestrus cycle when administered at different stages of the cycle with older animals exhibiting more cycling irregularities than the younger ones. These observations suggest that higher levels of histamine may be liable to

cause some changes or damage to the clock mechanism (suprachiasmatic nucleus) controlling the gonadotropin secretion and thus affect reproductive functions. It is also likely that histamine might contribute to the aging process of the hypothalamic - pituitary-ovarian system more or less inducing irregularities in the oestrous cycle.

H_2 receptor antagonists e.g cimetidine can produce transient disturbance of the oestrous cycle through increase prolactin levels (Arakelian and Libertun, 1977). The effect of H_1 receptor antagonists on the oestrous cycle of the rat was found to be variable. While some antihistamines like chlorpheniramine can produce changes in the oestrous cycle and thus affect cervical mucus, others like triprolidine have no effect on the oestrous cycle. The mechanism whereby some antihistamines on H_1 receptors affected oestrous rhythm while others do not is not fully understood. This phenomenon needs further investigation and it will be premature to suggest an explanation based on our data.

It is concluded that some antihistamines should be taken with caution during pregnancy as they are capable of affecting reproductive functions as we have seen from this work. A known case of abortion was reported in ABU Sick Bay - Main Campus after a woman had taken some tablets of Promethazine theoclate

(Avomine^{R}) to stop vomiting common with some women during the first trimester of pregnancy.

SUGGESTION FOR FURTHER WORK

Data presented in this work show that sex hormones can alter histamine receptors and affinity of (3H) mepyramine to histamine receptors in the central nervous system. In order to understand the physiological role of sex hormone on histaminergic pathways, it is here suggested that further work be set up to examine the levels of histamine in the brain during the different stages of the oestrous cycle, during ovarictomy and when sex hormones are exogenously administered. This aspect of the work can be done using HPLC.

Previous findings show that the level of histamine in the rat brain is high at birth but decreases as neural maturation proceeds (Schwartz et al, 1971). Suggestion is made base on our results, that children who are prone to petit mal attack may suffer from lower levels of histamine in the brain during this developmental stage. This suggestion can be confirmed by examining the levels of histamine in children suffering from petit mal attack and compare with that of normal children. The levels of histamine in normal children should also be compared with that of adult.

The mechanism whereby some antihistamines e.g chlorpheniramine produce changes in the oestrous cycle while others like triprolidine do not is not fully understood. This needs further investigation.

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APPENDIX 1

Preparation of Buffer (KREBS HENSELEIT)

Assay buffer was composed of:

	G/L	Molarity
NaCl	6.92	118.00
Kcl	0.354	4.75
$CaCl_2$	0.374	2.55
$MgSo_4$	0.294	1.20
KH_2Po_4	0.162	1.14
$NaHCO_3$	2.10	25.00
Glocose	2.00	11.00

APPENDIX II

STATISTICS

1. CASIO fx - 180P scientific calculator was used for the statistical calculations

2. Formula for Student's t test for two normal populations

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{1/n_1 + 1/n_2} \times \sqrt{\frac{(S_1)^2 n_1 - 1 + (S_2)^2 n_2 - 1}{n_1 + n_2 - 2}}}$$

t = Probability

$\bar{X}$ = Mean of each population

S = Standard deviation

n = Number of animals in each population

$n_1 + n_2 - 2$ = Degree of freedom

3. Formular for Standard Error Mean (SEM)

$$SEM = \frac{S}{\sqrt{n}}$$

APPENDIX III

APPROXIMATE COMPOSITION OF LOCALLY FORMULATED FEED

Nutrient %	Ladokun-Pfizer	ABU-Pharm Feed. (Locally Formulated feed)
Moisture	8.2±1.31	6.2±1.04
Ash	7.7±0.49	4.9±0.05
Lipids	8.3±2.70	12.4±3.92
Total Carbohydrate*	56.0	58.6
Energy(Kcal/100g)	407.0	553.0

Data are mean ±SD

* Total carbohydrate was determined by difference and these values include Crude fibre.