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BRAIN NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE METABOLISM DURING ALTERED STATES OF THYROID FUNCTION

A Thesis

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CONTENTS

LIST OF ABBREVIATIONS

ATP; adenosine triphosphate

COMT; catechol-o-methyl transferase

CSF; cerebrospinal fluid

DMI; desmethylimipramine

DOPA; 3-(3,4-dihydroxyphenyl)-L-alanine

DOPAD; dihydroxyphenylalanine decarboxylase

$DMPH_4$; 6,7-dimethyl-5,6,7,8-tetrahydropteridine

DβH; dopamine β-hydroxylase

5-HIAA; 5-hydroxyindoleacetic acid

5-HT; 5-hydroxytryptamine

5-HTPD; 5-hydroxytryptophan decarboxylase

MAO; monoamine oxidase

NSD-1034; N-methyl-N-3-hydroxybenzyl hydrazine

NE; norepinephrine

RNA; ribonucleic acid

TPH; tryptophan hydroxylase

TP; tryptophan

TR; tyrosine

T_3; L-triiodothyronine

TH; tyrosine hydroxylase

BH_4; tetrahydrobiopterin

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"The thyroid gland, of which no use whatever has hitherto been hinted by physiologists, is intended in part to serve as a diverticulum in order to avert from the brain a part of the blood which ..... might disorder or destroy the functions of that important organ."

Caleb Parry (1) (1825)

1. INTRODUCTION

The past twenty years have witnessed an information explosion in the area of developmental neurobiology encompassing experiments designed to pinpoint those important variables whose manipulation early in life results in long-lasting, perhaps permanent, effects upon brain function and ultimately behaviour. The scope of the interdisciplinary nature of these research endeavours has emerged from an emphasis upon structure and function of the brain in various species including man, through interactions between genetic and external factors, to an emphasis upon the biochemical basis for genetic expression of control mechanism(s) of neurotransmitter metabolism and function(s).

An impressive body of evidence has emerged which emphasizes the participation of various hormones in the process of myelination and organization of cellular architecture of the central nervous system as well as in the establishment of behavioural patterns. It is generally agreed that administration of cortisol results in a biphasic effect on the nervous system. Treatment of neonatal rats with adrenal steroids during the first 7 days of life has been shown to decrease brain excitability (2). Studies also indicate that cortisol decreases protein synthesis of key enzymes during the critical period of brain development. Conversely, administration of this adrenal hormone, after the sensitive period of brain development is over, enhances the maturation of the nervous system and increases brain excitability (3). That gonadal hormones are indispensable to brain maturation also has been well established by numerous experimental and clinical studies. Timiras (4) demonstrated that estrogens regulate the development of the central nervous system by their so-called "organizing action." Estradiol administration produced age-dependent alterations in amino acids and electrolyte contents

in developing brain (4). Additionally, testosterone has been shown to manipulate the electrophysiological activity of the brain (5).

It is known that thyroid hormone plays a critical role in the regulation of metabolism and in processes underlying maturation and differentiation of nervous tissue. Thyroid deprivation in early life results in marked interference of the normal ontogenesis of lipid (6,7), carbohydrate (8), nucleic acid (9,10) and protein metabolism (11) as well as in altered activity of a variety of enzymes of intermediary metabolism (12,13) in maturing nervous tissue. Balazs and Richter (14) have recently shown that the normal biochemical changes which occur during the transition from immature to the adult metabolic pattern in brain tissue, are markedly impaired in neonatally thyroidectomized animals. Coincident with biochemical changes, a marked hypoplasia of axonal and dendritic components of cortical neuropil also has been reported. Analysis of the changes in cortical structure which normally occur with advancing age suggests that the developmental abnormalities seen in hypothyroidism represent a distortion rather than a retardation of growth which may, in turn, account for 80% of the reduction in the probability of axodendritic interaction between adjacent neurons (15). Furthermore, altered thyroidal activity has been linked with severe and frequently irreversible behavioural perversions in both animals and man. Rats exposed to exogenous thyroid hormone during vulnerable periods of post-natal life show accelerated growth, precocious biochemical maturation and neurophysiological development (16). Conversely, animals deprived of thyroid hormone by administration of radio-iodine or antithyroid drugs in neonatal life appear listless, irresponsive to external stimuli, show lack of interest to environmental events and do not learn from experience. Similar changes in the demeanour of their human counterparts which suffer from disorders of this endocrine

gland have been reported. Excessive thyroid secretion has been demonstrated to produce hyperexcitability, irritability, restlessness, exaggerated responses to environmental stimuli and emotional instability, which if untreated, may ultimately lead to full blown psychosis. The symptoms of myxedema, on the other hand, make an insidious appearance of the patient and are generally characterized by apathy, listlessness, lack of energy, slowness of speech, lethargy, social withdrawal, reduced sensory capacity and somnolence (17). It is intriguing that several of these symptoms are not only characteristics of cretinism, but are also observed during "endogenous" depression (18), an affective disorder for which the "monoamine theory" envisaging changes in norepinephrine and 5-hydroxytryptamine has been proposed by Kety (19). Prange et al. (20) demonstrated that the efficacy of imipramine, a tricyclic antidepressant, in the treatment of clinically depressed euthyroid patient was enhanced when the drug was administered along with small doses of L-triiodothyronine and suggested that L-triiodothyronine may potentiate the sensitivity of norepinephrine receptors. Evidence also indicates that thyroidectomy in adult life results in a decreased sensitivity of the adrenoceptors in cardiac tissue (21) as well as in the central nervous system (22). This hyposensitivity may in turn, lead to an increased synthesis of the neurotransmitters. Despite the fact that extensive investigations have been carried out to elucidate the role of thyroid hormone in the morphological, biochemical and physiological maturation of the central nervous system, any unequivocal evidence relating thyroid dysfunction to mental illness and affective disorders is still lacking.

The present dissertation is concerned with elucidation of those alterations in neuronal substrates which are brought about by early thyroid malfunctions and may be relevant to behavioural and mental changes seen during

the so-called "cretinoid" syndrome as well as hyperthyroidism. In particular, the effects of neonatal hypothyroidism, induced either by radio-iodine or an anti-thyroidal agent, methimazole, were investigated on brain tyrosine hydroxylase, tryptophan hydroxylase as well as on the concentrations of norepinephrine, 5-hydroxytryptamine and a major indoleamine metabolite, 5-hydroxyindoleacetic acid. Since relationship seems to exist between the degree of mental retardation seen during hypothyroidism and the age of onset of this endocrine disorder (17), the influence of delayed radiothyroidectomy on norepinephrine and 5-hydroxytryptamine metabolism also was examined. In order to determine whether the changes observed in these putative neurotransmitters were specific to thyroid hormone, we investigated the effect of replacement therapy with L-triiodothyronine on the metabolism of these neurohormones in thyroid-deficient rats. The effects of desmethylimipramine on the metabolism of norepinephrine and 5-hydroxytryptamine were studied to elucidate whether the alterations (if any) in receptor sensitivity may modify the neurochemical effects of this imipramine congener during thyroid hormone deficiency. Additionally, in order to examine the influence of thyroid gland hyperactivity on brain amine metabolism, the effects of neonatal and adult hyperthyroidism were investigated on norepinephrine and 5-hydroxytryptamine metabolism. Excess of thyroid hormone in neonatal life produces neurochemical and behavioural alterations which are not only characteristics of hyperthyroidism, but are also commonly observed during mania in man (17). It was therefore deemed of interest to examine the effect of lithium on the metabolism of these central amines in hyperthyroid rats. Recently, lithium has been increasingly used in the treatment of mania and psychosis as well as an effective prophylactic agent against recurrent manic depressive disorders (23). This metal is also known to produce

hypothyroidism in man and laboratory animals as a side effect by interfering with thyroxin production in a manner which still needs clarification (24). Results of the present study demonstrate that thyroid hormone plays a vital role in the regulation of norepinephrine and 5-hydroxytryptamine metabolism in developing brain and raise the possibility that aberrant behaviour seen during "cretinoid" syndrome and hyperthyroidism may be related to abnormal metabolism of these putative neurohumors. Furthermore, a critical period seems to exist in early neonatal life during which thyroid hormone must be present in order to permit the normal ontogeny of the metabolism of norepinephrine and 5-hydroxytryptamine.

2. LITERATURE REVIEW

I. ROLE OF CATECHOLAMINES AND 5-HYDROXYTRYPTAMINE IN BRAIN FUNCTION AND BEHAVIOUR

The discovery of chemical neurotransmission in the brain is now legendary. The demonstration that several different transmitters exist in the brain that are localized in different anatomical systems has encouraged the neuropsychologists to investigate the functional significance of these neurotransmitter systems in the control of brain function and behaviour. A wealth of methodology, ranging from the well-tried lesion and stimulation techniques of neuropsychology to the extensive pharmacological knowledge of the interaction between drugs and neurotransmitters is now available to study the implications of catecholamines and indoleamines in a variety of behavioural responses.

A. Behavioural Arousal

It has been demonstrated that intraventricular administration of 6-hydroxydopamine, a chemical analogue of catecholamines which destroys the noradrenergic and dopaminergic neurons, blocks the stereotyped behaviour induced by amphetamine (25). Similar findings have been observed in rats pretreated with α-methyl-p-tyrosine, an agent known to prevent the synthesis of both dopamine and norepinephrine by inhibiting tyrosine hydroxylase, the rate-limiting enzyme in the pathway from tyrosine to DOPA (26). Rech et al. (27) demonstrated that oral or parenteral administration of α-methyl-p-tyrosine in non-toxic doses impaired the avoidance responding rotarod performance and spontaneous locomotor activity. Thus, it is conceivable that the depletion of brain norepinephrine and dopamine might be expected to impair the unconditioned behaviour and skeletal muscle tone and co-ordination, as well as conditioned responses. Pharmacological studies with reserpine which

is known to deplete the neuronal stores of catecholamines as well as 5-hydroxytryptamine, have demonstrated that this alkaloid produces severe depression of mood (28) and such drug-induced depression has been considered to be a possible pharmacological model of the naturally occurring disorder. Depression has also been reported in patients treated with tetrabenazine, a drug which is similar to reserpine in many of its pharmacological properties including the depletion of catecholamines (29). In animals, reserpine induces sedation which is associated with decreased levels of brain norepinephrine, dopamine and serotonin (30). Interference in norepinephrine synthesis without any change in 5-hydroxytryptamine by α-methyl tyrosine produces sedation and results in significant impairment of motor activity in animals. It has also been demonstrated that locomotor activity is enhanced following the administration of sympathomimetic drugs which are capable of accelerating the catecholaminergic activity (31). Conversely, when norepinephrine and dopamine stores are depleted following the use of enzyme inhibitors or storage blockers, behavioural arousal is suppressed (32). These findings have led to the suggestion that catecholamines might represent the neurochemical substrates of behavioural arousal (33).

Brodie et al. (34) suggested that 5-hydroxytryptamine is a putative neurotransmitter which manifests its central effect via trophotrophic system whose stimulation brings about behavioural suppression and hypo-activity. However, it was later demonstrated that under a certain set of conditions, increased concentrations of brain 5-hydroxytryptamine invoke excitation (35, 36). Conversely, low levels of 5-hydroxytryptamine lead to drowsiness and hypoactivity. Hole (37) demonstrated that administration of p-chlorophenylalanine in developing rats suppressed behavioural arousal as manifested by decreased exploratory activity, fast habituation to new environment and

low reactivity. A reduction in paradoxical sleep occurred at 10 and 14 days of age without any alteration in the total sleeping time (38). In fact, 5-hydroxytryptamine has been implicated as an important neurochemical correlate in certain types of depressive illnesses and low levels of its principal metabolite, 5-hydroxyindoleacetic acid have been noted in patients suffering from endogenous depression (39,40).

B. Regulation of Body Temperature

Feldberg and Myers (41,42) demonstrated that intraventricular injection of 5-hydroxytryptamine to unanaesthetized cats raised body temperature. In contrast, adrenaline and noradrenaline administration lowered the temperature either from control or after it had been elevated by 5-hydroxytryptamine or pyrogens. Although these experimental findings and their interpretation as to the involvement of 5-hydroxytryptamine in hyperthermia or in opposing hypothermia could be attractive, one has to be aware that the amounts of 5-hydroxytryptamine injected intraventricularly were extremely high i.e. usually in the hundreds of micrograms, an order of magnitude which is many times higher than the concentration found in the ventricles and required to elicit other physiological effects. In contrast to Feldberg's observations, Kulkarni (43) and Cooper et al. (44) noted that intraventricular injection of this indolealkylamine in doses from 30 to 500 µg, induced a fall in body temperature in the cat and rabbit. Bligh (45) noted a temperature drop in sheep treated with 5-hydroxytryptamine. In view of all these reports, it seems doubtful whether, at present, a physiological involvement of 5-hydroxytryptamine in the regulation of body temperature proposed by Feldberg should be contemplated.

C. Modulation of Pain

5-Hydroxytryptamine also has been implicated as an important modulator

of pain. Harvey and Yunger (46) demonstrated that lesions destructive to the ascending serotonergic system produce an increased pain sensitivity to electric foot shock which correlates well with the associated diminution of 5-hydroxytryptamine. Furthermore, the repletion of lowered 5-hydroxytryptamine by 5-hydroxytryptophan reverses the hyperalgesia. p-Chlorophenylalanine, which is known to inhibit the biosynthesis of brain 5-hydroxytryptamine, also increases pain sensitivity in normal rats (47). However, p-chlorophenylalanine administered to rats with lesions in the medial forebrain bundle does not produce a significant additive effect. This non-additive effect of the lesion and drug on pain thresholds indicate that both p-chlorophenylalanine and lesions in the medial forebrain bundle manifest their actions through the same mechanism i.e. by lowering the telencephalic content of 5-hydroxy-tryptamine (48). In addition, evidence indicates that 5-hydroxytryptamine depletion by p-chlorophenylalanine may be involved with auditory, visual, gustatory, olfactory and especially social stimuli. Depending on the experimental protocol, this heightened reactivity may be manifested behaviourally as enhanced avoidance, escape of approach behaviour, disrupted habituation, heightened motor activity, aberrant hypersexuality, aggressiveness, increased exploration, reduced seizure threshold or antagonism of drugs that reduce responsiveness to sensory inputs.

D. Sexual Behaviour

In recent years, considerable reports have appeared dealing with the neurochemical basis of male sexual behaviour. Two important findings contributed to stimulate these studies: the aphrodisiac effects of p-chloro-phenylalanine in male animals and of 3-(3,4-dihydroxyphenyl)-L-alanine in a number of parkinsonian patients. In rats, administration of p-chloro-phenylalanine, an irreversible inhibitor of tryptophan hydroxylase, induced

hypersexuality which was maximal at the time when brain 5-hydroxytryptamine levels were greatly depleted (49). Recently, Da Prada and his co-workers (50) studied sexual behaviour in male rats treated with 5-6 dihydroxytryptamine and demonstrated that this cytotoxic agent enhanced the copulatory behaviour which was associated with the deficiency of 5-hydroxytryptamine levels in brain tissue. Additional evidence may be derived from experiments in which the effects of p-chlorophenylalanine induced homo- and hetero-sexual behaviour were suppressed in rats, rabbits and cats by 5-hydroxytryptophan, the direct precursor of 5-hydroxytryptamine (49). Hyppa et al. (51) noted that p-chlorophenylalanine administration in young female rats delayed the time of vaginal opening even though the vaginal cytological cycles appeared to be normal. The females showed less ear wiggling activity during both spontaneous and hormone-induced copulatory behaviour and a tendency to shortening of mount latencies during hormone-induced masculine sexual behaviour. Evidence also indicates that brain dopamine plays an important role in the stimulation of sexual behaviour in male animals. Da Prada et al. (52) demonstrated that repeated administration of 3-(3,4-dihydroxyphenyl)-L-alanine to male rats pretreated with RO 4-4602 increased their homosexual mounting behaviour. Since 3-(3,4-dihydroxyphenyl)-L-alanine injection to rats treated with the peripherally acting decarboxylase inhibitors not only increases brain dopamine but also decreases 5-hydroxytryptamine, the stimulant effect on male copulatory behaviour may be ascribed to either mechanism. Since apomorphine, which is considered to act as a direct stimulant of dopamine receptors in brain also stimulates the copulatory behaviour in male rats, it is conceivable that brain dopamine also plays a stimulatory role in male sexual behaviour (53).

E. Sleep

5-Hydroxytryptamine has also been implicated in the neural control of sleep, particularly slow wave sleep (54) and a variety of waking behaviours. For example, depletion of brain 5-hydroxytryptamine by inhibition of synthesis with p-chlorophenylalanine has been shown to suppress sleep, enhance perception of pain (47) and augment social grooming and sexual behaviour (55).

II. NOREPINEPHRINE AS A CENTRAL SYNAPTIC NEUROTRANSMITTER

There is a large body of evidence to suggest that norepinephrine is an important amine for the normal brain functioning. It is generally agreed that the following criteria must be met for the identification of a neurotransmitter: (a) localization of the substance along with its synthesizing and catabolizing outfits within the nerve endings; (b) release of the substance in response to a nerve impulse; (c) production by the compound, in concentrations consistent with endogenous release of an effect which is identical to the response produced on the effector cell by presynaptic neuronal stimulation and finally, (d) the action of the applied neurohumor, as is true for effects of nerve stimulation, should be rapidly terminated. The development of histochemical techniques for direct visualization of the fluorescent derivatives of the transmitter (56) provided the first definitive evidence that in the central nervous system, amines are contained solely within the neurons. Evidence that nerve stimulation elicits release of norepinephrine was readily obtained in the peripheral nervous system using perfused organs with intact nerve supply. Demonstration that nerve stimulation causes release of amines by neurons in the brain has been more difficult to obtain. Attempts to use push-pull cannulae (57), ventricular perfusion (58) or cups affixed to the brain surface (59) have met with varying degrees of success. These techniques have provided evidence that

stimulation of central neurons is associated with increased quantities of neurotransmitters or their metabolites in the perfusion fluid; but in some cases, substance such as urea or inulin can also be "released", presumably as a consequence of alterations in tissue fluid dynamics (60). Likewise, it has been difficult to demonstrate in brain that the response to transmitter release during nerve stimulation is identical to that seen after direct application of the putative transmitter. Iontophoretic techniques similar to those reported by Connor (61) are among the more successful approaches to satisfying the third criterion for a neurotransmitter.

III. DEVELOPMENT OF THE CENTRAL CATECHOLAMINERGIC NEURONS

The developing nervous system provides a scenario in which the complex relationships among neurons take form. This process involves cell multiplication, cell migration, outgrowth of neuronal processes and synaptogenesis. Thus, it can be said that the development of the brain is a victorial process characterized by changes in time and space as well as magnitude (62). Research on the development of mammalian brain has been oriented primarily toward structural analysis and the correlation between morphological and biochemical differentiation has remained an elusive goal.

A. Morphogenesis of Central Neurons

Autoradiographic studies of the incorporation of ^{3}H-thymidine have proved to be an accurate method for dating the time of origin of neurons since the radio-labeled thymidine is only incorporated into the DNA of dividing cells (63). By combining the histofluorescent technique with ^{3}H-thymidine autoradiography, it has been possible to demonstrate when the central catecholaminergic neurons undergo cell division. The noradrenergic neurons in the locus coeruleus and the dopaminergic neurons in the substantia nigra exhibit a brief period of cell division between 12 and 14 days of

gestation and do not divide subsequently (64,65). Furthermore, it also has been demonstrated that neurons do not undergo differentiation until after they have ceased dividing (62). Catecholamine-induced fluorescence can be observed in cells that form the noradrenergic nucleus, the locus coeruleus, at 14 days of gestation; over the next 4 days, the neurons migrate caudally to their ultimate position in the pons (66). Fluorescence of the cell bodies of the dopaminergic neurons of the mid brain is apparent at 18 days of gestation, whereas the dopaminergic cell bodies in the hypothalamus first exhibit catecholamine fluorescence early in the postnatal period (67, 68). Thus, there is an apparent caudal-to-rostral sequence of appearance of catecholamine-induced fluorescence in the cell bodies that give rise to the various aminergic neuronal pathways of the brain.

Loizou (69) examined the post-natal proliferation of the catecholaminergic neurons in the rat brain. At birth, there is a striking scarcity of noradrenergic terminals, whereas scattered islands of dopaminergic terminals are already present in the caudate nucleus. Thus, in newborn rats, catecholamine fluorescence is limited to noradrenergic cell bodies in the medulla-pons and the dopaminergic cell bodies in the substantia nigra, to presumed dopaminergic terminals in the telencephalon, and to a few scattered noradrenergic terminals in the brain stem, hypothalamus and spinal cord (70). During the first week after birth, there is a marked increase in size and fluorescent intensity of the catecholaminergic cell bodies. Between the second and third week after birth, the noradrenergic terminals attain an adult pattern of density and intensity of fluorescence in the spinal cord and medulla-pons, whereas the adult pattern is established in the more rostral regions only by the fourth to fifth postnatal week. Although the diffusely intense fluorescence of the dopaminergic terminals in the striatum

is maximal by 4 weeks after birth, there is a shift in the fluorescent spectrum during subsequent maturation suggesting further increase in the dopamine concentration. The apparently more rapid maturation of the noradrenergic neurons when compared to the dopaminergic neurons is substantiated by the fact that norepinephrine attains adult concentration prior to dopamine (71-73). Thus, two phases in the morphogenesis of the catecholaminergic neurons seem to exist: a fetal and early post-natal period when the cell bodies acquire catecholamine fluorescence and then a period, primarily post-natal, when there is a centrifugal outgrowth of axons and terminals from the cell bodies.

Since the fluorescent intensity of the catecholaminergic neurons is related to their content of catecholamines (74), the effects of drugs on the catecholamine fluorescence may provide some indication of functional maturity of the neurons. The marked loss of catecholamine fluorescence produced by reserpine, a drug that disrupts the ability of intraneuronal storage vesicles to store catecholamines (from the earliest time the catecholamines can be detected in the hypothalamus neurons of the rat) suggests that the vesicular storage mechanism is functional even in the immature neurons although it might not be very efficient (75). Administration of α-methyl noradrenaline, a catecholamine that is not degraded by monoamine oxidase, results in increased fluorescence of the cell bodies, axons and terminals of the catecholaminergic neurons in neonatal rat brain (69). These findings indicate that the catecholamine uptake mechanism(s) are active in the membrane of the differentiating neuron. Furthermore, α-methyl-p-tyrosine, which inhibits the initial step in the biosynthetic pathway for catecholamines, causes a time-dependent depletion of catecholamine fluorescence that appears to be related to neuronal activity (76). Following administration

of α-methyl-p-tyrosine to new born rats, there is a marked loss in fluorescence of the terminals and cell bodies similar to that observed in adult rats, although the duration of depletion is more prolonged in the neonates (70). Thus, it might be conceived that catecholaminergic neurons are physiologically active well before they have completed morphologic differentiation.

B. Norepinephrine: Ontogenesis and Distribution

Extensive investigations have been carried out concerning alterations in the endogenous levels of brain catecholamines with maturation. There is general agreement that the concentration of norepinephrine in the whole rat brain increases in a linear fashion from 15 to 20% of the adult level at birth to the adult concentration by about 5-6 weeks (72, 77-79). By using an extremely sensitive enzymatic assay for norepinephrine, Coyle and Henry (80) have been able to demonstrate its presence as early as 15 days of gestation in the fetal rat brain; the concentration increased 5-fold until 19 days of gestation, after which it remained constant until birth. Because of changes in the rate of brain growth after birth, the developmental changes in total brain norepinephrine content were represented by a sigmoid curve with a 50-fold increase between birth and adulthood (72). In guinea pigs and chicks, where birth occurs at later stages of development, brain levels of catecholamines at the time of birth were similar to those seen in adults (81,82).

As expected, there are significant differences in regional changes in the concentration of brain norepinephrine with development. In the rat, there is an age-dependent linear increase in norepinephrine levels of the pons-medulla. Adult levels are reached by 1 month in the oblongata, but in the rostral regions, including the mesencephalon-diencephalon and telen-

cephalon, there is a much more gradual increase in the concentration of catecholamines (75). Kellogg and Lundborg (83) demonstrated that norepinephrine containing neurons within the brain became functionally mature at an earlier age than did the dopamine-containing neurons. They observed a significant decrease in norepinephrine content in whole brain of one-day old rats in response to inhibition of tyrosine hydroxylase, however, no pronounced changes were noted in dopamine levels until 4 days of neonatal life. Loizou (69) observed that histochemical fluorescence of noradrenergic terminals in the brain reached an adult pattern of density and fluorescence intensity at a slightly earlier period than did the fluorescence of dopamine terminals. Studies combining the histofluorescent method with ^{3}H-thymidine autoradiography have demonstrated that noradrenergic neurons in the locus coeruleus cease cell division approximately 1 week earlier than do dopamine cell bodies in the substantia nigra (63). Evidence therefore seems to exist that there is a more rapid maturation of noradrenaline neurons as compared to that of dopamine neurons, both morphologically and functionally.

C. Enzymes Involved in the Biosynthesis and Inactivation of Catecholamine

The dopaminergic and noradrenergic neurons have in common the first two enzymes in the biosynthetic pathway for their neurotransmitters, tyrosine hydroxylase, and dopa decarboxylase (84). The noradrenergic neurons possess an additional enzyme, dopamine β-hydroxylase, which converts dopamine to norepinephrine.

(i) Tyrosine hydroxylase: ontogenesis and distribution

Evidence indicates that tyrosine hydroxylase activity is present in the whole fetal rat brain at 15 days of gestation with a specific activity of about 7% of the adult level (85). This coincides closely with the time established by autoradiographic study as the period when differentiation

of the noradrenergic neurons commences (63). Tyrosine hydroxylase catalyzes the conversion of tyrosine to dopa. This enzyme in soluble form requires molecular oxygen and reduced pteridine as co-factors, is inhibited by peroxidases and stimulated by dihydropteridine reductase (86). Evidence indicates that particle-bound brain tyrosine hydroxylase is unaffected or slightly inhibited by $DMPH_4$ (87), whereas the soluble enzyme prepared from adrenal tissue is stimulated 15-fold or more in the presence of this co-factor (88). These findings do not prove that the particle-bound brain hydroxylases do not involve $DMPH_4$ in their actions. However, it might be conceived that the particles in question either contain sufficient endogenous $DMPH_4$ or are impermeable to the added co-factor. Tyrosine hydroxylase shows a triphasic increase in specific activity during development with a 4-fold rise between 15 and 18 days of gestation, then a period of relatively constant specific activity until 1 week after birth and finally a linear increase to adult levels by 5-6 weeks after birth (72,85). The triphasic pattern of increase in the specific activity of tyrosine hydroxylase with development, resembles the increase in the concentration of brain dopamine (72,78).

During post-natal development, a relatively small increase in the specific activity of tyrosine hydroxylase has been observed in the mid-brain, hypothalamus and in the pons-medulla area in which the cell bodies of the catecholaminergic neurons are located. Conversely, the regions where the terminals of catecholaminergic neurons are located, such as the cerebral cortex, cerebellum and corpus striatum, striking increases in the specific activity of tyrosine hydroxylase have been observed during brain development (85,89). Thus, with maturation, a centrifugal movement of tyrosine hydroxylase seems to be involved in the biosynthesis of catecholamines away from the regions of the brain that contain cell bodies to the regions that

receive the terminals.

The temporal-spatial changes in tyrosine hydroxylase activity during development are accompanied by changes in the subcellular distribution. For example, tyrosine hydroxylase in adult rat brain behaves as a soluble enzyme because it is released in the supernatant with the cytosol when the brain is homogenized in hypotonic buffer (90). However, when the adult brain is homogenized in isotonic sucrose and fractionated according to the method of Whittaker (91), most of the tyrosine hydroxylase is entrapped in the sediments with the synaptosomes, indicating a high degree of localization in the nerve terminals (90). Furthermore, when fetal brain is fractionated in the same manner, most of the tyrosine hydroxylase is released into the supernatant, suggesting a non-terminal localization of the enzyme in immature neurons. It seems therefore that with age, there is a progressive redistribution of the enzyme from soluble to the synaptosomal form (85). This regional and subcellular translocation of enzyme activity during development corresponds closely with the outgrowth of the catecholaminergic processes as demonstrated by histofluorescent techniques.

An important distinction must be made between the density of innervation and the total amount of terminals in a particular region. This issue is especially relevant in the case of the cerebellum, a region that develops primarily after birth. The specific activities of both tyrosine hydroxylase and dopamine β-hydroxylase increase about 2-fold between birth and adulthood in the cerebellum, which suggests only a small change in the density of noradrenergic innervation. However, the weight of the cerebellum increases 35-fold during the same period. Therefore, the actual increase in enzymatic activity and presumably the number of noradrenergic terminals in the cerebellum is in the order of 70-fold.

(ii) Dopa decarboxylase and/or 5-hydroxytryptophan decarboxylase: ontogenesis and distribution

The second enzyme in the biosynthetic pathway for catecholamines is dopa decarboxylase. The enzyme requires pyridoxal phosphate as the co-factor and is widely distributed in mammalian tissues. On the basis of immunologic studies, it appears that this enzyme is responsible for the decarboxylation of both dopa and 5-hydroxytryptophan (92). Karki et al. (81) demonstrated that the activity of brain dopa-decarboxylase in one-day old rats was 80% of the adult levels. Using 5-hydroxytryptophan as a substrate, Bennett and Giarman (93) found that enzyme activity doubled during the week before birth reaching 70% of the adult level, then declined to half of the adult values within a few days after birth, and finally rose steadily but never attained adult values before 5 weeks of age. A similar developmental pattern was observed by Lamprecht and Coyle (94) using dopa as the substrate, although the enzyme activity at birth was only 40% of the adult values. In brain, this enzyme has been found to be present in both the soluble as well as the particulate form. Similar to tyrosine hydroxylase, dopa decarboxylase activity also exhibited a caudal to rostral shift with maturation. Whereas enzyme activity doubled between birth and adulthood in the pons-medulla and mid brain-hypothalamus, (the regions containing cell bodies of aminergic neurons) it increased 5-7 fold in the cerebellum, corpus striatum and cerebral cortex, regions which receive terminals of aminergic neurons (94).

(iii) Dopamine β-hydroxylase: ontogenesis and distribution

The third enzyme which converts dopamine to norepinephrine in noradrenergic neurons is dopamine β-hydroxylase. Dopamine β-hydroxylase is quite fastidious, requiring ascorbate, fumarate, and catalase for optimal activity as well as Cu^{++}, N-ethylmaleimide or p-chloromercuribenzoate (95). The

enzyme is localized in amine storage vesicles in adrenal medulla, splenic nerve and brain (96,97). Evidence suggests that at 15 days of gestation, specific activity of dopamine β-hydroxylase is one-tenth of the adult level in whole fetal brain. During maturation, dopamine β-hydroxylase shows a linear increase, attaining 80% of adult specific activity by 4 weeks after birth (98). At 17 days of gestation, two-thirds of dopamine β-hydroxylase activity in the brain is localized in the pons medulla, where the cell bodies of the noradrenergic neurons are located. With maturation, there is a progressive rostral shift in dopamine β-hydroxylase activity, so that in adulthood only one-fifth of the total dopamine β-hydroxylase activity is found in the pons-medulla. Since this caudal to rostral translocation of enzyme activity during maturation is related to the outgrowth of noradrenergic axons and terminals, it is not surprising that there is a concurrent increase in the amount of dopamine β-hydroxylase in the synaptosomal fraction of homogenates.

(iv) Monoamine oxidase and catechol-o-methyl transferase: ontogenesis and distribution

The two enzymes primarily responsible for the catabolism of the catecholamines are monoamine oxidase and catechol-o-methyl transferase. Among many studies on the developmental profile of monoamine oxidase in rat brain, there is agreement that a sharp increase in specific activity occurs during the last week of gestation when it reaches 40% of the adult values. During the first week of neonatal life, enzyme activity remains fairly stable, followed by a linear rise to attain near adult levels by 4 weeks after birth (81,93,99,100). Throughout development, monoamine oxidase activity in the brain has been found to remain primarily associated with the mitochondria (79). The maturational changes in monoamine oxidase are probably not limited

to quantitative increases in activity, since there are multiple forms of the enzyme with different substrate specificities (101,102). Shih and Eiduson (103) have demonstrated that the isozymal pattern of monoamine oxidase, as determined by disc gel electrophoresis, is different in new born and adult rat brain. Furthermore, as demonstrated by histochemical techniques, the regional distribution of the enzyme changes with maturation; the activity is limited to the pons-medulla in the fetus and spreads anteriorly with age (104).

The information about the developmental pattern of catechol-o-methyl transferase in rat brain is still scanty. However, a very small amount of normetanephrine, o-methylated metabolite of norepinephrine, has been found in new born rat brain (78). It probably appears that this enzyme is not an efficient enzyme to metabolize catecholamines in the central nervous system of developing rats where it represents only 20% of the adult activity (105).

D. Development of Uptake and Storage Mechanisms in Catecholaminergic Neurons

Glezer's (106) study of the ultrastructural development of rat cerebral cortex indicated that the synaptic boutons are first formed and then subsequently filled with synaptic vesicles. In order to determine whether the central noradrenergic nerve endings develop prior to the capacity to store norepinephrine in vesicles, Coyle and Axelrod (79) studied the endogenous levels of norepinephrine and its sensitivity to reserpine. At 19 days of gestation, the day after saturable uptake of norepinephrine was demonstrated, desmethylimipramine inhibited the active uptake of L-(^{3}H) norepinephrine by 60%, a value which was not significantly different from that found in adult animals (79). However, pretreatment with reserpine produced no

inhibitory effect on the accumulation of L-(^{3}H) norepinephrine at 17 days of gestation. At 19 days of gestation, reserpine inhibited the 20 minute accumulation of L-(^{3}H) norepinephrine by only 30%. Reserpine treatment produced progressively greater inhibition with increasing age, but the inhibition of the accumulation of L-(^{3}H) norepinephrine was still significantly less for the brain of the 2 week old rats than that seen in the adults. Vernadakis (107) reported similar observations in developing chick and demonstrated that cocaine inhibited the accumulation of (^{3}H) norepinephrine as early as 10 days, whereas reserpine treatment inhibited the storage of radioactive norepinephrine in cerebral hemesphere after 20 days of embryonic age. These findings suggest that the development of the granular storage mechanism lags behind the development of the specific membrane mechanism for the uptake of norepinephrine.

Evidence also indicates that there are regional differences in the uptake of norepinephrine in the rat brain. At birth, the cerebral cortex, cerebellum, brain stem and hypothalamus-mid brain all exhibited the specific norepinephrine uptake mechanism. Between birth and adulthood, the cerebral cortex showed the largest increase in uptake, whereas the cerebellum showed the least change. The hypothalamus- mid brain and brain stem showed intermediate increases in the uptake between birth and adulthood.

IV. BRAIN 5-HYDROXYTRYPTAMINE

5-Hydroxytryptamine was first isolated in mammalian tissues by Rapport et al. in the year 1948 (108). Shortly after its discovery, 5-hydroxytryptamine was demonstrated by Twarog and Page (109) to occur in brain, but the significance of this finding was not appreciated until Gaddum (110) proposed that lysergic acid diethylamide (LSD) acted as a hallucinogen by virtue of its antagonistic action on 5-hydroxytryptamine. Working independently,

Woolley and Shaw (111) suggested that 5-hydroxytryptamine might be involved in mentation and any disturbance in its metabolism may lead to mental diseases. Shore et al. (112) demonstrated that reserpine changes brain 5-hydroxytryptamine from bound to the free state. Later, Brodie et al. (34) suggested that 5-hydroxytryptamine is a neurohormone acting on what Hess (113) called the trophotrophic functions of the diencephalon which integrate with the parasympathetic system. Trophotrophic stimulation results in drowsiness and sleep, increased parasympathetic activity, decreased skeletal muscle tone and activity and lowered responsiveness to external stimuli. However, gradual unfolding of the ubiquitous importance of this hormone and the development of techniques to assay enzymes involved in its metabolism led to the discovery that tranquilization effect seen in animals treated with reserpine may not solely be due to activation of serotonergic receptors by free 5-hydroxytryptamine. The most important point which the earlier workers had failed to consider while implicating 5-hydroxytryptamine as a neuro-hormone for trophotrophic system, is its deamination. It might be conceived that following administration of reserpine, 5-hydroxytryptamine from the storage granules is released and subsequently deaminated by intraneuronal monoamine oxidase without ever reaching to the post-synaptic receptors in the serotonergic neurons, consequently, sedation might be due, at least in part, to a lack of 5-hydroxytryptamine in the synaptic cleft. Indeed, Dewhurst (35) and Grahame-Smith (36) demonstrated that under certain set of conditions, increased levels of brain 5-hydroxytryptamine invoke excitation rather than drowsiness and hypoactivity. Furthermore, several investigators have implicated 5-hydroxytryptamine as an important neurochemical correlate in certain types of depressive illnesses (39,40).

The fluorescence histochemical studies in adult rats indicated that

whereas most of the cell bodies of serotonergic neurons are localized in the raphe region, a few can be noted outside this region i.e. the ventromedial reticular formation of the pons and mesencephalon (114). The 5-hydroxytryptamine containing nerve terminals in the central nervous system originate from cell bodies mainly concentrated in different raphe nuclei in the brain stem and mesencephalon. From these sites, ascending and descending axons project to the more rostral parts of the central nervous system and to the spinal cord. 5-hydroxytryptamine containing nerve terminals appear to be present in most parts of the central nervous system (115,116). The ascending axons reach the rostral parts of the brain and form nerve terminals in hypothalamus, limbic forebrain and neocortex. Dahlstrom and Haggendal (117) observed an accumulation of 5-hydroxytryptamine containing granules on the proximal side of the lesion which may represent a proximo-distal transport of amine granules storing 5-hydroxytryptamine. The 5-hydroxytryptamine containing nerve endings in the central nervous system are of varying sizes. In certain parts like the spinal cord, the varicosities of terminals are fairly large, 1 to 2 μ in size, and contain enough 5-hydroxytryptamine. However, in most parts of the brain, the varicosities appear to be much smaller (118).

V. DEVELOPMENT OF SEROTONERGIC NEURONS IN THE CENTRAL NERVOUS SYSTEM

Indolealkylamine-induced fluorescence can be observed in the 5-hydroxytryptamine containing nucleus at 12 days of gestation (119). Furthermore, these investigators observed an enhanced fluorescence of 5-hydroxytryptamine neurons of fetus whose mothers had been given an inhibitor of the enzyme, monoamine oxidase. These findings suggest that like the catecholaminergic neurons, serotonergic neurons not only develop early in prenatal life, but are also biochemically functional.

In neonatal rats, the developmental pattern of 5-hydroxytryptamine containing neurons in the brain is similar to that described earlier for catecholamines. By histofluorescent techniques, 5-hydroxytryptamine containing nerve terminals could be observed in some regions of the lower brain stem and spinal cord at the end of the first post-natal week. An abundance of these can be seen at 2 weeks, the time when 5-hydroxytryptamine containing cell bodies are also increased in number and intensity of fluorescence (69). It also was demonstrated that administration of nialamide further augmented the fluorescence intensity of 5-hydroxytryptamine containing cell bodies and nerve terminals . The distribution and density of 5-hydroxytryptamine containing nerve terminals attain the adult pattern by the end of the third post-natal week, which is slightly earlier as compared to dopaminergic neurons in which the adult pattern is achieved by 4 weeks of post-natal life (69).

The histofluorescent results described above seem to be quite consistent with the biochemical data. The brain 5-hydroxytryptamine concentration is quite low at birth (about 25-30% of the adult levels) which rapidly increased during the first 3-4 weeks of life (120), the period during which there is a rapid dendritic proliferation of serotonergic neurons (69). Furthermore, the activity of tryptophan hydroxylase, the rate-limiting enzyme, which was significantly low at birth also dramatically increased between 7 and 30 days postpartum (121,122). Thus, the low levels of 5-hydroxytryptamine in face of considerably higher levels of the precursor amino acid, tryptophan (123) might, in part, be due to impaired hydroxylation of the substrate. The activity of 5-hydroxytryptophan decarboxylase, the second enzyme in the biosynthetic pathway of 5-hydroxytryptamine, is unlikely to be a rate-limiting enzyme. Studies have indicated that the activity of this

enzyme was quite high in immature rats (93) and that intraperitoneal administration of 5-hydroxytryptophan raised the levels of 5-hydroxytryptamine whereas tryptophan treatment failed to exert any appreciable effect on this indoleamine in brains of young rats. However, certain other factors merit consideration while explaining the low levels of 5-hydroxytryptamine in face of relatively higher levels of its chief metabolite, 5-hydroxyindoleacetic acid in brains of developing rats. The storage system(s) within the neurons might be inadequate, thus exposing 5-hydroxytryptamine to the action of monoamine oxidase. Indeed, a fairly high activity of this enzyme has been demonstrated to be present in serotonergic neurons of rats right from the time of birth (69). Furthermore, the high levels of 5-hydroxyindoleacetic acid in neonates might, in part, be due to impaired mechanism(s) for the removal of 5-hydroxyindoleacetic acid from brain. Atack et al. (124) have demonstrated that homovanillic acid and 5-hydroxyindoleacetic acid and perhaps even bilirubin are removed from the immature rat brain to the blood largely via the cerebro-spinal fluid by means of a carrier-mediated mechanism with limited capacity probably localized at the choroid plexus. However, in adult rats, major avenue for the removal of 5-hydroxyindoleacetic acid seems to be an active transport mechanism located in the tissue parenchyma, presumably at glia-capillary interphase (124). Therefore, it seems reasonable to assume that the higher levels of 5-hydroxyindoleacetic acid seen in developing rat brain might be due to saturated transport sites in the cerebro-spinal fluid system.

VI. REGULATION OF BRAIN NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE

The catecholamines and 5-hydroxytryptamine are in a state of flux, yet they maintain a constant level in their corresponding neurons. This is made possible by a variety of self regulatory systems involving changes

in their biosynthesis, storage, release and metabolism within the neurons as well as modifications of the pre- and/or post-synaptic receptors. The levels of norepinephrine within the neuron can be regulated at several sites: cell body, where enzyme molecules are renovated by de novo protein synthesis; the axon, which transports the biosynthetic enzymes made in the cell body; storage vesicles of nerve terminals; the neuronal membrane, through which vesicles release their contents by exocytosis, and which also plays a vital role in the uptake of released neurotransmitters; and finally, the pre- and/or post-synaptic receptors.

A. Hydroxylation of Tyrosine and Tryptophan

Tyrosine and tryptophan are the two amino acids that serve as precursors in the biosynthesis of catecholamines and indolealkylamines, respectively. The first step in the biosynthesis of these two central amines involves ring hydroxylation by two mixed function oxygenases, tyrosine hydroxylase and tryptophan hydroxylase (Fig. 1, 2). It should be mentioned that the regulation of catecholamines and indolealkylamines in their corresponding neurons differ significantly. For example, there is not enough tryptophan in the brain to saturate tryptophan hydroxylase, while tyrosine hydroxylase is saturated with the substrate, tyrosine.

As described earlier, tyrosine hydroxylase occurs partially free and partially bound to membranes. The enzyme molecules are presumed to have the same amino acid sequence because the fraction that is bound depends upon the homogenization conditions (125,126). However, the conformations of the two forms are probably different because their catalytic properties are not identical. According to Kuczenski and Mandell (125), the membrane bound form of tyrosine hydroxylase, compared with the soluble form, exhibits a smaller apparent Km for $DMPH_4$ and a lower Ki for dopamine. The ratio of

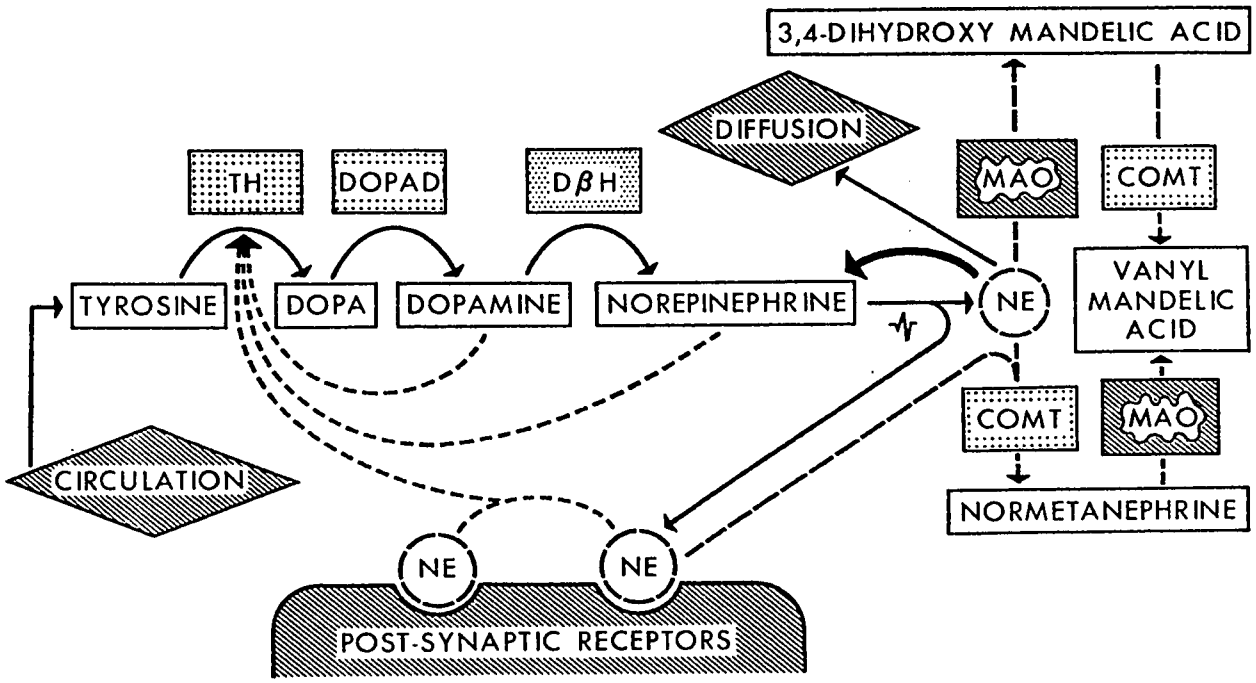

Fig. 1. The pathways of norepinephrine metabolism in brain. Abbreviations: tyrosine hydroxylase (TH); dihydroxyphenyl alanine (DOPA); dihydroxyphenyl alanine decarboxylase (DOPAD); dopamine β-hydroxylase (DβH); norepinephrine (NE); monoamine oxidase (MAO); catechol-o-methyl transferase (COMT).

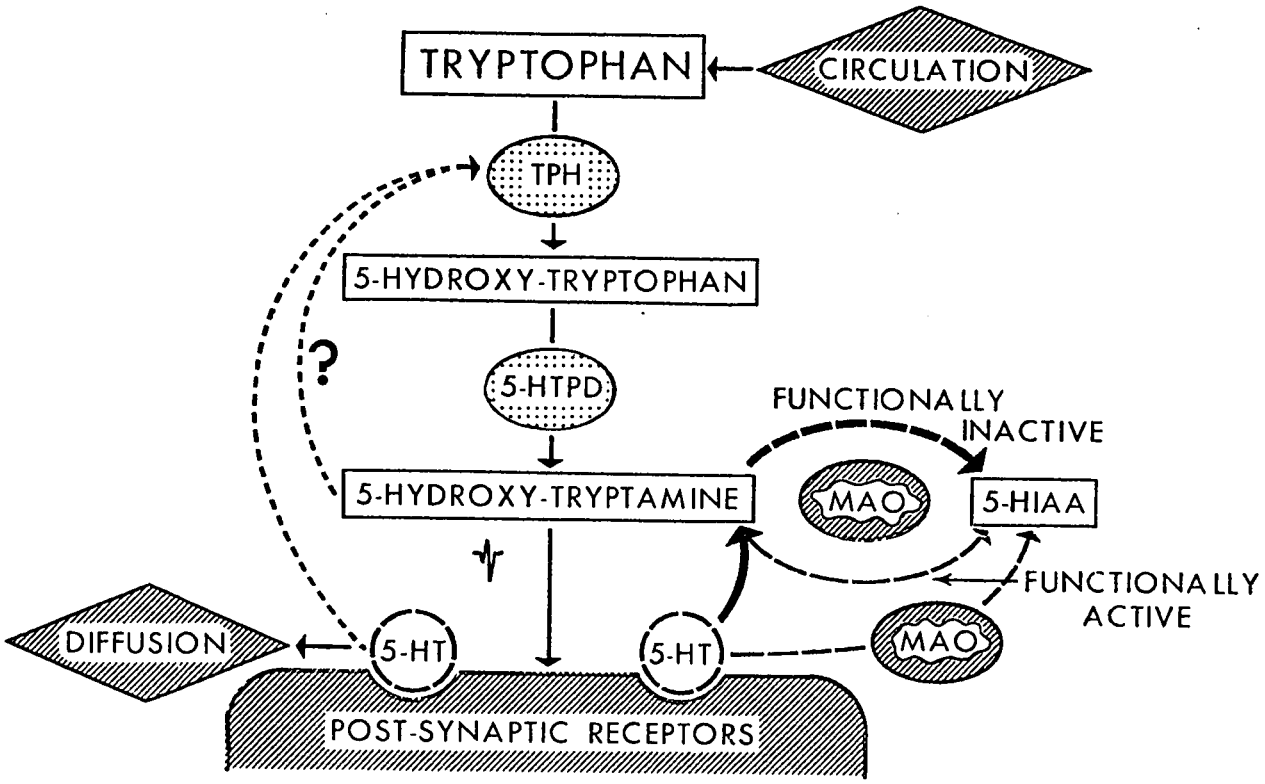

Fig. 2. The pathways of 5-hydroxytryptamine metabolism in brain. Abbreviations used: tryptophan hydroxylase (TPH); 5-hydroxytryptophan decarboxylase (5-HTPD); 5-hydroxytryptamine (5-HT); monoamine oxidase (MAO); 5-hydroxyindoleacetic acid (5-HIAA).

free to bound tyrosine hydroxylase can be changed by pharmacological manipulations. A single dose of methamphetamine (2.5 mg/kg) produced a slight but significant shift in localization of tyrosine hydroxylase from the soluble to the particulate form (127). A similar effect also has been described following the administration of reserpine and α-methyl tyrosine (127).

The occurrence of two forms of tryptophan hydroxylase in the brain and their varying regional distribution have been studied by Knapp and Mandell (128-130). It was found that particulate tryptophan hydroxylase activity in brain is most prominent in areas in which serotonergic nerve endings are in high concentration such as the frontal cortex, septal area, caudate and the lumbosacral cord. On the other hand, the soluble form of the enzyme is more prominent in mid-brain and pons-medulla, areas where the serotonergic cell bodies have been reported (115). The particulate form of tryptophan hydroxylase is not activated by the synthetic co-factor, $DMPH_4$. It appears that the particles to which the enzyme is presumably bound either contain adequate amount of $DMPH_4$ or are impermeable to the added co-factor (131).

B. Decarboxylation of DOPA and 5-Hydroxytryptophan

The decarboxylation of DOPA in catecholaminergic neurons and of 5-hydroxytryptophan in serotonergic neurons is carried out by L-aromatic amino acid decarboxylases, more commonly called dopa decarboxylase or 5-hydroxytryptophan decarboxylase (Fig. 1 and 2). There have been some reports suggesting that these decarboxylation reactions are carried out by two different and distinct enzymes but weight of evidence indicates that the same enzyme is involved in both reactions although it may be composed of several electrophoretically distinct forms (132). Evidence also suggests that the activity of L-aromatic amino acid decarboxylases varies towards different substrates. DOPA, for example, is decarboxylated about 5 times

as fast as is 5-hydroxytryptophan by what is presumed to be the same enzyme from various areas of cat brain (133). As is the case with other decarboxylases, brain L-aromatic amino acid decarboxylase also requires pyridoxal phosphate as a coenzyme.

C. Hydroxylation of Dopamine

In noradrenergic neurons an additional step is involved in the synthesis of norepinephrine. Dopamine is hydroxylated by dopamine β-hydroxylase into norepinephrine (Fig. 1). The cofactors for dopamine β-hydroxylase are ascorbic acid and copper. Dopamine β-hydroxylase is sequestered in storage vesicles of the neurons. Recently, it has been demonstrated that dopamine β-hydroxylase, localized in storage vesicles, is synthesized in the perikarya of the neurons and is transported down the axon of the rat sciatic nerve to nerve endings (134). Some of the dopamine which is formed from dopa is taken up into vesicles and converted into norepinephrine while some is deaminated by the intraneuronal enzyme, monoamine oxidase. Thus, a competition seems to exist between deamination and uptake/β-hydroxylation in the neuronal vesicle. It might be conceived that anything that increases the capacity of the neuron to β-hydroxylate dopamine should enhance the rate of norepinephrine biosynthesis.

D. Release of Norepinephrine and 5-Hydroxytryptamine from Nerve Terminals

The putative neurotransmitter, norepinephrine, is contained in a membrane bound vesicle (135). Its discharge from the nerve after depolarization might occur by the process of exocytosis even though the evidence for exocytosis is derived from studies with adrenal medulla. The mechanism for exocytosis requires fusion of the vesicular and neuronal membranes by an unknown process. The possibility can be raised that a number of discrete steps may be involved in the sequence of events which lead from

membrane depolarization to calcium entry, to approximation of the vesicle and nerve terminal membrane and finally to membrane fusion i.e. opening of a channel to the synaptic cleft, extrusion of the vesicular contents and termination of the release process with reconstitution or disposal of the remainder of the vesicle (136). Smith et al. (137) demonstrated that when sympathetic nerve to spleen is stimulated, dopamine β-hydroxylase is released together with norepinephrine. A similar mechanism for the release of 5-hydroxytryptamine can be speculated although it would differ from that of norepinephrine in the sense that the release of brain 5-hydroxytryptamine is not calcium-dependent (138).

E. Effects of Precursor Concentration on the Metabolism of Brain Norepinephrine and 5-hydroxytryptamine

Previous studies have demonstrated that the synthesis of 5-hydroxytryptamine might be accelerated by administration of its precursor, tryptophan. Although tryptophan hydroxylase is the rate-limiting enzyme in the biosynthesis of 5-hydroxytryptamine, tryptophan, owing to its low concentration in brain, determines the rate of formation of 5-hydroxytryptamine. Jequier et al. (139) demonstrated that the Km of tryptophan hydroxylase for tryptophan in the presence of $DMPH_4$ as a cofactor was in the range of 300 μM. This is considerably higher when compared with the endogenous concentrations of tryptophan present in the brain being about 30 μM. These investigators, therefore suggested that "the enzyme may not be fully saturated with substrate in normal brain and that the overall rate of 5-hydroxytryptamine synthesis may be partially dependent upon availability of tryptophan." A series of studies have since been undertaken and it has been observed that the Km of tryptophan hydroxylase is much lower (50 μM) when BH_4 is used as a co-factor (140). This Km value seemed to be consistent with their data from experiments with tryptophan loading. Administration of tryptophan

in a dose of 50 mg/kg intraperitoneally, caused brain tryptophan concentration to increase to about 100 μM (3-fold normal), and of brain 5-hydroxytryptamine to nearly double the normal values (141). Larger doses of this amino acid increased brain tryptophan levels proportionately, but produced no further increase in the concentration of 5-hydroxytryptamine. Grahame-Smith (36) noted a maximal rise in this indoleamine with a dose of 120 mg/kg tryptophan which also produced a rise in brain tryptophan concentration to approximately 350 μM. Thus, 5-hydroxytryptamine formation *in vivo* is probably maximal when brain tryptophan concentration is elevated by 3-6 times. Similar conclusion was derived by Carlsson and his associates (142) who estimated the *in vivo* activity of tryptophan hydroxylase. Therefore, it appears that tryptophan hydroxylase activity is not saturated *in vivo* and that by changing the brain tryptophan concentrations, it is possible to control the rates of 5-hydroxytryptamine synthesis and accumulation in brain.

Brain tryptophan is, of course, derived from plasma and after a tryptophan load, the concentrations in brain and plasma are directly proportional to each other (36). In addition, tryptophan is the only essential amino acid which is partially bound to albumin in the plasma (143). Because binding, in general, implies storage, several investigators have suggested that the quantity of tryptophan which reaches the cerebral tissue *via* the blood brain barrier is closely dependent on the small proportion of the amino acid still free in plasma (144). Under these circumstances, the possibility exists that factors which are capable of disturbing the equilibrium between the bound and free forms of tryptophan in plasma, may rapidly modify the availability of tryptophan to the brain. Recently, it has been demonstrated that tryptophan is almost totally in a free state in the plasma of new born animals (145). This free state of amino acid is not solely related to the

presence of high levels of fatty acids in the blood, but also to the differences in the binding capacity of albumin in new born animals. Consequently, salicylates, which stimulate 5-hydroxytryptamine synthesis in the adult by displacing tryptophan from the binding sites of albumin, failed to increase the formation of 5-hydroxytryptamine in new born rats (145). However, in addition to plasma levels of free tryptophan, several other factors merit consideration since it has been demonstrated that probenecid (146) and chlordiazepoxide (145) rapidly increase the levels of free tryptophan without eliciting any stimulatory effects on 5-hydroxytryptamine synthesis (147,148). For instance, the intraneuronal uptake of tryptophan plays a vital role in the regulation of 5-hydroxytryptamine synthesis. Reserpine (149) or lithium (130) stimulates the formation of 5-hydroxytryptamine by virtue of being able to activate the transport of tryptophan in synaptosomal preparations (150). In contrast, imipramine (151), chlorimipramine (152) and cocaine inhibit both amino acid transport and 5-hydroxytryptamine (128, 145). Fernstrom and Wurtman (141) also have examined the factors that affect brain and plasma tryptophan concentrations. Plasma tryptophan was found to be subject to diurnal variations (21 µg/ml at noon; 32 µg/ml at midnight) due to variations in diet, corticosteroid levels and the activity of hepatic catabolizing enzymes. Intraperitoneal doses of tryptophan which changed plasma tryptophan concentrations by an extent smaller than the circadian variation, also produced a significant rise in brain 5-hydroxytryptamine (141). However, since a rise in free plasma tryptophan did not necessarily elevate brain tryptophan, Fernstrom and Wurtman (153) suggested that it may be the ratio of tryptophan and its competing amino acids (tyrosine, phenylalanine, leucin, isoleucin and valine) which actually regulates the levels of brain tryptophan. These investigators further demonstrated that

diets containing high concentrations of protein (such as 40% casein, or as high a proportion of protein as would be consumed in a steak) which are known to increase the levels of competing amino acids in plasma, decreased the synthesis of 5-hydroxytryptamine in the brain (154). Additional support to this view can be gained by the earlier findings which indicated that excess phenylalanine when present in the diet, given acutely (155) or in phenylketonuria (156,157), decreased brain 5-hydroxytryptamine concentrations. Conversely, a carbohydrate-rich diet by releasing insulin, elevated the ratio of plasma tryptophan to competing amino acids and in turn raised brain tryptophan uptake and finally the synthesis of 5-hydroxytryptamine (153).

It may be important to mention that although the levels of tryptophan are considerably high in various brain regions of new born rats, the amount of 5-hydroxytryptamine is very low (123). This lower concentration of 5-hydroxytryptamine may, in part, be due to decreased activity of tryptophan hydroxylase in new born animals (120,121). Since the rate of 5-hydroxy-tryptamine accumulation in corresponding neurons is limited by their capacity for uptake and storage into granules, the possibility also remains that these mechanisms might be under-developed in brains of immature rats. In presence of excess substrate, an increased amount of 5-hydroxytryptamine might be synthesized that is rapidly metabolized by intraneuronal monoamine oxidase, without ever being stored in the synaptic vesicles and taking part in neural transmission in young brain. This 5-hydroxytryptamine can then be presumed as "nonfunctional," in contrast to the "functional pool" of 5-hydroxytryptamine which is stored in the synaptic vesicles and is released upon nerve stimulation before being finally metabolized by the deaminating enzyme (Fig. 2). Thus, it might be conceived that administration of tryptophan or 5-hydroxytryptophan does not necessarily raise the levels

of "functional" 5-hydroxytryptamine in the brain. Moir and Eccleston (158) observed that acute loading doses of 5-hydroxytryptophan markedly elevated the levels of 5-hydroxyindoleacetic acid without appreciably altering the levels of brain 5-hydroxytryptamine. A kind of "shunt" mechanism has been hypothesized by these investigators in which 5-hydroxytryptophan was rapidly metabolized to 5-hydroxyindoleacetic acid without eliciting any effect on the levels of 5-hydroxytryptamine. However, caution must be exercised while extrapolating these findings to chronic situations. The possibility can be raised that with an acute loading dose, the rate of accumulation of 5-hydroxytryptamine in serotonergic neurons is limited by the capacity for uptake into the storage granules, whereas with continuous administration, the storage capacity might be augmented.

The effects of tyrosine on catecholamines synthesis in brain tissue also have been studied. Tyrosine is present in an entirely free form in the plasma (159) and exists in high concentrations in brain. Therefore, the rate-limiting enzyme tyrosine hydroxylase always appears to be saturated (160). Evidence also indicates that very high tyrosine concentrations inhibit catechol formation in vitro (86), however, in vivo studies failed to demonstrate any such effect (161). Thus, it would appear that the precursor tryptophan, in contrast to tyrosine, plays a vital role in the control of corresponding brain amine (5-hydroxytryptamine) synthesis. These studies also support the view that alterations in 5-hydroxytryptamine synthesis induced by various drugs and exogenous factors might be secondary to their effects on the concentration of brain tryptophan. Therefore, it is important to monitor tryptophan levels while studying the effects of a given drug or hormone on the synthesis of 5-hydroxytryptamine.

F. End Product Regulation of Norepinephrine and 5-Hydroxytryptamine Synthesis

In addition to several other mechanisms, the intraneuronal levels of putative neurotransmitters (norepinephrine, dopamine and 5-hydroxytryptamine) also play an important role in the control of their biosynthesis in brain. Udenfriend et al. (162) showed that a variety of catechols, including naturally occurring catecholamines, acted as inhibitors of tyrosine hydroxylase in vitro by virtue of competitive inhibition with the $DMPH_4$ cofactor system. This has led to the proposal that control of catecholamine synthesis in vivo is achieved by feedback inhibition of the product on the pteridine cofactor (160). In other words, the limitation on catecholamine synthesis is not set by the amount of tyrosine or by total tyrosine hydroxylase protein but by the amount of reduced protein produced by the pteridine cofactor. The cofactor acts as a hydrogen donor to the oxidized protein (tyrosine hydroxylase). Neff and Costa (163,164) demonstrated that monoamine oxidase inhibitors substantially reduced in vivo norepinephrine synthesis in brain and heart from tyrosine but enhanced catecholamine production from dopa indicating that increased concentrations of catecholamines in nerve endings suppressed the hydroxylation of tyrosine.

Using an isolated guinea pig vas deferens preparation, Weiner et al. (165) demonstrated that the reduction in tyrosine hydroxylase activity induced by monoamine oxidase inhibitors or by indirectly acting sympathomimetic amines, could be antagonized by the addition of excess pteridine cofactor to the bathing medium suggesting that in response to over stimulation of catecholamine receptors at post-synaptic membrane, the feedback mechanisms or servo mechanisms are activated, which in turn decrease the activity of tyrosine hydroxylase by acting on the cofactor system (Fig. 1). These workers have suggested a more precise means by which the end product mech-

anism may possibly operate. It has been conceived that norepinephrine in nerve endings exists in two free and bound pools. The free pool is cytoplasmic while the bound pool is enclosed in the synaptic vesicles. On nerve stimulation, it is norepinephrine in the bound pool which is released into synaptic cleft (165). The newly synthesized norepinephrine can be released into the free pool of axoplasm where it can either be taken up by synaptic vesicles, excreted into the extraneuronal space, or destroyed by intraneuronal monoamine oxidase. It also has been suggested that free norepinephrine acts to suppress tyrosine hydroxylation by end product inhibition. However, norepinephrine stored in synaptic vesicles remains isolated from tyrosine hydroxylase and therefore does not produce end product inhibition. If synaptic vesicles become depleted through nerve stimulation, newly synthesized norepinephrine is preferentially taken up into the vesicles reducing the size of free pool and decreasing the end product inhibition of tyrosine hydroxylase. If this is the case, then dopamine should be considered even more important than norepinephrine in regulating the activity of tyrosine hydroxylase, because this catecholamine is formed in the axoplasm and is presumably not stored in the vesicles (166-168). In fact, Weiner et al. (169) observed that dopamine was a more effective inhibitor of tyrosine hydroxylase (Fig. 1) than norepinephrine since the Ki of the former was approximately one half that of the latter compound. However, Kehr et al. (170) suggested that there is a receptor-mediated feedback control for striatal tyrosine hydroxylase based on the amount of dopamine liberated into the synaptic cleft. Moreover, Dairman et al. (171) observed a diminished activity of tyrosine hydroxylase in adrenal glands of rats treated with high doses of L-dopa and suggested that the observed suppression of tyrosine hydroxylation was not due to dopa itself, but might be due to norepinephrine or some other metabolic product.

The electrophysiological experiments recording the rate of firing of cell bodies of dopaminergic neurons in substantia nigra demonstrated that phenothiazine, which blocks the dopamine receptors, increased the firing rate of dopaminergic neurons (172). Conversely, monoamine oxidase inhibitors which raise the transmitter amines concentration by blocking their destruction, decreased the firing rate of serotonergic and dopaminergic neurons. Apomorphine, which stimulates dopamine receptors, slowed the firing rate of dopaminergic neurons as well as the synthesis of the corresponding amine (173). Similarly, lysergic acid diethylamide, which is claimed to be a serotonergic agonist, suppressed the firing rate of serotonergic neurons and decreased the turnover rate of 5-hydroxytryptamine. Furthermore, chronic administration of desmethylimipramine, which raises the levels of norepinephrine and 5-hydroxytryptamine within the synaptic clefts by blocking their neuronal uptake, decreased the activity of tyrosine hydroxylase (127) and tryptophan hydroxylase (151,174) in rat brain. Additional support to the view that 5-hydroxytryptamine levels in the synaptic cleft control the synthesis of this indoleamine by feedback mechanism has been gained by the findings of Macon *et al.* (175) and Hamon *et al.* (176), who observed a decreased rate of 5-hydroxytryptamine synthesis in rats treated with monoamine oxidase inhibitors.

In contrast to catecholamines, which inhibit the activity of soluble tyrosine hydroxylase, tryptophan hydroxylase does not seem to be sensitive to competitive feedback control by its ultimate product, 5-hydroxytryptamine. However, this indoleamine at high concentrations (10^{-3}M) does inhibit the enzyme in a non-competitive manner (130). Additionally, Macon *et al.* (175) demonstrated that 5-hydroxytryptamine synthesis was reduced both *in vivo* and *in vitro* when intraneuronal concentrations of the amine attained 2.5 times normal levels following inhibition by monoamine oxidase (177). Synthesis of this indoleamine is also inhibited in slices when intraneuronal stores

of 5-hydroxytryptamine in serotonergic terminals are increased with exogenous 5-hydroxytryptamine. This inhibitory process seems to occur during the first step of the indoleamine synthesis, since no reduction in ^{3}H-5-hydroxytryptamine formation could be noted in rats injected with monoamine oxidase inhibitors when ^{3}H-5-hydroxytryptophan was substituted for ^{3}H-tryptophan (145). Recently, Carlsson and his colleagues (142) observed a decrease in the rate of 5-hydroxytryptophan accumulation in brains of mice injected with monoamine oxidase inhibitors following blockade of the enzyme, 5-hydroxytryptophan decarboxylase. These findings indicate that there indeed may be an end product regulation of 5-hydroxytryptamine synthesis in serotonergic neurons. However, whether or not 5-hydroxytryptamine, like the catecholamines, actually reduces the hydroxylation by competing with pteridine cofactor (BH_4) still remains unclear.

VII. HORMONAL IMPACT ON BIOCHEMICAL MATURATION OF THE CENTRAL NERVOUS SYSTEM

Extensive studies have been carried out to demonstrate that the rate of brain maturation can be altered by a number of external and internal factors, particularly during the critical periods of post-natal development. Among the internal factors, hormones play a vital role in the maturation of the central nervous system and in the establishment of subsequent psychophysiological processes of the organism.

A. Sex Hormones

Neonatal administration of estradiol accelerates the functional brain maturation of young rats (178). This action is, in part, reflected biochemically by alterations in electrolyte distribution (179) and precocious laying down of the myelin sheath around the nerve fibers in brain tissue (180,181). Estradiol treatment in developing rats significantly elevated the brain protein content and the activity of cholineacetyl transferase

and acetylcholinesterase. Conversely, ovariectomy in neonatal rats retarded the electrophysiological, behavioural and biochemical maturation of the brain (182). Evidence indicates that ovariectomy at 2 weeks of age reduced brain protein, lipid, cerebroside and sulphatide levels which became statistically significant at 21 days of age. Furthermore, a significantly lower activity of brain cholineacetyl transferase and acetylcholinesterase was observed in ovariectomized rats (183). Administration of testosterone to developing rats markedly elevated the levels of brain norepinephrine (184). Thus, evidence exists that both male and female sex hormones influence brain maturation and the development of behavioural processes.

B. Adrenocortical Hormones

Adrenal steroids also have been implicated to play an important role in the maturation of brain and behaviour. The augmented brain excitability seen in developing rats treated with cortisol during critical period of growth might, in part, be associated with increased myelination. In fact, Casper et al. (181) demonstrated that cortisol treatment significantly enhanced the levels of cerebrosides, an important constituent of myelin. Later, it was shown that this corticosteroid significantly elevated the chloride content in spinal cord of developing rats and it was suggested that the altered excitability might be associated with elevated chloride levels (179).

C. Thyroid Hormones

Extensive studies in experimental animals have demonstrated that thyroid hormone plays a very critical role in the biochemical maturation of the central nervous system. Alterations in the activity of thyroid gland lead to profound changes in the metabolism of several important constituents of brain and spinal cord, particularly protein, carbohydrate, lipid, electrolytes

and biogenic amines, which may be associated with altered neurophysiology and behaviour seen during neonatal hypo- and hyperthyroidism.

(i) Effect of thyroid hormone on nucleic acid and protein metabolism

There is abundant clinical and physiological evidence to suggest the intimate role of the thyroid hormone in the regulation of body protein metabolism. In man, cretinism is usually associated with dwarfism, and thyroidectomy in immature animals results in retarded growth which can be corrected by L-thyroxine (185). Gelber et al. (186) observed that whereas administration of L-thyroxine significantly enhanced the ^{14}C-leucine incorporation into protein in developing brain, it elicited no appreciable effect on protein biosynthesis in adult animals. Functional dissimilarities in mitochondrial fractions have been presumed to be responsible for the varying effects of adult and immature rats since the mitochondria of mature brain have been shown to be rather insensitive to the action of thyroxine (187). Thyroid hormone stimulates nuclear RNA polymerase activity and the synthesis of ribosomal and perhaps messenger RNA. These chain of reactions ultimately enhance the cellular contents of functional ribosomes which then lead to increased synthesis of proteins (188).

In contrast, neonatal thyroidectomy has been shown to decrease the metabolism of protein and nucleic acids (189). A decreased incorporation of isotopic leucine into protein has been shown in the cerebral cortex of rats made hypothyroid at 1 day of age (190). Since the incorporation of labeled amino acid into microsomal or ribosomal protein in vitro is markedly dependent on Mg^{+2}, K^{+} and Na^{+} concentrations, the suggestion has been raised that the reduced incorporation of amino acids into proteins in neonatally thyroidectomized rats might be associated with altered Na^{+}-K^{+} pump (190).

Several investigators have also demonstrated marked alterations in

enzyme activity of the nervous system in thyroid-deficient animals. Geel and Timiras (191) found a decrease in the activity of cholinesterase in the cerebral cortex and in acetylcholinesterase activity of the hypothalamus. Garcia Argiz et al. (192) reported that thyroid deprivation at birth leads to an altered developmental pattern of glutamate decarboxylase, Mg^+ and Na^+-K^+ ATPase and γ-aminobutyric acid transaminase in rat cerebral cortex and of γ-aminobutyric acid transaminase and Na^+-K^+ ATPase in the cerebellum. The decreased levels of several of these brain enzymes in neonatally thyroid-ectomized rats were restored following administration of thyroid hormone (193). Siegel and Sisler (194) demonstrated that cycloheximide, an inhibitor of protein synthesis, prevented the L-triiodothyronine-induced rise in the activities of several brain enzymes in neonatally hypothyroid rats, reinforcing the view that thyroid hormone enhances protein biosynthesis in brain. Since cycloheximide inhibits the protein synthesis by preventing the transfer of aminoacetyl-transfer RNA to ribosomes, it is conceivable that thyroid hormone affects brain protein synthesis at the translation level.

(ii) Influence of thyroid hormone on carbohydrate metabolism

Schwark et al. (12) demonstrated that thyroid hormone is also involved in the regulation of enzymes which catalyze several important pathways of carbohydrate metabolism in the developing brain. The activities of 3 key glycolytic enzymes as well as of 2 hexose monophosphate shunt enzymes were markedly reduced in rats made hypothyroid by administering ^{131}I at 1 day of age (13).

(iii) Effect of thyroid hormone on myelination

The process of myelination is an important aspect of brain development. Deposition of myelin accounts for considerable increase seen in brain dry weight post-natally. The timing of myelination seems to be specified in the

genetic code since it always occurs at a particular time in a given species. In rats, it usually starts somewhere between 12 to 20 days of life (180,195). As early as 1948, Barnett (196) had reported that hypothyroidism in developing rats markedly retarded the process of myelination. Thyroid hormone deprivation in early life also reduced the cerebroside, sulphatide and cholesterol content of the brain as well as delayed the onset of sulphatide biosynthesis (6). Balazs et al. (197) have assessed myelin deposition by determining cholesterol content and showed that the amount of myelin was significantly reduced in thyroid-deprived rats. In contrast, administration of thyroxine to normal animals during early life resulted in an accelerated myelination (198).

VIII. CRETINISM - MAGNITUDE OF OCCURRENCE

Thyroxine and 3,5,3'-triiodothyronine (T_3) are the two active thyroid hormones that are known to influence a diversity of metabolic processes in the body. Diseases of the thyroid gland are manifested by qualitative or quantitative alterations in hormonal secretion or enlargement of the thyroid (Goiter) or both. Clinically, hypothyroidism results from a wide variety of structural or functional abnormalities that lead to insufficient synthesis of thyroid hormone. A permanent retardation in the development of the skeleton or the central nervous system resulting from thyroid deficiency during fetal or early neonatal life has been termed as "cretinism." However, the term "myxedema" denotes a form of hypothyroidism in which there is accumulation of hydrophilic mucopolysaccharides in the ground substance of the dermis as well as other tissues, leading to thickening of the facial features and doughy induration of the skin.

A. "Endemic" vs. "Sporadic" Goiter

There has been some confusion concerning the terms "sporadic" and

"endemic" goiter. The term "sporadic cretinism" was first employed by the English clinician Fagge (199) to describe a condition of thyroid deficiency associated with retarded physical and mental development. This condition may be associated either with goiter as in the classical biosynthetic defects first recognized by Stanbury and Querido (200), or without goiter when associated with a failure of development of the thyroid during fetal life, or with the presence of only a fragmentary thyroid at the base of the tongue. This condition of "sporadic cretinism" is widespread throughout the world - it exhibits no geographical localization. In contrast, "endemic cretinism" is geographically associated with endemic goiter and iodine deficiency. It includes two clinically different entities: (a) neurologic cretinism - characterized by mental retardation, deafness, disorders of gait and stance and (b) hypothyroid cretinism - which is in fact clinically indistinguishable from "sporadic cretinism."

Available information regarding the neuropathology of neurologic cretinism suggests that cerebral cortical and eighth nerve damage are responsible for mental retardation and deafness (201). The frequent presence of squint in cretinism suggests that it is also associated with brain stem damage. However, none of these features are generally seen in "sporadic" hypothyroid cretinism. Neurological cretinism rather represents a distinct entity - "iodine-deficient embryopathy." "Endemic" cretinism therefore includes two distinct clinical entities with different etiology and pathogenesis. The common entity, neurologic cretinism, predominates in the Himalayas, New Guinea and South America. The less common entity is hypothyroid cretinism which seems to predominate in the African Congo (202).

IX. THYROID DYSFUNCTION AND AFFECTIVE DISORDERS

A. Hypothyroidism vs. Depression

It is now apparent from both clinical as well as animal studies that

a number of metabolic and psychic disturbances may be common to thyroid disorder and affective illness. One might speculate that these biological insights provide a rudimentary basis for an understanding of greater suppression of mood in hypothyroidism or conversely the apparent protection from sadness which the hyperthyroid state provides. Biochemical studies have demonstrated that both show a diminished response to infused norepinephrine (203,204) and both probably show a high urinary output of catecholamines and their metabolites (205,206). Disturbances in sodium, potassium and calcium metabolism have been found in both affective illness (207) and thyroid dysfunction (208, 209). Psychological studies also have suggested that the symptoms of myxedema, a severe form of hypothyroidism, make an insidious appearance and are generally characterized by listlessness, lack of energy, slowness of speech, reduced sensory capacity, impairment of memory, somnolence, social withdrawal and an altered sleep pattern (17,210). Several of these psychological symptoms are commonly seen in depressed patients as well e.g. somnolence, slowness of speech, reduced sensory capacity, lack of energy, social withdrawal and altered sleep pattern (18,210-212). In addition, there is indirect evidence which suggests that depressed patients may have actually less thyroid hormone levels than are normally required (20,213,214). Indeed, Dewhurst et al. (215) have observed abnormally high levels of thyrotropic hormone in the blood of depressed patients, although it was suggested that the emotional stress associated with psychiatric illness might have been the cause of the elevated level of this hormone. Thus, although there is some evidence to implicate thyroid dysfunction with depression, it is difficult at present to point whether abnormal thyroid function is the result or the cause of affective disorder.

B. Thyroid-Imipramine Interaction and the Use of Thyroid Hormone in Depressive Illness

It was in 1957 when Kuhn (216) first reported the therapeutic usefulness of imipramine in the treatment of depression. Later, the similarity between the symptoms of depression and hypothyroidism as well as the pharmacology of imipramine prompted Prange and Lipton (217) to conduct a systematic study of the relationship of the action of imipramine and thyroid function. Subsequently, a concept emerged that the controlled administration of a thyroid hormone as an adjunct to imipramine therapy might lead to an enhanced antidepressant activity. In 1968, Prange and associates (20,218) reported the therapeutic advantage of combining L-triiodothyronine and imipramine in the treatment of depression. It was demonstrated that L-triiodothyronine did not enhance the therapeutic efficacy of imipramine by altering the metabolism of this tricyclic antidepressant (219). However, it was suggested that L-triiodothyronine enhanced the sensitivity of adrenergic receptors and imipramine elevated the effective concentration of this putative neurotransmitter by blocking the neuronal uptake of norepinephrine, thus manifesting an additive or synergistic effect in depressed patients. Thyrotropic hormone also has been shown to potentiate the action of tricyclic antidepressants presumably by releasing thyroid hormone (214,220). Indeed, Emlen et al., (22) demonstrated that surgical thyroidectomy in adult animals results in decreased sensitivity of pre- and/or post-synaptic receptors in the catecholaminergic neurons which in turn, increased the synthesis of norepinephrine probably via the positive feedback mechanism.

C. Thyrotropin-Releasing Hormone: An Antidepressant Agent

In addition to thyroid hormone, thyrotropin releasing hormone has been reported to ameliorate mental depression (220,221). However, Plotnikoff

et al. (222) demonstrated that the activity of thyrotropin releasing hormone was independent of pituitary or thyroid hormone. The anti-depressant activity of this hormone is due to its ability to enhance the rate of norepinephrine turnover and release in brain tissue (223,224).

D. Hyperthyroidism vs. Elation

Studies also indicate that the symptoms of hyperthyroidism have several features in common with elation. Excessive thyroid secretion is known to be associated with hyperexcitability, irritability, anxiety, fatigue, restlessness, exaggerated responses to environmental stimuli and emotional instability, symptoms which may be of sudden onset and if left untreated, may lead to psychosis (17,212). In addition, it is generally recognized that elation is associated with the excess of norepinephrine (225) and 5-hydroxytryptamine at the corresponding receptor sites. Recently, it has been demonstrated that hyperthyroidism in mice produced significant increases in the turnover of brain norepinephrine and 5-hydroxytryptamine (226). Thus, there appears to be some relationship between hyperthyroidism and elation, but a great deal of further research is needed to delineate the causal effect relationship, if any, between these two conditions.

X. EFFECT OF LITHIUM ON THYROID FUNCTION

Lithium is being used increasingly in the treatment of mania and in the prophylaxis against recurrent manic-depressive illness (227). During lithium treatment, the development of goiter has occasionally been observed. However, the mechanism by which lithium interferes with thyroid function is still obscure. It has been demonstrated that thyroid shows an avidity for lithium and following 2 weeks of administration of this anti-manic agent in rats, the concentration of lithium in thyroid gland was three times whereas in liver, brain, muscle and kidney, between 0.6 and 1.7 times

the serum concentration (228). Since protein bound iodine values remained normal or low, it is possible that lithium primarily inhibits hormone production in the thyroid which in turn leads to a compensatory increase of thyrotropic hormone secretion in pituitary with resultant hyperplasia of the thyroid gland.

In summary, the foregoing survey of the literature drawn from clinical and experimental studies indicate that thyroid hormone exerts a profound influence on the maturation of the central nervous system as reflected by changes in cerebral morphology, biochemical compartmentation of protein, lipid, nucleic acids and carbohydrates, electrical activity of brain and in behavioural patterns. However, evidence is lacking to show how these various metabolic abnormalities finally culminate in learning deficits and depressed behaviour seen in cretinous subjects. The present study was undertaken to investigate the effects of neonatal hypo- and hyperthyroidism on the metabolism of norepinephrine and 5-hydroxytryptamine for which there are suggestions that they play an important role in neural transmission and behaviour.

3. MATERIALS AND METHODS

I. ANIMALS

Rats of the Sprague-Dawley strain, maintained on Purina Laboratory Chow and water ad libitum were used throughout the course of this investigation. Pregnant females were obtained from Bio-Breeding Laboratories of Canada Ltd. and maintained under constant environmental conditions (24°C temperature, 60% relative humidity and regular alternate cycles of 12 hours light and darkness). Litter sizes were reduced to a maximum of 8 by discarding the extra number of pups. The runts were not included. The litters were weaned at 22 days of age. No attempt was made to distinguish the sex of neonatal animals used in various phases of the present study. The following experimental procedures were undertaken.

II. THYROIDECTOMY

A. Radiothyroidectomy

Animals were thyroidectomized by treatment with ^{131}I according to the method of Goldberg and Chaikoff (229). This method is based on the ability of the thyroid gland to concentrate ^{131}I with consequent radio-destruction of the thyroid tissue by β-emissions. The animals were injected intraperitoneally with varying dosages of ^{131}I (obtained from Atomic Energy of Canada, Ltd. Ottawa) in a volume of 0.05 ml through the muscles of the rump using a 30 gauge needle in order to prevent leakage from the site of injection. Littermate controls were given an equal volume of physiological saline. Electron microscopic studies by Schwark (230) have earlier demonstrated that a dose of 200 uCi of ^{131}I produces discrete destruction of thyroid gland without affecting the parathyroid gland. The mortality rate with this dose of ^{131}I was approximately 25 to 30%.

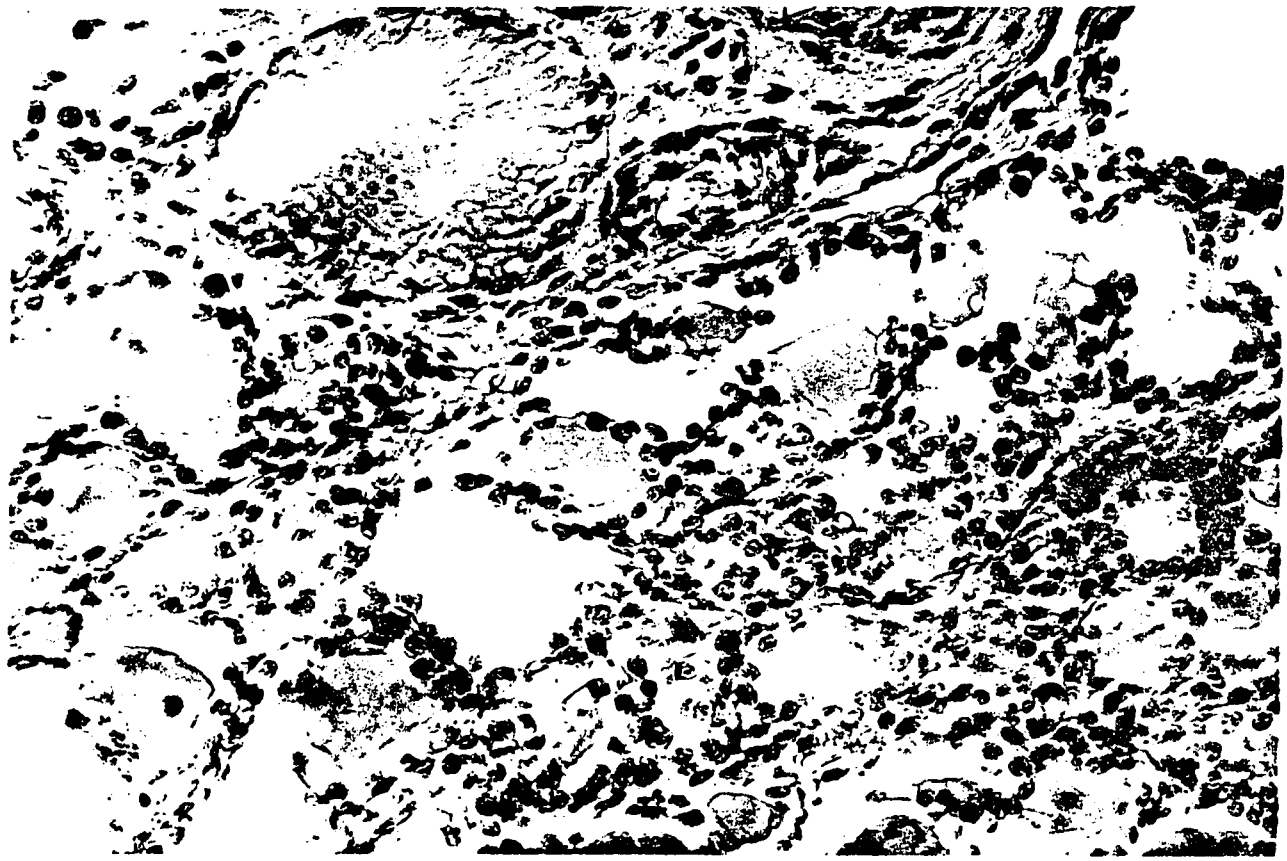

Fig. 2A. Photomicrograph showing a section of the thyroid region of a rat injected with methimazole (0.2 mg from day 1-7 and 0.4 mg from day 8-30). Based on the height of the epithelium and the relative frequency of follicles of various sizes, the thyroid appears to have remained normal. (Bouine's fixation, Hemotoxylin-Eosin staining x 200) Prepared through the kind courtesy of Dr. S. Jande, Department of Histology and Embryology, University of Ottawa.

B. Chemical Thyroidectomy

Chemical thyroidectomy was induced by administering a potent anti-thyroid drug, methimazole, which is known to produce an effective hypothyroidism (231). This thiourylene goiterogen elicits its effect by suppressing the synthesis of thyroid hormone. New born rats were injected intraperitoneally with "low" or "high" doses of methimazole. The schedule for low dosage of methimazole consisted of 0.1 mg from day 1-10, 0.2 mg from day 11-20 and 0.4 mg from day 21-30; whereas that for the high dose included 0.2 mg of the anti-thyroid agent from day 1-7 and 0.4 mg from day 8-30 as described earlier (232,233). The littermate controls were injected with an equal volume (0.05 ml) of physiological saline. The mortality rate in chemically thyroidectomized rats was about 20%, slightly lower than that seen in radiothyroidectomized rats. The method of inducing hypothyroidism by methimazole was relatively tedious since it required daily injection of the drug for an extended period of time. The physical and behavioural alterations seen in chemically thyroidectomized rats were similar to those observed following radiothyroidectomy.

III. HYPERTHYROIDISM

Hyperthyroidism was induced by daily administration of L-triiodothyronine subcutaneously in a dose of 10 µg/100 g body weight for a period of 30 days (230). Neonatal hyperthyroidism was produced by beginning L-triiodothyronine treatment in one-day old rats, whereas 120-day old animals were employed for producing adult hyperthyroidism. The mortality rate in both neonatal as well as adult hyperthyroid rats was almost negligible.

IV. MEASUREMENT OF SPONTANEOUS LOCOMOTOR ACTIVITY

The spontaneous locomotor activity was quantitated with the use of a Selective Activity Meter, model SE (Columbus Instruments, Columbus, Ohio).

This was always done under identical conditions at the same time of the day in a temperature controlled, sound proof room. One experimental or control rat was removed and placed in a cage on the Selective Activity Meter for 5 minutes for exploration before the actual recording of the activity over a 30 minute session.

V. BIOCHEMICAL DETERMINATIONS

A. Tissue Preparation

Animals were sacrificed using the 'near-freezing' technique of Takahashi and Aprison (234). Following decapitation, the skin overlying the skull was cut in the midline and reflected laterally. One tip of a pair of scissors was introduced into the foramen magnum and a midline cut was made through the sagittal suture of the skull. Perpendicular cuts were then made through the coronal sutures and the bone flaps were reflected. The exposed brain was teased out of the cranial vault with the tip of a spatula, stripped of adherent meningeal tissue and grossly visible blood vessels and weighed on a torsion balance (Federal Pacific Electric Co., Newark, N.J.). The whole brain (minus olfactory tubercles, cerebellum and medulla oblongata) was divided longitudinally into two halves; each left half was used to determine the activity of tyrosine hydroxylase and tryptophan hydroxylase as well as the levels of tyrosine and tryptophan, whereas the right half of the brain was employed for measuring the concentrations of norepinephrine, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid.

B. Dissection of Brain into Different Regions

In order to study the regional distribution of norepinephrine and 5-hydroxytryptamine in hypo- and hyperthyroid rats, the brain was separated into seven specific areas according to the procedure of Glowinski and Iversen (235). The various regions examined included cerebellum, pons-medulla,

hypothalamus, mid-brain, striatum, hippocampus and finally, cerebro-cortex. Since 4 of these regions contain more than one distinct anatomical structure, it is appropriate to describe them more precisely. The 'pons-medulla' corresponds to medulla oblongata and pons; 'hypothalamus' to thalamus and subthalamus (the latter structure being a part of the diencephalon), whereas the 'striatum' incluced the putamen nucleus, caudate nucleus (striatum) and the globus pallidus nucleus (i.e. the basal ganglia of the telencephalon without the amygdala). The 'cerebro-cortex' corresponds to the telencephalon without the 'striatum' and included white and gray matter of the cerebral cortex (235).

The dissection of the brain was performed as follows: first the rhombencephalon was separated by a transverse section from the rest of the brain and dissected into two parts; the 'cerebellum' and the 'pons-medulla.' Another transverse section was then made at the level of 'optic chiasma' which delimits the anterior part of the hypothalamus and passes through the anterior commissure. This section separated the cerebrum into two parts, anterior and posterior. The posterior part of the cerebrum was dissected into 5 regions. First the 'hypothalamus' was dissected by taking the anterior commissure as a horizontal reference and the line between the posterior hypothalamus and the mammillary bodies as the caudal limit. The 'striatum' was dissected with external walls of the lateral ventricles as internal limits and the corpus callosum as external limits. The frontal parts of the 'striatum', which are in the anterior portion of the cerebrum, were dissected separately and combined with the 'striatum' removed from the posterior part of the cerebrum. The 'mid-brain' was gently separated from the remaining part of the brain. The hippocampus was then dissected. The remaining posterior part of the cerebrum was combined with the remaining anterior part of the cerebrum to form the 'cerebro-cortex.' The 'striatum'

dissected from the left half of the brain was employed to determine the activity of tyrosine hydroxylase and tyrosine level, whereas the striatum obtained from the right half of the brain was frozen, stored and later used for measuring the concentrations of biogenic amines. The 'mid-brain' was divided longitudinally into two halves. The left half was employed for assaying the activity of tryptophan hydroxylase as well as the endogenous levels of tryptophan whereas the right half was frozen and stored for future determination of monoamine levels. The remaining brain regions were quickly frozen by immersing in liquid nitrogen and then stored at -20°C for no longer than 3-4 days before the levels of norepinephrine, 5-hydroxytryptamine and its chief metabolite, 5-hydroxyindoleacetic acid were determined. Mention may be made of the fact that quick freezing and storing the brain tissue at -20°C for as long as 3 weeks produced no effect on the concentrations of these biogenic amines. The 'cerebro-cortex' was employed to assay the activity of monoamine oxidase and catechol-o-methyl transferase enzymes immediately after the dissection.

C. Homogenization and Sample Preparation for Enzyme Assays

Homogenization was carried out in a chilled Potter-Elvehjem glass homogenizer (Fisher Scientific Co., Fairlawn, N.J.) tightly fitted with a teflon pestle turning at about 700 r.p.m. in 20 volumes of ice-cooled, 0.28 M sucrose solution for exactly 60 seconds.

(i) Tyrosine hydroxylase

The activity of tyrosine hydroxylase was measured according to the method of McGeer et al. (87), and expressed as nmoles DOPA formed/g wet weight of brain tissue/hour. The following components were added to the incubation mixture (final volume, 0.6 ml) in the given sequence: 0.4 ml 0.28 M potassium phosphate buffer (pH 6.2) containing 140,000-165,000 disintegrations/

minute of L-(^{14}C) tyrosine (uniformly labelled; specific activity of 485-507 mCi/mmol), 10^{-3}M N-methyl-N-3-hydroxybenzylhydrazine (NSD-1034) and 0.2 ml brain homogenate. Since preliminary studies demonstrated that the presence of pteridine cofactor does not significantly affect tyrosine hydroxylase activity in crude brain homogenates, this was not added in the incubation mixture (87,236). Tissue blanks were run in duplicate by adding to the reaction mixture, 0.2 ml of the homogenate which has been boiled at 100°C for 15 minutes and then cooled on ice. The reaction was initiated by incubating the tubes at 37°C for 30 minutes in a water bath shaker (Eberback Corp., Ann Arbor, Mich.) and terminated by adding 4 ml of a 1:1 mixture of 0.4 N perchloric acid and 0.2 N acetic acid. The tubes were spun for 10 minutes at 7,000 r.p.m. in a refrigerated Sorvall centrifuge, model RC2-B (Ivan Sorvall, Norwalk, Conn.). The supernatant was then transferred into a 10 ml beaker containing 1.5 ml of 0.2 M EDTA and 3 ml of 0.28 M KH_2PO_4 (pH 6.2). The pH was adjusted within the range of 8.8 - 9.2 and adsorption of ^{14}C-labeled DOPA on alumina was carried out by adding 200 mg neutral alumina and gently spinning a bar magnet for exactly 4 minutes. The mixture was transferred to a glass column plugged at the bottom with cotton. ^{14}C-labeled DOPA was then eluted with 4 ml of 0.5 N acetic acid. Fifteen ml of Bray's mixture (237) was added and the resulting solution counted in a Beckman LS-150 Scintillation Counter (Beckman Instruments Inc., Fullerton, Calif.).

(ii) Tryptophan hydroxylase

Tryptophan hydroxylase activity was measured in brain homogenates according to the method of Peters et al. (131) and expressed as nmoles/g wet weight of brain tissue/hour. The assay was based upon ion-exchange separation of the labeled serotonin produced from incubation of L-(^{14}C)

tryptophan with tissue homogenates. L-(^{14}C) tryptophan was hydroxylated to 5-hydroxytryptophan which in turn was enzymatically decarboxylated to 5-hydroxytryptamine by the large excess of 5-hydroxytryptophan decarboxylase normally present in brain. Destruction of serotonin was prevented by inclusion of a monoamine oxidase inhibitor. The following components were added to the reaction mixture (final volume 0.6 ml) in the given sequence: 0.04 ml of L-(^{14}C) tryptophan uniformly labeled 11x10^{6} disintigrations/minute (50 mCi/mmol); 0.1 ml of 0.5 M Tris acetate buffer (pH 7.8); 0.12 mg pargylene, 0.26 ml distilled water and 0.2 ml brain homogenate. Tissue blanks were run in duplicate by adding to the reaction mixture, 0.2 ml of the homogenate which has been boiled at 100^{o}C for 15 minutes and then cooled on ice. The reaction was initiated by incubating the tubes at 37^{o}C for 30 minutes in a water bath shaker (Eberbach Corp., Ann Arbor, Mich.) and was stopped by adding 2 ml of a 1:1 mixture of 0.4 N perchloric acid and 0.2 N acetic acid. The tubes were spun for 10 minutes at 7,000 r.p.m. in a refrigerated Sorvall centrifuge, model RC2-B (Ivan Sorvall, Norwalk, Conn.). The supernatants were transferred into 15 ml beakers containing 2 ml of 0.5 M Tris buffer and brought to a pH of 7.0. For the ion exchange columns, glass tubes (0.6 cm internal diameter and a length of 12 cm) topped by a 50 ml reservoir and plugged with cotton at the tip were used. The superantant fluid was passed through columns of CG-50 type I resin with a bed of 2.5 cm. The ^{14}C 5-hydroxytryptamine was eluted from the columns with 5.0 ml of 4 N acetic acid. Fifteen ml of Bray's mixture (237) was added and the resulting solution counted in a Beckman LS-150 scintillation counter (Beckman Instruments Inc., Fullerton, Calif.).

(iii) Monoamine oxidase

Monoamine oxidase activity was determined by measuring the deaminated

^{14}C-metabolites of ^{14}C-tryptamine according to the method of Wurtman and Axelrod (238). In a typical assay, 40 µl of homogenate was mixed with 0.1 ml 0.5 M Tris-acetate buffer, pH 7.4, 0.3 ml water and 0.1 ml ^{14}C tryptamine (10^6 disintegrations/minute; 60 mCi/mmol) and incubated at 37°C for 15 minutes. The reaction was terminated by the addition of 2 N HCl. The ^{14}C-deaminated metabolites were extracted into toluene. After centrifugation, 2 ml aliquot of the organic layer was transferred to a counting vial, followed by addition of toluene based phosphor solution and counted in a scintillation counter. Tissue blanks were run in duplicate by incubating ^{14}C-tryptamine with boiled homogenates as in the case of tyrosine hydroxylase and tryptophan hydroxylase assays.

(iv) Catechol-O-methyl transferase

The activity of brain catechol-O-methyl transferase was measured according to the method of McCaman (239) adapted from D'Iorio (240). S-Adenosyl L-methionine-iodide (methyl-^{14}C) was used as the methyl donor and 3,4-dihydroxybenzoic acid as the substrate. The method was based on the measurement of ^{14}C-O-methylated product. The following components at the designated final concentrations were added to the reaction mixture (final volume, 125 µl) in the given sequence: potassium phosphate (pH 7.8), 0.08 M; $MgCl_2$, 5×10^{-3}M; 3,4-dihydroxybenzoic acid, 10^{-3} M; S-adenosyl L-methionine-iodide (methyl-^{14}C), 6×10^{-5}M and brain homogenate, 20 µl. The tubes were incubated at 37°C for 30 minutes and the reaction terminated by the addition of 3 N HCl. ^{14}C-O-methylated metabolites were extracted in ethyl acetate. After centrifugation, an aliquot of the organic layer was transferred to a scintillation counting vial, a toluene based phosphor solution added and the radioactivity determined. Results were corrected for blank values obtained by carrying out a similar incubation procedure but without any

added substrate.

D. Sample Preparation for Assaying Endogenous Levels of Amino Acids

Brain tissues were homogenized in 20 volumes of 0.28 M ice-cooled sucrose (freshly prepared) in a glass homogenizer fitted with a teflon pestle. Aliquots of 0.5 ml homogenate were transferred into tubes containing 2 ml of 8% trichloroacetic acid, mixed and left on ice for 10 minutes and then centrifuged in a clinical centrifuge (International Equipment Co., Boston, Mass.). The supernatant fluids were decanted into glass tubes and 0.5 ml aliquots employed to determine the endogenous levels of tyrosine and tryptophan.

(i) Tyrosine

Brain tyrosine levels were determined essentially according to the procedure of McGeer et al. (87), adapted from Waalkes and Udenfriend (241). The values are expressed as micrograms of tyrosine per g wet weight of brain tissue.

(ii) Tryptophan

Endogenous brain tryptophan levels were measured according to the method of Hess and Udenfriend (242) and also expressed as micrograms per g wet weight of the tissue. 0.5 ml aliquots of deproteinized supernatants were transferred into glass tubes followed by the addition of 2 ml water and 0.1 ml 18% formaldehyde. The tubes were stoppered loosely and immersed in a boiling water bath for 20 minutes. 0.1 ml of 5% hydrogen peroxide was then added to each tube and the resultant mixtures were reheated for a period of 20 minutes. After cooling to room temperature, fluorescence was measured using an Aminco-Bowman spectrophotofluorometer (American Instrument Co. Inc., Silver Spring, Md.) by reading samples at 365 nm while activating at 320 nm. Blanks were always run in duplicate using an equal volume of trichloroacetic acid.

E. Homogenization and Extraction Procedures for Determining Biogenic Amines and Metabolites

The whole brain was homogenized in 10 ml of chilled 0.1% acidified butanol. Homogenization was effected with a chilled Potter Elvehjem homogenizer (Fisher Scientific Co., Fairlawn, N.J.) fitted with a teflon pestle spinning at about 700 r.p.m. for exactly 90 seconds. The extraction procedures for norepinephrine, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid were similar to those described by Maickel et al. (243) and Curzon and Green (244). The homogenate was centrifuged at about 6,000 r.p.m. using a clinical centrifuge (International Equipment Co., Boston, Mass.). The supernatant was decanted and an aliquot of 7.5 ml was transferred to a 50 ml centrifuge tube containing 15 ml n-heptane and 1.5 ml 0.1 N HCl. The amines were extracted in the acidic layer. The tubes were centrifuged and the aqueous layer was removed to determine the levels of norepinephrine and 5-hydroxytryptamine. A volume of 15 ml of the organic layer was transferred to another set of tubes containing 2 ml of 0.5 M Tris acetate (pH 7.0) to extract 5-hydroxyindoleacetic acid. The reagent blanks were always carried out in duplicate using 7.5 ml 0.1% acidified butanol.

For determining amine concentrations in specific brain regions, these were separately homogenized in 3 ml 0.1% acidified butanol and 2.5 ml of the supernatant was extracted with 5 ml n-heptane and 1.5 ml 0.1 N HCl. After the amine extraction, 5-hydroxyindoleacetic acid, an indoleamine metabolite, was extracted into 0.6 ml of 0.5 M Tris acetate (pH 7.0) from 5 ml volume of the organic layer.

(i) Norepinephrine

The levels of this brain catecholamine were determined according to the method of Laverty and Taylor (245). The method is based on the prod-

uction of a fluorescent indole derivative ("lutin") by means of oxidation and subsequent rearrangement. 0.5 ml portion of the acidic extract was then transferred to a glass tube. To form a fluorophor, 0.05 ml of iodine solution was added, followed by 0.5 ml alkaline sulfite reagent after 4 minutes and 0.2 ml glacial acetic acid 5 minutes later. The tubes were left at room temperature for 20 minutes and fluorescence measured in an Aminco-Bowman spectrophotofluorometer (American Instrument Co. Inc., Silver Spring, Md.) by reading at 380 nm while activating at 480 nm. Blanks, in duplicate, were run simultaneously.

(ii) 5-Hydroxytryptamine

The levels of this indoleamine were measured according to the method of Curzon and Green (244). The procedure is based on the fact that 3,5-substituted indoles form highly fluorescent complexes with o-phthalaldehyde. 0.5 ml portion of the acidic extract was pipetted into a tube to which were added 1.2 ml of 0.004% o-phthalaldehyde in 10 N HCl and 0.02 ml of 1% cysteine. After mixing, the tubes were heated in a water bath at 60°C for 15 minutes, cooled and fluorescence measured in an Aminco-Bowman spectrophotofluorometer (American Instrument Co. Inc., Silver Spring, Md.) by reading at 360 nm while activating at 470 nm. Blanks, in duplicate, were run simultaneously.

(iii) 5-Hydroxyindoleacetic acid

Brain 5-hydroxyindoleacetic acid levels were determined by the method of Curzon and Green (244). 0.4 ml portion of the Tris-acetate extract was transferred to a tube and the following reagents were added in the given sequence: 0.05 ml 1% cysteine; 1.0 ml concentrated HCl; 0.05 ml 0.1% o-phthalaldehyde in methanol and 0.05 ml .02% sodium periodate. The tubes were immersed in a water bath at 60°C for 15 minutes and after cooling to room temperature, fluorescence was measured in an Aminco-Bowman spectro-

photofluorometer (American Instrument Co. Inc., Silver Spring, Md.) by reading at 360 nm while activating at 470 nm. Blanks, in duplicate, were run simultaneously.

F. Chemicals

All reagents were of the purest grade available and dissolved in glass distilled water. DL-Arterenol, L-tyrosine, 6,7-dimethyl 5,6,7,8-tetrahydropteridine ($DMPH_4$), 3-(3,4-dihydroxyphenyl)-L-alanine, serotonin cretinine sulfate, 5-hydroxyindoleacetic acid, L-tryptophan, o-phthalaldehyde were purchased from Calbiochem (La Jolla, Calif.). L-Cysteine, S-adenosyl-L-methionine iodide were purchased from Sigma Chemical Co. (St. Louis, Mo.). 3,4-Dihydroxybenzoic acid was purchased from J.T. Baker Chemical Co. (Philipsburg, N.J.). Amberlite resin CG-50(H) was obtained from British Drug House (Montreal, Que.). L-(methylene-^{14}C) tryptophan, L-(^{14}C) tyrosine and S-adenosyl-L-methionine-iodide (methyl-^{14}C) were purchased from Amersham/Searle Corp. (Arlington Heights, Ill.). Tryptamine bisuccinate (2-^{14}C) was purchased from New England Nuclear (Boston, Mass.). N-Methyl-N-3-hydroxybenzylhydrazine (NSD-1034) was purchased from Smith and Nephew Ltd., (Lachine, Que.). 2-Mercaptoethanol was purchased from Eastman Kodak Co. (Rochester, N.Y.) whereas pargyline was kindly donated by Abbott Laboratories, Chicago, Ill.

Methimazole was obtained through the kind courtesy of Dr. Dolman, Eli Lilly and Co., Toronto, Ont. Desmethylimipramine was provided by Geigy Pharmaceuticals, Dorval, Quebec and was freshly dissolved in physiological saline just prior to injection. L-triiodothyronine (Sigma Chemical Co.) was dissolved in 0.02 N NaOH and administered by the subcutaneous route. ^{131}I was obtained from Atomic Energy of Canada, Ottawa and injected intraperitoneally. Lithium carbonate was dissolved in physiological saline

and the pH was adjusted to 7.4 with citric acid. In each case, control animals were injected with an equal volume of the appropriate vehicle solution.

VI. STATISTICAL ANALYSIS

The results were subjected to statistical evaluation using the Student's "t-test," and significant differences between the means (calculated as p values) are shown. No statistical significance is indicated when the p value was >0.05.

4. RESULTS

I. INFLUENCE OF RADIOTHYROIDECTOMY

A. Effect of Neonatal Administration of ^{131}I on Physical and Behavioural Development

The treatment of one-day old rats with a dose of 200 µCi of ^{131}I induced typical signs of hypothyroidism as evidenced by retarded growth, eyelid dysjunction, elevation of the pinnae and snout elongation. The fur was dry and coarse and some animals exhibited a bilaterally symmetrical alopecia of the abdominal and flank regions. The hypothyroid rats also showed altered demeanour when compared to that of control littermates. These animals appeared listless and relatively immobile. There was retardation in the development of righting and placing reflexes in thyroidectomized rats. Some animals exhibited a peculiar high-stepping gait while others showed fine tremors of the head and limbs. Figure 3 shows a photograph comparing the appearance of a normal 15 day old rat with that of a litter-mate treated with 200 µCi of ^{131}I at 1 day of age. There appeared to be no noticeable difference in the appearance and growth of neonatally thyroidectomized rats at 15 days when compared to controls. However, at 30 days of age, ^{131}I-treated rats displayed typical signs of cretinism as described above (Fig. 4). Results illustrated in Figure 5 show the developmental pattern of body and brain weights between 1 and 60 days of neonatal life in normal and thyroidectomized rats. These data show that in all groups examined, there was a statistically significant decrease in body and brain weights of hypothyroid rats when compared with the corresponding values of littermate controls. The decrease in body and brain weights become quite apparent at 28 days of age, after which only a slight change was noted in either parameter in hypothyroid animals.

Fig. 3. Photograph comparing the appearance of a normal 15 day old rat with its litter mate treated with 200 μCi of ^{131}I at 1 day of age. Normal - left; ^{131}I-treated - right.

Fig. 4. Photograph comparing the appearance of a normal 30 day old rat with its litter mate treated with 200 μCi of ^{131}I at 1 day of age. Normal - left; ^{131}I-treated - right.

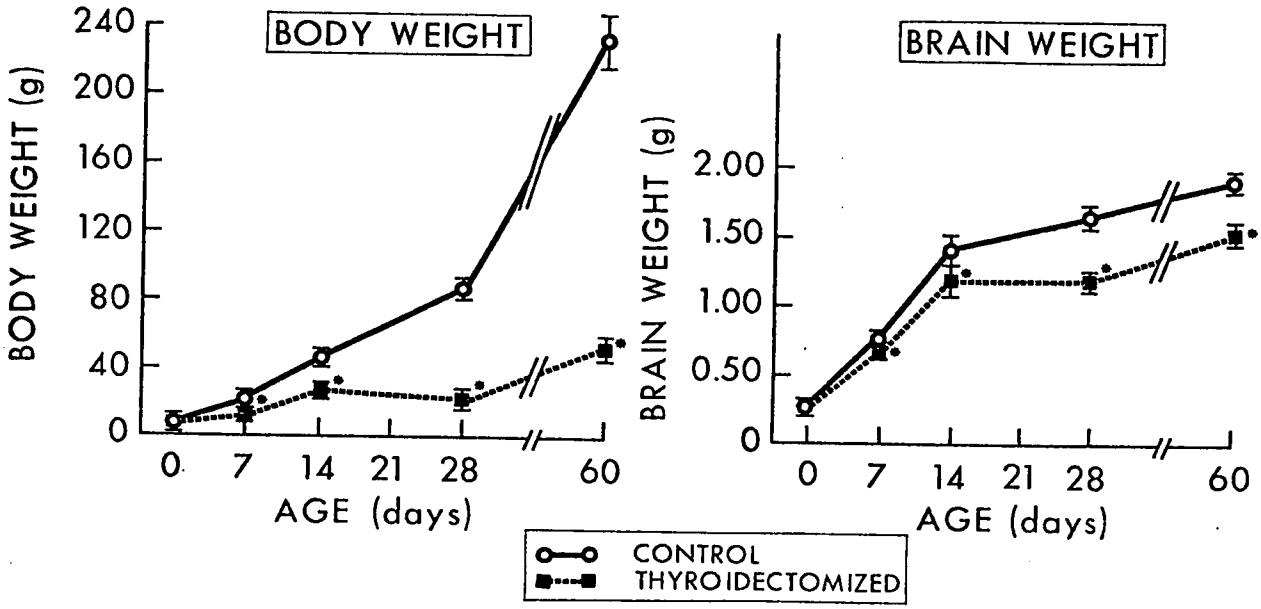

Fig. 5. Influence of neonatal thyroidectomy on body and brain weights. Each point represents the mean ± S.E.M. of 6 values in the group. One-day old rats were injected intraperitoneally with 200 μCi of ^{131}I and killed after 0, 7, 14, 28 or 60 days.

*Statistically significant differences in comparison to the values seen in littermate controls of the corresponding age group ($p < 0.05$).

The ontogenic pattern of spontaneous locomotor activity in normal and thyroidectomized rats are shown in Figure 6. At 3 days of age, the spontaneous locomotor activity of normal rats was only about 3% of the adult values. Rapid increases in the locomotor activity were noted during the first 30 days of life, after which it seemed to drop. Results in Figure 6 also demonstrate that radiothyroidectomy led to marked interference in the normal ontogeny of spontaneous locomotor activity. Hypothyroid rats displayed maximal suppression in their mobility at 15 days of age, when the spontaneous locomotor activity was only 26% of the corresponding controls. Although the spontaneous locomotor activity of 45 and 60 day old hypothyroid rats remained at a lower level than that seen in the corresponding controls, the changes were statistically non-significant.

B. Alterations in Whole Brain Norepinephrine and 5-Hydroxytryptamine Metabolism

The developmental changes in brain norepinephrine level and the activity of tyrosine hydroxylase are shown in Figure 7. At birth, norepinephrine levels were only about 24% of the adult values. The largest increase was observed between the 7th and the 28th day when the concentration of this catecholamine attained almost the adult values. Rapid increases were observed in brain tyrosine hydroxylase activity during the first 15 days of life; thereafter, the enzyme activity seemed to remain relatively unchanged. In addition, results of Figure 7 demonstrate that neonatal thyroidectomy resulted in marked interference of ontogenic increases in brain norepinephrine and tyrosine hydroxylase activity observed in normal rats. However, the activity of brain tyrosine hydroxylase was slightly higher in neonatally hypothyroid rats than in controls during the first week of life which started to decline at around 10 days of age.

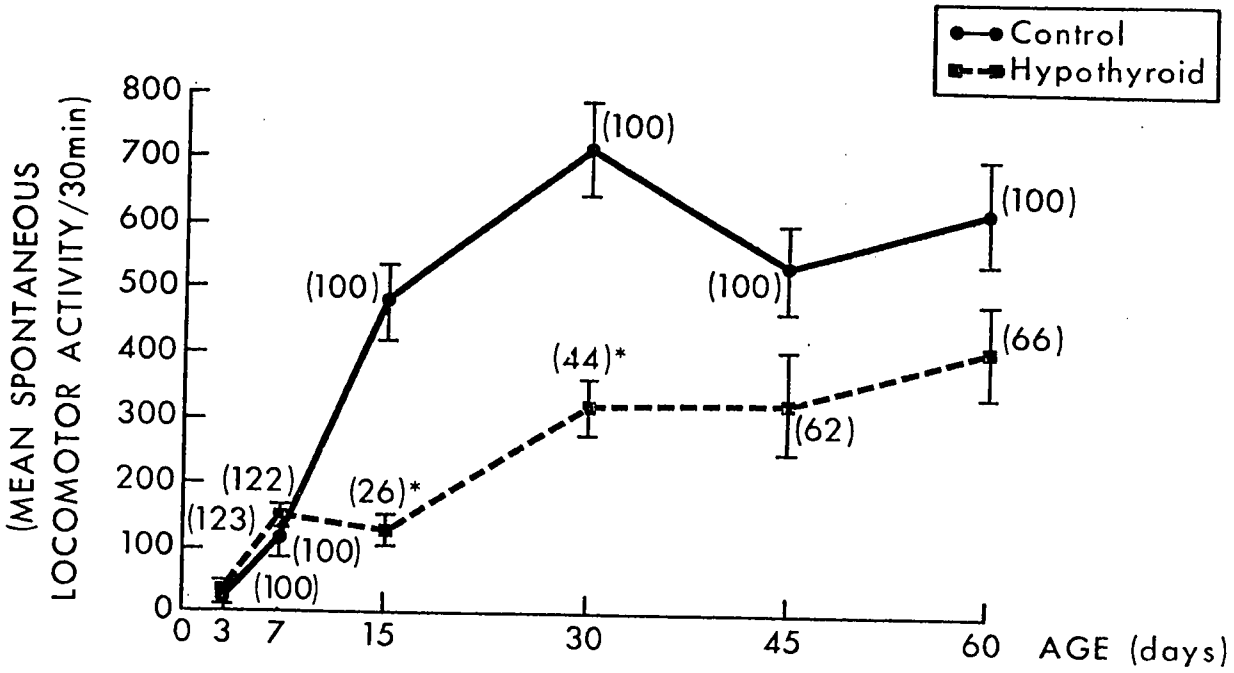

Fig. 6. Effect of neonatal hypothyroidism on the ontogenesis of spontaneous locomotor activity. Each point represents the mean ± S.E.M. of 8 rats. One-day old rats were injected intraperitoneally with 200 µCi of ^{131}I. Control rats received an equal volume of physiological saline. The spontaneous locomotor activity was determined for a 30 min session. Data in parentheses express results in percantages taking the values for corresponding control animals as 100%.

*Statistically significant differences when compared with the values of littermate controls of the corresponding age group ($p < 0.05$).

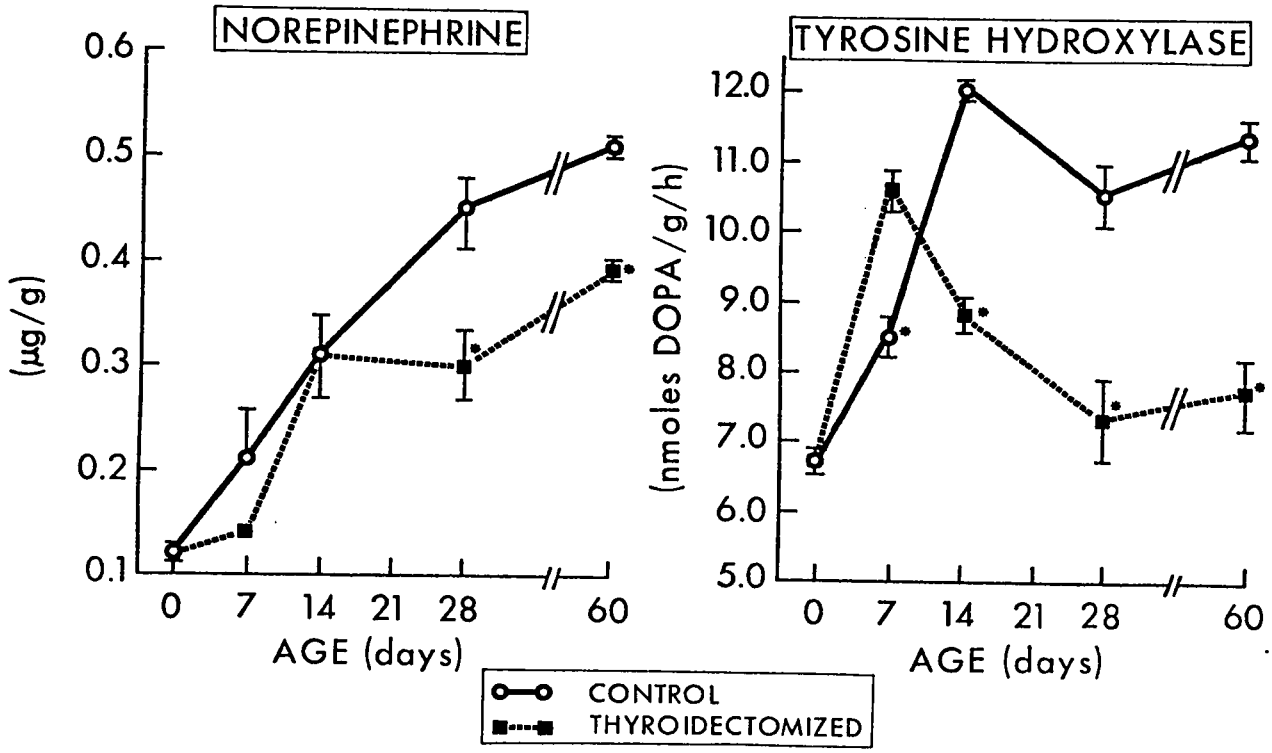

Fig. 7. Effects of neonatal thyroidectomy on the ontogenesis of brain NE and TH activity. Each point represents the mean ± S.E.M. of 6 values in the group. Rats at one day of age were treated intraperitoneally with 200 µCi of ^{131}I and killed after 0, 7, 14, 28 or 60 days.

*Statistically significant alterations in comparison to the values seen in littermate controls of the corresponding age group ($p < 0.05$).

Since a functional interplay seems to exist between various neurotransmitters, any modification in noradrenergic pathway would be expected to affect the function of other central amine systems. Therefore, the effect of radiothyroidectomy on the developmental pattern of 5-hydroxytryptamine also was examined. Figure 8 illustrates the developmental changes in 5-hydroxytryptamine levels and the activity of brain tryptophan hydroxylase. Concentration of brain 5-hydroxytryptamine seemed to be low at early stages of development, being only about one-third of the adult values on the first day of postnatal life. The largest increase was observed between the 7th and the 28th day of age when the level of 5-hydroxytryptamine attained almost the adult values. The activity of brain tryptophan hydroxylase increased approximately 2½-fold during the first 2 weeks of life and gradually reached its peak value by 60 days. The level of brain 5-hydroxyindoleacetic acid exhibited a pronounced increase between 7 and 14 days postpartum, when it seemed to attain adult values (Fig. 9). An impairment of the ontogenic increases in brain tryptophan hydroxylase activity as well as in 5-hydroxytryptamine levels was seen in rats subjected to radiothyroidectomy at birth which became more pronounced as the animals became older. The levels of 5-hydroxyindoleacetic acid, however, seemed to be relatively higher in brains of thyroidectomized rats and were significantly increased in 28-day old hypothyroid rats when compared to the control values (Fig. 8, 9). In contrast to the observed changes in 5-hydroxytryptamine and norepinephrine, the concentrations of brain tyrosine and tryptophan remained unaltered in hypothyroid rats when compared with control animals of the corresponding age group. Data presented in Table 1 demonstrate that the brains of 30-day old thyroid-deficient rats contained slightly lower levels of these two amino acids although the changes were statistically non-significant ($p < 0.05$).

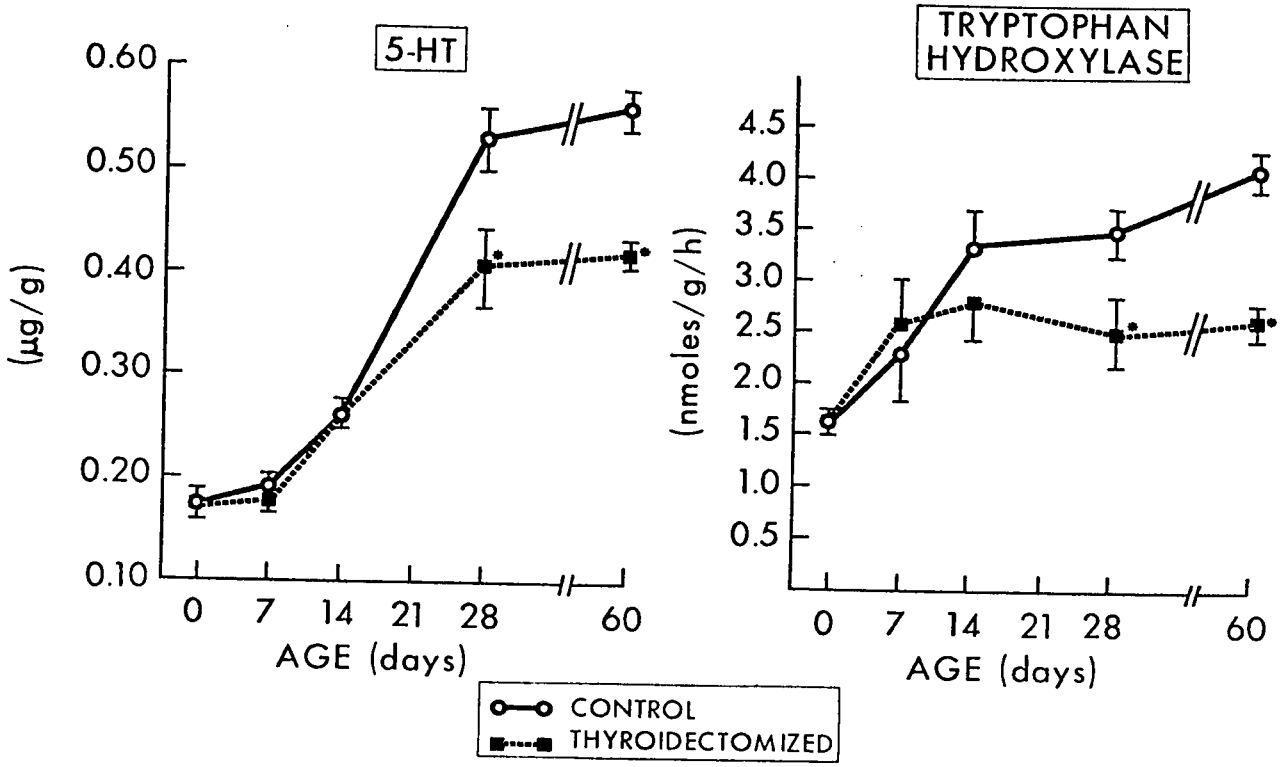

Fig. 8. Influence of neonatal thyroidectomy on the ontogenesis of brain 5-HT and TPH activity. Each point represents the mean ± S.E.M. of 6 values in the group. One-day old rats were injected intraperitoneally with 200 µCi of ^{131}I and killed after 0, 7, 14, 28 or 60 days.

*Statistically significant alterations when compared to the values of littermate controls of the corresponding age group ($p < 0.05$).

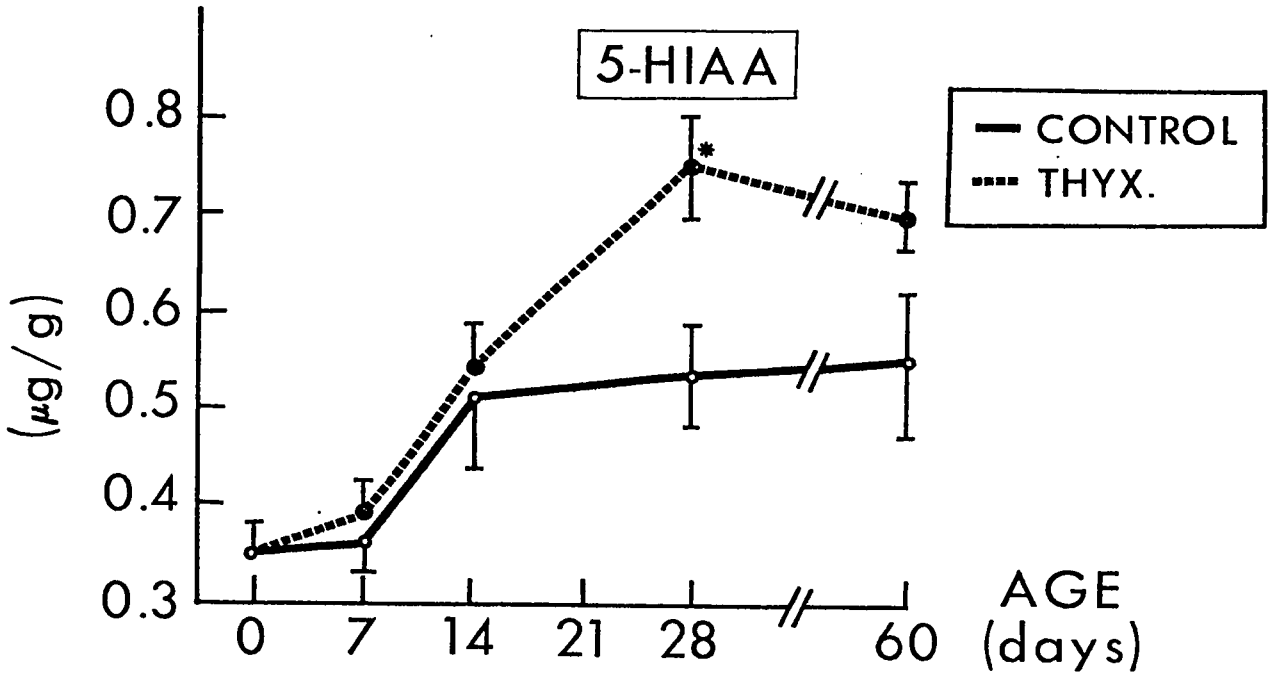

Fig. 9. Effect of neonatal thyroidectomy on the ontogenesis of brain 5-HIAA. Each point represents the mean ± S.E.M. of 6 animals in the group. One-day old rats were injected intraperitoneally with 200 μCi of ^{131}I or saline and killed after 0, 7, 14, 28 or 60 days.

*Statistically significant difference when compared with normal values of corresponding age group ($p < 0.05$).

TABLE 1

EFFECT OF NEONATAL HYPOTHYROIDISM ON BRAIN TR AND TP

Values represent the means ± S.E.M. of 6 rats in each group. One-day-old rats were injected i.p. with 200 μCi of ^{131}I and the levels of TR and TP were determined at 30 days of age. Control animals received an equal volume of physiological saline. Data in parentheses express the results in percentages of controls taken as 100%.

Treatment	TR (μg/g)	TP (μg/g)
Control	18.3±1.13 (100)	2.3±0.09 (100)
^{131}I	16.3±1.82 (89) θ	2.16±0.11 (94) θ

θStatistically non-significant difference when compared with the values of control animals ($p > 0.05$).

C. Influence of Varying Doses of ^{131}I

Data in Table 2 demonstrate the influence of varying dosages of ^{131}I on body and brain weights as well as on norepinephrine levels and tyrosine hydroxylase activity. Whereas 50 μCi of ^{131}I exerted only little effect, 100 and 200 μCi doses of the radioisotope produced marked interference with the ontogenic increases in brain norepinephrine levels and the activity of tyrosine hydroxylase. As expected, the impairment of body and brain growth was also more marked in rats treated with the 200 μCi dose of the radioisotope. Histological studies by Schwark (230) had earlier demonstrated that whereas ^{131}I in a dose of 200 μCi produced discrete destruction of thyroid gland without affecting the parathyroid, lower doses of ^{131}I failed to produce total thyroidectomy. Data presented in Table 3 show that alterations in 5-hydroxytryptamine metabolism also were related to the dose of the radio-isotope. When ^{131}I was administered in a dosage of 50 μCi, changes in various neurochemical parameters related to 5-hydroxytryptamine metabolism did not significantly differ from controls. However, increasing the dose of ^{131}I to 200 μCi resulted in marked interference of the ontogenic changes in brain tryptophan hydroxylase activity as well as 5-hydroxytryptamine and 5-hydroxyindoleacetic acid levels at 30 days of age. These data indicate that the observed decreases in the metabolism of norepinephrine and 5-hydroxytryptamine in the developing brain are related to the degree of thyroid gland dysfunction produced by radio-iodine. The maximal suppression in norepinephrine and 5-hydroxytryptamine levels as well as in the activity of synthesizing enzymes was noted in the group of animals treated with 200 μCi of ^{131}I, a dose which has previously been found to produce total thyroidectomy in rats.

TABLE 2

EFFECT OF VARYING DOSAGES OF ^{131}I ON BODY AND BRAIN WEIGHTS AS WELL AS BRAIN NE AND TH ACTIVITY

Values represent the means ± S.E.M. of 5 animals in each group. One-day-old rats were injected intraperitoneally with varying dosages of ^{131}I and killed at 30 days of age. Data in parentheses indicate the percentages of control values which are taken as 100%.

Dose of ^{131}I (μCi/rat)	Body wt. (g)	Brain wt. (g)	NE (μg/g)	TH (nmoles DOPA/g/h)
Control	86±5.0 (100)	1.63±0.02 (100)	0.45±0.03 (100)	10.55±0.45 (100)
50	75±1.83 (87)	1.45±0.02 (89)	0.42±0.03 (93)	9.50±0.40 (90)
100	32.25±2.1 (37)*	1.28±0.03 (78)*	0.39±0.03 (87)	7.81±0.27 (74)*
200	23.8±2.43 (27)*	1.18±0.03 (72)*	0.30±0.03 (66)*	7.30±0.60 (69)*

*Statistically significant difference when compared with the values of control rats ($p < 0.05$).

TABLE 3

EFFECT OF VARYING DOSAGES OF ^{131}I ON BRAIN TPH ACTIVITY AS WELL AS ON THE CONCENTRATION OF 5-HT AND 5-HIAA

Each value represents the mean ± S.E.M. of 5-6 animals in each group. One-day-old rats were injected i.p. with varying doses of ^{131}I and killed at 30 days of age. Data in parentheses express results in percentages taking the values of control rats as 100%.

Dose of ^{131}I (μCi/rat)	TPH (nmoles/g/h)	5-HT (μg/g)	5-HIAA (μg/g)
Control	3.45±0.24 (100)	0.53±0.03 (100)	0.52±0.05 (100)
50	4.06±0.54 (118)	0.47±0.02 (89)	0.53±0.04 (102)
100	3.17±0.46 (92)	0.44±0.02 (83)*	0.58±0.05 (111)
200	2.53±0.34 (73)*	0.41±0.04 (77)*	0.72±0.05 (136)*

*Statistically significant difference when compared with the control values ($p < 0.05$).

D. Changes in Norepinephrine and 5-Hydroxytryptamine Metabolism in Discrete Brain Regions

It has been suggested that studies using the whole brain generally reflect changes of larger brain regions such as the cortex which may mask even the most pronounced alterations seen in specific brain regions. We were therefore prompted to examine the effect of neonatal thyroidectomy on norepinephrine and 5-hydroxytryptamine metabolism in various brain regions. Data presented in Figure 10 demonstrate that norepinephrine was distributed unevenly in various brain regions of control animals. Hypothalamus contained the highest level of this putative neurotransmitter with a decreasing order of concentration in mid-brain, cerebellum, pons-medulla and striatum. Neonatal radiothyroidectomy markedly reduced the endogenous levels of this catecholamine in hypothalamus, pons-medulla and striatum. Whereas hypothyroidism in developing rats failed to exert any appreciable effect on norepinephrine content of cerebellum, a 22% rise in the level of this neurohumor was noted in mid-brain. In addition, hypothyroidism led to a marked decrease in the activity of striatal tyrosine hydroxylase. The concentration of striatal tyrosine, the precursor of catecholamines, seemed to remain at a slightly lower level although the change was statistically non-significant.

Results presented in Figures 11-13 demonstrate that in control animals, the hypothalamus contained the highest level of 5-hydroxytryptamine and its chief metabolite, 5-hydroxyindoleacetic acid; the mid-brain being the next and the cerebellum showed the lowest levels. Figures 11-13 also show that neonatal hypothyroidism resulted in significant decreases in the levels of 5-hydroxytryptamine in cerebellum, mid-brain and striatum. Whereas the concentration of this amine remained unchanged in hypothalamus, a significantly higher level of 5-hydroxytryptamine was noted in pons-medulla of

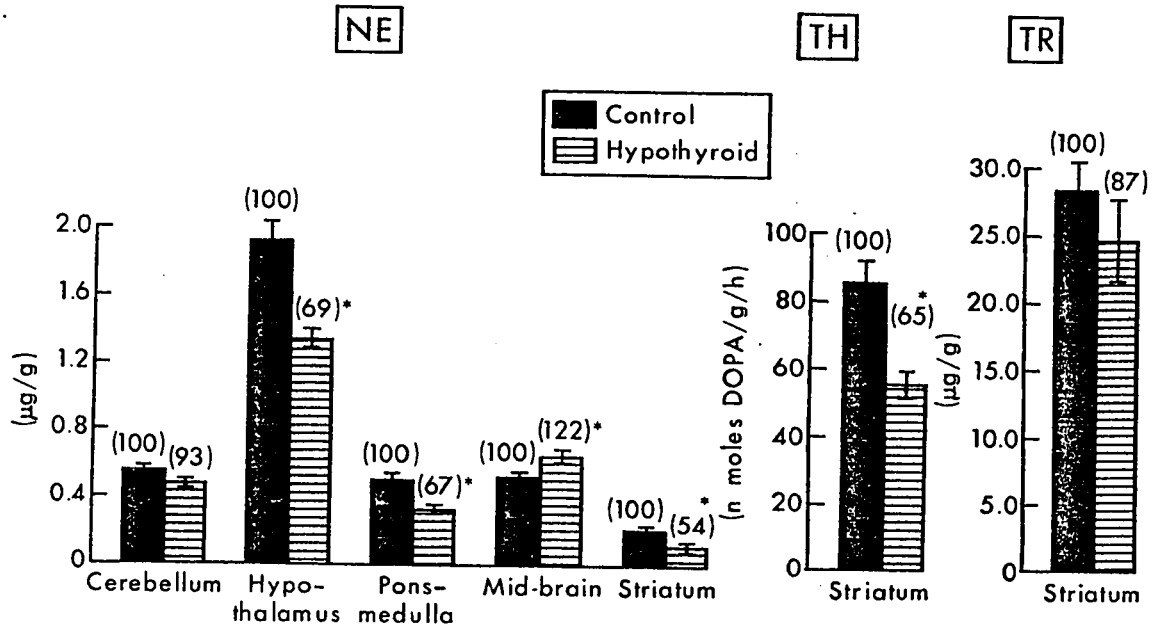

Fig. 10. Effect of neonatal hypothyroidism on NE levels in certain brain regions and on striatal TH and TR. Each bar represents the mean ± S.E.M. of 6 animals in each group. One-day old rats were injected intraperitoneally with 200 µCi of ^{131}I and killed at 30 days of age. Control rats received an equal volume of physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

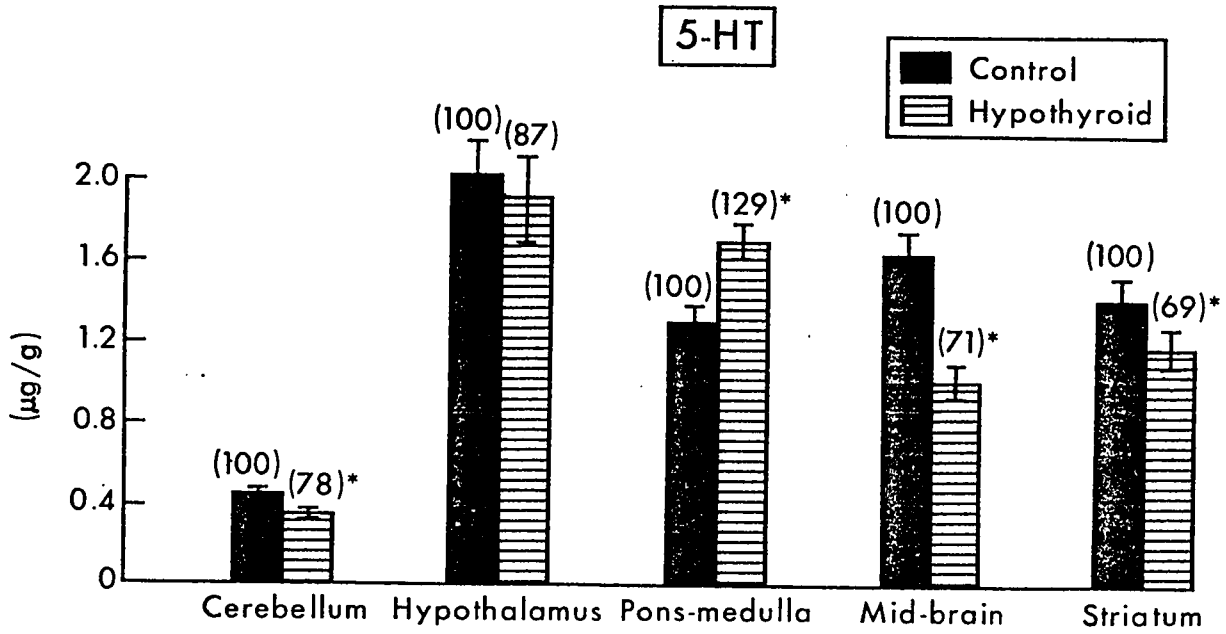

Fig. 11. Effect of neonatal hypothyroidism on 5-HT levels in certain brain regions. Bars represent the means ± S.E.M. of 6 animals in each group. One-day old rats were injected intraperitoneally with 200 μCi of ^{131}I and killed at 30 days of age. Control rats received an equal volume of physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control rats ($p < 0.05$).

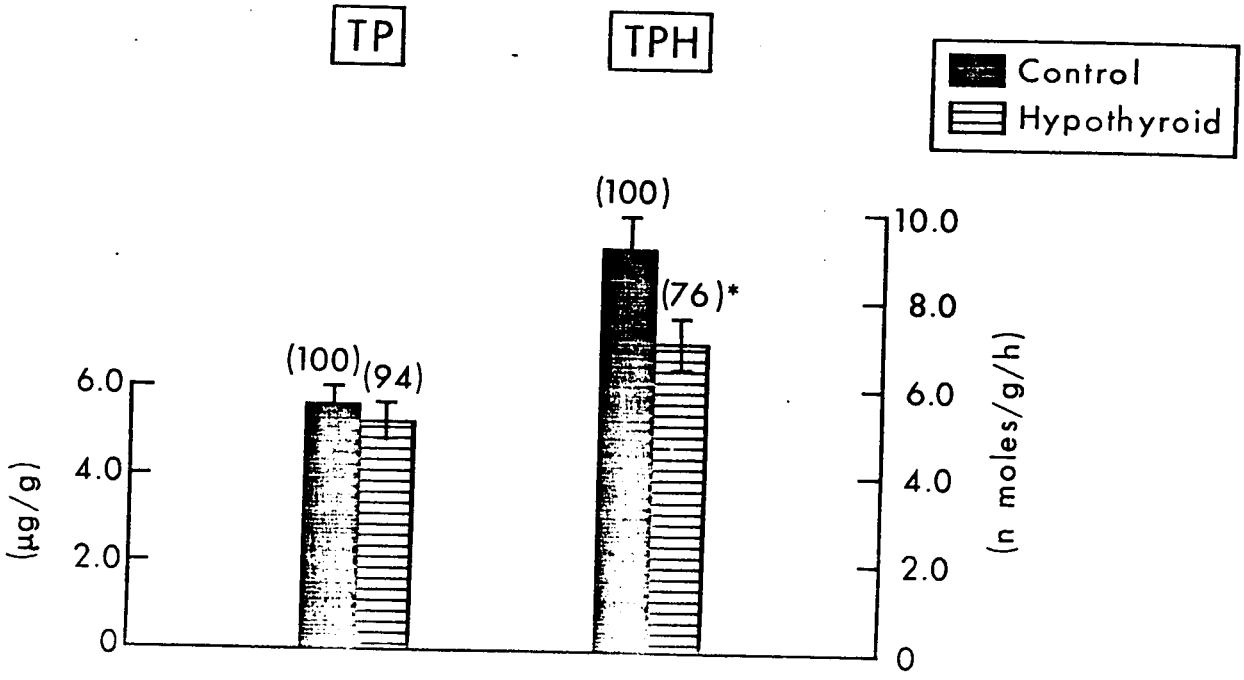

Fig. 12. Effect of neonatal hypothyroidism on mid-brain TPH and TP. Bars represent the means ± S.E.M. of 6 animals in each group. One day old rats were injected intraperitoneally with 200 µCi of ^{131}I and killed at 30 days of age. Control rats received an equal volume of physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control rats ($p < 0.05$).

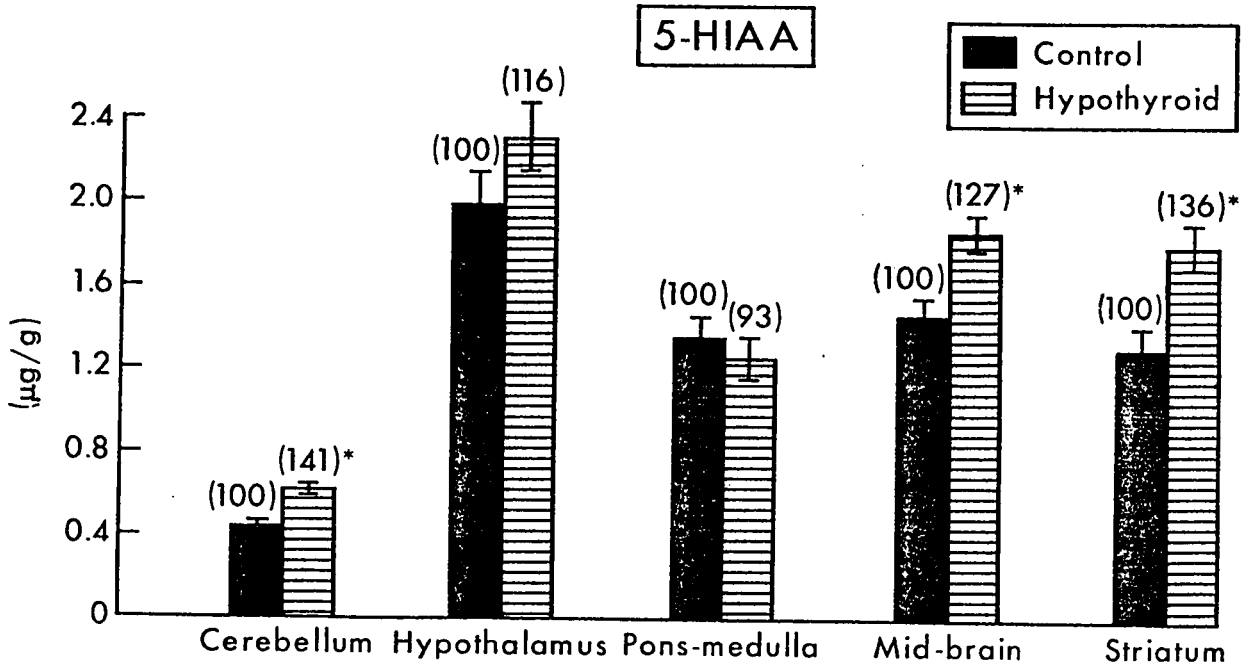

Fig. 13. Effect of neonatal hypothyroidism on 5-HIAA levels in certain brain regions. Bars represent the means ± S.E.M. of 6 animals in each group. One-day old rats were injected intraperitoneally with 200 μCi of ^{131}I and killed at 30 days of age. Control rats received an equal volume of physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control rats ($p < 0.05$).

thyroid deficient rats. The activity of tryptophan hydroxylase was decreased by 24% in mid-brain of radio-isotope treated rats. Of greater interest are the changes in 5-hydroxyindoleacetic acid levels which were found to be markedly enhanced in all brain regions examined except the hypothalamus and pons-medulla of hypothyroid rats (Fig. 13).

Since the Michaelis constant (Km) of tryptophan hydroxylase, unlike that of tyrosine hydroxylase, is much higher than the concentration of tryptophan present in the brain, it is possible that the synthetic rate of 5-hydroxy-tryptamine might change in response to altered levels of its precursor. In order to examine whether the observed decrease in the rate of synthesis of the indoleamine might be related to the lack of available precursor, the effect of neonatal thyroidectomy was studied on tryptophan levels of mid-brain. Data in Figure 12 show that neonatal thyroidectomy produced no significant change in the concentration of this important amino acid in mid-brain region.

E. Effects on Monoamine Oxidase and Catechol-o-Methyl Transferase Activity in Certain Discrete Brain Regions

In order to gain deeper insight into the mechanism(s) responsible for the elevated levels of 5-hydroxyindoleacetic acid in the face of low levels of 5-hydroxytryptamine in thyroid-deficient rats, changes in the activity of the catabolizing enzymes, monoamine oxidase, were studied in various regions of the brain. Whereas radiothyroidectomy at birth decreased the level of monoamine oxidase in the hypothalamus, the activity of this deaminating enzyme was significantly enhanced in mid-brain. However, hypothyroidism failed to produce any appreciable effect on monoamine oxidase activity of the cortex, brain stem and striatum (Table 4). The activity of catechol-o-methyl transferase in certain regions of the brain of normal and neonatally thyroidectomized rats is presented in Table 5. In control animals, whereas

TABLE 4

EFFECT OF NEONATAL HYPOTHYROIDISM ON MAO ACTIVITY IN DIFFERENT BRAIN REGIONS

Values represent the means ± S.E.M. of 6 animals in each group. One-day-old rats were injected i.p. with 200 µCi of ^{131}I and killed at 30 days of age. Control rats received an equal volume of physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

Parameter	Region	Treatment	
		Control	^{131}I
MAO (nmoles/g/h)	Cortex	172±6.0 (100)	168±7.0 (99)
	Brain stem	176±6.0 (100)	176±8.0 (100)
	Striatum	144±11.4 (100)	156±6.6 (108)
	Hypothalamus	164±6.0 (100)	144±9.3 (86)*
	Mid-brain	192±8.4 (100)	216±5.1 (114)*

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

TABLE 5

EFFECT OF NEONATAL HYPOTHYROIDISM ON COMT ACTIVITY IN DIFFERENT BRAIN REGIONS

Values represent the means ± S.E.M. of 6 animals in each group. One-day-old rats were injected i.p. with 200 µCi of ^{131}I and killed at 30 days of age. Control rats received an equal volume of physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

Parameter	Region	Treatment	
		Control	^{131}I
COMT (nmoles/g/h)	Cortex	9300±320 (100)	8900±180 (95)
	Brain stem	10280±860 (100)	12920±980 (126)*
	Striatum	5440±460 (100)	6660±540 (122)*
	Hypothalamus	5460±380 (100)	3280±360 (60)*
	Mid-Brain	12260±300 (100)	14600±300 (119)*

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

the mid-brain had the highest activity of catechol-o-methyl transferase, the striatum and hypothalamus showed the lowest activity of this o-methylating enzyme. Neonatal thyroidectomy led to significant enhancement in the activity of catechol-o-methyl transferase in brain stem, striatum and mid-brain whereas the activity of this enzyme was lowered in the hypothalamic region. In contrast, cerebrocortical catechol-o-methyl transferase activity remained unaffected following neonatal radiothyroidectomy.

F. Influence of Delayed Thyroidectomy on Brain Norepinephrine and 5-Hydroxytryptamine

Since the severity of brain dysfunction accompanying hypothyroidism is related to the age at which this endocrine disorder becomes apparent (246), it was of interest to investigate the effect of delaying the administration of radio-iodine on the metabolism of central amines. Figure 14 illustrates the effect of delayed thyroidectomy on body and brain weights. Thyroidectomy at 1 day of age resulted in approximately 70% and 30% interference with the normal increases of body and brain weights, respectively. However, less pronounced changes in both of these parameters were noted when ^{131}I administration was delayed for 5 or 10 days of age. Furthermore, delaying the thyroidectomy to 20 days after birth, produced no apparent change in growth or physical appearance when compared with control rats of the corresponding age group.

Thyroidectomy at 1-day of age resulted in a decrease of approximately 30% in the activity of TH and 34% in the levels of brain norepinephrine (Fig. 15). However, smaller decreases in norepinephrine and its synthesizing enzyme were observed when thyroidectomy was delayed in early neonatal life of the animal. Administration of ^{131}I at 5 days of age produced 25% inhibition of brain norepinephrine and tyrosine hydroxylase. In contrast,

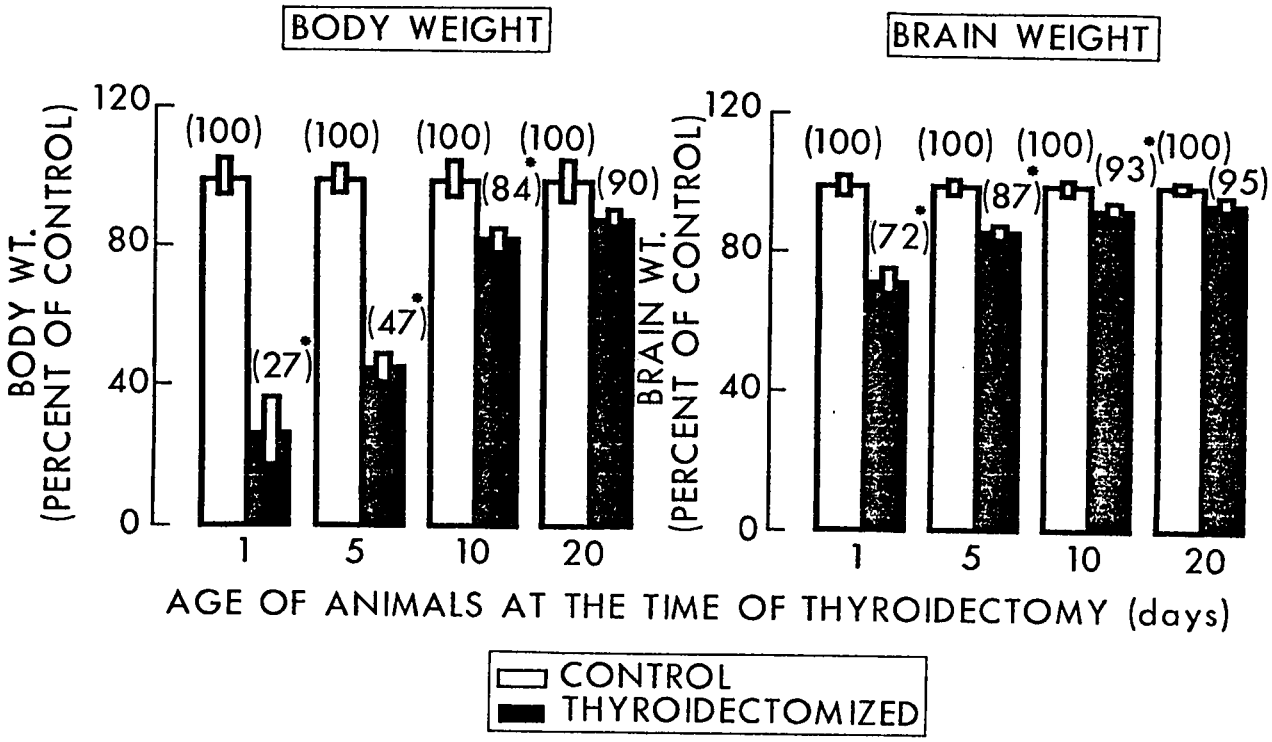

Fig. 14. Effect of delayed thyroidectomy on body and brain weights. Bars represent the means ± S.E.M. of 5-6 values in each group. Animals were injected intraperitoneally with 200 μCi of ^{131}I at 1, 5, 10 or 20 days of age and killed 30 days after the treatment. Data in parentheses indicate the percentages of corresponding control values taken as 100%.

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

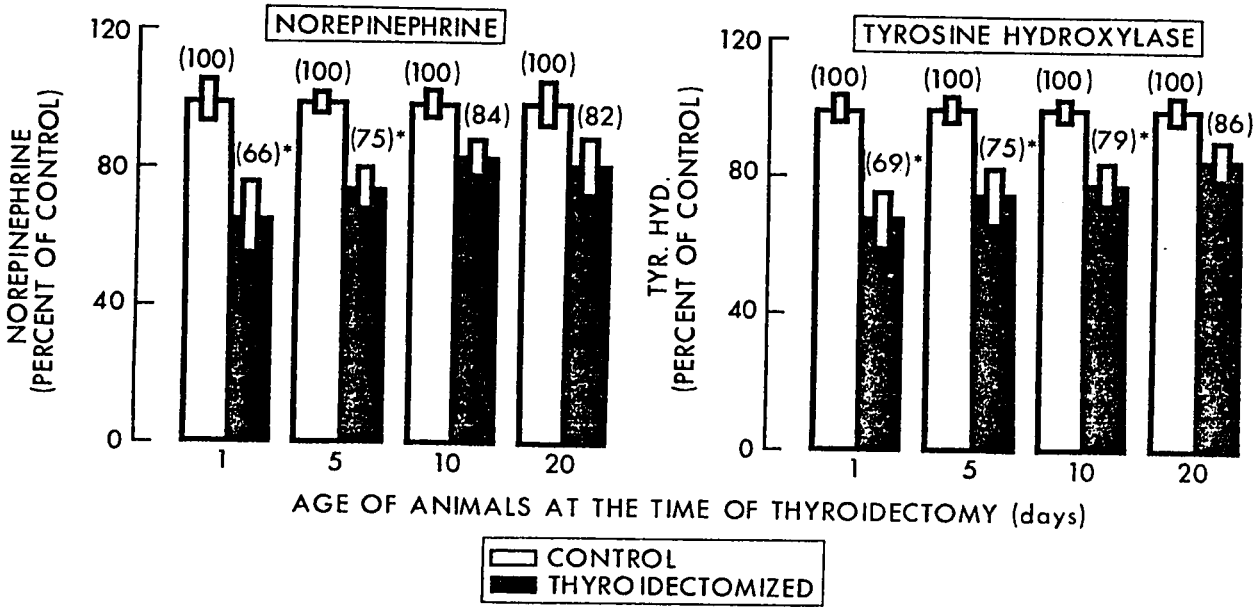

Fig. 15. Effect of delayed thyroidectomy on brain NE and TH activity. Bars represent the means ± S.E.M. of 5-6 animals in each group. Animals were injected intraperitoneally with 200 μCi of ^{131}I at 1, 5, 10 or 20 days of age and killed 30 days after the treatment. Data in parentheses indicate the percentages of corresponding control values taken as 100%.

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

when thyroidectomy was delayed for 20 days after birth, the differences in norepinephrine levels and brain tyrosine hydroxylase activity between control and thyroidectomized rats were statistically non-significant. Similarly, treatment with the radioisotope at 1 day of age resulted in significant inhibition of the activity of brain tryptophan hydroxylase and 5-hydroxytryptamine levels (Fig. 16), as well as elevation in 5-hydroxyindoleacetic acid (Fig. 17). However, delaying radiothyroidectomy for 5 or 10 days of age resulted in less pronounced changes in these neurochemical parameters. Furthermore, results presented in Figures 16 and 17 show that delaying thyroidectomy until 20 days of age resulted in no appreciable differences in tryptophan hydroxylase activity and 5-hydroxytryptamine and 5-hydroxyindoleacetic acid levels between the brains of control and hypothyroid rats.

II. EFFECTS OF L-TRIIODOTHYRONINE IN NEONATALLY THYROIDECTOMIZED RATS

Results described in the preceding section showed that administration of radio-iodine in neonatal life significantly altered the metabolism of brain norepinephrine and 5-hydroxytryptamine. In order to examine whether the observed changes were specific to thyroid hormone, the influence of replacement therapy with L-triiodothyronine on the metabolism of these central amines was studied in neonatally hypothyroid rats.

A. Time-Course Studies with L-Triiodothyronine

Results illustrated in Figure 18 demonstrate the effects of L-triiodothyronine treatment (10 µg/100 g/day) for various periods of time on body and brain weights as well as on norepinephrine and tyrosine hydroxylase in the brains of neonatally hypothyroid rats. Treatment with L-triiodothyronine for 10 days, initiating on the 20th day of age, failed to produce an appreciable effect not only on body and brain weights, but also on norepinephrine and its synthesizing enzyme, tyrosine hydroxylase. However,

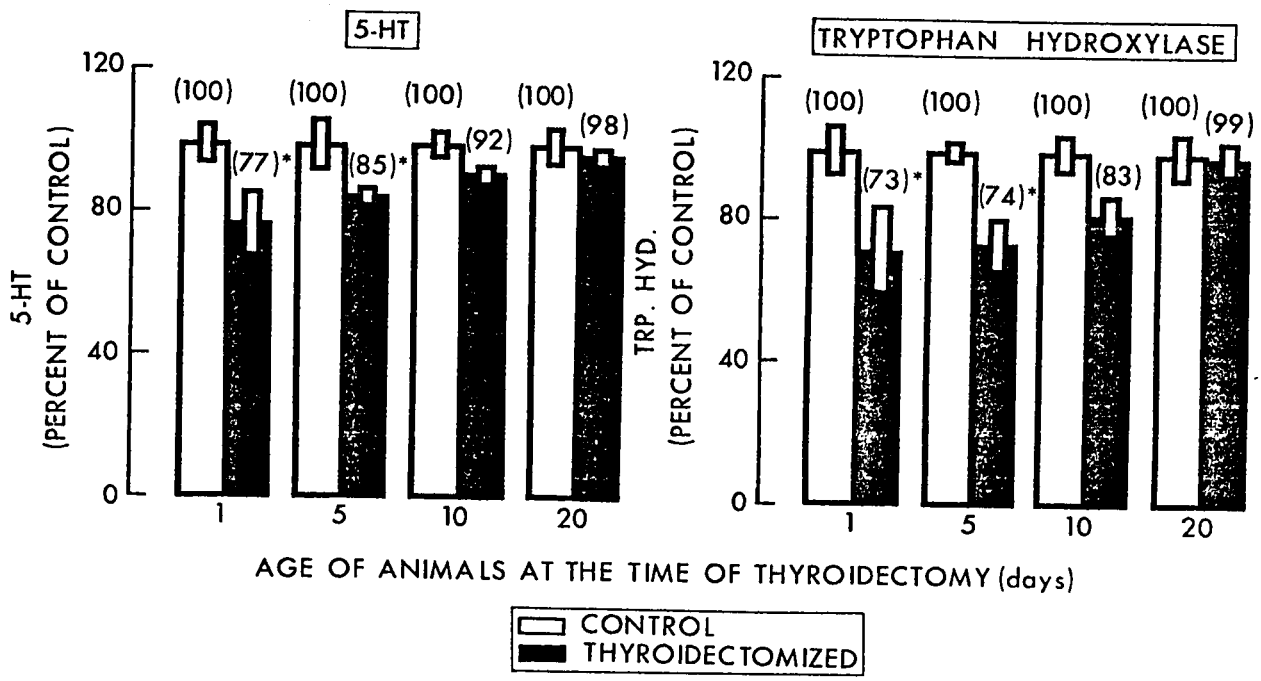

Fig. 16. Effect of delayed thyroidectomy on brain 5-HT and TPH activity. Bars represent the means ± S.E.M. of 5-6 values in each group. Animals were injected intraperitoneally with 200 μCi of ^{131}I at 1, 5, 10 or 20 days of age and killed 30 days after the treatment. Data in parentheses indicate the percentages of corresponding control values taken as 100%.

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

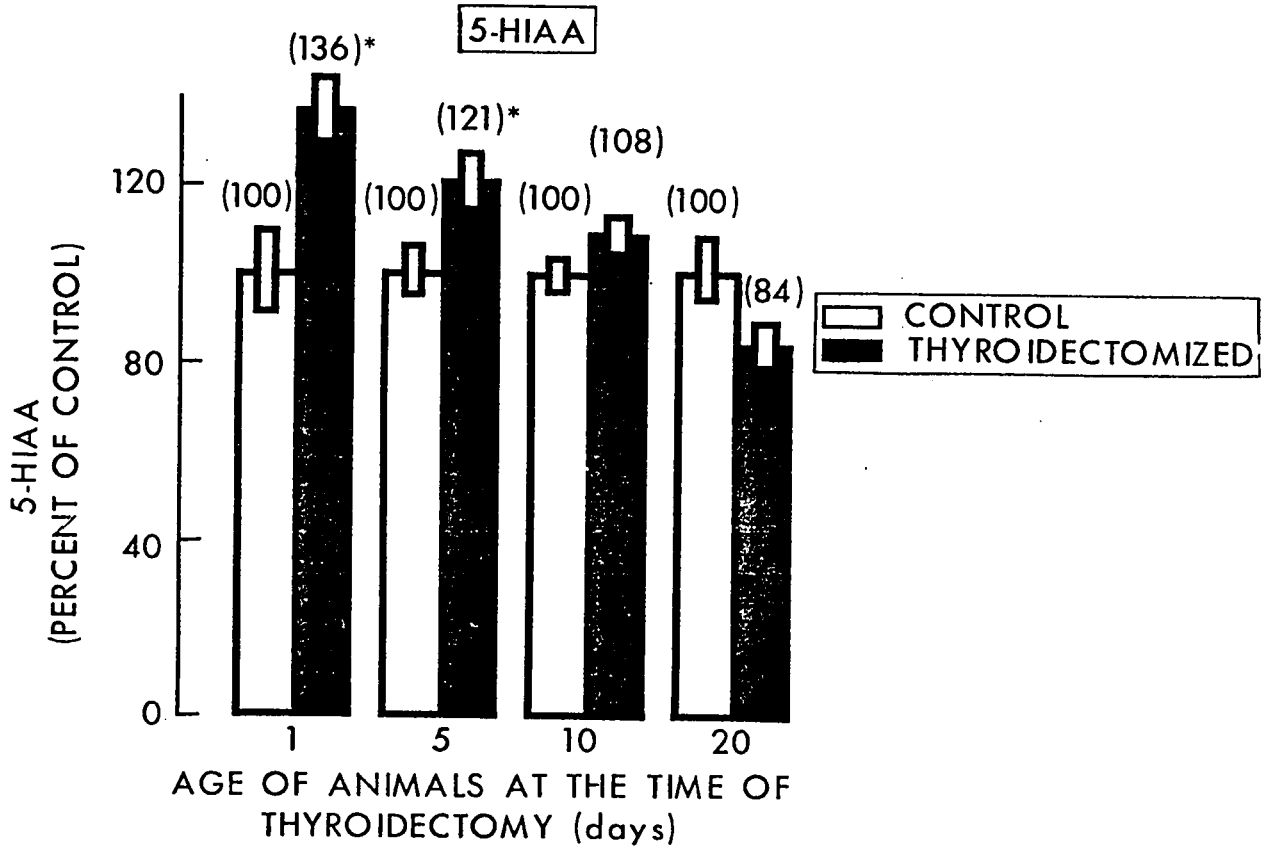

Fig. 17. Effect of delayed thyroidectomy on the concentrations of brain 5-HIAA. Bars represent the means ± S.E.M. of 5-6 values in each group. Animals were injected intraperitoneally with 200 μCi of ^{131}I at 1, 5, 10 or 20 days of age and killed 30 days after the treatment. Data in parentheses indicate the percentages of corresponding control values taken as 100%.

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

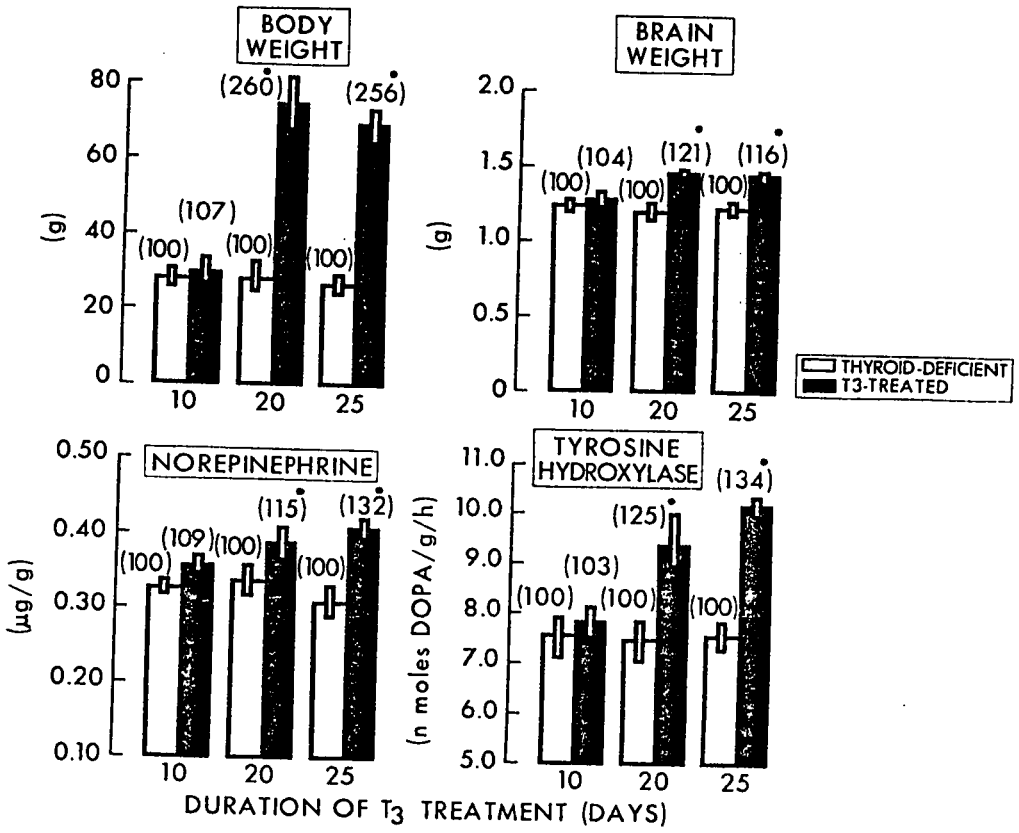

Fig. 18. Effect of treatment with T_3 on body and brain weight as well as on NE levels and the activity of brain TH. Each bar represents the mean ± S.E.M. of 6 rats in each group. Animals were thyroidectomized by the administration of 200 µCi of ^{131}I intraperitoneally at 1 day of age. Groups of thyroidectomized rats were then treated daily with T_3 (10 µg/100 g/day) for either 10, 20 or 25 days and killed at 30 days of age. Data in parentheses represent the percentages of the values for untreated thyroidectomized animals taken as 100%.

*Statistically significant difference when compared with the values of control rats ($p < 0.05$).

when the replacement therapy with L-triiodothyronine was extended for 20 days (beginning from the 10th day of age) or 25 days (beginning from the 5th day of age), body and brain weights markedly increased by about 160% and 20% respectively, and attained values close to those seen in normal rats of the corresponding age group. Likewise, norepinephrine levels and tyrosine hydroxylase activity in thyroid-deficient rats was restored to normal values in neonatally thyroidectomized rats which received replenishment therapy for 20 or 25 days.

Data presented in Figure 19 demonstrate that whereas L-triiodothyronine treatment for 10 or 20 days failed to exert any significant effect on brain 5-hydroxytryptamine levels, administration of the hormone for 25 days restored the levels of this indoleamine to normal. Figure 19 also shows that administration of L-triiodothyronine for 20 or 25 days markedly increased the activity of tryptophan hydroxylase in neonatally hypothyroid rats. Conversely, the levels of 5-hydroxyindoleacetic acid were decreased in rats treated with L-triiodothyronine and attained almost the normal values in rats which received L-triiodothyronine in early post-natal life for an extended period of time.

B. Effects of L-Triiodothyronine on Norepinephrine and 5-Hydroxytryptamine Metabolism in Certain Discrete Brain Regions of Neonatally Thyroidectomized Rats

Results presented in the foregoing section indicate that replacement therapy in thyroid-deficient rats may restore the metabolism of norepinephrine and 5-hydroxytryptamine to virtually normal state, provided that the treatment is initiated at an appropriate time in early neonatal life. Data in Table 6 further show that norepinephrine levels were markedly augmented in the hypothalamus, cerebellum and striatum. However, regions like the midbrain and pons-medulla in hypothyroid rats appeared to be somewhat irresp-

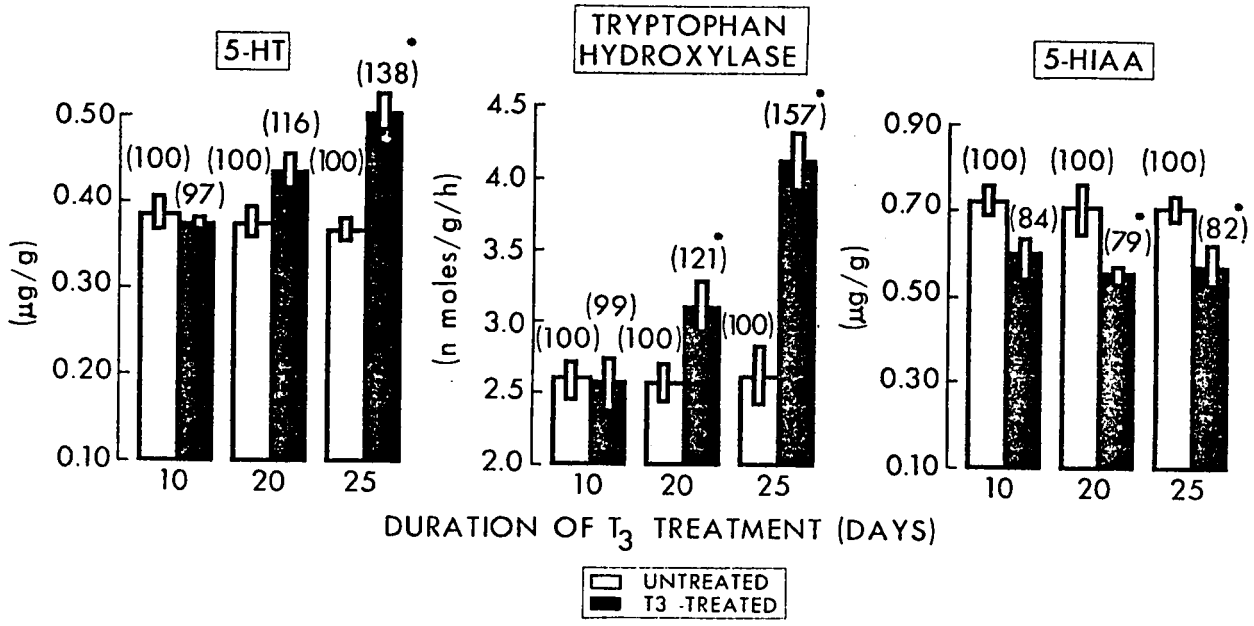

Fig. 19. Influence of varying periods of T_3 treatment on brain TPH activity as well as on 5-HT and 5-HIAA levels in neonatally thyroidectomized rats. Each bar represents the mean ± S.E.M. of 6 rats in the group. Animals were thyroidectomized by an intraperitoneal injection of 200 µCi of ^{131}I at 1 day of age. Groups of thyroidectomized rats were then treated daily with T_3 (10 µg/100 g/day) for either 10, 20 or 25 days and killed at 30 days of age. Data in parentheses indicate the percentages taking control values as 100%.

*Statistically significant alterations when compared to the values seen in littermate controls of the corresponding age group ($p < 0.05$).

TABLE 6

EFFECT OF TREATMENT WITH T_3 ON NE LEVELS IN CERTAIN BRAIN REGIONS AND ON STRIATAL TH AND TR IN NEONATALLY HYPOTHYROID RATS

Values represent the means ± S.E.M. of 6 rats in each group. Animals were thyroidectomized by an i.p. injection of 200 µCi of ^{131}I at 1 day of age. Groups of thyroidectomized rats were then treated daily with T_3 (10 µg/100 g/day) for 25 days beginning from 5 days of age, and killed at 30 days of age. Data in parentheses indicate the percentages taking control values as 100%.

Parameter	Region	Treatment	
		Control	T_3
NE (µg/g)	Cerebellum	0.41±0.02 (100)	0.54±0.02 (132)*
	Hypothalamus	1.38±0.09 (100)	1.92±0.08 (139)*
	Pons-Medulla	0.34±0.01 (100)	0.31±0.02 (91)
	Mid-brain	0.58±0.02 (100)	0.54±0.02 (93)
	Striatum	0.15±0.01 (100)	0.19±0.01 (126)*
TH (nmoles DOPA/g/h)	Striatum	53.25±4.32 (100)	86.80±5.81 (163)*
TR (µg/g)	Striatum	26.30±2.80 (100)	33.50±3.43 (127)*

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

onsive to the hormonal treatment. The activity of striatal tyrosine hydroxylase was increased by 63% in hypothyroid rats treated with L-triiodothyronine. Table 6 also demonstrates that replacement therapy with L-triiodothyronine significantly raised the concentration of tyrosine in the striatum of hypothyroid rats.

Results presented in Table 7 show that replacement therapy for 25 days markedly elevated the levels of 5-hydroxytryptamine in the striatum, mid-brain, cerebellum and hypothalamus, but produced no change in this amine in the pons-medulla of neonatally hypothyroid rats. The activity of tryptophan hydroxylase and the concentration of its substrate, tryptophan, were elevated by about 35% in the mid-brain. As expected, the levels of 5-hydroxyindoleacetic acid remained significantly low in the mid-brain and cerebellum. Whereas the levels of this metabolite were augmented in pons-medulla, the concentration of 5-hydroxyindoleacetic acid remained relatively unchanged in hypothalamus and striatum. It is of interest that administration of L-triiodothyronine for 25 days, initiated from the 5th day of age, apparently also changed the physical appearance of neonatally thyroidectomized rats. The animals became relatively mobile and displayed physical appearance which was comparable to that of their normal counterparts of the same age group.

C. Effect of L-Triiodothyronine on Cerebrocortical Monoamine Oxidase and Catechol-o-Methyl Transferase in Neonatally Thyroidectomized Rats

The possibility whether L-triiodothyronine treatment in hypothyroid rats could have lowered the levels of 5-hydroxyindoleacetic acid via its ability to interfere with the activity of the catabolizing enzyme, monoamine oxidase, was also investigated. Results in Table 8 demonstrate that administration of exogenous thyroid hormone to 5-day old thyroidectomized

TABLE 7

EFFECT OF T_3 TREATMENT ON 5-HT AND 5-HIAA LEVELS IN CERTAIN BRAIN REGIONS AND ON MID-BRAIN TPH AND TP

Values represent the means ± S.E.M. of 6 rats in each group. Animals were thyroidectomized by an i.p. injection of 200 µCi of ^{131}I at 1 day of age. Groups of thyroidectomized rats were then treated daily with T_3 (10 µg/100 g/day) for 25 days beginning from 5 days of age, and killed at 30 days of age. Data in parentheses indicate the percentages taking control values as 100%.

Parameter	Region	Treatment	
		Control	T_3
5-HT (µg/g)	Cerebellum	0.32±0.01 (100)	0.45±0.02 (141)*
	Hypothalamus	1.78±0.16 (100)	2.03±0.11 (114)*
	Pons-Medulla	1.72±0.11 (100)	1.64±0.13 (95)
	Mid-Brain	1.18±0.09 (100)	1.45±0.11 (123)*
	Striatum	1.00±0.06 (100)	1.51±0.09 (151)*
TPH (nmoles/g/h)	Mid-Brain	6.89±0.47 (100)	9.23±0.56 (134)*
TP (µg/g)	Mid-Brain	6.60±0.19 (100)	8.90±0.69 (135)*
5-HIAA (µg/g)	Cerebellum	0.55±0.01 (100)	0.31±0.01 (56)*
	Hypothalamus	2.38±0.17 (100)	2.16±0.18 (91)
	Pons-Medulla	1.21±0.04 (100)	1.59±0.08 (131)*
	Mid-Brain	1.68±0.04 (100)	0.99±0.06 (59)*
	Striatum	1.61±0.04 (100)	1.89±0.15 (118)

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

TABLE 8

EFFECT OF T_3 TREATMENT ON CEREBROCORTICAL MAO AND COMT ACTIVITY IN NEONATALLY HYPOTHYROID RATS

Values represent the means ± S.E.M. of 6 rats in each group. Animals were thyroidectomized by an i.p. injection of 200 µCi of ^{131}I at 1 day of age. Groups of thyroidectomized rats were then treated daily with T_3 (10 µg/100 g/day) for 25 days beginning from 5 days of age, and killed at 30 days of age. Data in parentheses indicate the percentages taking control values as 100%.

Parameter	Treatment	
	Control	T_3
MAO (nmoles/g/h)	172±5.7 (100)	188±7.0 (109)
COMT (nmoles/g/h)	8080±160 (100)	10100±170 (125)*

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

rats for 25 days produced no appreciable effect on monoamine oxidase activity of the cerebral cortex. However, the activity of catechol-o-methyl transferase in hypothyroid rats did increase by 25% following treatment with L-triiodothyronine.

D. Dose-Response Studies with L-Triiodothyronine

Since the administration of L-triiodothyronine in a dose of 10 µg/100 g for varying periods of time produced a time-dependent rise in brain norepinephrine and 5-hydroxytryptamine synthesis in neonatally thyroidectomized rats, a dose-response study was undertaken to determine whether larger doses of this hormone for a shorter duration also could elicit any significant responses. Daily administration of thyroid hormone at the dose of 25, 100 or 250 µg/100 g for 3 consecutive days initiated from the 27th day of age, failed to exert any appreciable effect on body and brain weights (Table 9). Treatment with 100 or 200 µg/100 g dose of the hormone produced dose-dependent enhancement in the levels of brain norepinephrine and its synthesizing enzyme, tyrosine hydroxylase in neonatally thyroidectomized rats. The effects of short-term treatment with higher doses of triiodothyronine on brain 5-hydroxytryptamine metabolism was also studied and the data are shown in Table 10. L-Triiodothyronine treatment for 3 days at a daily dose of 25 µg/100 g failed to produce any change in brain tryptophan hydroxylase, 5-hydroxytryptamine or 5-hydroxyindoleacetic acid. Administration of 100 µg/100 g dose of L-triiodothyronine elevated the concentration of 5-hydroxytryptamine but produced no significant change in brain tryptophan hydroxylase or 5-hydroxyindoleacetic acid. In contrast, this hormone significantly reduced brain 5-hydroxyindoleacetic acid concentration when given at the dose level of 250 µg/100 g daily for 3 days.

TABLE 9

INFLUENCE OF SHORT TERM T_3 TREATMENT (3 DAYS) ON BODY AND BRAIN WEIGHTS AS WELL AS BRAIN NE AND TH ACTIVITY

Values represent the means ± S.E.M. of 6 animals in each group. Rats were thyroidectomized by an i.p. injection of 200 µCi of ^{131}I at 1 day of age. Beginning from the 27th day of age, rats were treated daily with subcutaneous injection of T_3 (25, 100 or 250 µg/100 g) for 3 days and killed 24 h after the last injection. Data in parentheses indicate percentages of the values for control rats taken as 100%.

Dosage of T_3 (µg/100 g/day)	Body wt. (g)	Brain wt. (g)	NE (µg/g)	TH (nmoles DOPA/g/h)
Control	29.9±4.1 (100)	1.25±0.02 (100)	0.33±0.03 (100)	7.59±0.65 (100)
25	30.5±1.11 (102)	1.26±0.01 (101)	0.34±0.02 (103)	7.36±0.33 (97)
100	31.4±1.63 (105)	1.31±0.02 (105)	0.37±0.00 (112)*	8.67±0.20 (114)*
250	33.2±1.52 (111)	1.35±0.03 (108)	0.40±0.02 (121)*	9.02±0.27 (119)*

*Statistically significant difference when compared with the control values ($p < 0.05$).

TABLE 10

EFFECT OF SHORT-TERM T_3 TREATMENT (3 DAYS) ON BRAIN TPH ACTIVITY, 5-HT AND 5-HIAA LEVELS IN NEONATALLY THYROIDECTOMIZED RATS

Each value is the mean ± S.E.M. of 6 animals in the group. Rats were thyroidectomized by an i.p. injection of 200 μCi of ^{131}I at 1 day of age. Beginning from the 27th day, rats were injected s.c. with either 25, 100 or 250 μg/100 g dose of T_3 daily for 3 days and killed 24 hours after the last injection. Data in parentheses express results in percentages taking the values of control rats as 100%.

Dose of T_3 (μg/100 g/day)	TPH (nmoles/g/h)	5-HT (μg/g)	5-HIAA (μg/g)
Control	2.65±0.23 (100)	0.38±0.02 (100)	0.72±0.05 (100)
25	2.68±0.13 (101)	0.41±0.01 (108)	0.74±0.02 (103)
100	2.84±0.19 (107)	0.52±0.03 (137)*	0.71±0.02 (98)
250	2.98±0.14 (112)	0.49±0.03 (129)*	0.56±0.02 (78)*

*Statistically significant difference when compared with the control values ($p < 0.05$).

E. Influence of Delayed L-Triiodothyronine Treatment on Norepinephrine and 5-Hydroxytryptamine Metabolism in Brains of Neonatally Thyroidectomized Rats

The question whether the observed L-triiodothyronine stimulated changes in body and brain growth as well as amine metabolism also were related to the age at which L-triiodothyronine is initiated was examined by delaying the beginning of exogenous thyroid hormone administration. The results presented in Figure 20 show that although L-triiodothyronine treatment (10 µg/100 g) during early neonatal life (5 days of age) markedly increased the body and brain weights, this hormone produced no effect on these parameters when the replacement therapy was delayed until adulthood (120 days). Figure 20 also demonstrates that administration of L-triiodothyronine in early neonatal life restored brain tyrosine hydroxylase activity and norepinephrine levels. In contrast, when L-triiodothyronine was injected for 25 days initiating from 120 days of age, no apparent change in the metabolism of this neurohumor could be noted in brains of thyroidectomized rats.

Data in Figure 21 demonstrate that although L-triiodothyronine treatment during early neonatal life (5 days of age) significantly augmented brain tryptophan hydroxylase activity and 5-hydroxytryptamine levels to 157% and 138% of the control values, respectively, this hormone produced no appreciable effect on these parameters when the replacement therapy was delayed until adulthood (120 days). Furthermore, whereas in young thyroidectomized rats, the concentration of 5-hydroxyindoleacetic acid was significantly reduced by L-triiodothyronine treatment, it seemed to remain at higher levels when the hormone treatment was postponed until the hypothyroid rats had become 120 days old.

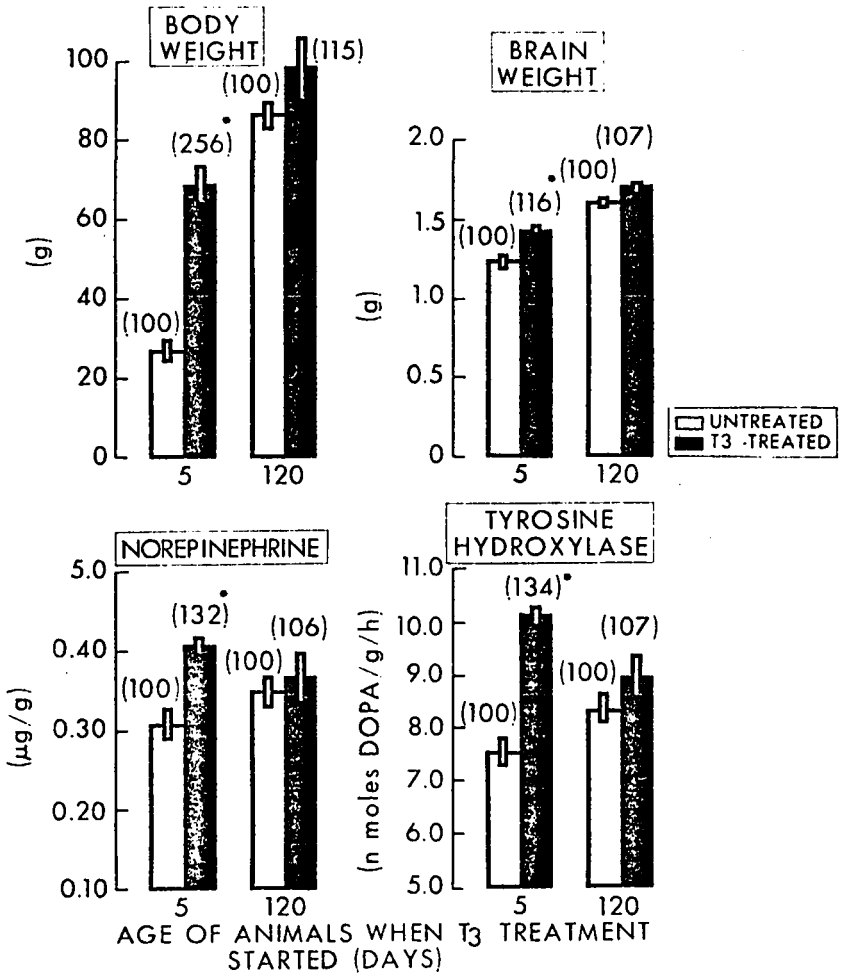

Fig. 20. Influence of delayed T_3 treatment on body and brain weight as well as on NE levels and the activity of TH in neonatally thyroidectomized rats. Bars represent the means ± S.E.M. of 5-6 rats in each group. One-day old rats were radiothyroidectomized by an intraperitoneal injection of 200 µCi of ^{131}I. Groups of thyroidectomized rats then received daily injections of T_3 (10 µg/100 g/day) for 25 days starting either at 5 or 120 days of age and were killed 24 hr after the last injection. Values in parentheses represent the percentages of the corresponding control values taken as 100%.

*Statistically significant differences when compared with the values of control rats ($p < 0.05$).

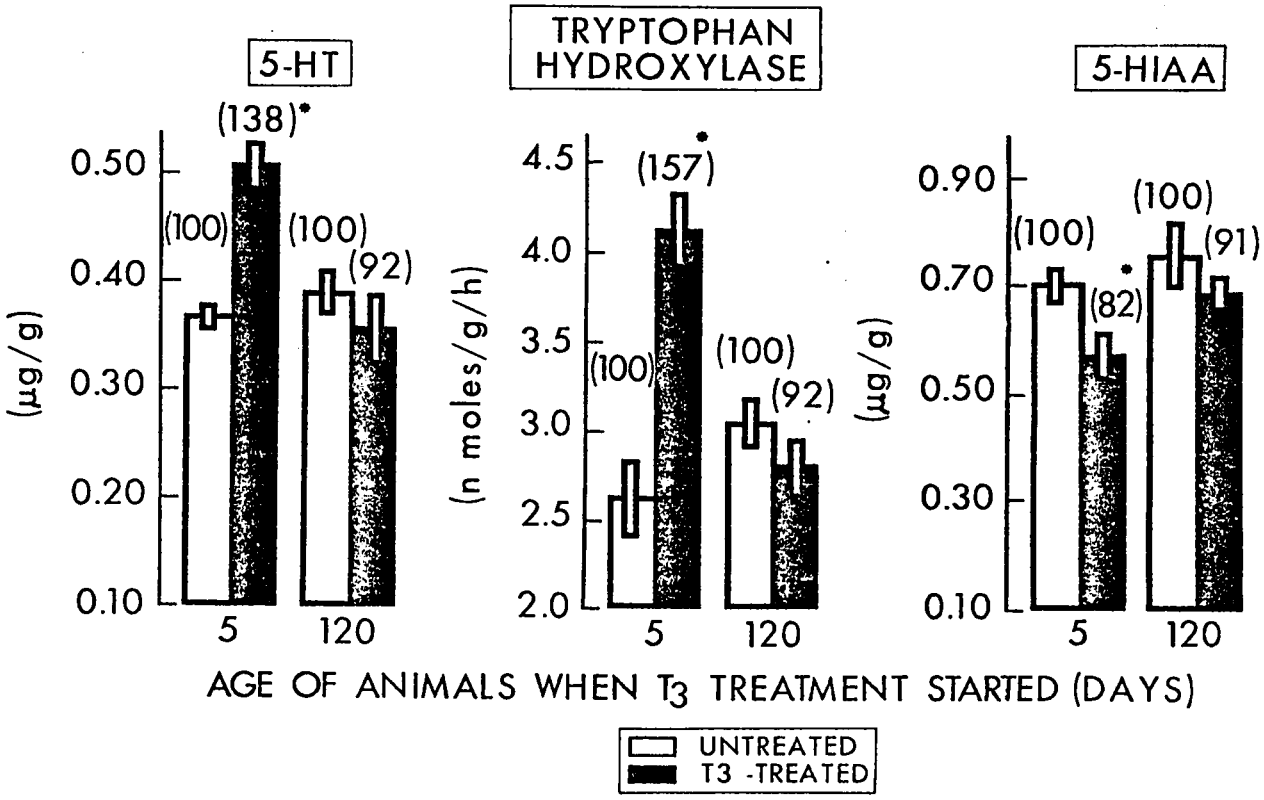

Fig. 21. Influence of delayed T_3 treatment on brain TPH activity as well as on 5-HT and 5-HIAA levels in neonatally thyroidectomized rats. Bars represent the means ± S.E.M. of 5-6 rats in each group. One-day old rats were radiothyroidectomized by an intraperitoneal injection of 200 µCi of ^{131}I. Groups of thyroidectomized rats then received daily injections of T_3 (10 µg/100 g/day) for 25 days starting either at 5 or 120 days of age and were killed 24 hr after the last injection. Values in parentheses represent the percentages of the corresponding control values taken as 100%.

*Statistically significant differences when compared with the values of control rats ($p < 0.05$).

F. Influence of Delayed L-Triiodothyronine Administration on Norepinephrine and 5-Hydroxytryptamine Metabolism in Certain Discrete Brain Regions of Neonatally Thyroidectomized Rats

In order to gain better knowledge of the effect of delayed L-triiodothyronine treatment on norepinephrine metabolism, the levels of this catecholamine as well as of tyrosine hydroxylase activity were determined in specific regions of the brain of neonatally hypothyroid rats. Data in Figure 22 demonstrate that delaying the initiation of L-triiodothyronine treatment for 120 days produced no appreciable change in norepinephrine concentration in several regions of the brain. In point of fact, the hypothalamus of thyroidectomized rats in which replacement therapy was delayed for 120 days showed the level of norepinephrine that was even lower than that of the untreated group. Whereas L-triiodothyronine treatment in young rats increased the activity of tyrosine hydroxylase and tyrosine levels by 63% and 27% respectively, this hormone failed to produce any significant change in these parameters when the replacement therapy was postponed until adulthood. Like norepinephrine, 5-hydroxytryptamine levels remained unaltered in cerebellum, hypothalamus, mid-brain and striatum of hypothyroid rats treated with L-triiodothyronine at 120 days of age (Fig. 23). However, the level of this indoleamine was significantly reduced in the pons-medulla of thyroidectomized rats treated with L-triiodothyronine in adulthood. The effect of delayed L-triiodothyronine treatment on mid-brain tryptophan hydroxylase and tryptophan also was examined, and the results are illustrated in Figure 24. Whereas L-triiodothyronine during early neonatal life (5 days) augmented the mid-brain tryptophan hydroxylase activity by 34%, this hormone produced slight but non-significant increases in the activity of this enzyme in hypothyroid rats receiving L-triiodothyronine in adulthood. Delayed

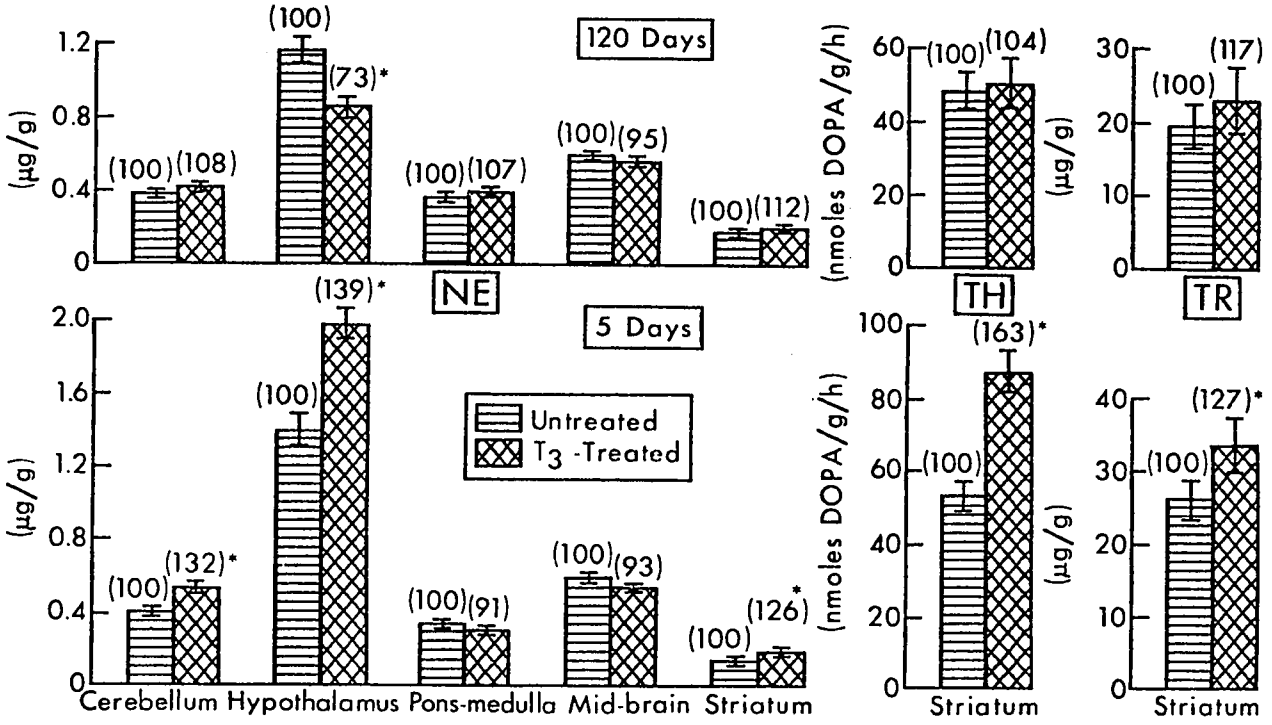

Fig. 22. Influence of delayed T_3 treatment on NE levels in certain brain regions and on striatal TH and TR in neonatally hypothyroid rats. Bars represent the means ± S.E.M. of 5-6 rats in each group. One-day old rats were radiothyroidectomized by an intraperitoneal injection of 200 μCi of ^{131}I. Groups of thyroidectomized rats then received daily injections of T_3 (10 μg/100 g/day) for 25 days starting either at 5 or 120 days of age and were killed 24 hr after the last injection. Values in parentheses represent the percentages of the corresponding control values taken as 100%.

*Statistically significant differences when compared with the values of control rats ($p < 0.05$).

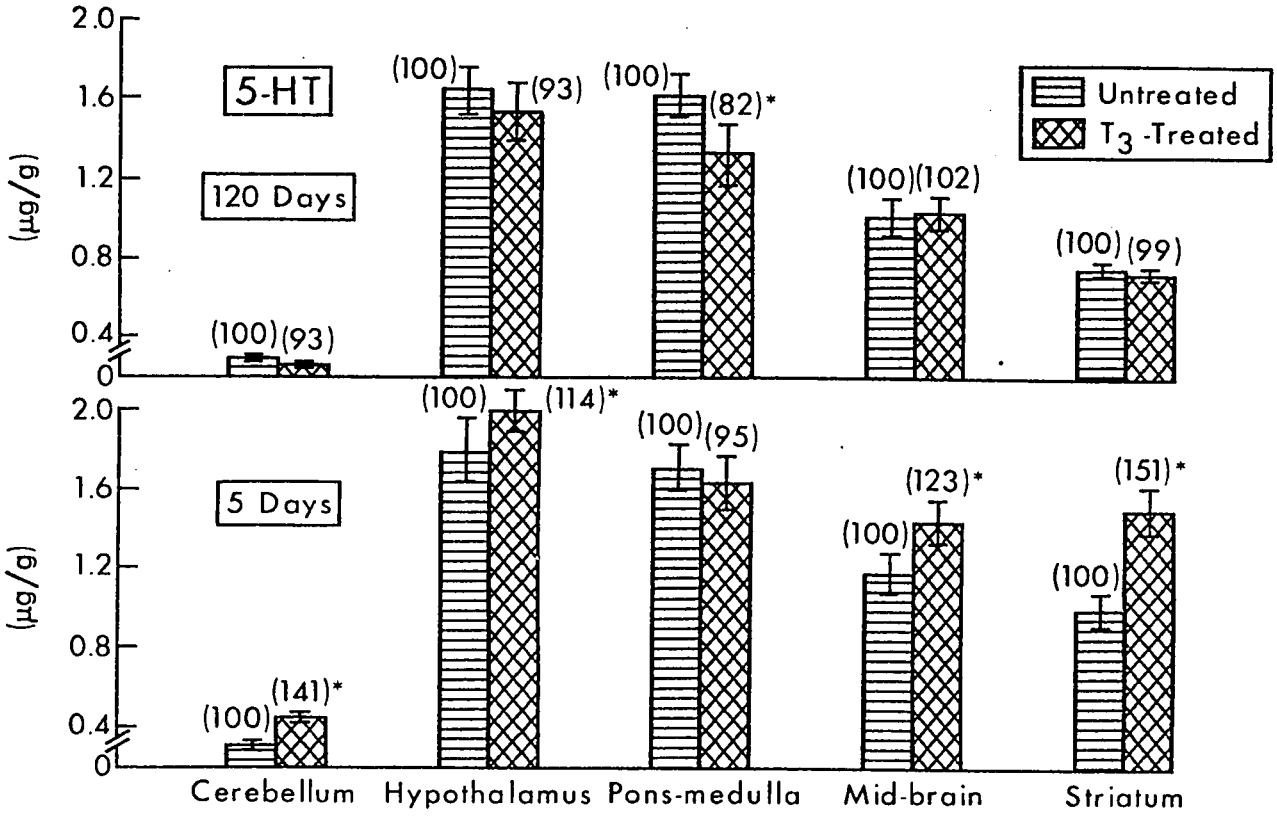

Fig. 23. Effect of delayed T_3 treatment on 5-HT levels in certain brain regions of neonatally hypothyroid rats. Bars represent the means ± S.E.M. of 5-6 rats in each group. One-day old rats were radiothyroidectomized by an intraperitoneal injection of 200 µCi of ^{131}I. Groups of thyroidectomized rats then received daily injections of T_3 (10 µg/100 g/day) for 25 days starting either at 5 or 120 days of age and were killed 24 hr after the last injection. Values in parentheses represent the percentages of the corresponding control values taken as 100%.

*Statistically significant differences when compared with the values of control rats ($p < 0.05$).

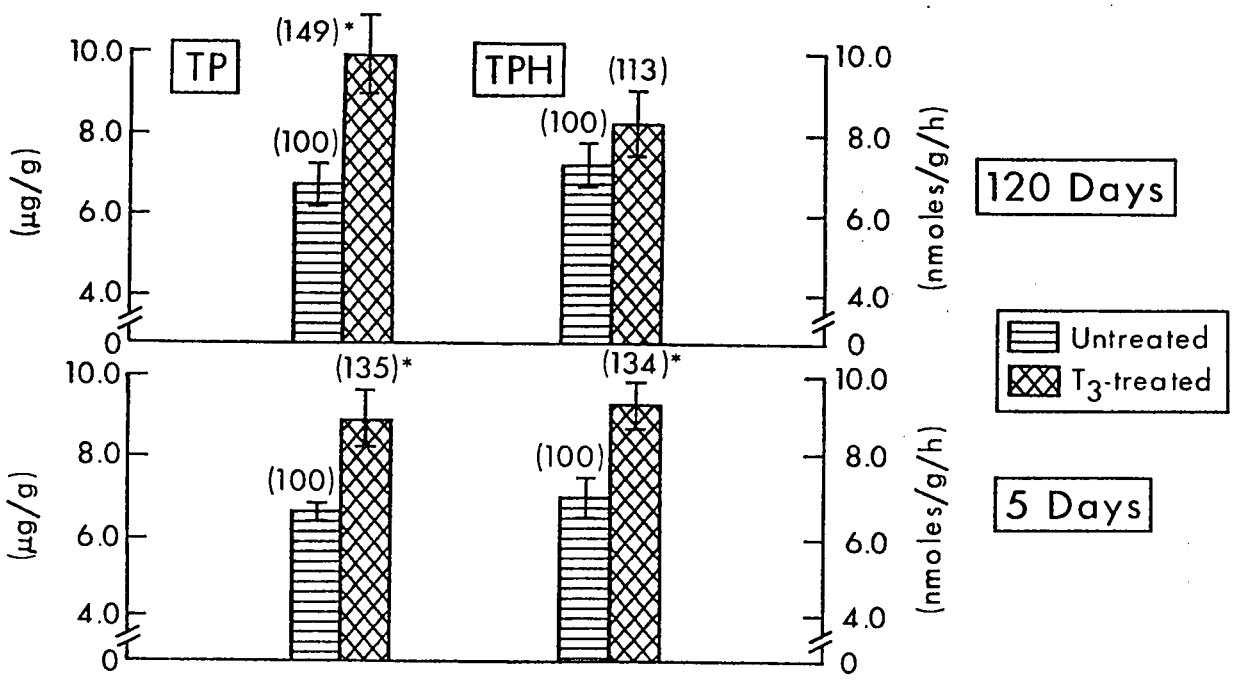

Fig. 24. Effect of delayed T_3 treatment on mid brain TPH and TP in neonatally hypothyroid rats. Bars represent the means ± S.E.M. of 5-6 rats in each group. One-day old rats were radiothyroidectomized by an intraperitoneal injection of 200 µCi of ^{131}I. Groups of thyroidectomized rats then received daily injections of T_3 (10 µg/100 g/day) for 25 days starting either at 5 or 120 days of age and were killed 24 hr after the last injection. Values in parentheses represent the percentages of the corresponding control values taken as 100%.

*Statistically significant differences when compared with the values of control rats ($p < 0.05$).

L-triiodothyronine treatment raised the concentration of tryptophan in mid-brain by 49%. Results presented in Figure 25 demonstrate that while in young thyroidectomized rats, the concentration of 5-hydroxyindoleacetic acid was significantly reduced in several regions of the brain by L-triiodothyronine treatment, it remained elevated in all regions examined except in the pons-medulla (where the levels of this metabolite were significantly decreased) when the hormone treatment was postponed until the hypothyroid rats had become 120 days old.

G. Effect of Delayed L-Triiodothyronine Administration on Monoamine Oxidase and Catechol-o-Methyl Transferase Activity in the Cerebral Cortex of Neonatally Hypothyroid Rats

In contrast to changes in the activities of tyrosine hydroxylase and tryptophan hydroxylase, L-triiodothyronine treatment in young and adult cretinous rats seemed to produce a similar change in cerebrocortical monoamine oxidase and catechol-o-methyl transferase activity. Results in Figure 26 show that the activity of catechol-o-methyl transferase was raised significantly in the cerebrocortex even though the treatment with L-triiodothyronine was delayed in thyroid-deficient animals for 120 days. It is of interest that monoamine oxidase activity did not change appreciably no matter when the administration of this hormone was initiated.

III. INFLUENCE OF CHEMICAL THYROIDECTOMY

The foregoing observations suggested that a critical period exists in the early neonatal life during which thyroid hormone exerts its most marked influence upon the neurochemical maturation of developing animals. It was deemed of interest to study the metabolism of these putative neurotransmitters in another model of hypothyroidism in which rats were made thyroid-deficient by giving an anti-thyroid drug. Methimazole is presumed to be a potent anti-thyroid agent which has been used extensively to produce chemical

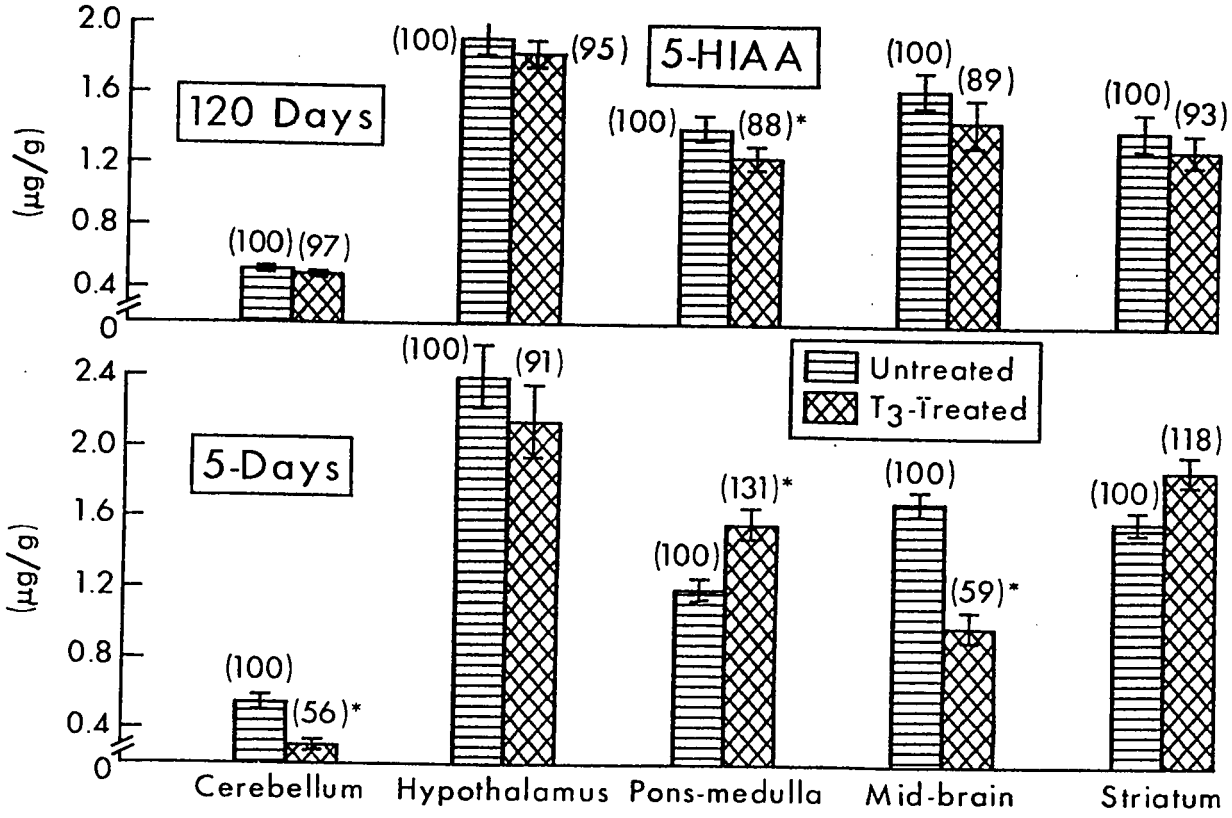

Fig. 25. Effect of delayed T_3 treatment on 5-HIAA levels in certain brain regions of neonatally hypothyroid rats. Bars represent the means ± S.E.M. of 5-6 rats in each group. One-day old rats were radiothyroidectomized by an intraperitoneal injection of 200 μCi of ^{131}I. Groups of thyroidectomized rats then received daily injections of T_3 (10 μg/100 g/day) for 25 days starting either at 5 or 120 days of age and were killed 24 hr after the last injection. Values in parentheses represent the percentages of the corresponding control values taken as 100%.

*Statistically significant differences when compared with the values of control rats ($p < 0.05$).

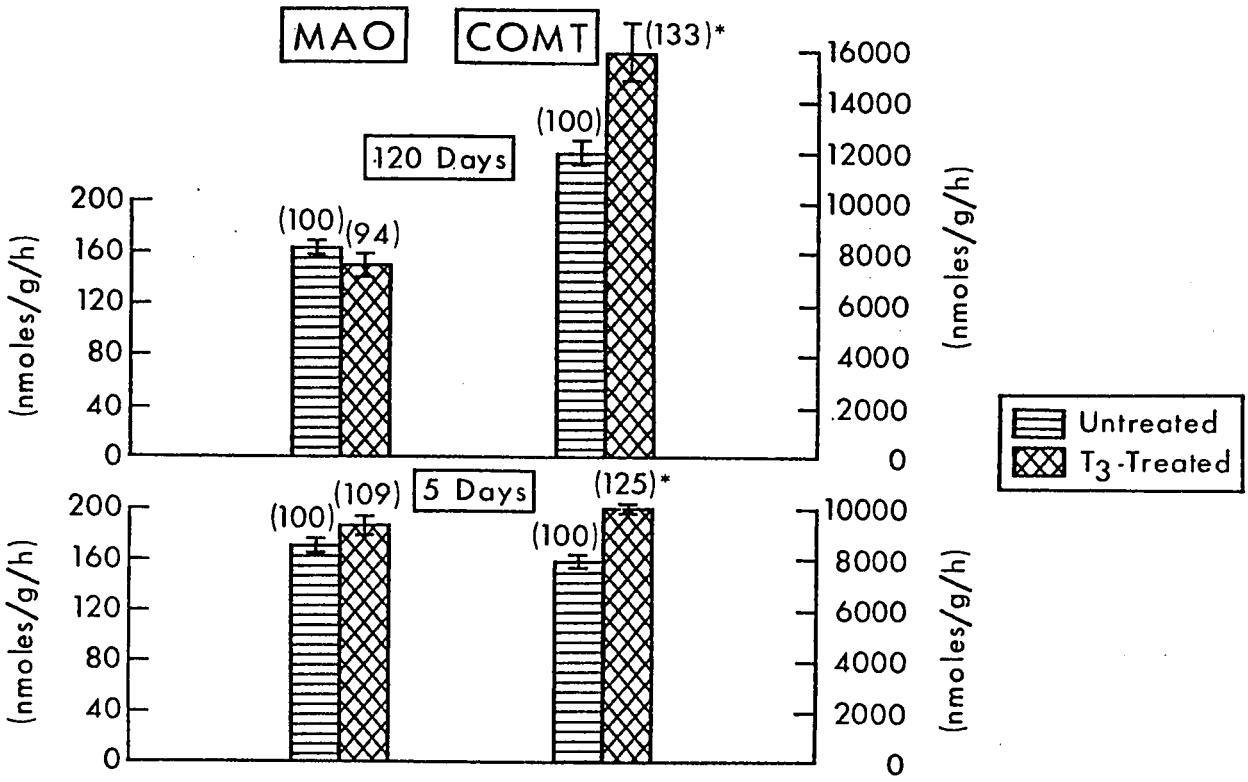

Fig. 26. Effect of delayed T_3 treatment on cerebrocortical MAO and COMT activity in neonatally hypothyroid rats. Bars represent the means ± S.E.M. of 5-6 rats in each group. One-day old rats were radiothyroidectomized by an intraperitoneal injection of 200 µCi of ^{131}I. Groups of thyroidectomized rats then received daily injections of T_3 (10 µg/100 g/day) for 25 days starting either at 5 or 120 days of age and were killed 24 hr after the last injection. Values in parentheses represent the percentages of the corresponding control values taken as 100%.

*Statistically significant differences when compared with the values of control rats ($p < 0.05$).

thyroidectomy by virtue of its ability to depress thyroid hormone synthesis (231). In addition, this drug is also used clinically in the management of hyperthyroidism.

A. Effect of Methimazole Treatment on Physical and Behavioural Development

Figure 27 shows a photograph comparing the appearance of a normal 30 day old rat with its littermate treated with a low dosage of methimazole. The schedule for treatment with low dosage of methimazole consisted of 0.1 mg from day 1-10, 0.2 mg from day 11-20 and 0.4 mg from day 21-30. It seems that methimazole administration in low dosage produced only slight alterations in the growth and appearance of 30 day old rats when compared with controls of the corresponding age group. However, treatment of developing rats with a high dosage of methimazole (0.2 mg from day 1-7 and 0.4 mg from day 8-30) induced chemical thyroidectomy as reflected among other things, by significant impairment of physical growth (Fig. 28). The hair was dry and coarse and the eyelid dysjunction was delayed by 2-3 days as compared to control rats. The snout elongation and elevation of pinnae also were considerably retarded in methimazole-treated animals.

Figure 29 shows the effect of low and high dosages of methimazole treatment on body and brain weights. Administration of this goiterogen in low dosage for 15 days produced no appreciable effect on body and brain growth; however, the changes became statistically significant when the drug was given for 30 days. It may be noted that methimazole in high dosages produced greater alterations in both body and brain weights which became statistically significant even after 15 days of drug treatment.

Results illustrated in Figure 30 demonstrate that administration of methimazole in a high dosage for 7 days slightly increased (24%) the locomotor activity. However, exposure to this antithyroid drug for an extended

Fig. 27. Photograph comparing the appearance of a normal 30 day old rat with its littermate treated with low doses of methimazole (0.1 mg from day 1-10, 0.2 mg from day 11-20 and 0.4 mg from day 21-30). Normal - left; methimazole-treated - right.

Fig. 28. Photograph comparing the appearance of a normal 30 day old rat with its littermate treated with high doses of methimazole (0.2 mg from day 1-7 and 0.4 mg from day 8-30). Normal - right; methimazole-treated - left.

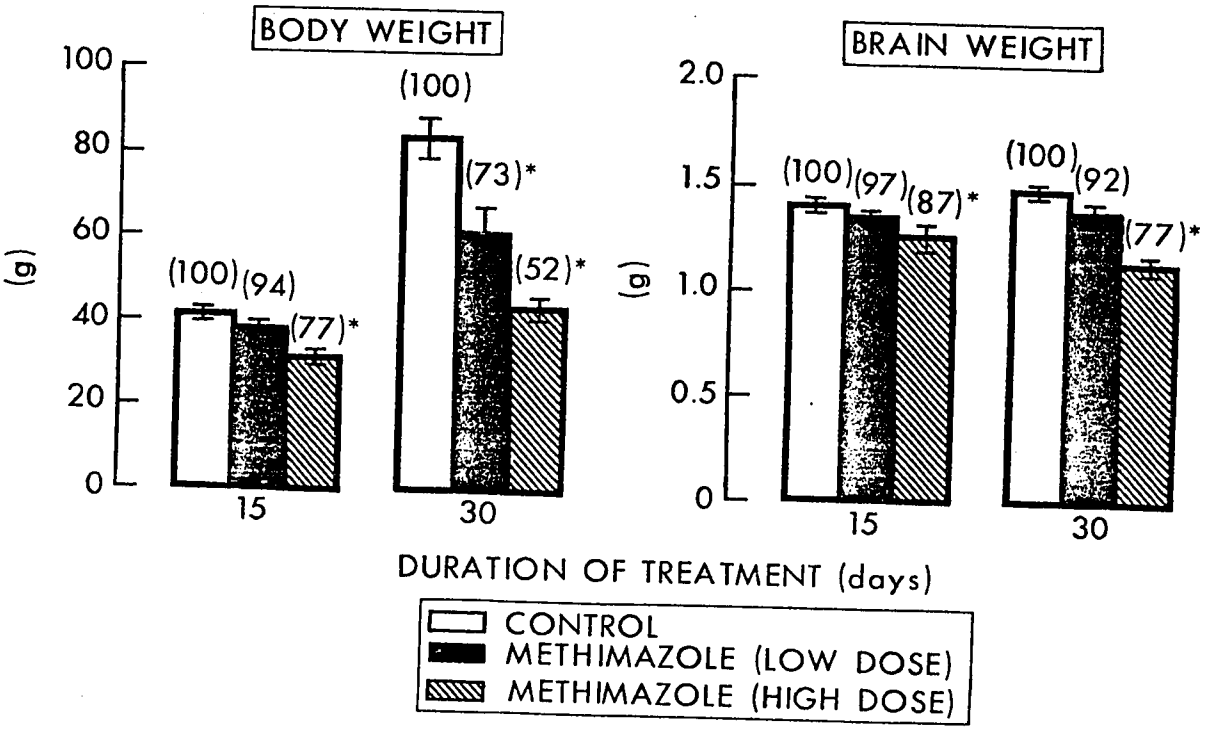

Fig. 29. Effect of methimazole treatment on body and brain weights. Each bar represents the mean ± S.E.M. of 6 values in the group. One-day old rats were injected with low or high doses of methimazole for 15 or 30 days. Data in parentheses indicate percentages taking the values of corresponding control rats as 100%.

*Statistically significant alterations when compared to the values for control animals ($p < 0.05$).

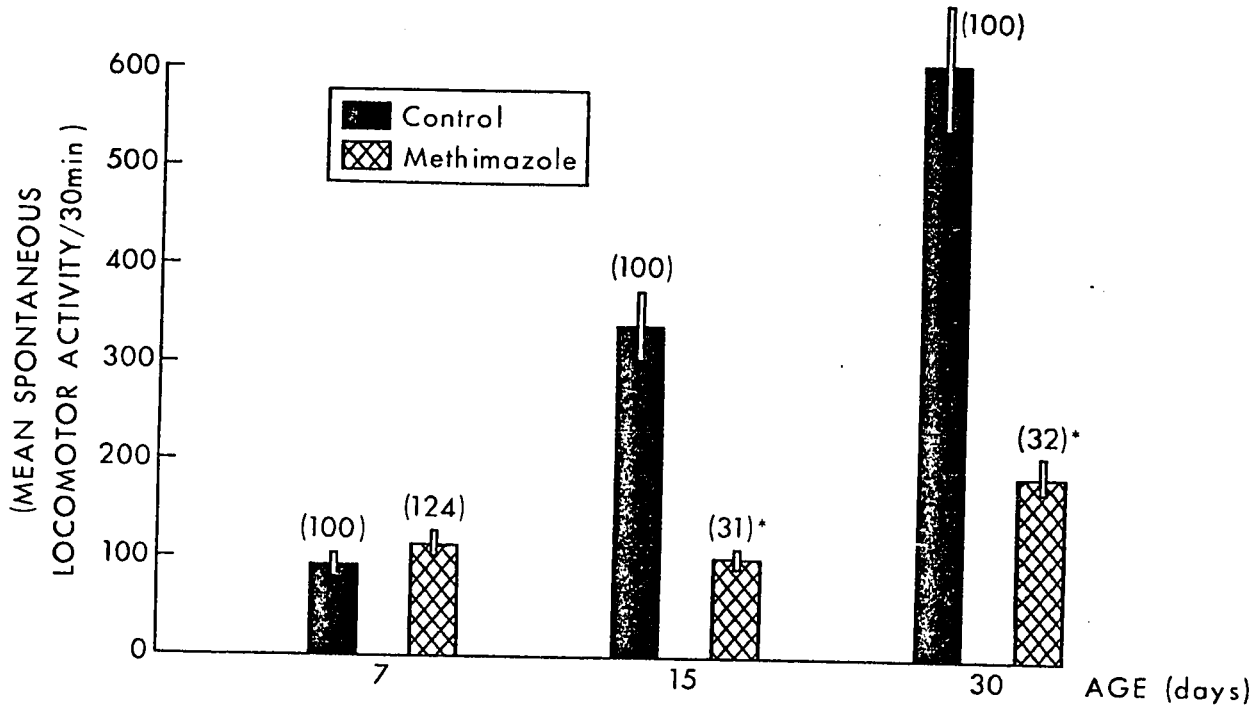

Fig. 30. Effect of methimazole-treatment on the developmental pattern of spontaneous locomotor activity in developing rats. Bars represent the means ± S.E.M. of 6 rats in each group. One-day old rats were injected with high doses of methimazole for 30 days. Control rats received an equal volume of physiological saline. The spontaneous locomotor activity was quantitated in a 30 min session. Data in parentheses express results in percentages taking the control values as 100%.

*Statistically significant difference when compared with the control values ($p < 0.05$).

period of time led to profound impairment in the ontogenic increases of spontaneous locomotor activity. The changes became statistically significant at 15 days of post-natal life when the spontaneous activity was decreased by 69%; the locomotor activity remained equally suppressed (68%) in hypothyroid rats at 30 days of age. These results suggest that methimazole administration in high dosages for 30 days induced chemical thyroidectomy which was manifested by marked interference both in growth and behaviour. Furthermore, the methimazole-induced alterations seemed to be comparable to those seen in radiothyroidectomized rats.

B. Methimazole-Induced Alterations in Brain Norepinephrine and 5-Hydroxytryptamine Metabolism

Results in Figure 31 demonstrate that methimazole administered in low dosages for 30 days significantly decreased the activity of brain tyrosine hydroxylase. The changes in the activity of this brain enzyme were quite pronounced even in 15 day old rats receiving high dosages of the anti-thyroid drug. Treatment with this goiterogen produced an almost similar decrease in the concentration of brain norepinephrine. Data presented in Figure 31 also show that methimazole produced pronounced alterations in the concentration of brain norepinephrine which was reduced by 24% in 30 day old rats given high dosages of the anti-thyroid drug. Whereas exposure to methimazole for 15 days produced a statistically-nonsignificant change in the activity of brain tryptophan hydroxylase, it was decreased to 82% in rats given high dosages of methimazole for 30 days (Fig. 32). Methimazole treatment also produced consistent changes in the levels of brain 5-hydroxytryptamine. Although methimazole given for 15 days produced no appreciable effect on brain 5-hydroxyindoleacetic acid levels, these were significantly elevated in 30 day old rats receiving high dosages of methimazole.

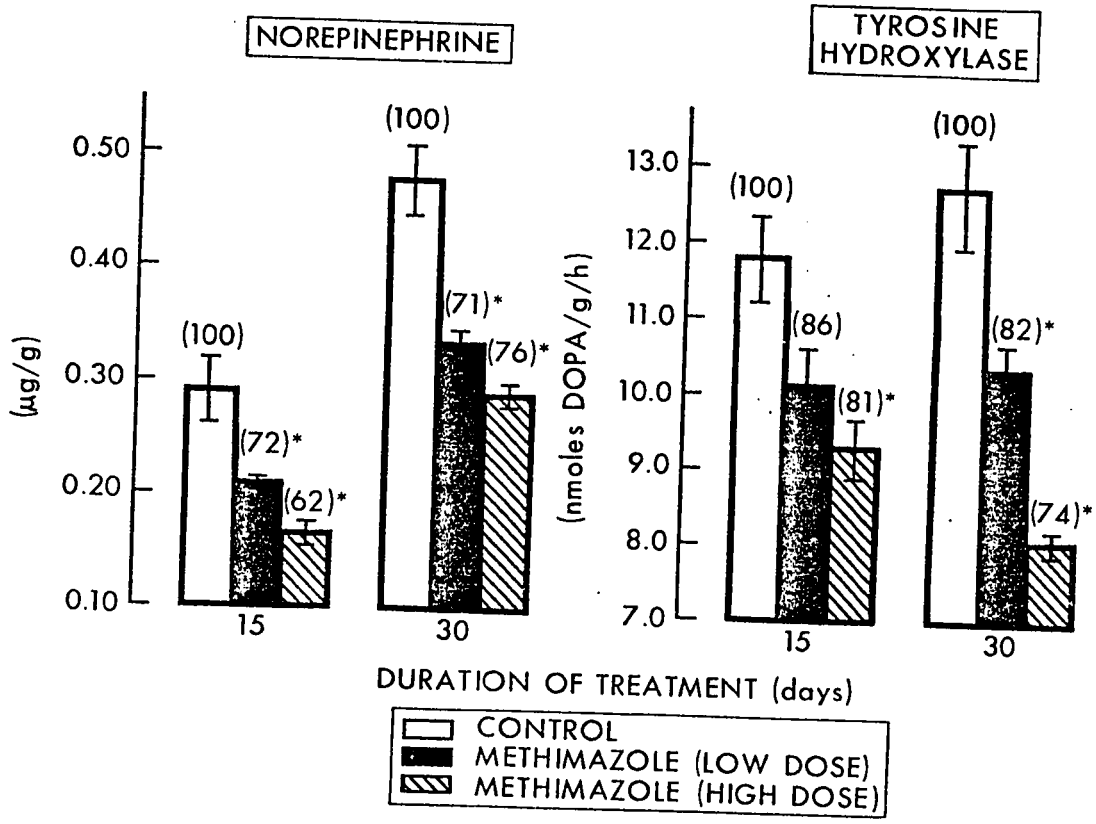

Fig. 31. Effect of methimazole-treatment on brain NE and TH activity. Each bar represents the mean ± S.E.M. of 6 values in the group. One-day old rats were injected with low or high doses of methimazole for 15 or 30 days. Data in parentheses indicate percentages taking the values of corresponding control rats as 100%.

*Statistically significant alterations when compared to the values for control animals ($p < 0.05$).

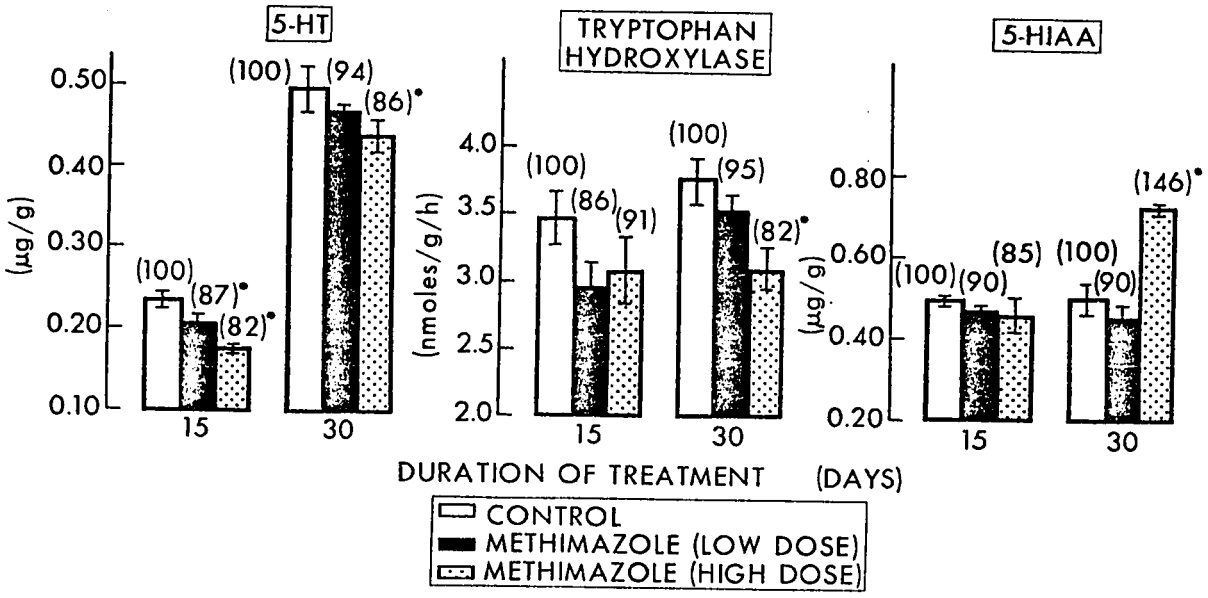

Fig. 32. Effect of methimazole-treatment on brain TPH activity as well as on 5-HT and 5-HIAA levels. Each bar represents the mean ± S.E.M. of 6 values in the group. One-day old rats were injected with low or high doses of methimazole for 15 or 30 days. Data in parentheses indicate percentages taking the values of corresponding control rats as 100%.

*Statistically significant alterations when compared with the values of control animals ($p < 0.05$).

Since the level and the turnover of brain 5-hydroxytryptamine are profoundly influenced by the rate of tryptophan uptake in nervous tissue (141), the effect of chemical thyroidectomy on the concentrations of this amino acid also was investigated. Data presented in Table 11 suggest that exposure to methimazole (high dosages) for a period of either 15 or 30 days exerted no significant effect on the concentrations of brain tryptophan, the substrate for the enzyme tryptophan hydroxylase.

C. Effect of Methimazole on Brain Monoamine Oxidase and Catechol-o-Methyl Transferase

Data in Table 12 demonstrate that chemical thyroidectomy by methimazole tended to increase the activity of monoamine oxidase in 30 day old rats; however, the changes were statistically non-significant ($p > 0.05$). In contrast, catechol-o-methyl transferase activity was significantly augmented in the brains of rats exposed to the high dosages of methimazole in early neonatal life.

IV. DOSE RESPONSE STUDIES WITH L-TRIIODOTHYRONINE

A. Influence of L-Triiodothyronine Treatment on Chemically Thyroidectomized Rats

The question whether administration of L-triiodothyronine to chemically thyroidectomized rats could restore some of the alterations seen in brain amine metabolism was next investigated. Daily administration of L-triiodothyronine in doses of either 25, 100 or 250 µg/100 g for 3 days virtually failed to produce any appreciable change in body and brain weights (Table 13). The levels of norepinephrine and tyrosine hydroxylase were elevated slightly in hypothyroid rats treated with low doses (25 or 100 µg/100 g) of L-triiodothyronine, although the changes were statistically non-significant However, increasing the dose of L-triiodothyronine to 250 µg/100 g markedly

TABLE 11

EFFECT OF METHIMAZOLE TREATMENT ON BRAIN TP LEVELS

Values represent means ± S.E.M. of 6 rats in each group. One-day old rats were injected with high doses of methimazole for a period of either 15 or 30 days. Control animals received an equal volume of physiological saline. The values of tryptophan are expressed as µg/g. Data in parentheses express results in percentages taking the control values as 100%.

Treatment	Age (Days)	
	15	30
Control	1.12±0.11 (100)	1.84±0.12 (100)
Methimazole	1.15±0.10 (103)[θ]	1.78±0.14 (97)[θ]

θ Statistically non-significant difference when compared with the values of control animals ($p > 0.05$).

TABLE 12

EFFECT OF METHIMAZOLE TREATMENT ON BRAIN MAO AND COMT ACTIVITY

Values represent means ± S.E.M. of 6 rats in each group. One-day old rats were injected with high doses of methimazole for 30 days. Control rats received an equal volume of physiological saline. Data in parentheses express results in percentages taking the control values as 100%.

Treatment	MAO (nmoles/g/h)	COMT (nmoles/g/h)
Control	128±20 (100)	8710±618 (100)
Methimazole	148±24 (115)	11077±729 (118)*

*Statistically significant difference when compared with the control values ($p < 0.05$).

TABLE 13

EFFECT OF T_3 TREATMENT ON BODY AND BRAIN WEIGHTS AS WELL AS ON NE LEVELS AND TH ACTIVITY IN BRAINS OF METHIMAZOLE-TREATED RATS

Each value represents the mean ± S.E.M. of 6 animals in the group. Rats pretreated with methimazole (H.D.) for 30 days were injected s.c. with T_3 in a dose of either 25, 100 or 250 µg/100 g daily for 3 days. Data in parentheses express results in percentages taking the values for control (methimazole-treated) animals as 100%.

Dosage of T_3 (µg/100 g/day)	Body wt. (g)	Brain wt. (g)	NE (µg/g)	TH (nmoles DOPA/g/h)
Control	45.2±3.28 (100)	1.32±0.04 (100)	0.32±0.02 (100)	7.91±0.47 (100)
25	47.5±6.96 (105)	1.36±0.03 (103)	0.35±0.02 (109)	8.33±0.41 (105)
100	47.3±3.02 (105)	1.45±0.05 (110)	0.36±0.03 (113)	9.18±0.26 (116)
250	53.3±7.08 (118)	1.50±0.03 (114)	0.44±0.02 (138)*	9.65±0.13 (122)*

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

increased the concentration of norepinephrine as well as the activity of its synthesizing enzyme, tyrosine hydroxylase. Similarly, this dose of L-triiodothyronine restored the levels of 5-hydroxytryptamine and the activity of tryptophan hydroxylase in brains of methimazole-treated animals. Furthermore, the concentration of brain 5-hydroxyindoleacetic acid in L-triiodothyronine-treated (250 µg/100 g) rats was significantly decreased and appeared to attain normal values seen in the corresponding age group (Table 14).

V. INFLUENCE OF ACUTE DESMETHYLIMIPRAMINE TREATMENT ON ALTERED BRAIN AMINE METABOLISM SEEN DURING CHEMICAL THYROIDECTOMY

Since striking similarities seem to exist between the symptomatology of thyroid deficiency and of certain affective disorders such as depression (18), it was of interest to examine whether desmethylimipramine, an efficient clinically used anti-depressant, can influence the changes in norepinephrine and 5-hydroxytryptamine metabolism seen in the brains of chemically thyroidectomized rats. Results presented in Figure 33 demonstrate that acute desmethylimipramine treatment (10 mg/kg) significantly decreased the levels of brain 5-hydroxytryptamine in normal rats. It may be of interest to note that the level of this indoleamine was further reduced in the brains of chemically thyroidectomized rats receiving a single injection of this imipramine congener. The activity of brain tryptophan hydroxylase also was reduced, although the decrease was statistically significant only in the group of normal animals. Following the administration of this tricyclic antidepressant, the concentration of brain 5-hydroxyindoleacetic acid was reduced in both normal and chemically thyroidectomized animals. Data presented in Figure 34 show that acute treatment with desmethylimipramine failed to alter the activity of brain tyrosine hydroxylase and the level of norepinephrine in both normal as well as thyroid-deficient rats.

TABLE 14

EFFECT OF T_3 TREATMENT ON TPH ACTIVITY AS WELL AS ON 5-HT AND 5-HIAA LEVELS IN BRAINS OF METHIMAZOLE-TREATED RATS

Each value represents mean ± S.E.M. of 6 animals in the group. Rats pretreated with methimazole (H.D.) for 30 days were injected subcutaneously with T_3 in a dose of either 25, 100 or 250 µg/100 g daily for 3 days. Data in parentheses express results in percentages taking the values for control (methimazole-treated) animals as 100%.

Dosage of T_3 (µg/100 g/day)	5-HT (µg/g)	TPH (nmoles/g/h)	5-HIAA (µg/g)
Control	0.42±0.01 (100)	2.95±0.18 (100)	0.76±0.07 (100)
25	0.45±0.03 (107)	2.92±0.14 (99)	0.74±0.02 (97)
100	0.50±0.02 (119)*	3.39±0.35 (115)	0.57±0.09 (75)
250	0.51±0.02 (121)*	3.48±0.12 (118)*	0.50±0.06 (66)*

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

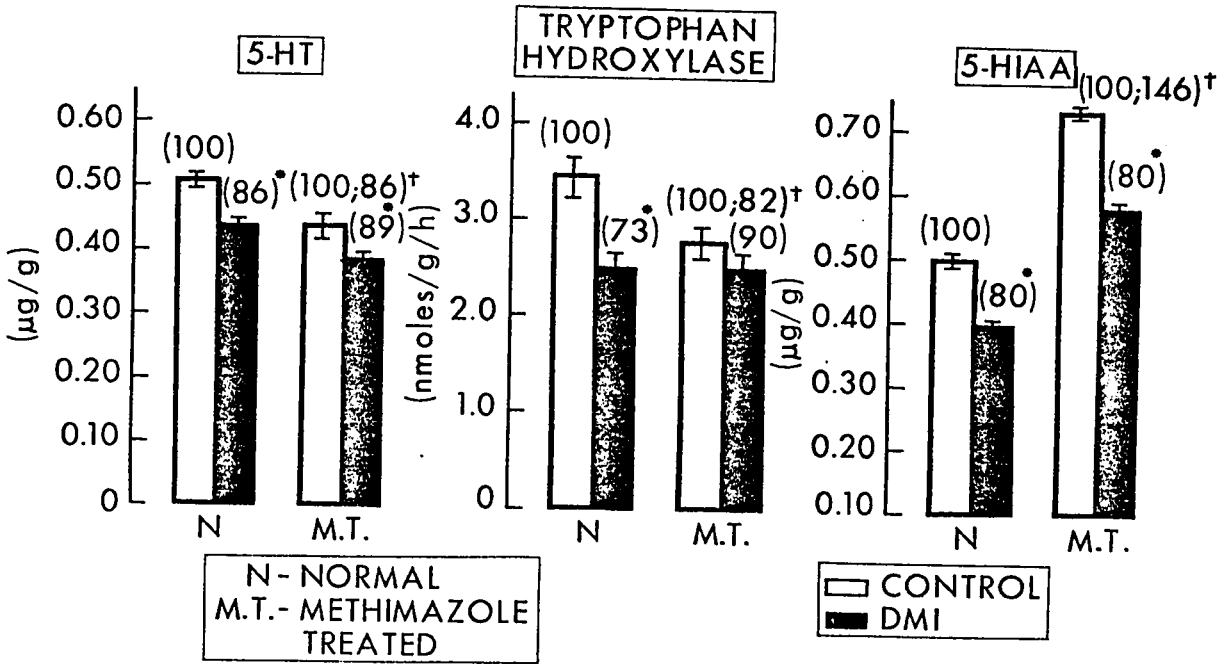

Fig. 33. Effects of DMI on brain TPH activity as well as on 5-HT and 5-HIAA levels in normal and methimazole-treated rats. Bars represent the means ± S.E.M. of 5 rats in each group. One-day old rats were treated intraperitoneally with high doses of methimazole for 30 days. Control animals received an equal volume of physiological saline. Groups of 30 day old normal and methimazole-treated rats were injected with DMI (10 mg/kg, i.p.) or saline and killed 60 min later. Data in parentheses express the results in percentages taking the values of control animals as 100%.

*Statistically significant difference when compared with corresponding controls ($p < 0.05$).

†Statistically significant difference when compared with control values of normal rats ($p < 0.05$).

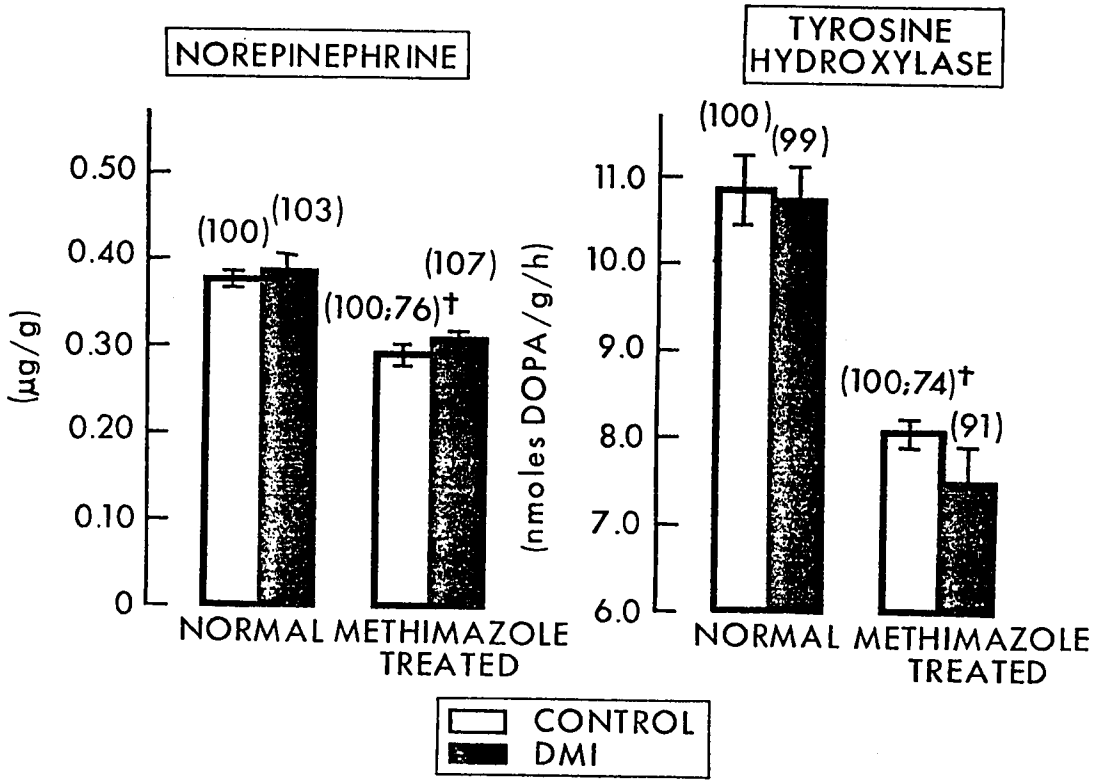

Fig. 34. Effects of DMI on brain TH activity and the levels of NE in normal and methimazole-treated rats. Bars represent the means ± S.E.M. of 5-6 rats. One-day old rats were treated intraperitoneally with high doses of methimazole for 30 days. Control animals received an equal volume of physiological saline. Groups of 30 day old normal and methimazole-treated rats were injected with DMI (10 mg/kg, i.p.) or saline and killed 60 min later. Data in parentheses express results in percentages taking the values of control animals as 100%.

†Statistically significant difference when compared with control values of normal rats ($p < 0.05$).

VI. EFFECT OF HYPERTHYROIDISM

Results of the preceding section demonstrated that the ontogeny of norepinephrine and 5-hydroxytryptamine metabolism is profoundly influenced by the deficiency of thyroid hormone in early neonatal life. Furthermore, a critical period appeared to exist in early post-natal life during which this hormone must be present in order to permit the normal developmental patterns of these putative neurotransmitters. These findings prompted an investigation of the influence of excess thyroid hormone on the metabolism of these neurohumors in neonates as well as in adult animals.

A. Effect of Neonatal Hyperthyroidism on Physical and Behavioural Development

In neonates, hyperthyroidism induced by daily administration of L-triiodothyronine (10 µg/100 g) accelerated the development of a variety of physical and behavioural characteristics which accompany maturation of young rats. L-Triiodothyronine treatment advanced the first appearance of incisors and opening of eyes by 2-3 days. The elevation of pinnae and snout elongation also were advanced as compared to the littermate controls. After 15 days of L-triiodothyronine treatment, the animals had the general appearance of a "miniature" adult. However, administration of exogenous thyroid hormone tended to decrease the body (82%) and brain weights (86%) although the changes were statistically nonsignificant ($p > 0.05$) (Table 15). The neurophysiological precocity was evidenced by earlier appearance of startle, righting and placing reflexes and a more co-ordinated and aggressive feeding behaviour. The animals were hyperactive and a few exhibited circling movement followed by transient tremors. Results presented in Figure 35 illustrate the ontogenesis of spontaneous locomotor activity in rats treated with L-triiodothyronine daily for 45 days and then subsequently withdrawn for 60

TABLE 15

EFFECT OF NEONATAL HYPERTHYROIDISM ON BODY AND BRAIN WEIGHTS

Values represent the means ± S.E.M. of 6 animals in each group. One-day-old rats were injected s.c. with T_3 (10 µg/100 g/day) for 30 days and killed 24 h after the last injection. Control rats received an equal volume of the vehicle (0.02 N NaOH). Data in parentheses indicate the percentages taking the values for control rats as 100%.

Treatment	Body wt. (g)	Brain wt. (g)
Control	60.0±2.9 (100)	1.12±0.06 (100)
Hyperthyroid	49.4±3.3 (82)θ	0.97±0.1 (86)θ

θStatistically non-significant difference when compared with the values of control animals ($p > 0.05$).

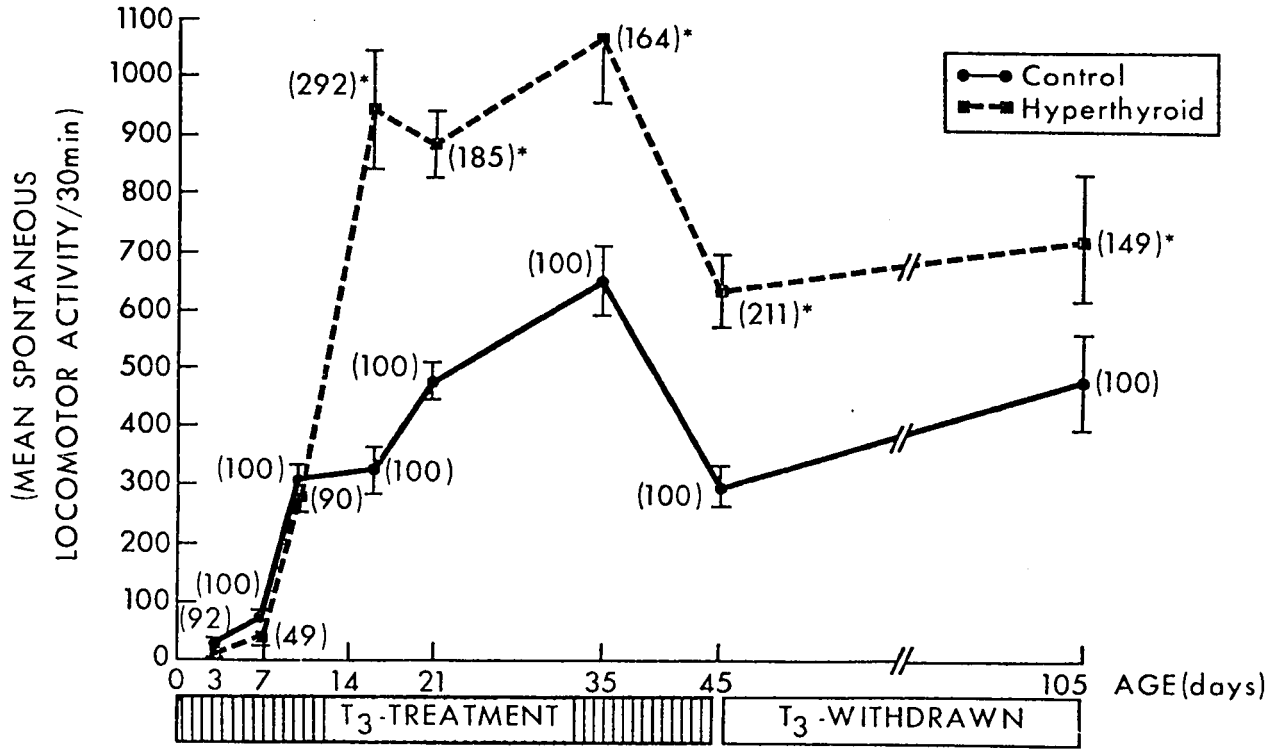

Fig. 35. Effect of 45-day T_3 treatment and 60-day withdrawal on the ontogenesis of spontaneous locomotor activity. Each point represents the mean ± S.E.M. of 8 rats in the group. One-day old rats were injected subcutaneously with T_3 (10 µg/100 g/day) for 45 days and then subsequently withdrawn from T_3 for 60 days. The spontaneous locomotor activity was determined for 30 min session. Data in parentheses express results in percentages taking the values for corresponding controls as 100%.

*Statistically significant difference when compared with the values for control animals ($p < 0.05$).

days. Administration of L-triiodothyronine during the first 10 days of life seemed to slightly slow down the movement of rats. However, at 15 days of age, the hyperthyroid rats showed the most marked increase (292%) in their motor activity which remained elevated throughout the entire course of thyroid hormone administration. It is of interest that hyperthyroidism produced in early life seems to leave a fairly permanent effect on the locomotor activity which remained at significantly higher levels even 60 days after the discontinuation of L-triiodothyronine treatment.

B. Effect of Neonatal Hyperthyroidism on Norepinephrine and 5-Hydroxytryptamine Metabolism in Certain Discrete Brain Regions

Results in Table 16 demonstrate that neonatal hyperthyroidism increased the activity of striatal tyrosine hydroxylase by 36%. The concentration of tyrosine, the substrate for the enzyme tyrosine hydroxylase, also was significantly enhanced. In contrast, the steady state levels of norepinephrine remained unchanged in hypothalamus, pons-medulla, mid-brain, striatum and hippocampus. However, in cerebellum, the levels of this catecholamine were increased by 65%. The effects of excess thyroid hormone on the metabolism of 5-hydroxytryptamine in brains of developing rats are shown in Table 17. Whereas pons-medulla, mid-brain, striatum and hippocampus showed no significant alterations, the levels of 5-hydroxytryptamine were slightly decreased in cerebellum and hypothalamus of rats receiving L-triiodothyronine from birth. Conversely, the activity of tryptophan hydroxylase and tryptophan levels in mid-brain were increased by 28% and 41% respectively. The concentration of indoleamine metabolite, 5-hydroxyindoleacetic acid, was markedly increased in all regions of the brain examined except in cerebellum where the increase was statistically nonsignificant. These results demonstrate that neonatal hyperthyroidism in rats led to significant changes in the

TABLE 16

EFFECT OF NEONATAL HYPERTHYROIDISM ON NE LEVELS IN CERTAIN BRAIN REGIONS AND ON STRIATAL TH AND TR

Values represent the means ± S.E.M. of 6 animals in each group. One-day-old rats were injected s.c. with T_3 (10 µg/100 g/day) for 30 days and killed 24 h after the last injection. Control rats received an equal volume of the vehicle (0.02 N NaOH). Data in parentheses indicate the percentages taking the values for control rats as 100%.

Parameter	Region	Treatment	
		Control	Hyperthyroid
NE (µg/g)	Cerebellum	0.32±0.03 (100)	0.53±0.07 (165)*
	Hypothalamus	2.31±0.18 (100)	2.52±0.23 (109)
	Pons-Medulla	0.61±0.04 (100)	0.65±0.06 (107)
	Mid-brain	0.63±0.05 (100)	0.54±0.08 (85)
	Striatum	0.35±0.03 (100)	0.34±0.04 (96)
	Hippocampus	0.41±0.04 (100)	0.46±0.03 (113)
TH (nmoles DOPA/g/h)	Striatum	83.78±6.58 (100)	114.31±8.8 (136)*
TR (µg/g)	Striatum	31.27±2.0 (100)	37.89±2.7 (121)*

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

TABLE 17

EFFECT OF NEONATAL HYPERTHYROIDISM ON 5-HT AND 5-HIAA LEVELS IN CERTAIN BRAIN REGIONS AND ON MID-BRAIN TPH AND TP

Values represent the means ± S.E.M. of 6 animals in each group. One-day-old rats were injected s.c. with T_3 (10 µg/100 g/day) for 30 days and killed 24 h after the last injection. Control rats received an equal volume of the vehicle (0.02 N NaOH). Data in parentheses indicate the percentages taking the values for control rats as 100%.

Parameter	Region	Treatment	
		Control	Hyperthyroid
5-HT (µg/g)	Cerebellum	0.55±0.03 (100)	0.47±0.03 (86)*
	Hypothalamus	2.49±0.17 (100)	1.97±0.18 (79)*
	Pons-Medulla	1.48±0.07 (100)	1.45±0.08 (98)
	Mid-brain	1.76±0.09 (100)	1.71±0.11 (97)
	Striatum	1.52±0.18 (100)	1.16±0.17 (76)
	Hippocampus	1.32±0.12 (100)	1.19±0.15 (90)
TPH (nmoles/g/h)	Mid-brain	10.91±0.93 (100)	13.97±1.01 (128)*
TP (µg/g)	Mid-brain	6.4±0.45 (100)	9.04±1.16 (141)*
5-HIAA (µg/g)	Cerebellum	0.41±0.03 (100)	0.48±0.07 (116)
	Hypothalamus	1.95±0.19 (100)	2.96±0.24 (152)*
	Pons-Medulla	1.36±0.12 (100)	1.81±0.13 (133)*
	Mid-brain	1.64±0.07 (100)	1.98±0.09 (121)*
	Striatum	1.37±0.08 (100)	2.32±0.11 (169)*
	Hippocampus	1.26±0.10 (100)	1.49±0.09 (118)*

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

metabolism of both brain norepinephrine and 5-hydroxytryptamine.

C. Effect of Neonatal Hyperthyroidism on Monoamine Oxidase and Catechol-o-Methyl Transferase Activity

Data presented in Table 18 demonstrate that administration of L-triiodothyronine in early life resulted in no appreciable effect in the activity of the deaminating enzyme, monoamine oxidase. However, the cerebrocortical catechol-o-methyl transferase activity was significantly decreased (18%) in hyperthyroid rats, which seems to be opposite to the effect of neonatal thyroidectomy produced by methimazole or radioactive iodine.

D. Effect of Adult Hyperthyroidism on Norepinephrine and 5-Hydroxytryptamine Metabolism in Certain Discrete Brain Regions

The influence of the administration of L-triiodothyronine in adult rats on the metabolism of brain norepinephrine and 5-hydroxytryptamine also was investigated. Daily administration of L-triiodothyronine (10 µg/100 g) for 30 days initiated at 120 days of age, significantly reduced (84%) body growth without eliciting much effect on brain weights (Table 19). In addition, L-triiodothyronine treatment during adulthood failed to produce any apparent alterations in the physical appearance of animals.

Data in Table 20 show that treatment of adult rats with L-triiodothyronine for 30 days significantly decreased the levels of norepinephrine in hypothalamus, pons-medulla and mid-brain. The magnitude of change was maximal in the hypothalamic region in which norepinephrine level was reduced to 0.95 µg/g from the control values of 2.15 µg/g. The concentrations of this catecholamine increased slightly in the hippocampus, but remained unchanged in cerebellum and striatum of adult hyperthyroid rats. Unlike neonatal hyperthyroidism, exogenous thyroid hormone treatment of adult rats failed to stimulate the activity of striatal tyrosine hydroxylase.

TABLE 18

EFFECT OF NEONATAL HYPERTHYROIDISM ON CEREBROCORTICAL MAO AND COMT ACTIVITY

Values represent the means ± S.E.M. of 6 animals in each group. One-day-old rats were injected s.c. with T_3 (10 µg/100 g/day) for 30 days and killed 24 h after the last injection. Control rats received an equal volume of the vehicle (0.02 N NaOH). Data in parentheses indicate the percentages taking the values for control rats as 100%.

Parameter	Treatment	
	Control	Hyperthyroid
COMT (nmoles/g/h)	8400±400 (100)	6880±620 (82)*
MAO (nmoles/g/h)	156±9.2 (100)	156±7.0 (100)

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

TABLE 19

EFFECT OF ADULT HYPERTHYROIDISM ON BODY AND BRAIN WEIGHTS

Values represent the means ± S.E.M. of 6 animals in each group. 120-day-old rats were injected s.c. with T_3 (10 µg/100 g/day) for 30 days and killed 24 h after the last injection. Control rats received an equal volume of the vehicle (0.02 N NaOH). Data in parentheses indicate the percentages taking the values for control rats as 100%.

Treatment	Body wt. (g)	Brain wt. (g)
Control	411.4±11 (100)	1.61±0.14 (100)
Hyperthyroid	347.0±6.08 (84)*	1.68±0.1 (103)

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

TABLE 20

EFFECT OF ADULT HYPERTHYROIDISM ON NE LEVELS IN CERTAIN BRAIN REGIONS AND ON STRIATAL TH AND TR

Values represent the means ± S.E.M. of 6 animals in each group. 120-day-old rats were injected s.c. with T_3 (10 µg/100 g/day) for 30 days and killed 24 h after the last injection. Control rats received an equal volume of the vehicle (0.02 N NaOH). Data in parentheses indicate the percentages taking the values for control rats as 100%.

Parameter	Region	Treatment	
		Control	Hyperthyroid
NE (µg/g)	Cerebellum	0.34±0.01 (100)	0.31±0.02 (91)
	Hypothalamus	2.15±0.14 (100)	0.95±0.09 (44)*
	Pons-Medulla	0.58±0.03 (100)	0.52±0.01 (89)*
	Mid-brain	0.53±0.04 (100)	0.41±0.02 (77)*
	Striatum	0.37±0.03 (100)	0.36±0.03 (98)
	Hippocampus	0.32±0.02 (100)	0.37±0.03 (116)
TH (nmoles DOPA/g/h)	Striatum	76.8±3.2 (100)	89.9±4.8 (117)
TR (µg/g)	Striatum	27.18±1.31 (100)	33.45±2.6 (123)*

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

However, the concentration of striatal tyrosine was significantly increased in rats made hyperthyroid during adult life. L-Triiodothyronine treatment in adult animals produced no appreciable effect on the endogenous levels of 5-hydroxytryptamine in various brain regions except in cerebellum and striatum where the concentration of this indoleamine was reduced by 28% and 30% respectively (Table 21). The activity of mid-brain tryptophan hydroxylase was significantly stimulated by 37%. In addition, a significant augmentation in the levels of mid-brain tryptophan was observed in adult hyperthyroid rats. Results in Table 21 also show that in contrast to the increased levels of 5-hydroxyindoleacetic acid in neonatally hyperthyroid animals, administration of thyroid hormone in adult rats produced virtually no change in the level of this metabolite, except in cerebellum and striatum where it was decreased by almost 15%.

E. Effect of Adult Hyperthyroidism on Monoamine Oxidase and Catechol-o-Methyl Transferase Activity

Administration of L-triiodothyronine in adult animals produced qualitatively similar changes in the activities of monoamine oxidase and catechol-o-methyl transferase as those noted for the brains of young hyperthyroid rats. Whereas the activity of monoamine oxidase remained unaltered in the cerebrocortices of adult hyperthyroid rats, catechol-o-methyl transferase activity was significantly reduced during adult hyperthyroidism (Table 22).

VII. EFFECT OF LITHIUM TREATMENT IN HYPERTHYROID ANIMALS

The above studies suggested that an excess of thyroid hormone in early neonatal life produced a variety of neurochemical and behavioural changes which are not only characteristic of hyperthyroidism, but are also observed during mania in man (17). It has been shown by previous workers that there is an increased turnover of catecholamines manifested by increased

TABLE 21

EFFECT OF ADULT HYPERTHYROIDISM ON 5-HT AND 5-HIAA LEVELS IN CERTAIN BRAIN REGIONS AND ON MID-BRAIN TPH AND TP

Values represent the means ± S.E.M. of 6 animals in each group. 120-day-old rats were injected s.c. with T_3 (10 µg/100 g/day) for 30 days and killed 24 h after the last injection. Control rats received an equal volume of the vehicle (0.02 N NaOH). Data in parentheses indicate the percentages taking the values for control rats as 100%.

Parameter	Region	Treatment	
		Control	Hyperthyroid
5-HT (µg/g)	Cerebellum	0.35±0.02 (100)	0.25±0.02 (72)*
	Hypothalamus	1.78±0.20 (100)	1.55±0.18 (87)
	Pons-Medulla	1.32±0.06 (100)	1.10±0.10 (83)
	Mid-brain	1.66±0.07 (100)	1.51±0.08 (91)
	Striatum	1.43±0.09 (100)	1.00±0.08 (70)*
	Hippocampus	1.10±0.08 (100)	1.03±0.04 (94)
TPH (nmoles/g/h)	Mid-brain	7.21±0.54 (100)	9.88±0.73 (137)*
TP (µg/g)	Mid-brain	7.19±0.57 (100)	10.67±0.97 (148)*
5-HIAA (µg/g)	Cerebellum	0.25±0.02 (100)	0.21±0.02 (84)*
	Hypothalamus	1.46±0.14 (100)	1.52±0.12 (104)
	Pons-Medulla	1.19±0.07 (100)	1.20±0.09 (101)
	Mid-brain	1.38±0.06 (100)	1.13±0.05 (82)
	Striatum	1.33±0.08 (100)	1.16±0.07 (87)*
	Hippocampus	0.98±0.10 (100)	1.04±0.09 (106)

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

TABLE 22

EFFECT OF ADULT HYPERTHYROIDISM ON CEREBROCORTICAL MAO AND COMT ACTIVITY

Values represent the means ± S.E.M. of 6 animals in each group. 120-day-old rats were injected s.c. with T_3 (10 µg/100 g/day) for 30 days and killed 24 h after the last injection. Control rats received an equal volume of the vehicle (0.02 N NaOH). Data in parentheses indicate the percentages taking the values for control rats as 100%.

Parameter	Treatment	
	Control	Hyperthyroid
COMT (nmoles/g/h)	8920±520 (100)	7760±280 (87)*
MAO (nmoles/g/h)	160±8.0 (100)	160±6.2 (100)

*Statistically significant difference when compared with the values of control animals ($p < 0.05$).

levels of metabolites in the urine and cerebrospinal fluid of manic patients (247). During recent years, lithium has been employed in the treatment of mania as well as in the prophylaxis of recurrent manic depressive disorders. This antimanic agent is also known to produce hypothyroidism as a side effect (248). Studies on the effect of lithium on norepinephrine and 5-hydroxytryptamine metabolism were therefore undertaken in the brains of young as well as adult hyperthyroid rats.

A. Effect of Lithium on Body and Brain Weights as Well as on the Spontaneous Locomotor Activity of Neonatally Hyperthyroid Rats

Results in Table 23 show that daily administration of lithium in a dose of 60 mg/kg for 6 consecutive days in young rats which had been pretreated with L-triiodothyronine for 24 days, produced virtually no change in body and brain weights. However, lithium treatment in young hyperthyroid rats decreased the spontaneous locomotor activity by 38%, a value which was not significantly different from that seen in 30 day old control animals (Fig. 36). Results presented in Figure 36 also demonstrate that administration of lithium in normal rats, by itself, produced no significant change in their mobility.

B. Effect of Lithium on Norepinephrine and 5-Hydroxytryptamine Metabolism in Certain Discrete Brain Regions of Neonatally Hyperthyroid Rats

As can be seen in Figure 37, the levels of norepinephrine were markedly enhanced in the hypothalamus, mid-brain and striatum, but remained unchanged in the cerebellum and pons-medulla of young hyperthyroid rats treated with lithium. In contrast, the activity of striatal tyrosine hydroxylase was decreased and was restored almost to the levels seen in normal rats of the corresponding age group. Lithium-treated rats tended to exhibit a decrease (11%) in tyrosine concentration of the striatum, although the change was

TABLE 23

EFFECT OF LITHIUM TREATMENT ON BODY AND BRAIN WEIGHTS OF NEONATALLY HYPERTHYROID RATS

Values represent means ± S.E.M. of 6 rats in each group. One-day old rats were injected subcutaneously with T_3 (10 µg/100 g/day) for 30 days. A group of rats treated with T_3 for 24 days was subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express the results in percentages taking the values of control animals as 100%.

Treatment	Body Wt. (g)	Brain Wt. (g)
Hyperthyroid	49.4±3.3 (100)	0.97±0.1 (100)
Hyperthyroid + Lithium	47.8±3.88 (95)$^{\theta}$	1.01±0.06 (105)$^{\theta}$

$^{\theta}$Statistically non-significant difference when compared with the values of control animals ($p < 0.05$).

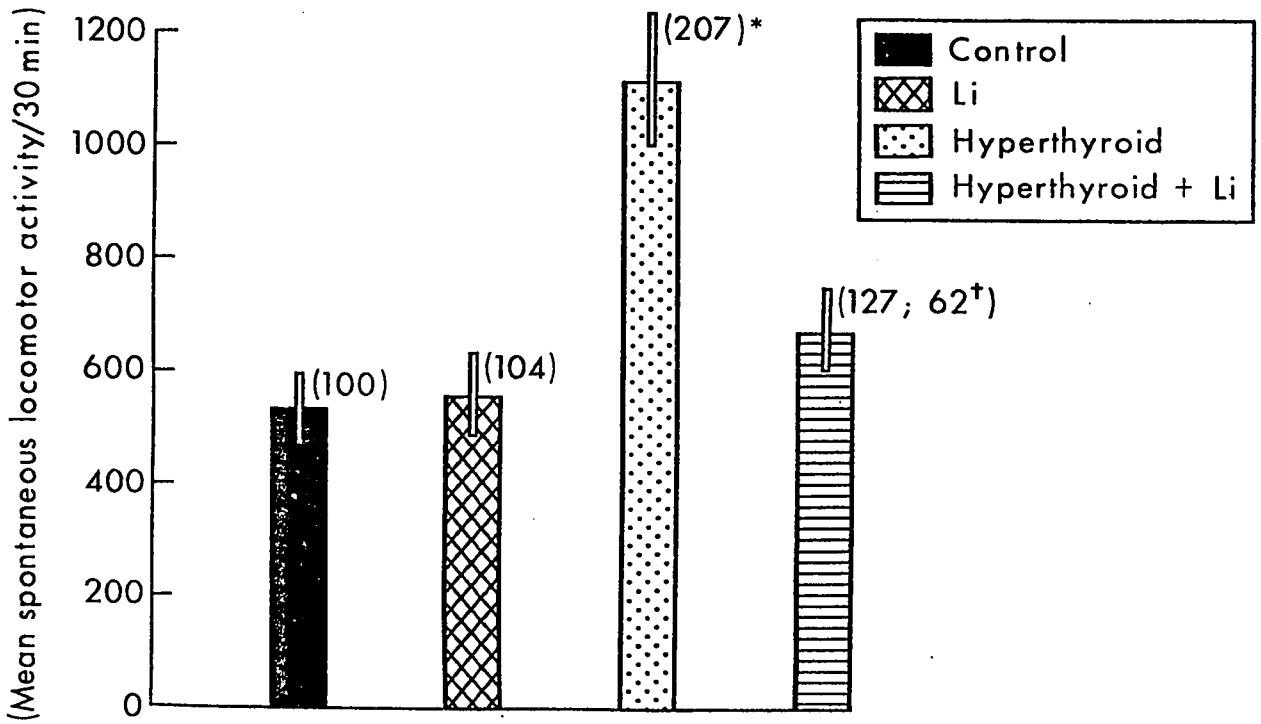

Fig. 36. Effect of lithium on spontaneous locomotor activity of normal and neonatally hyperthyroid rats. Bars represent the means ± S.E.M. of 8 rats in each group. One-day old rats were injected subcutaneously with T_3 (10 µg/100 g/day) for 30 days. Groups of rats treated with T_3 or the vehicle (0.02 N NaOH) for 24 days were subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline.

*Statistically significant difference when compared with the values of control rats ($p < 0.05$).

†Statistically significant difference when compared with the values of hyperthyroid rats ($p < 0.05$).

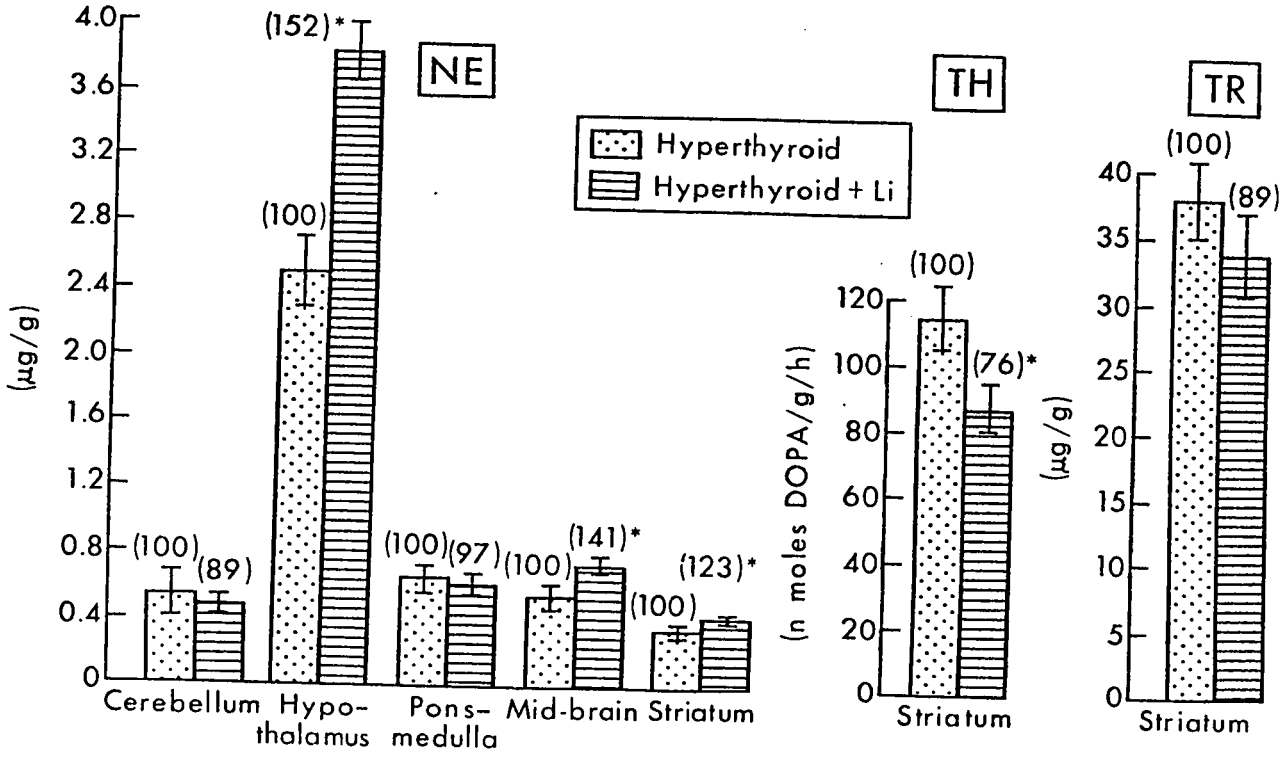

Fig. 37. Effect of lithium treatment on NE levels in certain brain regions and on striatal TH and TR in neonatally hyperthyroid rats. Bars represent the means ± S.E.M. of 6 rats in the group. One-day old rats were injected subcutaneously with T_3 (10 μg/100 g/day) for 30 days. A group of rats treated with T_3 for 24 days was subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control (hyperthyroid) rats ($p < 0.05$).

statistically nonsignificant.

Lithium treatment in young hyperthyroid rats significantly raised the levels of 5-hydroxytryptamine in all regions of brain examined except in the hypothalamus where the change was statistically nonsignificant (Fig. 38). The mid-brain tryptophan levels were significantly elevated (23%) in lithium-treated young hyperthyroid rats (Fig. 39). In contrast, the activity of tryptophan hydroxylase was markedly decreased (50%) from 9.04 to 4.53 nmoles/g/hour. The results illustrated in Figure 40 demonstrate that 5-hydroxyindoleacetic acid concentrations were significantly decreased in all of the brain regions examined, although the magnitude of change (61%) was maximal in the hypothalamus.

C. Effect of Lithium on Monoamine Oxidase and Catechol-o-Methyl Transferase Activity in Neonatally Hyperthyroid Animals

The results presented in Figure 41 serve to demonstrate that lithium treatment exerted no appreciable influence on the activity of monoamine oxidase. However, this antimanic agent was capable of restoring the activity of the o-methylating enzyme, catechol-o-methyl transferase, to the values seen in normal animals of the corresponding age group.

D. Effect of Lithium Administration on Physical and Behavioural Changes Seen in Adult Hyperthyroid Rats

The administration of lithium (60 mg/kg) for 6 days in adult rats, which had been pretreated with L-triiodothyronine for 24 days, produced only a slight but statistically significant increase in body weights (Table 24). However, the brain weights remained relatively unchanged. Figure 42 shows the effect of lithium on the spontaneous locomotor activity of normal and adult hyperthyroid rats. It can be seen that administration of L-triiodothyronine during adulthood increased the locomotor activity, although the

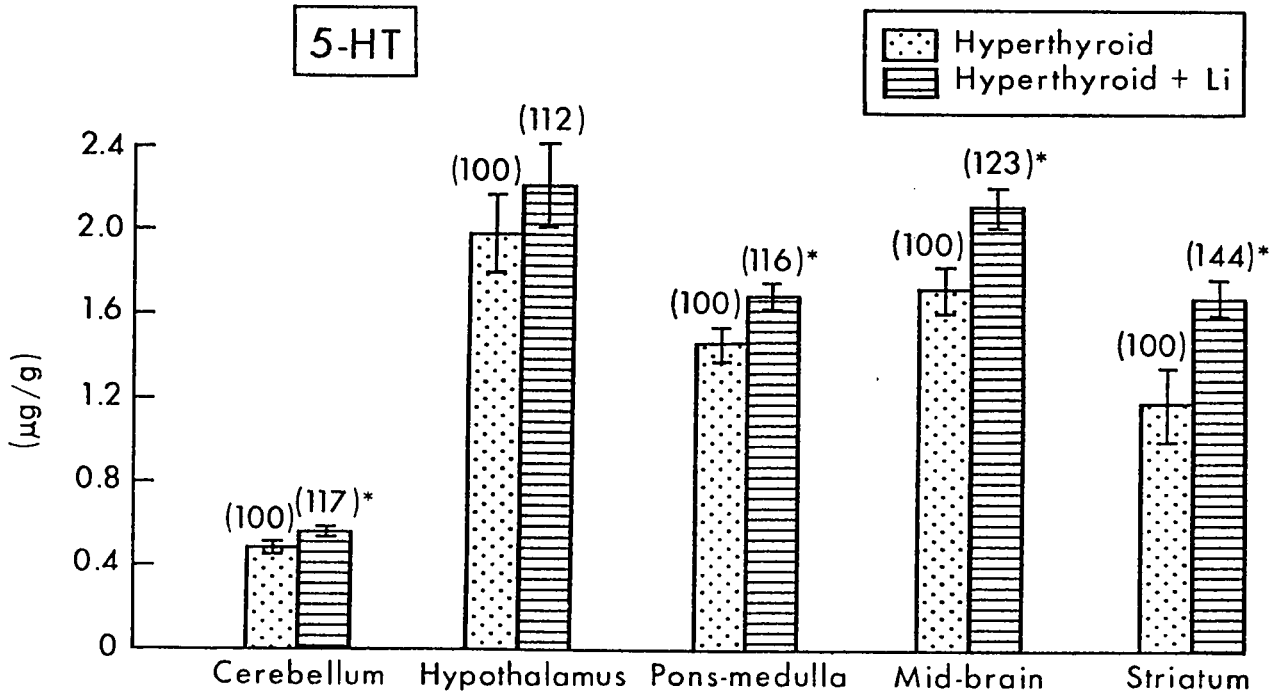

Fig. 38. Effect of lithium treatment on 5-HT levels in certain brain regions of neonatally hyperthyroid rats. Bars represent the means ± S.E.M. of 6 rats in the group. One-day old rats were injected subcutaneously with T_3 (10 μg/100 g/day) for 30 days. A group of rats treated with T_3 for 24 days was subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control (hyperthyroid) rats ($p < 0.05$).

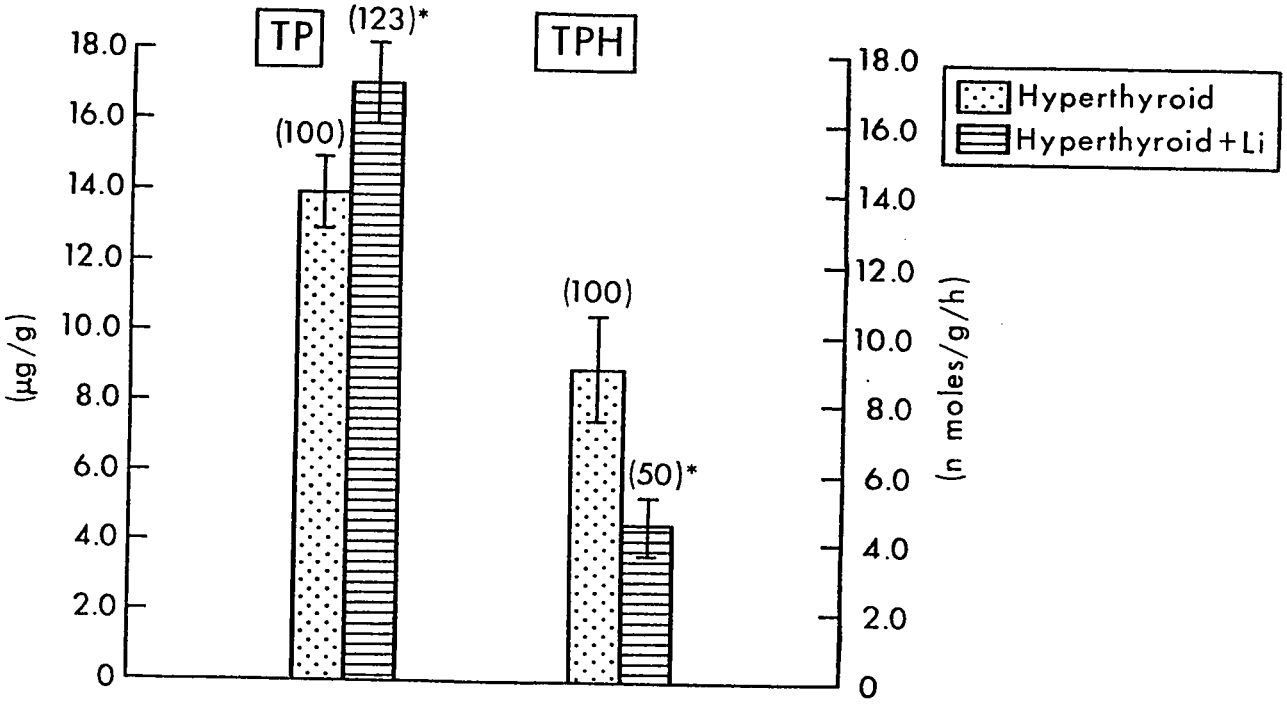

Fig. 39. Effect of lithium treatment on TP and TPH activity in midbrain of neonatally hyperthyroid rats. Bars represent the means ± S.E.M. of 6 rats in the group. One-day old rats were injected subcutaneously with T_3 (10 µg/100 g/day) for 30 days. A group of rats treated with T_3 for 24 days was subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control (hyperthyroid) rats ($p < 0.05$).

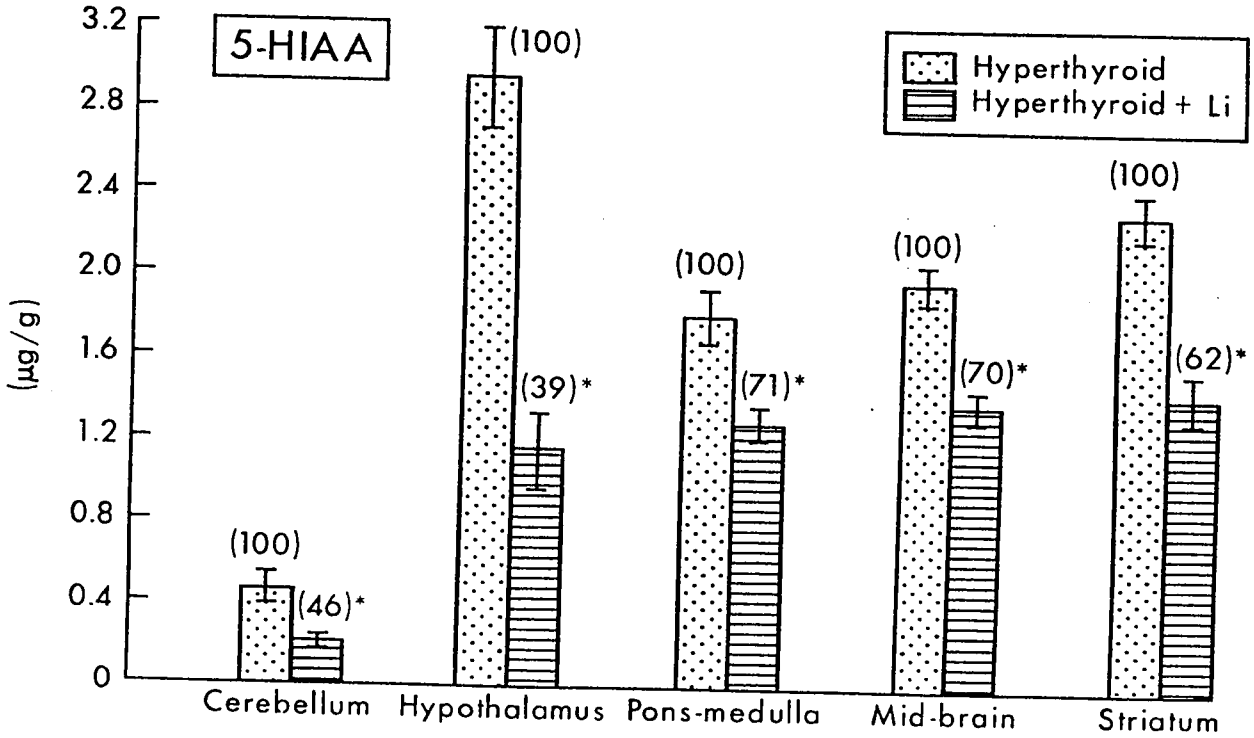

Fig. 40. Effect of lithium treatment on 5-HIAA levels in certain brain regions of neonatally hyperthyroid rats. Bars represent the means ± S.E.M. of 6 rats in the group. One-day old rats were injected subcutaneously with T_3 (10 μg/100 g/day) for 30 days. A group of rats treated with T_3 for 24 days was subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control (hyperthyroid) rats ($p < 0.05$).

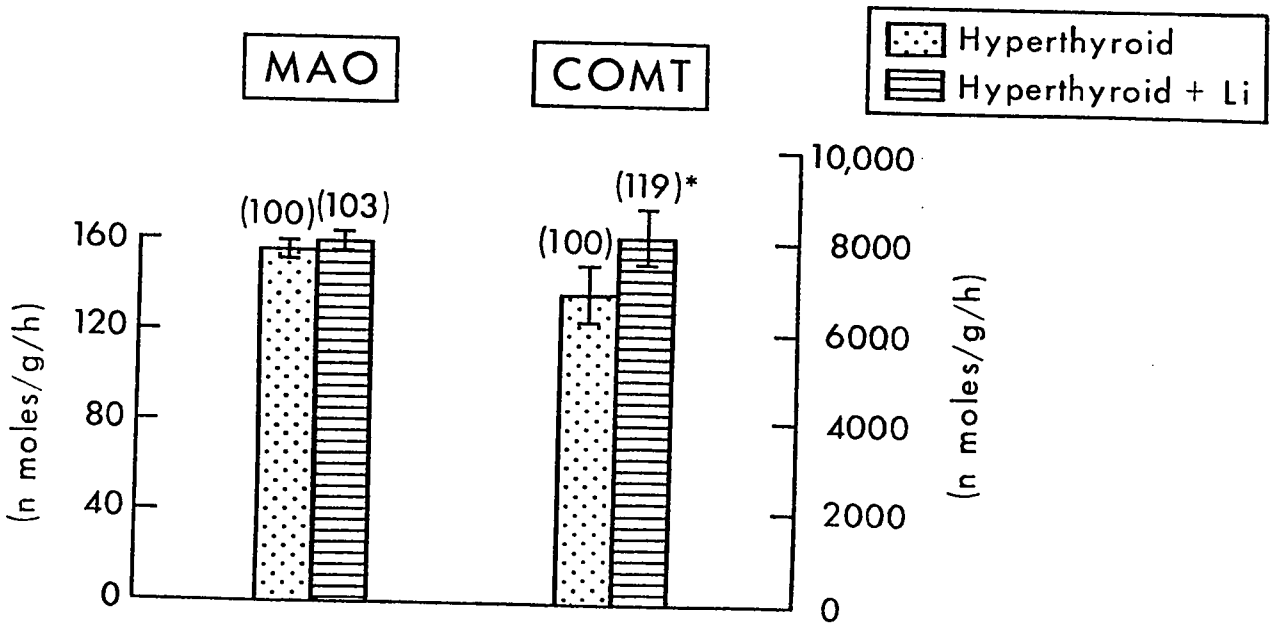

Fig. 41. Effect of lithium treatment on cerebrocortical MAO and COMT in neonatally hyperthyroid rats. Bars represent the means ± S.E.M. of 6 rats in the group. One-day old rats were injected subcutaneously with T_3 (10 µg/100 g/day) for 30 days. A group of rats treated with T_3 for 24 days was subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control (hyperthyroid) rats ($p < 0.05$).

TABLE 24

EFFECT OF LITHIUM TREATMENT ON BODY AND BRAIN WEIGHTS OF ADULT HYPERTHYROID RATS

Values represent means ± S.E.M. of 6 rats in the group. Adult rats were injected s.c. with T_3 (10 g/100 g/day) for 30 days. A group of rats treated with T_3 for 24 days was subsequently injected with lithium intraperitoneally (60 mg/kg body wt.) for 6 days. Control animals received an equal volume of the physiological saline. Data in parentheses express the results in percentages taking the values of control animals as 100%.

Treatment	Body Wt. (g)	Brain Wt. (g)
Hyperthyroid	347±6.08 (100)	1.68±0.1 (100)
Hyperthyroid + Lithium	392±9.16 (113)*	1.56±0.08 (93)

*Statistically significant difference when compared with the values for control animals ($p < 0.05$).

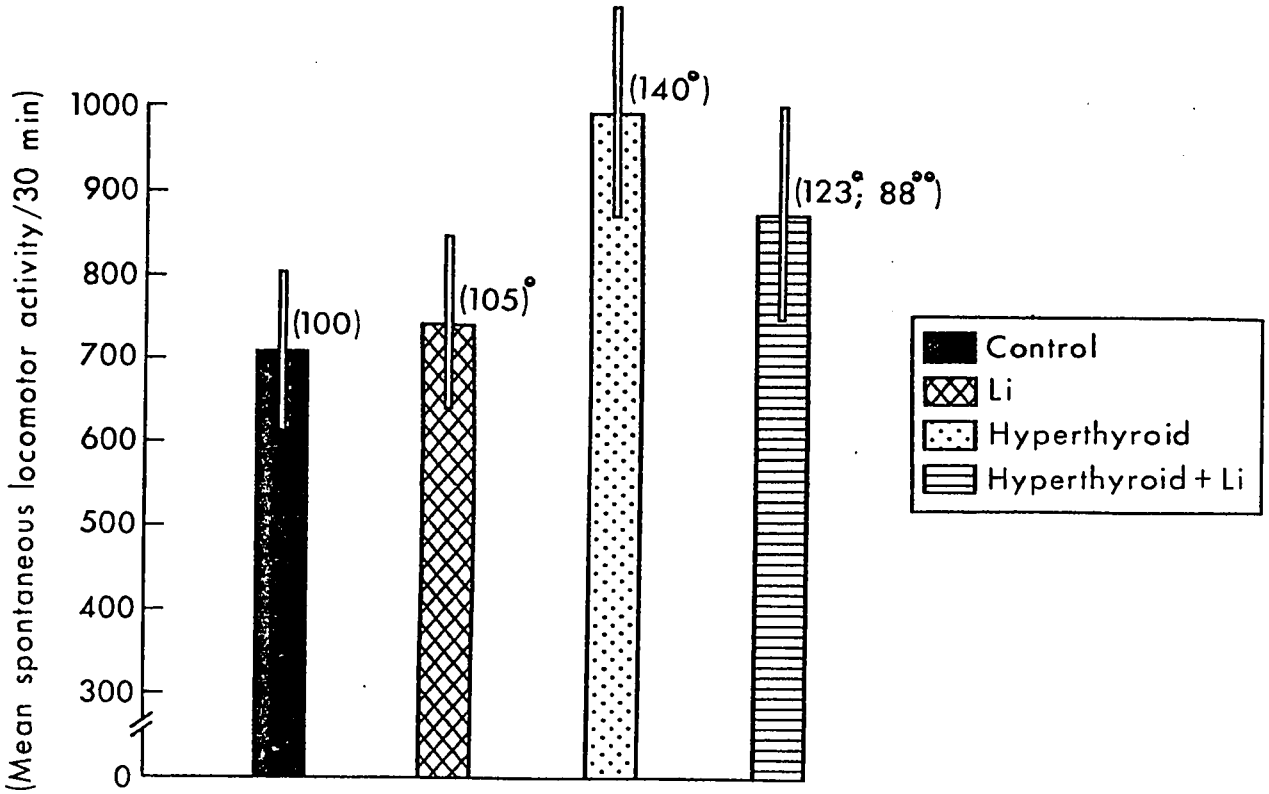

Fig. 42. Effect of lithium on spontaneous locomotor activity of normal and adult hyperthyroid rats. Bars represent the means ± S.E.M. of 8 rats in each group. Adult rats were injected with T_3 (10 µg/100 g/day) for 30 days. Groups of rats treated with T_3 or vehicle (0.02 N NaOH) for 24 days were subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the corresponding vehicle.

θ Statistically non-significant difference when compared with the values of control rats ($p < 0.05$).

θθ Statistically non-significant difference when compared with the values of hyperthyroid rats.

changes were not quite as marked as those seen in young hyperthyroid rats. Furthermore, lithium treatment in hyperthyroid rats decreased the spontaneous locomotor activity by 12%; the change being statistically nonsignificant ($p < 0.05$). Results in Figure 42 also show that lithium treatment in adult animals, by itself, failed to produce any apparent change in their mobility.

E. Effect of Lithium on Norepinephrine and 5-Hydroxytryptamine Metabolism in Certain Discrete Brain Regions of Adult Hyperthyroid Rats

Treatment of adult hyperthyroid rats with lithium significantly raised the concentrations of norepinephrine in the striatum, pons-medulla and hypothalamus (Fig. 43). The levels of this catecholamine also were increased in the cerebellum and mid-brain of hyperthyroid rats treated with lithium, although the changes were statistically nonsignificant. In contrast, the activity of striatal tyrosine hydroxylase was decreased (29%) from 89.9 to 63.8 nmoles DOPA/g/hr, a value which was even lower than that seen in normal adult rats of the corresponding age group. Results in Figure 43 also show that chronic lithium treatment failed to alter the concentration of striatal tyrosine in adult hyperthyroid animals.

The effect of lithium treatment on brain 5-hydroxytryptamine metabolism of adult hyperthyroid rats also was examined. Data in Figure 44 demonstrate that whereas the levels of this indoleamine markedly rose in the striatum, hypothalamus and cerebellum, statistically significant decreases were observed in the mid-brain and pons-medulla. The activity of tryptophan hydroxylase and the concentration of tryptophan in mid-brains of lithium-treated rats showed no significant deviation from the corresponding control values (Fig. 45). It may be of interest to note that the pons-medulla and the mid-brain which contained significantly low levels of 5-hydroxytryptamine also showed significantly low concentrations of 5-hydroxyindoleacetic acid

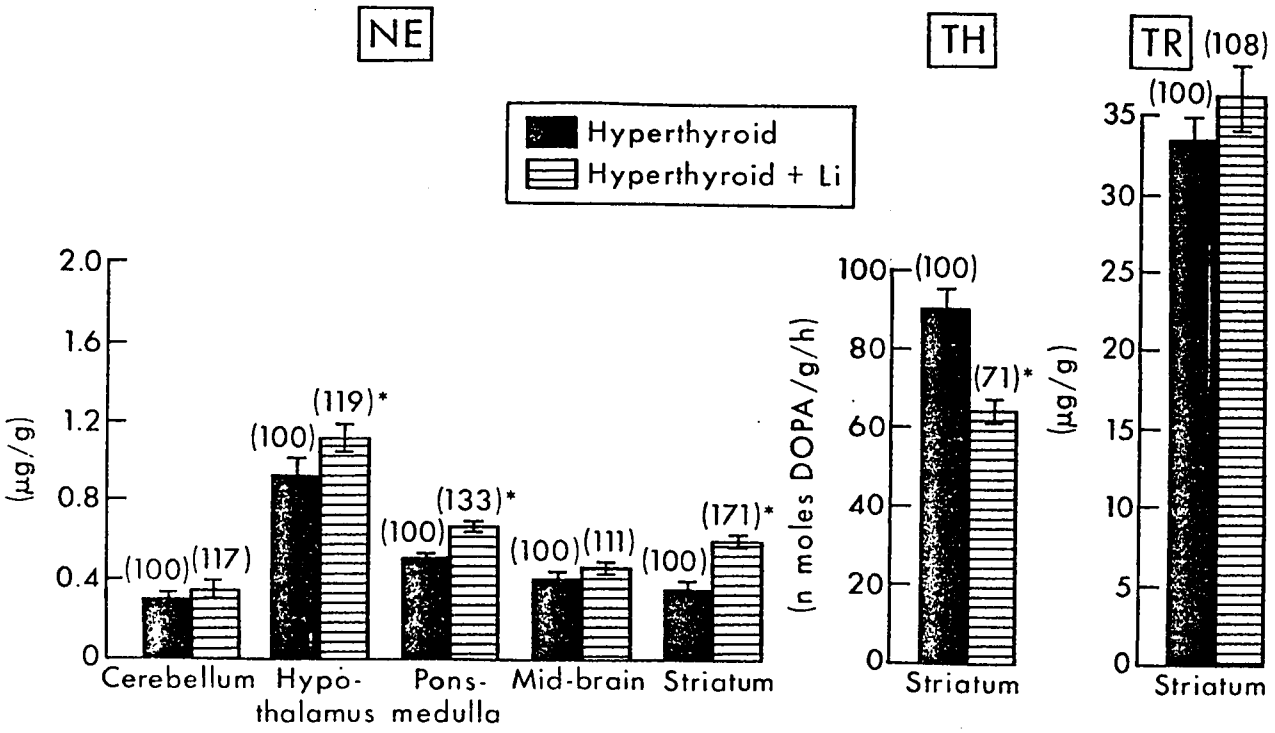

Fig. 43. Effect of lithium treatment on NE levels in certain brain regions and on striatal TH and TR in adult hyperthyroid rats. Bars represent the means ± S.E.M. of 6 rats in the group. Adult rats were injected subcutaneously with T_3 (10 μg/100 g/day) for 30 days. Groups of rats treated with T_3 for 24 days were subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control (hyperthyroid) rats ($p < 0.05$).

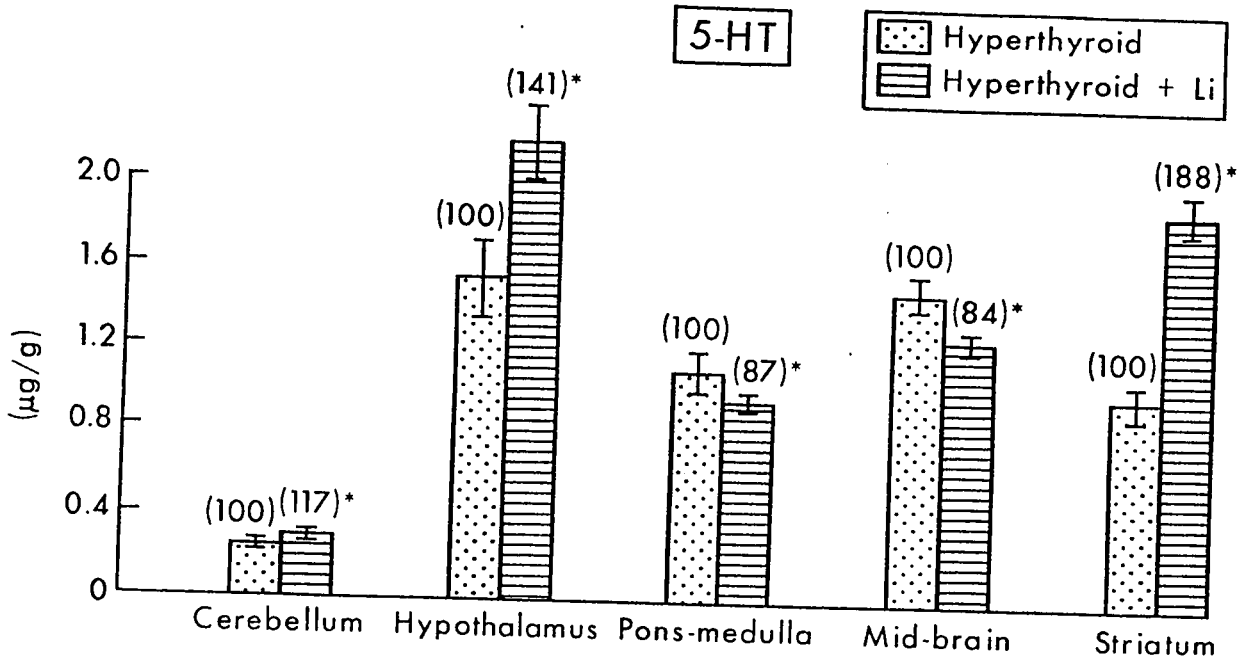

Fig. 44. Effect of lithium treatment on 5-HT levels in certain brain regions of adult hyperthyroid rats. Bars represent the means ± S.E.M. of 6 rats in the group. Adult rats were injected subcutaneously with T_3 (10 μg/100 g/day) for 30 days . Groups of rats treated with T_3 for 24 days were subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control (hyperthyroid) rats ($p < 0.05$).

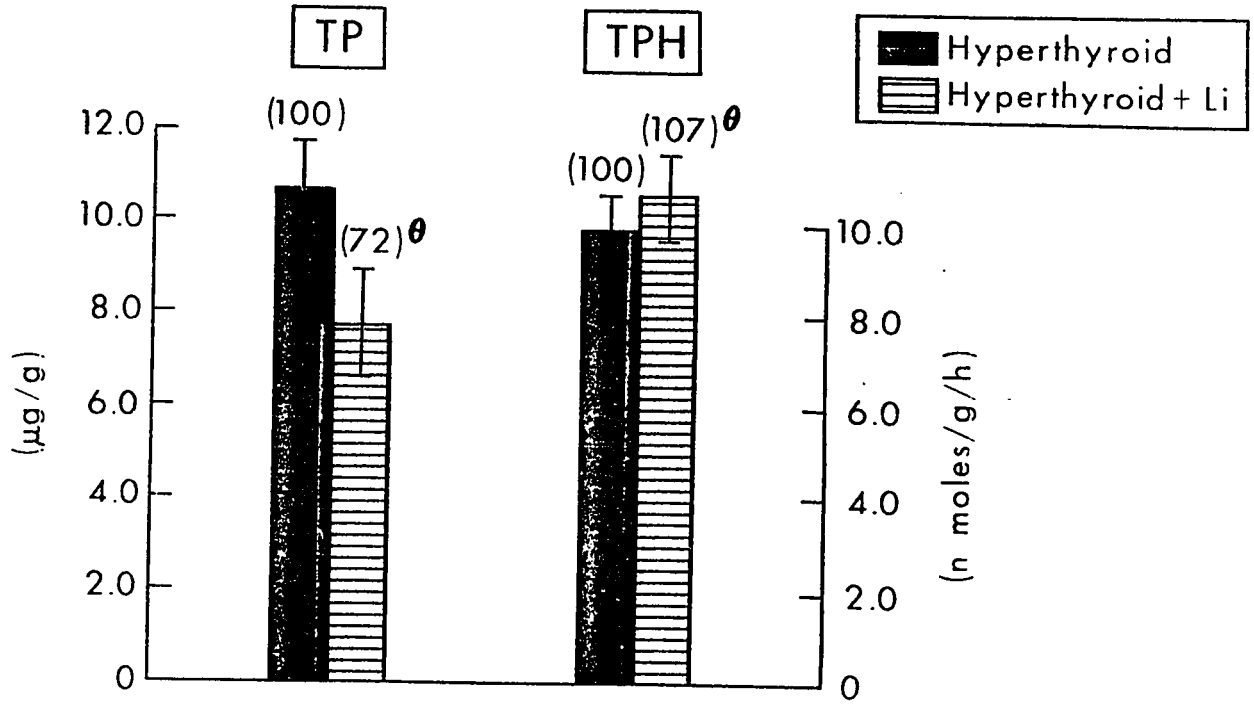

Fig. 45. Effect of lithium treatment on TP and TPH activity in mid brain of adult hyperthyroid rats. Bars represent the means ± S.E.M. of 6 rats in the group. Adult rats were injected subcutaneously with T_3 (10 µg/100 g/day) for 30 days. Groups of rats treated with T_3 for 24 days were subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

θStatistically non-significant difference when compared with the values of control (hyperthyroid) rats ($p < 0.05$).

(Fig. 46). In contrast, other brain regions such as the cerebellum, hypothalamus and striatum which contained higher amounts of 5-hydroxytryptamine also showed significantly augmented levels of this indoleamine metabolite.

F. Effect of Lithium on Monoamine Oxidase and Catechol-o-Methyl Transferase Activity in Adult Hyperthyroid Rats

Data presented in Figure 47 show that administration of lithium to adult hyperthyroid rats failed to exert any appreciable effect on the activities of the two catabolizing enzymes, monoamine oxidase and catechol-o-methyl transferase in the cerebrocortical region.

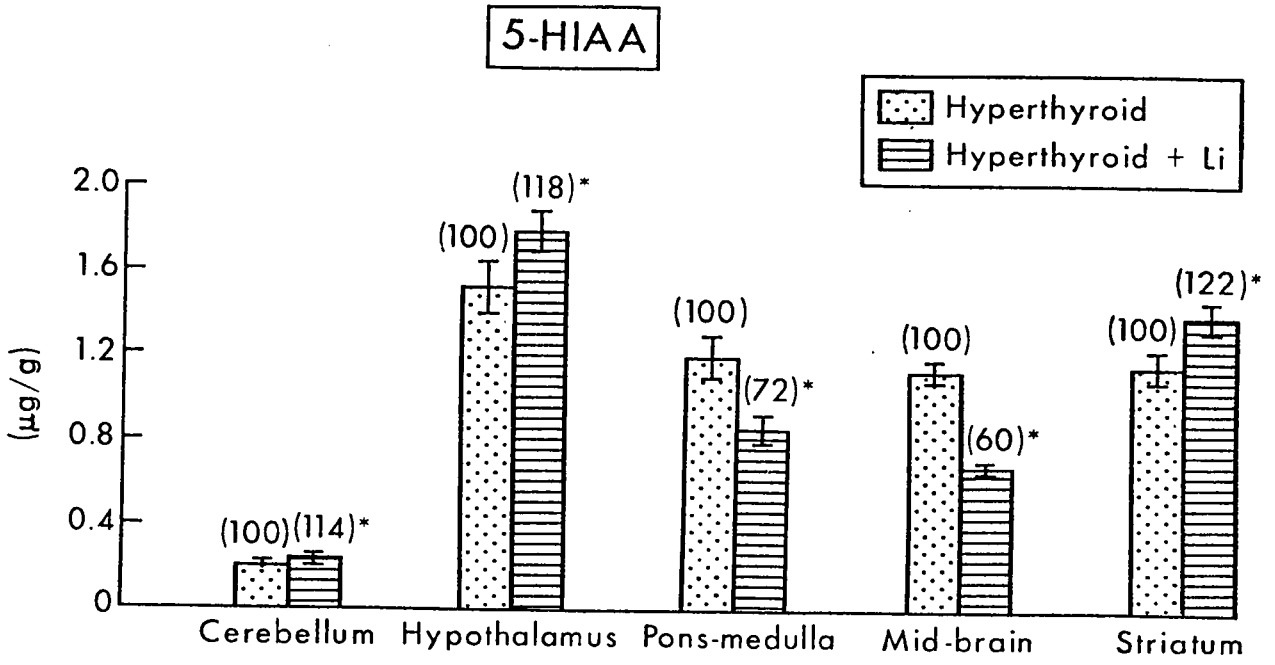

Fig. 46. Effect of lithium treatment on 5-HIAA levels in certain brain regions of adult hyperthyroid rats. Bars represent the means ± S.E.M. of 6 rats in the group. Adult rats were injected subcutaneously with T_3 (10 μg/100 g/day) for 30 days. Groups of rats treated with T_3 for 24 days were subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

*Statistically significant difference when compared with the values of control (hyperthyroid) rats ($p < 0.05$).

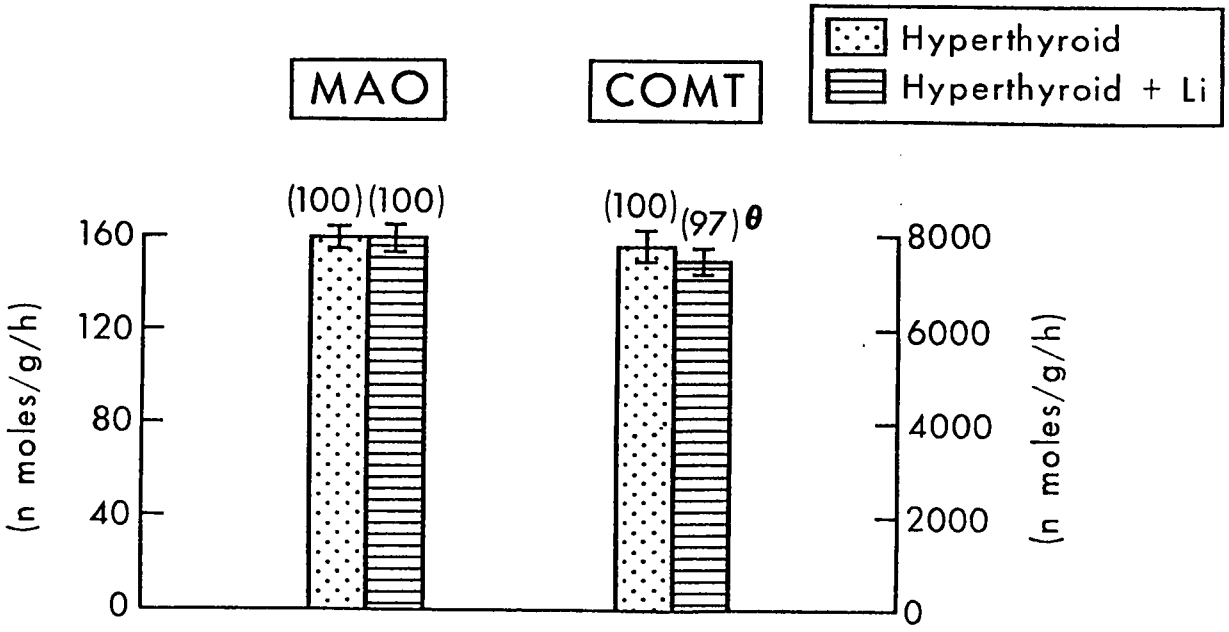

Fig. 47. Effect of lithium treatment on cerebrocortical MAO and COMT in adult hyperthyroid rats. Bars represent the means ± S.E.M. of 6 rats in the group. Adult rats were injected subcutaneously with T_3 (10 µg/100 g/day) for 30 days. Groups of rats treated with T_3 for 24 days were subsequently injected with lithium (60 mg/kg) by the intraperitoneal route for 6 days. Control rats received an equal volume of the physiological saline. Data in parentheses express results in percentages taking the values of control rats as 100%.

$^{\theta}$Statistically non-significant difference when compared with the values of control (hyperthyroid) rats (p 0.05).

5. DISCUSSION

The brain of most mammalian species, including man, is largely underdeveloped at birth and undergoes morphological, functional and biochemical maturation in early post-natal life. The post-natal maturation does not merely represent growth, but continued differentiation. Eayrs (16) carried out extensive studies on the post-natal morphogenesis and functional development of rat brain. In this species, brain is quite underdeveloped at birth and maturation is achieved subsequently in the first 5-6 weeks of life. Normally, the brain size increases approximately 6-7 fold during this period, but the most dramatic changes take place in the microscopic appearance of the tissue. The neurons increase in size mainly because of an increase in perikaryonal cytoplasm. There is a marked proliferation of axonal and dendritic processes and the density of axodendritic connections is strikingly increased, particularly in the neuropil. Myelin is laid down around axons and the nerve cell bodies become less densely packed as the spaces between them get filled with axonal and dendritic processes.

The morphological alterations in brain tissue are paralleled by extensive biochemical changes (249). Some of these reflect the altered morphological state whereas others result from continued post-natal differentiation of the tissue. The amount of protein and RNA increases and the perikaryonal cytoplasm enlarges. Cell proliferation proceeds for only a short time after birth and thereafter, the nuclei do not increase significantly in size or amount; consequently, the concentration of DNA falls as the constant amount of DNA becomes diluted by the increasing cytoplasmic mass and myelin. Lipid composition, which reflects to a large extent the deposition of myelin, is markedly altered. Myelination in rat generally starts somewhere between 12 to 20 days of age. That biochemical regulatory mechanisms underlie the overall maturational

process is evidenced by the extensive realignment of brain enzyme patterns that takes place during this period of post-natal life.

I. EFFECT OF NEONATAL THYROIDECTOMY ON NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE METABOLISM IN DEVELOPING BRAIN

The present study demonstrates that maturation of the neuronal tissue is accompanied by significant increases in the activities of tyrosine hydroxylase and tryptophan hydroxylase, enzymes which play a rate-limiting role in the synthesis of norepinephrine and 5-hydroxytryptamine, respectively. A sharp rise was observed in tyrosine and tryptophan hydroxylases between the first and the 14th day of age, the period during which there is a rapid increase in both the volume and the weight of neurons (250). Several other events such as appearance of synaptic junctions, dendritic proliferation and increased interneuronal interactions are known to occur during this period of brain maturation (15,251). In addition, electrical activity in brain assumed the adult pattern somewhere between the 3rd and the 4th week of post-natal life (252). There was an apparent developmental lag between brain norepinephrine levels and the activity of tyrosine hydroxylase. Coyle and Axelrod (98) demonstrated that dopamine β-hydroxylase activity in neonatal rat brain was markedly lower than that seen in adult rats and it seems likely that dopamine β-hydroxylase may well be responsible for low norepinephrine levels in infant brain. Another possibility also merits consideration. Kulkarni and Shideman (77) demonstrated that neonatal rat brain is more sensitive to the catecholamine depleting action of reserpine than adult brain. Indeed, electron microscopic studies of developing rat cerebral cortex revealed that the synaptic boutons are laid out first which are subsequently filled with synaptic vesicles (106). Suggestion has been made that granular storage in rat adrenals is more fragile at birth and that the storing capacity

augments with age. In addition, the active uptake mechanism for norepinephrine at birth was 2.5 times as inferior as that observed in adulthood (79). Similar possibilities may also be offered for low concentrations of 5-hydroxytryptamine and relatively higher levels of its major metabolite, 5-hydroxyindoleacetic acid, seen in brains of newborn rats. Loizou and Salt (73) demonstrated that the number of serotonergic neurons per unit weight of neural tissue is lower in newborn rats and that the adult pattern of distribution is attained only after the 3rd week of life.

The cognizance for the regulatory influence of thyroid hormone on norepinephrine and 5-hydroxytryptamine metabolism was demonstrated in experiments in which radiothyroidectomy or chemical thyroidectomy in neonatal life was found to markedly impair the normal ontogeny of tyrosine hydroxylase and tryptophan hydroxylase. The effects of the lack of thyroid hormone on enzyme activity were first manifested around 10-15 days of life, the period when cortical neuroblasts assume the characteristics of mature neurons. The activities of tyrosine hydroxylase and tryptophan hydroxylase were markedly decreased in striatum and mid-brain respectively, the regions enriched with the nerve endings of noradrenergic and cell bodies of serotonergic neurons. It has been demonstrated that neonatal thyroid deficiency has a profound effect on the maturation of nerve cells producing a severe retardation of dendritic ramification (246,253). Ultrastructural studies further revealed that the number of terminals per nerve cell is reduced in the visual cortex of thyroid-deficient rats (254). Since fairly large amounts of tyrosine hydroxylase and tryptophan hydroxylase are present in nerve endings, it is conceivable that the decrease in the activity of these enzymes in thyroidectomized animals might, at least in part, be associated with a reduction in the number of nerve terminals in catecholaminergic and serotonergic neurons.

Another aspect of this investigation is the evidence that the degree of enzyme inhibition was dose-dependent since greater alterations in brain tyrosine hydroxylase and tryptophan hydroxylase activities were noted when the dose of ^{131}I was augmented to 200 μCi/rat. This dose of ^{131}I, which produced total thyroidectomy (230) and maximal suppression of the arborization of nerve terminals (249) also resulted in maximal changes in tyrosine hydroxylase and tryptophan hydroxylase of developing brains. Additionally, administration of methimazole, which induced chemical thyroidectomy by suppressing thyroid hormone synthesis, produced dose- and time-dependent decreases in the normal ontogenic patterns of brain tyrosine hydroxylase and tryptophan hydroxylase. Methimazole in high dosage for 30 days resulted in considerably greater alterations in tyrosine hydroxylase and tryptophan hydroxylase activity as well as in norepinephrine and 5-hydroxytryptamine levels than those seen in rats given the low dosage of this anti-thyroid agent.

Radiothyroidectomy was found to be as effective as methimazole in interfering with the normal ontogenic changes in norepinephrine and 5-hydroxytryptamine levels of developing brains. Toth and Csaba (255) also reported a significant reduction in 5-hydroxytryptamine levels in brain stem and blood of thyroidectomized rabbits. Greater alterations were observed in norepinephrine levels of those regions of the brain which are either rich in the cell bodies of noradrenergic neurons (e.g. pons-medulla) or contain abundance of nerve endings (e.g. hypothalamus). Although the striatum contains mostly dopaminergic nerve fibers, a small amount of norepinephrine observed in this region may be that which is taken up from adjacent regions by neuronal uptake mechanisms. Since neonatal hypothyroidism markedly depleted dopamine levels in striatum and several other brain regions, it is possible that the observed decrease in striatal norepinephrine may be associated with underdeveloped dopaminergic nerve endings of this region. Despite the fact that cerebellum

develops mostly after birth, neonatal thyroid deficiency produced no appreciable effect on norepinephrine concentration of this brain region.

Valcana (256) observed no change in acetylcholinesterase activity in cerebellum of ^{131}I-treated rats but the activity of this enzyme was significantly reduced in cerebral cortex. Like norepinephrine, 5-hydroxytryptamine concentrations also were altered in mid-brain, striatum (regions enriched with serotonergic cell bodies and nerve endings respectively) and cerebellum. In contrast to the observed changes in 5-hydroxytryptamine, the concentration of 5-hydroxyindoleacetic acid was significantly increased in whole brain as well as in discrete regions such as mid-brain, striatum and cerebellum of hypothyroid rats. Several mechanisms can be adduced to explain the higher concentrations of 5-hydroxyindoleacetic acid in face of low levels of 5-hydroxytryptamine in hypothyroid brain. Firstly, brains of hypothyroid animals which are deficient both anatomically and biochemically might have inadequate storage mechanism(s). If so, then a major proportion of 5-hydroxytryptamine synthesized would be deaminated without ever taking part in the neuronal processes. Secondly, Trendelenburg (257) noted a marked decrease in the activity of hepatic monoamine oxidase in rats fed desiccated thyroid gland. It is therefore possible that the augmented levels of 5-hydroxyindoleacetic acid might be due to increased activity of brain monoamine oxidase in thyroid-deficient rats. Attempts to examine whether such was the case revealed that chemical thyroidectomy tended to increase the activity of brain monoamine oxidase although, the change was statistically non-significant. In radiothyroidectomized rats, monoamine oxidase activity significantly increased in mid-brain whereas it was decreased in the hypothalamus. Thus, elevated levels of 5-hydroxyindoleacetic acid in mid-brain could be due, at least in part, to increased activity of monoamine oxidase. Moreover, the hypothalamus which showed no significant change in 5-hydroxyindoleacetic

acid levels showed a lowering in the activity of monoamine oxidase in thyroid-deficient animals. Finally, it may be assumed that the brains of thyroid-deficient rats have an impaired efflux mechanism(s). Recently, Atack et al. (124) demonstrated that the process of elimination of organic acids from brains of newborn animals is not quite as efficient as in adults. These investigators observed that the rate of elimination of 5-hydroxyindoleacetic acid involving the mechanism of bulk flow in the cerebrospinal fluid system was only 6% of its endogenous rate of formation in brains of 4 day old rats. Hence, it is not unlikely that the elevated levels of 5-hydroxyindoleacetic acid in both radio- and chemically thyroidectomized rats whose brains are immature morphologically, neurophysiologically as well as biochemically, may be associated with a sluggish rate of bulk flow of cerebrospinal fluid which can hardly serve as a major avenue for removal of this metabolite. Recently, it has been demonstrated that radiothyroidectomy increased both homovanillic acid and dihydroxyphenylacetic acid levels in the striatum of 30 day old rats by 79% and 40%, respectively. The elevated levels of homovanillic acid in face of low levels of dopamine in hypothyroid rats might, in part, be associated with enhanced activity of catechol-o-methyl transferase since it has been shown that homovanillic acid is produced extraneuronally in contrast to dihydroxyphenylacetic acid which is formed intraneuronally. Since monoamine oxidase activity remained unchanged in striatum of young thyroid-deficient rats, enhanced levels of dihydroxyphenylacetic acid and homovanillic acid in brains of cretinous rats might also be associated with inadequate efflux mechanism(s). Although the pharmacological actions of these organic metabolites are still unexplored, it is reasonable to suggest that the aberrant behaviour seen in cretinous rats may be related to the toxic effects manifested by intracranial accumulation of 5-hydroxyindoleacetic acid, homovanillic acid, dihydroxyphenylacetic acid and perhaps even other acid metabolites.

Results of the present study also show that delaying radiothyroidectomy for 10 days after birth produced less conspicuous alterations in various parameters related to norepinephrine and 5-hydroxytryptamine metabolism than those seen after neonatal thyroidectomy. Furthermore, administration of radioiodine on the 20th day of age produced virtually no significant change in norepinephrine, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid levels as well as in the activities of tyrosine and tryptophan hydroxylases. These data seem to be in line with the previous report of Eayrs (258) in which he demonstrated that the severity of changes in various behavioural parameters was related to the age of onset of thyroid deficiency. The changes related to learning ability and adaptive behaviour also were shown to be less marked in rats thyroidectomized at later stages of growth. Furthermore, electrophysiological changes were less significant in brains of animals thyroidectomized in adulthood when compared to those noted in rats thyroidectomized on the day of birth (259).

The observed neurochemical changes seem to be specific to thyroid hormone since administration of L-triiodothyronine to hypothyroid rats produced time- and dose-dependent changes in the concentrations of norepinephrine, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid as well as in the activities of tyrosine hydroxylase and tryptophan hydroxylase. Restoration by thyroid hormone of not only body and brain weights, but also of tyrosine hydroxylase and tryptophan hydroxylase in striatum and mid-brain respectively, as well as in whole brain of hypothyroid rats suggests that this hormone is capable of resetting protein synthesis to almost normal limits provided that the therapy is instituted in early life. Cycloheximide, an inhibitor of protein synthesis, effectively inhibited the L-triiodothyronine-stimulated increases in several key glycolytic and hexose monophosphate shunt enzymes in brains of radiothyroidectomized rats (12,13,260). Although the influence of an

inhibitor of protein synthesis was not examined in the present study, the possibility remains that de novo protein synthesis may be involved in the observed L-triiodothyronine-stimulated increases in striatal tyrosine hydroxylase and mid-brain tryptophan hydroxylase activity of neonatally thyroidectomized animals. The experiments in which L-triiodothyronine treatment of adult hypothyroid rats failed to affect body and brain weights as well as norepinephrine and 5-hydroxytryptamine metabolism favor the existence of a critical period in early life during which thyroid hormone exerts most marked influence on the biochemical maturation of neuronal tissue. Sokoloff (261) demonstrated that thyroid hormone stimulates protein synthesis in brain only during the period of its growth and development and manifests no such effect in fully mature brain. In addition, Bradley et al. (259) demonstrated that thyroid deficiency in adult rats does not alter the electrical activity of the brain. An analogy seems to exist between present data and the clinical situation in which permanent brain dysfunction in a child can be prevented only if treatment is initiated no later than the 6th month (preferably 3rd month) after birth. However, should the first year of life be allowed to pass without any thyroid hormone therapy, the cretinous children may improve to some extent, but they seldom fully make up their mental arrears.

II. INFLUENCE OF DESMETHYLIMIPRAMINE ON NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE METABOLISM IN HYPOTHYROID RATS.

Evidence that neonatal hypothyroidism failed to alter the responsiveness of receptor sites was derived from experiments in which acute desmethylimipramine was shown to produce similar changes in norepinephrine, 5-hydroxytryptamine and acetylcholine in normal as well as chemically thyroidectomized rats. Desmethylimipramine is a potent blocker of neuronal uptake of 5-hydroxytryptamine resulting in increased levels of this amine within the

synaptic cleft which by negative feedback mechanism(s), slows down the firing of serotonergic neurons and decreases the activity of tryptophan hydroxylase. This would seem to be in line with the data reported by Bruinsvels (151). Since desmethylimipramine treatment produced similar changes in 5-hydroxytryptamine levels and in the activity of tryptophan hydroxylase in both normal and chemically thyroidectomized rats, it is possible that the sensitivity of serotonergic receptors at pre- and/or post-synaptic membrane remained unaltered. The decrease in 5-hydroxyindoleacetic acid levels observed after desmethylimipramine may, at least in part, be due to membrane blockade of 5-hydroxytryptamine reuptake and hence, inaccessibility to the deaminating enzyme, monoamine oxidase, which by itself, remained unaltered by desmethylimipramine (262). The observation that endogenous levels of norepinephrine remained unaltered following a single injection of desmethylimipramine in normal animals is consistent with the findings of Schildkraut et al. (263) and Tarlov et al. (264).

III. EFFECT OF THYROID HORMONE DEFICIENCY ON THE ONTOGENESIS OF SPONTANEOUS LOCOMOTOR ACTIVITY.

The present investigation also shows that hypothyroidism led to significant impairment of ontogenic increases in behavioural arousal. The observed rapid increase in locomotor activity in control animals during the first 30 days of life is in line with a previous report of Sauerhoff and Michaelson (265). The suppression of locomotor activity in radio- as well as chemically thyroidectomized rats became apparent at 15 days of age, a time at which the ontogenic increases in the activities of brain tyrosine hydroxylase and tryptophan hydroxylase also were suppressed. Evidence indicates that catecholamines might serve as the neurochemical substrate for behavioural arousal (32,33). It has been shown that locomotor activity is elevated following the administration of sympathomimetic drugs which are known to accelerate

catecholaminergic activity (31). Conversely, when norepinephrine and dopamine stores are depleted following the use of enzyme inhibitors or storage blockers, behavioural arousal is depressed (32). In addition, it has been shown that an anticholinergic drug, scopolamine (which blocks acetylcholine transmission by occupying post-synaptic receptor sites) produces marked increment in locomotor activity (266), whereas anticholinesterases (267) and cholinomimetic agents (268) depress arousal. It is therefore conceivable that suppressed locomotor activity in hypothyroid rats might be associated with impaired maturation of norepinephrine and dopamine-synthesizing systems. Since acetylcholine levels are known to vary inversly with the degree of functional activity of the brain and are higher than normal during certain states of behavioural depression (269,270), the possibility also remains that augmented levels of acetylcholine in radio-thyroidectomized rats (271) and/or increased ratio of acetylcholine to dopamine in brains of methimazole-treated animals (174, 272) might be related, at least in part, to depressed psychomotor activity and behaviour seen in "cretinoid" state.

IV. INFLUENCE OF NEONATAL HYPERTHYROIDISM ON BRAIN NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE METABOLISM

In contrast to the changes seen in hypothyroid animals, the activity of striatal tyrosine hydroxylase was significantly enhanced in neonatal rats made hyperthyroid with daily L-triiodothyronine treatment for 30 days. Despite an increase in the activity of brain tyrosine hydroxylase, the steady-state levels of norepinephrine remained unaltered in all regions of the brain examined except cerebellum. Although the present study may seem to suggest that synthesis and perhaps utilization of norepinephrine is increased in young hyperthyroid animals, no conclusion can be drawn without data on the changes in its metabolites. It is possible that excess of thyroid hormone in infancy

might lead to increased flow of norepinephrine to the corresponding pre- and/or post-synaptic receptor sites. These results are complimentary to those reported by Engstrom et al. (226) who observed a significant increase in the turnover rate of brain norepinephrine in hyperthyroid mice. Increased release of brain catecholamines, for example by amphetamine, has often been found to be associated with increased locomotor activity in rodents (273,274). Thus, it seems probable that the increase in spontaneous locomotor activity noted in neonatally hyperthyroid animals might be associated with an increased release of norepinephrine. Furthermore, in young hyperthyroid rats, norepinephrine might remain within the synaptic cleft for a slightly longer period of time owing to the decreased activity of catechol-o-methyl transferase and could thus promote further post-synaptic depolarization and neural transmission.

It is of interest that neonatal administration of L-triiodothyronine markedly elevated the concentration of brain tryptophan. Since the activity of tryptophan hydroxylase and the availability of the substrate are two important regulatory factors, elevation of mid brain tryptophan hydroxylase along with increased levels of tryptophan may be related to the increased synthesis of 5-hydroxytryptamine in neonatally hyperthyroid rats. The mechanism by which L-triiodothyronine produces this effect is not clearly understood. The fact that the plasma/brain ratio is 5:1 for tryptophan and 3:1 for tyrosine (275) suggests that the increase in brain levels of these amino acids may result from an increase in brain permeability. Several other possibilities also can be adduced. Since tryptophan is not synthesized in mammalian tissues, an increase in its concentration may be due to altered protein binding in serum. Serum tryptophan is mostly albumin-bound (143) and only a small fraction is free and available to the brain. Although the ratio between free and total tryptophan in the serum was not determined in the present study,

possibility remains that L-triiodothyronine might compete for the binding sites and thus displace tryptophan from albumin. Despite increased synthesis of 5-hydroxytryptamine, the steady-state levels of this indoleamine remained unchanged in several regions of the brain (pons–medulla, mid-brain, striatum and hippocampus), in hyperthyroid animals. In cerebellum and hypothalamus, the levels of 5-hydroxytryptamine were even decreased when compared to controls. These data suggest that neonatal hyperthyroidism significantly enhanced the synthesis as well as utilization of this putative neurotransmitter as evidenced by elevated levels of 5-hydroxyindoleacetic acid. Since increase of 5-hydroxyindoleacetic acid was more pronounced than the synthesis of 5-hydroxy-tryptamine, it can be suggested that utilization of indoleamine within the serotonergic neurons was more prominent than its synthesis. Thus, excess of thyroid hormone in early life augments the impulse flow rate in tryptaminergic neurons, which seems to be opposite to what might be expected in neonatally thyroid-deficient rats. At present, it is not clear whether the increase in 5-hydroxytryptamine synthesis is secondary to an increased release from serotonergic neurons or to an enhanced intraneuronal deamination of 5-hydroxytryptamine. However, available data seem to negate the latter possibility since monoamine oxidase activity remained unaltered in cerebrocortices of neonatally hyperthyroid animals.

V. EFFECT OF ADULT HYPERTHYROIDISM ON BRAIN NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE METABOLISM

Results of the present study demonstrate that hyperthyroidism in adult animals failed to enhance the activity of brain tyrosine hydroxylase. In contrast to neonatal hyperthyroidism, adult hyperthyroidism tended to decrease the steady-state levels of norepinephrine in several regions of the brain suggesting that hyperthyroidism in adult rats probably increased the utilization

of this brain catecholamine. Treatment with L-triiodothyronine also increased the cerebral transport of tyrosine and tryptophan as well as the activity of tryptophan hydroxylase. Thus, hyperthyroidism in adult rats also seemed to enhance the rate of synthesis of 5-hydroxytryptamine. Whereas the levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid were decreased in cerebellum and striatum, these remained unaltered in other regions of the brain examined. The possibility remains that adult hyperthyroidism accelerated utilization of 5-hydroxytryptamine more than the rate of its synthesis which may explain low levels of this indoleamine in discrete brain regions. Since adult hyperthyroidism failed to exert any appreciable influence on monoamine oxidase activity, it is unlikely that L-triiodothyronine treatment in adult animals enhanced the deamination of 5-hydroxytryptamine. The low levels of 5-hydroxyindoleacetic acid might be due to its enhanced removal from the brain and/or metabolite disposition. It has been demonstrated that 5-hydroxyindoleacetaldehyde is the substrate for the incorporation of radioactivity from labeled 5-hydroxytryptamine or tryptamine into an acid-insoluble fraction of the brain or liver and that the magnitude of this incorporation was markedly altered by drugs (276,277). At present, it is uncertain whether the decrease in 5-hydroxyindoleacetic acid and 5-hydroxytryptamine levels and increase in the activity of tryptophan hydroxylase found after L-triiodothyronine treatment can be equated with increased activity of the serotonergic system or some yet undefined mechanism(s). However, the present study shows that hyperthyroidism results in somewhat different effects on norepinephrine and 5-hydroxytryptamine metabolism in brains of neonate and mature animals.

VI. EFFECT OF LITHIUM ON NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE IN NEONATALLY HYPERTHYROID RATS

Previous studies have shown that chronic treatment of male rats with

L-triiodothyronine was protective against the toxic effects of lithium (278). This finding was relevant in as much as it offered therapeutic implications in minimizing the side effects of lithium in psychiatric patients. Both the antithyroid and goiterogenic effects of lithium have been well documented in man (24,228,279) and animals (280). The findings that protein bound iodine and thyroxine are decreased and that the uptake of radioactive iodine is increased in lithium-treated rats indicate that the gland fails to excrete sufficient thyroxine, thereby eliciting an increased secretion of thyroid stimulating hormone from the pituitary. However, there is still no direct evidence that thyroid stimulating hormone is elevated following chronic administration of lithium (24,281). Lithium neither interfered with iodide entry into the thyroid gland nor with its subsequent "organification" (24,282). In addition, Wolff et al. (283) reported that lithium inhibited the activity of adenyl cyclase and almost doubled the time necessary to release a given amount of iodine from the thyroid gland. Mania and hyperthyroidism seem to have certain common features such as hyperactivity and sleeplessness raising the possibility of an association between the antithyroid and antimanic properties of lithium. However, little is known about the mechanism by which lithium decreases the turnover of norepinephrine and 5-hydroxytryptamine in brains of neonatally hyperthyroid animals.

Schildkraut et al. (284) and Schanberg et al. (285) demonstrated that lithium facilitated the reuptake of norepinephrine by pre-synaptic neurons, thus enhancing the intraneuronal concentrations of this putative neurotransmitter. This is in line with the elevated levels of endogenous norepinephrine observed in the present study. Since the activity of tyrosine hydroxylase is regulated, in part, by the intraneuronal concentrations of free norepinephrine (165), the decreased striatal tyrosine hydroxylase in lithium-treated

hyperthyroid rats might be due to a feedback inhibition by elevated norepinephrine within the adrenergic neurons. Furthermore, lithium may decrease the levels of norepinephrine at pre- and/or post-synaptic receptor sites by accelerating the o-methylation of this monoamine since it significantly increased the activity of catechol-o-methyl transferase. The reduced concentration of norepinephrine within the synaptic cleft would prevent post-synaptic depolarization and neural transmission. This reduced firing of adrenergic neurons may perhaps lead to suppressed psychomotor activity in lithium-treated young hyperthyroid rats. Additional support to this view may be gained from the report that imipramine treatment in depressed patients occasionally results in manic-like symptoms as if it had over-compensated for the deficiency of synaptic catecholamines (286).

The present finding that lithium significantly elevated the levels of tryptophan in mid brain of developing hyperthyroid rats is in agreement with the previous reports of Perez-Cruet et al. (287) and Tagliamonte et al. (288). Since tryptophan is not synthesized in mammalian tissues, an increase in its concentration could be due to altered protein binding in serum, blockade of its catabolism or interference with uptake and transport to the brain. Administration of lithium to normal rats failed to alter the ratio between the free and total tryptophan in the serum (289). Furthermore, lithium in normal rats produced no significant alterations in the activity of liver tryptophan pyrrolase, the rate-limiting enzyme involved in the conversion of tryptophan to kynurenine (289,290,291). If such were the case in neonatally hyperthyroid rats, then the altered uptake and/or transport of the amino acid to the brain might be one mechanism responsible for increased levels of brain tryptophan (130). The activity of tryptophan hydroxylase in mid-brain (a region representative of serotonergic cell bodies) was reduced which is in line with the findings of Knapp and Mandell (130). The point has been raised

that since tryptophan hydroxylase is normally unsaturated, increases in substrate concentration produced by lithium may be responsible for increased 5-hydroxytryptamine biosynthesis. Data that lithium increased the steady-state levels of 5-hydroxytryptamine in several regions including the striatum, a region enriched in serotonergic nerve endings, appear to be consistent with the hypothesis of Knapp and Mandell (130) suggesting that lithium initially stimulates 5-hydroxytryptamine biosynthesis in nerve endings. A series of events might subsequently occur before the elevated levels of 5-hydroxytryptamine invoke a feedback reduction in cell body enzyme activity. Although further investigation on the effects of long term treatment with lithium on mid-brain and striatal tryptophan hydroxylase are needed, it is possible that restoration of locomotor activity by lithium in neonatally hyperthyroid animals may be due to a compensatory decrease in brain tryptophan hydroxylase activity following an initial increase in the uptake of tryptophan and subsequent conversion to 5-hydroxytryptamine in nerve endings. The decreased levels of its chief metabolite, 5-hydroxyindoleacetic acid in brains of lithium-treated rats are consistent with the findings of Goodwin et al. (292) in cerebrospinal fluid of man. The observed decrease in 5-hydroxyindoleacetic acid in face of elevated 5-hydroxytryptamine might be related to either decreased activity of monoamine oxidase or reduced turnover of indoleamine. The fact that monoamine oxidase activity remained unchanged in cerebrocortices of lithium-treated hyperthyroid rats, it is quite possible that lithium indeed decreased the utilization of this indoleamine.

VII. EFFECT OF LITHIUM ON NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE IN ADULT HYPERTHYROID RATS

Administration of lithium produced almost similar effects on the metabolism of brain norepinephrine in both adult and young hyperthyroid rats. Whereas lithium treatment in neonatally hyperthyroid rats decreased the levels

of this catecholamine within the synaptic cleft by augmenting the o-methylation, it failed to exert any appreciable influence on catechol-o-methyl transferase activity in adult hyperthyroid animals. It is of interest that in adult hyperthyroid rats, this antimanic agent failed to alter the levels of brain tryptophan. In neonates, the ratio of free to bound tryptophan in the plasma is known to be higher than in the adult (145). It is therefore conceivable that lithium enhanced the uptake and/or transport of tryptophan across the synaptosomal membrane in neonates but not in adult rats. Lithium decreased the levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in mid-brain and pons-medulla, regions representative of serotonergic cell bodies. In contrast, the concentrations of this indoleamine and metabolite were enhanced in striatum, hypothalamus and cerebellum, regions enriched in serotonergic nerve fibers. Several possibilities can be offered to explain the observed variations in response of discrete regions of the brain: (i) there might be differences in the access of lithium to different brain regions; (ii) the neuronal uptake mechanism(s) for the drug may not be equally efficient; this would seem to be consistent with the presence of relatively higher concentrations of lithium in the synaptosomes (293) and (iii) lithium, in synaptosome-rich regions like the striatum and hypothalamus, may enhance the uptake of 5-hydroxytryptamine by either altering the membrane permeability directly or by affecting an enzyme and/or transport mechanism(s).

It has been presumed that lithium exerts its depressant effect in hyperthyroid rats by reducing the turnover rate of brain norepinephrine and 5-hydroxytryptamine. In addition, lithium enhanced the o-methylation of norepinephrine by increasing the activity of catechol-o-methyl transferase and thus, decreases the amount of norepinephrine available for interaction with receptor sites. In order to understand the mode of action of lithium in depressing psychomotor activity in hyperthyroid animals, one must also

take into account its effect upon electrolyte balance. Lithium has been shown to actively displace sodium from the cells (294,295). Furthermore, thyroxine has been found to accelerate sodium transport across the isolated skin and bladder membrane (296,297). If such were the case in neuronal tissue as well, then L-triiodothyronine would be expected to enhance the intraneuronal levels of sodium. Support for this can be derived from the finding that sodium accumulation during manic episodes, which resembles hyperthyroidism, increases up to 200% (207,298,299). It is therefore possible that as lithium enters neuronal cells, it replaces intracellular sodium and gets "trapped in" since there is no "lithium pump." Lithium ions remain as non-functional substitute for other metabolically active ions such as sodium or potassium and thus interferes with membrane transport mechanisms and neural transmission in hyperthyroid animals. This effect of lithium upon neural transmission may also result in the suppression of psychomotor activity seen in hyperthyroid subjects. In addition, present data support the suggestion of Forrest (300) that lithium may be useful in the treatment of states of excessive hormone secretion, particularly thyrotoxicosis. Indeed, recent studies have shown that lithium is preferable over iodides in cases of thyrotoxicosis where rapid control of thyroid hormone secretion is required (301,302).

In evaluating the electrolyte and catecholamine data, one basic presumption has been that lithium somehow normalizes the state of neural excitability in hyperthyroid rats. Previous studies have suggested that a relationship might exist between calcium and norepinephrine release from rat brain slices when electrically stimulated <u>in vitro</u> (303). One may ask whether lithium elicits its effects by interfering with a calcium-dependent system of catecholamine release and reuptake. Lithium has also been shown to produce effects upon another bivalent cation, magnesium (304). Since alterations in

calcium and magnesium produce changes in the membrane transport system, it is conceivable that lithium may exert a stabilizing effect upon neural cell membrane of young hyperthyroid rats via changes in sodium and potassium or through its effects upon calcium and magnesium. Thus, at present, it remains obscure as to whether the effects of lithium on these neurohumors are secondary to the cation imbalance or are due to its effect on the circulating levels of thyroid hormone. It would also be important to know whether lithium invokes any effects on the responsiveness of receptor sites for norepinephrine and 5-hydroxytryptamine in hyperthyroid rats, as is the case with the responsiveness of invertebrate receptor sites to glutamate (305).

6. SUMMARY

It is generally recognized that thyroid hormone plays a vital role in the control of mammalian neurogenesis during a critical period of early post-natal development. Neonatal thyroidectomy, which induces a state of experimental cretinism, has been demonstrated to impair learning ability and delay skeletal and somatic maturation. Biochemical studies demonstrated that dysfunction of thyroid gland leads to profound changes in the metabolism of several important brain constituents, particularly protein, carbohydrate, lipid and electrolytes. Despite extensive investigations on the role of thyroid hormone in the morphological and biochemical maturation of the central nervous system, how various abnormalities finally culminate in an irreversible mental deficit and depressed behaviour in cretinous subjects remains obscure. The purpose of the present investigation was to examine the effects of neonatal hypo- and hyperthyroidism on the metabolism of brain norepinephrine and 5-hydroxytryptamine, substances which have been presumed to be involved in neural transmission and behaviour.

Experimental cretinism induced by a single intraperitoneal injection of 200 µCi of ^{131}I on the day of birth led to a marked impairment of body and brain growth and interfered with the normal developmental increases in the activity of tyrosine and tryptophan hydroxylase in striatum and mid-brain, regions enriched with noradrenergic nerve terminals and serotonergic cell bodies, respectively. The steady state levels of norepinephrine and 5-hydroxytryptamine also were significantly altered in several brain regions. Whereas 50 µCi of ^{131}I exerted no appreciable effect, 100 and 200 µCi doses of the radioisotope led to a significant impairment of normal ontogenic increases in tyrosine and tryptophan hydroxylase as well as the levels of norepinephrine and 5-hydroxytryptamine. In contrast, the concentrations of

5-hydroxyindoleacetic acid, the chief metabolite of 5-hydroxytryptamine, were consistently increased in several brain regions of thyroid-deficient rats. Whereas thyroid hormone deficiency in early life produced no appreciable change in whole brain monoamine oxidase activity, it was significantly enhanced in the mid-brain and decreased in the hypothalamus. In addition, the activity of catechol-o-methyl transferase was augmented in several brain regions of radiothyroidectomized animals.

Daily treatment of neonatal rats with methimazole (0.2 mg from day 1-7 and 0.4 mg from day 8-30) induced chemical thyroidectomy as reflected among other things, by significant decreases in body and brain weights. Administration of this goiterogenic agent produced dose- and time-dependent changes in the activities of tyrosine and tryptophan hydroxylase as well as the levels of norepinephrine and 5-hydroxytryptamine. Like radiothyroidectomy, methimazole treatment also elevated the levels of brain 5-hydroxyindoleacetic acid. The observed increase in 5-hydroxyindoleacetic acid appeared to be unrelated to any change in the deamination of intraneuronal 5-hydroxytryptamine, as the activity of monoamine oxidase remained unaffected in chemically thyroidectomized rats. Methimazole treatment significantly enhanced the activity of brain catechol-o-methyl transferase. It is of interest that radio- or chemical thyroidectomy did not exert any significant effect on the concentration of tryptophan, the amino acid presumed to be involved in controlling the rate of synthesis of 5-hydroxytryptamine.

Delaying radiothyroidectomy for 5 or 10 days after birth produced less pronounced changes in the metabolism of norepinephrine and 5-hydroxytryptamine. When thyroidectomy was delayed for 20 days after birth, no significant changes could be noted in the activity of tyrosine and tryptophan hydroxylase as well as the levels of norepinephrine and 5-hydroxytryptamine. The conc-

entration of 5-hydroxyindoleacetic acid also failed to increase appreciably in brains of rats receiving ^{131}I at 20 days of age. Thyroid hormone deficiency in early life markedly suppressed the ontogenesis of behavioural arousal and spontaneous locomotor activity. The maximal suppression was observed in 15 day old radio- and chemically thyroidectomized rats when the spontaneous locomotor activity was reduced to 26% and 31% of the control values, respectively.

The observed neurochemical changes appeared to be thyroid hormone specific, since replacement therapy with L-triiodothyronine for 25 days, beginning from 5 days of age, restored not only body and brain weights, but also the metabolism of norepinephrine and 5-hydroxytryptamine. However, when the replenishment therapy was postponed until adulthood, L-triiodothyronine failed to produce any restorative effects, further suggesting that a critical period exists in early post-natal life during which thyroid hormone must be present to permit normal developmental pattern of central amines.

Evidence also has been presented to show that acute treatment with desmethylimipramine (10 mg/kg i.p.) suppressed the activity of tryptophan hydroxylase and the levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in both normal and chemically thyroidectomized rats. However, this tricyclic antidepressant failed to exert any appreciable effect on the levels of norepinephrine and tyrosine hydroxylase activity. Data suggest that the responsiveness of the critical receptors at pre- and/or post-synaptic sites to the action of desmethylimipramine remains unaltered in hypothyroid rats.

In addition, data have been presented which demonstrate that neonatal hyperthyroidism induced by daily L-triiodothyronine treatment (10 µg/100 g s.c. for 30 days) increased the spontaneous locomotor activity; maximal rise being seen in 17 day old rats when it increased by 192% of the corresponding

controls. Administration of L-triiodothyronine in early life seemed to exert a rather prolonged effect on the spontaneous locomotor activity since it remained elevated in rats that were subsequently withdrawn from this treatment for as long as 60 days. Hyperthyroidism in developing rats increased the activity of striatal tyrosine hydroxylase and mid-brain tryptophan hydroxylase. Despite increases in the activities of these rate-limiting enzymes, the steady state levels of norepinephrine and 5-hydroxytryptamine remained unaltered. However, the levels of 5-hydroxyindoleacetic acid were significantly increased in the hypothalamus, pons-medulla, mid-brain, striatum as well as in hippocampus of neonatally hyperthyroid rats. Since hyperthyroidism in young rats produced no significant change in the activity of monoamine oxidase, it is possible that the elevated levels of 5-hydroxyindoleacetic acid may not be due to enhanced intraneuronal deamination of 5-hydroxytryptamine. Exposure of neonatal rats to L-triiodothyronine significantly decreased the activity of cerebrocortical catechol-o-methyl transferase. It may be noteworthy that hyperthyroidism in adult rats (120 days old) seemed to produce somewhat different effects on brain norepinephrine and 5-hydroxytryptamine metabolism when compared to those seen in young hyperthyroid animals.

Treatment with lithium carbonate (60 mg/kg i.p. for 6 days), an antimanic agent also known to suppress thyroid hormone production, significantly decreased not only the spontaneous locomotor activity, but also the synthesis of norepinephrine and 5-hydroxytryptamine in brains of neonatally hyperthyroid rats. Whereas lithium exerted no effect on monoamine oxidase, the activity of catechol-o-methyl transferase was significantly enhanced in cerebrocortices of young hyperthyroid rats. In adult hyperthyroid animals, this antimanic drug reduced the activity of striatal tyrosine hydroxylase but

increased the endogenous levels of norepinephrine in several brain regions, suggesting that lithium decreased the synthesis and probably utilization of this catecholamine. Administration of lithium produced no effect on mid-brain tryptophan hydroxylase, tryptophan levels or the activities of cerebrocortical catechol-o-methyl transferase and monoamine oxidase. The concentrations of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid were significantly increased in cerebellum, hypothalamus and striatum, but decreased in pons-medulla and mid-brain of lithium-treated adult hyperthyroid rats.

The present study demonstrates that alterations in thyroid function lead to marked changes in the metabolism of two important putative neurotransmitters, norepinephrine and 5-hydroxytryptamine. A critical period seems to exist in early post-natal life during which thyroid hormone must be present to permit the normal developmental pattern of these neurohumors. Data also indicate that a derangement in central amine metabolism might, at least in part, be involved in the etiology of the abnormal behaviour and learning deficits seen during "cretinoid syndrome." In contrast to hypothyroidism, the presence of excess thyroid hormone during infancy enhances the turnover rate of norepinephrine and 5-hydroxytryptamine in adrenergic and serotonergic neurons, respectively. Chronic treatment with lithium seems to decrease the locomotor activity as well as the synthesis of norepinephrine and 5-hydroxytryptamine in brains of young hyperthyroid rats. Additional work on the intraneuronal mechanisms such as transmitter storage and uptake, translocation of enzymes through axons and end product inhibition by receptor-mediated feedback mechanisms is essential in order to gain deeper insight into the thyroid hormone regulation of various central amines.

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Thyroid hormone control of 5-hydroxytryptamine metabolism in developing brain. J. Pharmacol. Expt. Therap. 191, 72-81 (1974).

Alterations in brain norepinephrine and tyrosine hydroxylase activity during experimental hypothyroidism in rats. Brain Res. 81, 253-266 (1974).

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ABSTRACTS

Effect of methimazole treatment on the metabolism of brain norepinephrine and 5-hydroxytryptamine. Pharmacologist 16, 655 (1974).

Inhibition of brain 5-hydroxytryptamine synthesis in developing rats following I^{131} administration. Fed. Proc. 34, 3140 (1975).

Neonatal hypothyroidism and impaired metabolism of brain norepinephrine, dopamine and 5-hydroxytryptamine. Proc. VIth International Congress of Pharmacology, IUPHAR, Helsinki, Finland. July (1975). In press.

ABSTRACT

Experimental hypothyroidism induced in neonatal rats either by ^{131}I or methimazole treatment produced marked decreases in body and brain weights. The spontaneous locomotor activity was reduced by almost 70% in 15 day old rats. Administration of radioiodine or methimazole resulted in significant interference with ontogenic changes in the activities of brain tyrosine hydroxylase and tryptophan hydroxylase as well as the levels of norepinephrine and 5-hydroxytryptamine. In contrast, the concentration of 5-hydroxyindoleacetic acid was consistently increased in several regions of the brain. Treatment of developing rats with ^{131}I or methimazole produced no significant effect on brain monoamine oxidase whereas the activity of catechol-o-methyl transferase was significantly enhanced. Delaying the process of radiothyroidectomy for 20 days after birth exerted no appreciable influence on the metabolism of brain norepinephrine and 5-hydroxytryptamine. Replacement therapy with L-triiodothyronine in early life restored not only body and brain weights, but also striatal tyrosine hydroxylase and mid-brain tryptophan hydroxylase as well as the levels of norepinephrine, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid to virtually normal limits. However, when the initiation of L-triiodothyronine treatment was delayed until the hypothyroid rats had reached adulthood, this hormone failed to produce any significant change in any of the parameters examined. Data suggest that a critical period exists in early post-natal life of the animal during which thyroid hormone must be present in order to permit the normal developmental patterns of these putative neurohumors. The results also indicate that a derangement in central amine metabolism might, at least in part, be involved in the etiology of the abnormal behaviour and learning deficits seen during "cretinoid syndrome." In addition, evidence has been presented to demonstrate that thyroid deficiency

in early life does not alter the responsiveness of pre- and/or post-synaptic receptor sites since acute treatment with desmethylimipramine, a clinically used anti-depressant, produced almost similar effects on 5-hydroxytryptamine and norepinephrine metabolism in normal and methimazole-treated rats.

Hyperthyroidism in young rats produced by daily injection of L-triiodothyronine (10 µg/100 g for 30 days) resulted in significant increases in spontaneous locomotor activity. The maximal rise was seen in 15 day old hyperthyroid rats when the spontaneous locomotor activity was augmented by 198%. The activity of striatal tyrosine hydroxylase and mid-brain tryptophan hydroxylase as well as the endogenous levels of tyrosine and tryptophan were significantly elevated in 30 day old hyperthyroid rats. Whereas the concentration of norepinephrine and 5-hydroxytryptamine remained unchanged in hyperthyroid animals, the levels of 5-hydroxyindoleacetic acid were increased in virtually all of the brain regions investigated. Since no significant effect was noted on monoamine oxidase activity, the present data suggest that hyperthyroidism in developing rats probably increased the synthesis and utilization of 5-hydroxytryptamine in serotonergic neurons. Administration of L-triiodothyronine to adult animals (120 days old) failed to produce any significant change in the activity of striatal tyrosine hydroxylase. In general, adult hyperthyroidism seemed to exert somewhat different effects on the metabolism of these putative neurohumors when compared to those observed during neonatal hyperthyroidism.

Treatment of young hyperthyroid rats with lithium carbonate (60 mg/kg daily for 6 days) significantly decreased not only the spontaneous locomotor activity, but also the synthesis of brain norepinephrine and 5-hydroxytryptamine. However, in adult hyperthyroid rats, this antimanic agent produced little or no effect on spontaneous locomotor activity which was initially increased

only by 40% of the control values. Furthermore, like young hyperthyroid rats, administration of lithium to adult hyperthyroid animals decreased the synthesis of norepinephrine. However, lithium produced no significant effect on mid-brain tryptophan hydroxylase as well as cerebrocortical monoamine oxidase and catechol-o-methyl transferase activities in adult hyperthyroid rats. The concentrations of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid were increased in cerebellum, hypothalamus and striatum, but decreased in pons-medulla and mid-brain.

Data presented in this dissertation support the concept that thyroid hormone exerts an important regulatory influence on the metabolism of norepinephrine and 5-hydroxytryptamine in developing brain. A derangement in central amine metabolism might, at least in part, be involved in the abnormal behaviour and learning deficits seen during "cretinoid syndrome." In contrast to hypothyroidism, the presence of excess thyroid hormone during infancy appeared to enhance the synthesis of norepinephrine and 5-hydroxytryptamine in adrenergic and serotonergic neurons, respectively.