THE PHARMACOLOGY OF CENTRALLY ACTING DRUGS IN ANIMALS OF ALTERED THYROID STATUS.

A thesis presented by

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ABSTRACT

The pharmacological effects of a number of centrally acting drugs have been compared in euthyroid mice and mice made hyperthyroid by pretreatment with sodium-l-thyroxine.

The potencies of two barbiturates, pentobarbitone and thiopentone - as indicated by the duration of their hypnotic actions and their acute toxicities - are increased in hyperthyroid mice. An acutely active uncoupler of phosphorylative oxidation is 2, 4-dinitrophenol, an agent which proved to be a potent hypnotic when administered intracerebrally. An attempt has been made to relate the mechanism of action of the barbiturates to the uncoupling effects of thyroxine and 2, 4-dinitrophenol.

The pharmacological effects of chlorpromazine, reserpine and amphetamine-like drugs have also been studied in hyperthyroid mice. After pretreatment with thyroxine, mice show a reduced tendency to become hypothermic after chlorpromazine or reserpine; in fact, under suitable laboratory conditions these agents produce a hyperthermic effect. Yet their known depressant effects upon locomotor activity were not substantially altered. Thus it appeared that depression of locomotor activity and hypothermia are not necessarily correlated, an observation at variance with previously held opinion. These results have been discussed in the light of our knowledge of the role of the thyroid gland in thermoregulation.

The actions of tremorine and its metabolite, oxotremorine, have also been examined. Hyperthyroid animals are less susceptible to both the hypothermia and tremor produced by these agents. An attempt is made to explain these observations, in view of the known mechanism of action of oxotremorine and the tremorgenic actions that thyroxine may have. A number of experimental methods have been used to study the anti-nociceptive (analgesic) effects of drugs in euthyroid and hyperthyroid mice. The sites and mechanisms of action of these drugs and the known actions of thyroxine have been discussed.

CONTENTS

SECTION I

INTRODUCTION

CHAPTER I

THE PHYSIOLOGICAL ROLE OF THE THYROID GLAND

a/HISTORICAL

Although the thyroid gland has been recognised since at least the time of Galen (Singer & Underwood, 1962), it was not until the middle of the nineteenth century that the first significant works appeared leading to an understanding of the precise physiological functions of the gland. In 1835 Graves described three cases of hyperthyroidism, noting the characteristic tachycardia, nervousness and occasional exophthalmos associated with the disease. In 1850 in a report of two postmortem examinations of mentally retarded children (cretins), Curling observed that both children showed an absence of thyroid tissue. In subsequent papers, Fagge (1871) and Gull (1873) described juvenile hypothyroidism, sporadic cretinism, associated with a wasting or absence of the thyroid, and the adult form of the disease - myxoedema.

The first real advance in our knowledge of thyroid function came in 1883 when Semon, having read papers by Reverdin (1882) and Kocker (1883) describing the postoperative effects of thyroidectomy, suggested that myxoedema, cretinism and related states were all due to a loss of thyroid function. Early attempts to treat myxoedema were confined to the transplantation of thyroid tissue from animals to man. The first real success came in 1892 when Murray reversed the symptoms of myxoedema by injections of a glycerine extract of sheep thyroid gland. Subsequently

both Mackenzie and Fox in 1892, working independently, reported that oral administration of such extracts was equally effective. Beadles (1893) subsequently reviewed the new forms of treatment available for myxoedema.

Baumann (1895) discovered that iodine was present in the active principles of the gland, and in 1914 Kendall isolated and named the thyroid hormone thyroxine. The hormone was later synthesised by Harrington and Barger in 1927.

The presence of another hormone 3, 5, 3'-triiodothyronine was demonstrated in 1952 by Gross and Pitt-Rivers and by Roche and co-workers.

b/STRUCTURE.

The thyroid gland is a bilobed structure which lies in front of and to either side of the trachea and lower larynx. Histologically the gland appears as a large number of vesicles, the walls of which are a single layer of secretory cuboidal epithelium. Within these vesicles is a structureless semifluid protein material, thyroglobulin, which contains the thyroid hormones in a protein-bound form. The gland has a rich blood supply and in the average man receives about 5 litres of blood per hour. Innervation of the gland is both parasympathetic (vagus) and sympathetic; whilst it is likely that these fibres are purely vasomotor in function, nevertheless they may influence the hormonal output by changing the rate of blood flow through the gland.

c/THYROID SECRETIONS.

The thyroid gland secretes two major hormones, namely 3,5,3',5' - tetraiodothyronine (thyroxine, T4.) which predominates, and 3,5,3' - triiodothyronine (T3). The synthesis of these hormones may be regarded essentially as a three stage process:-

1. The gland concentrates iodine from the blood, maintaining a high thyroid:serum iodine ratio. Oxidation of the iodide occurs within the gland and the resulting iodine is bound organically.

2. The iodine is combined with tyrosine to form the 3-mono and 3,5-diiodotyrosines.

3. Combination of these iodoamino acids leads to the formation of T4 and T3.

The formation of the thyroid hormones is shown diagramatically in Fig. 1.

More recently a third thyroid hormone, thyrocalcitonin, has been identified (Copp, 1962; Hirschi, 1963). This agent is a polypeptide with a molecular weight of about 4000 (Macintyre, 1967).

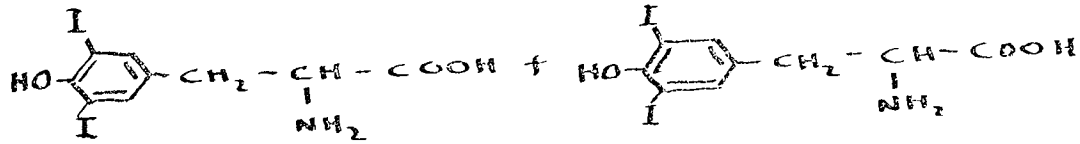
d/CONTROL OF THYROID SECRETION.

The control of thyroid hormone secretion is complex and the elucidation of the precise mechanisms concerned has involved a considerable number of studies (for reviews see: Bogdanove, 1962; Reichlin, 1966; Brown-Grant, 1966; Guillemin, 1967).

Control of thyroid hormone secretion appears to be exerted by two interrelated systems, one acting from the pituitary gland and the other from

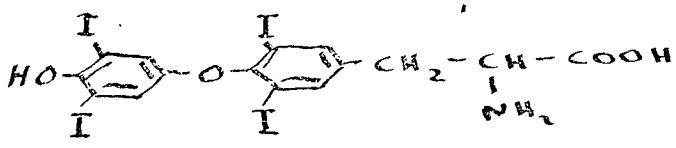
FIGURE 1.

Formation of thyroxine and triiodothyronine from iodinated tyrosine in the thyroid gland.



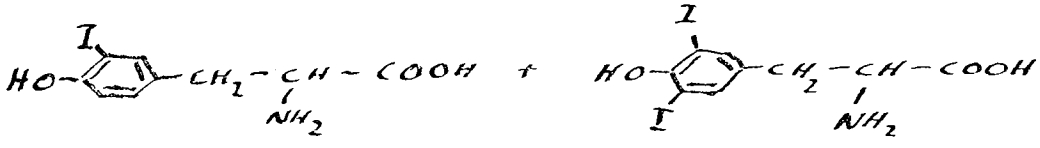
3, 5- DIIODOTYROSINE

3, 5 DIIODOTYROSINE



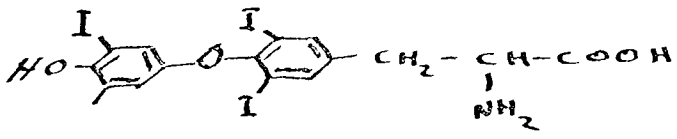
3, 5, 3', 5' - TETRAIODOTHYRONINE

(THYROXINE)



MONOIODOTYROSINE

DIIODOTYROSINE



3, 5, 3' - TRIIODOTHYRONINE

the hypothalamus via the pituitary gland.

The adenohypophysis secretes thyroid-stimulating hormone (TSH or thyrotrophin) in response to which the thyroid gland releases its hormones into the blood stream. Control of TSH secretion occurs through a negative feedback system; low blood levels of thyroid hormone (TH) cause an increased secretion of TSH which subsequently increases the blood TH; this in turn inhibits further TSH secretion.

The hypothalamus also exerts an important control over the secretion of TH although experimental evidence suggests that the adenohypophysis is itself directly sensitive to T4, and can still secrete TSH in the absence of an intact hypothalamo/hypophyseal connection (von Euler & Holmgren, 1956 a & b; D'angelo 1959; Bogdanove & Crabhill, 1961). The existence of thyrotrophin release factor (TRF) secreted by the hypothalamus has now been established (Guillemin, 1968; Schally, 1968). In response to this neurohumor the adenohypophysis releases TSH. Electrical stimulation or ablation studies on the hypothalamus indicate that it is the anterior hypothalamus which is responsible for this release of TRF (Bogdanove & Halmi 1953; Ganong, 1955; Harris & Woods, 1958; Campbell George and Harris, 1960; Knigge, 1960). The question of a T4-sensitive area in the hypothalamus controlling the release of TRF is rather more controversial, and present evidence is conflicting (von Euler & Holmgren 1956; Yamada & Greer, 1959). There can be no doubt, however, that the hypothalamus plays an important part in the control of thyroid function and its modification in response to changes in the animal's environment. Thus exposure to cold results in increased thyroid secretion mediated via the hypothalamus (Knigge, 1958; Andersson, 1963).

e/ACTIONS OF THE THYROID HORMONES.

Although the actions of the thyroid hormones have been the subject of extensive investigation, the precise mechanisms involved are still largely unknown (see reviews by Barker, 1966; Wolff & Wolff, 1966; Werner and Nauman, 1968). It is known that T4 stimulates (Roher, 1924), and thyroidectomy decreases (Forster, 1927), oxygen consumption by various tissues. The stimulant action of T4 on tissue respiration does not appear to be universal however, since certain tissues - for example, the brain and the gastrointestinal smooth muscle (Barker, 1955; Gordon, 1944; Weiss, 1957) - do not respond to T4 by an increase in respiratory rate. The absence of a respiratory response to T4 by adult amphia, coupled with the inability of their tissues to deiodinate T4, has led some workers to suggest that preliminary deiodination is necessary for the respiratory response to T4, (Galton and Ingbar, 1961; Tata, 1961).

Most work in the field has been directed towards the discovery of a single underlying mechanism whereby T4 gives rise to all its various effects. Many of these studies, perhaps unreasonably, have assumed that the primary effect of T4 is on respiratory rate. From the numerous studies carried out on this subject a number of effects directly attributable to T4 have been established:-

1. Metal binding:

T4 has been shown to form chelation complexes with a number of metal ions including Cu^{++}, Mn^{++}, Co^{++}, Fe^{++}, and Zn^{++} (Lardy, 1955). It is possible that T4 may to some extent influence enzyme activities by removal of essential or inhibitory metal ions from the surrounding medium.

2. Direct action on enzymes:

T4 inhibits the actions of several dehydrogenases, stimulates some peroxidase reactions and increases the rates of oxidation of a number of substrates both in vivo and in vitro (for references see Wolff & Wolff, 1966).

3. Oxidative phosphorylation.

A number of experiments have indicated that T4 can exert considerable influence over the tightness of coupling of oxidative phosphorylation. The possible significance of this property of T4 in relation to its pharmacological properties will be discussed later (Section 3, Chapter 2.).

4. Effects on intermediary metabolism.

The effects of T4 on intermediary metabolism are widespread. The thyroid hormones increase the rate of carbohydrate anabolism (Glock, Mclean & Whitehead, 1956; Spiro & Ball, 1958; Bargoni et al, 1961; Necheles et al, 1961), but the precise mechanisms involved are not completely understood (see Wolff & Wolff, 1966). Treatment with TH increases both the synthesis and catabolism of lipids (Marchi & Mayer, 1959; Critchevesky, 1960; Fletcher & Myant, 1960). In thyroidectomised animals acetate oxidation is inhibited leading to a build up of acetoacetate and cholesterol (Mookerjee & Sadhu, 1955). The anabolism of protein is increased by TH but in advanced cases of hyperthyroidism, unless large quantities of fat and carbohydrate are supplied in the diet, the increased calorific requirements are met partially by gluconeogenesis and a general wasting of body protein occurs (Barker, 1964

f/THYROID DYSFUNCTION.

Although many diseases and abnormalities of the thyroid gland are

described,for the purpose of this account these abnormalities will be considered in two broad groups - hyperfunction and hypofunction.

1. Hyperthyroidism.

As has been stated before, this condition was first described by Graves and is still often referred to as Graves' disease. The condition is more common in females than in males, and the abnormally high levels of circulating TH found in this disease are though to be maintained, not by normal pituitary TSH, but by a long-acting thyroid stimulator (LATS). Although similar in effect to TSH, LATS has a much more prolonged action (McKenzie, 1967). The aetiology of the disease is uncertain, but various reports have suggested that it can be precipitated by emotional stress (Mandelbrote & Wittkower, 1955; Bennet & Cambor, 1961; Lubart, 1964). Some workers (McKenzie, 1967) believe that Graves disease is autoimmune in origin, LATS acting as the antibody to some antigen the nature of which is still unknown.

As would be expected the disease is characterised by a high basal metabolic rate (BMR), the patient is usually hyperthermic and shows intolerance to heat. The pulse rate is high and atrial fibrillation or flutter may be present. The hyperthyroid patient is usually tense, nervous and given to sudden changes of mood. Although some weight gain may be noted at the beginning of the disease, advanced cases show a general wasting of body protein and fat is almost totally absent. The electroencephalogram (EEG) in hyperthyroidism may show paroxysmal spike discharges and seizure activity resembling epilepsy (Condon, Becka & Gibbs, 1965), and in some cases patients have suffered epileptic seizures for the first time with the onset of this disease. However, a definite causal relationship has not been established (Skanse & Nyman, 1956).

In addition to these clinical observations experimental studies in animals have shown that treatment with T3 or T4 increases the excitability of the brain and its capacity to sustain seizures (Timiras, 1955 and 1956).

The involvement of the sympathetic nervous system in the production of the symptoms of hyperthyroidism is a rather controversial matter. Several authors have reported that treatment with adrenergic -receptor blocking agents can control the cardiac symptoms of the disease (Gaffney, Braunwald & Kahler, 1961; Waldstein et al, 1964; Parsons & Jewitt, 1967), and it appears that the sympathetic nervous system has a definite role to play in the production of the symptoms. Whilst some studies suggest that the effects of the catecholamines are enhanced in hyperthyroidism (Brewster et al, 1956; Gaffney, Braunwald & Kahler, 1961), this hypothesis has since been questioned by a number of authors who failed to demonstrate any difference in response between euthyroid and hyperthyroid animals (Margolius, Gaffney & Werk, 1964; Margolius & Gaffney, 1965; Van der Schoot & Moran, 1965).

Changes in calcium metabolism are also reported in hyperthyroidism; Aub et al (1929) noted that calcium and phosphorus excretion were both increased in the disease, although they failed to show any elevation in the serum levels of either element. More recently Baxter (1966) has shown that hypercalcaemia is a common feature of hyperthyroidism. The resulting high levels of calcium excretion may ultimately result in osteoporosis.

2. Hypothyroidism.

A distinction must be made between two forms of this disease : hypothyroidism occurring in adult life is called myxoedema, a form of the disease which usually can be treated successfully with TH administration. Alternatively, cretinism is a form of the disease occurring in neonatal life and its effects are more far reaching than those of myxoedema since the central nervous symptoms in particular may be irreversible.
The symptoms of hypothyroidism arise as a result of inadequate levels of circulating thyroid hormones, a condition which may be brought about by a variety of factors - congenital, genetic, environmental or as a secondary result of other disorders.

As would be expected, myxoedema is characterised by a low BMR, the patient tends to be hypothermic and cannot tolerate exposure to cold, his general activity is slow and there is mental slowing. A common accompanying disorder is angina pectoris which may be due to a reduced cardiac output, which it is known may be insufficient to fill the coronary vessels (Macgreggor, 1966). The metabolic disturbances seen in myxoedema are widespread ; carbohydrate metabolism is affected since all oxidative processes are inhibited and hypoglycaemia may be present; the anabolism and catabolism of lipids is inhibited and there is a rise in serum lipid concentration and total body fat. Similarly, both the synthesis and breakdown of protein is inhibited causing a characteristically abnormal serum protein picture.

The central effects of myxoedema, though not as disastrous as those of cretinism, are well marked. The disease is characterised by mental sluggishness with a tendency to periods of depression. There

are numerous reports in the literature of psychiatric disorders occurring in myxoedema, (Brockman & Whitman, 1952; Jonas, 1952; Pitts & Guze. 1961; Glucksman & Stokes, 1967), although the incidence of such symptoms is relatively low. The incidence of psychiatric complications was much higher in the report of the Clinical Society of London in 1888, but it is likely that their report was dealing with a large proportion of advanced untreated cases, and it is possible that the psychological manifestations of the disease are more common in such cases. The symptoms may be so advanced as to give rise to frank psychoses with hallucinations, guilt feelings, and delusions of persecution; indeed Asher (1949) stated that "Myxoedema is one of the most important, one of the least known, and one of the most frequently missed causes of organic psychosis". When psychosis does occur, it appears that it can be reversed by adequate thyroid therapy (Glucksman & Stokes, 1967).

Cretinism is a condition arising from a neonatal deficiency of thyroid hormone. The disease can result from a variety of causes, and the effects are likely to be far more far reaching than those of myxoedema. Unless replacement medication is instituted soon after birth, the child remains a mentally retarded dwarf throughout life. If therapy is commenced early enough the patient will attain normal stature and may develop a normal degree of intelligence. The prognosis for normal mental development is, however, uncertain even if therapy is commenced early in extra-uterine life (Smith, Blizzard & Wilkins, 1957).

CHAPTER II

a/THE EFFECTS OF THE THYROID HORMONES ON BEHAVIOUR

In considering the effects of the thyroid hormones on behaviour thr relationship between thyroid status and the central nervous sustem (CNS) must be considered first:

1. The effects of thyroid status on cerebral development.

It has been recognised since the condition was first described, that cretinism is associated with extreme mental retardation. That this retardation is not simply the result of the metabolic effects of neonatal hypothyroidism is indicated by the fact that these mental effects cannot always be reversed by otherwise adequate thyroid hormone therapy, (Topper, 1951; Smith, Blizzard & Wilkins, 1957). In experimental work on neonatally-induced hypothyroidism in rats, it has been shown that there is a reduction in the axonal and dendritic density of various areas of the cerebral cortex as compared with euthyroid animals (Eayrs & Taylor, 1951; Horn, 1955; Eayrs & Horn, 1955; Eayrs, 1960). This change in cellular structure is accompanied by a reduction in the density of capillaries in the cortex (Eayrs 1954). Both these changes would be expected to exert a profound effect on the behaviour of the animal, and Eayrs (1960) has reported that, in the rat, they appear to be irreversible unless treatment is commenced before the tenth day of extra-uterine life.

2. The effects of thyroid status on brain metabolism.

The brain of the adult (Chapter 1 e) does not respond to TH as do most other tissues by an increase in oxygen consumption (Barker, 1952; Sensenbach et al, 1956; Sokoloff et al, 1953). This is not true of the brain of an immature animal (Reiss, 1956), where T4 causes an increased

respiratory rate and the brain gains weight more rapidly than that of the euthyroid animal. Despite the apparent lack of response in the adult brain, changes in metabolism have been reported to occur; Potop (1958) has shown that, in the immature rat, T4 stimulates the anaerobic metabolism of carbohydrates and accelerates the metabolism of pyruvate.

Experimental work on hypothyroidism indicates that cerebral oxygen consumption is decreased, but that it can be raised by treatment with T4 (Himwich et al, 1942; Scheinberg et al, 1950). The cerebral blood flow in hypothyroidism is reported to be reduced (Scheinberg et al, 1950), whilst conversly hyperthyroidism induced a moderate increase in cerebral blood flow (Sokoloff et al, 1953; Sensenbach et al, 1956). Both these factors may be significant in the production of the behavioural symptoms of thyroid dysfunction.

3. The effects of thyroid status on the electroencephalogram.

Thyroid dysfunction (Chapter 1, f) may lead to changes in the E.E.G. myxoedema giving rise to slow monomorphous rhythms (Condon, Becka & Gibbs, 1956; Lansing & Trunnel, 1963), whereas hyperthyroidism increases α-rhythm frequency (Lindsley, 1937; Rubin, 1937; Ross & Schwab, 1939; Herman & Quarton, 1964) and may give rise to seizure activity. Bradley et al (1960; 1961; 1964), working with cretinous rats, reported am increased latency of recruiting responses with an abnormally low amplitude of the EEG. The latency of these responses can be reduced by treatment with T3 but the reduced amplitude of the EEG remains unaltered. The authors interpreted these changes as being indicative of faulty development of neurones in the brains of such animals.

Investigations into the effects of raised levels of thyroid hormones on the development of immature rats has shown that this situation leads to

a more rapid maturation of the CNS as shown by the EEG (Schapiro & Norman, 1967).

Apart from the central changes seen in thyroid dysfunction, its peripheral effects are also intimately concerned with the production of the behavioural symptoms seen. Obviously the metabolic changes are important in the production of behavioural changes (Chapter 1, f), however in this section it is proposed to discuss the more specific effects of thyroid dysfunction likely to produce changes in behaviour.

4. The effects of thyroid status on peripheral nervous and muscular activity.

Myxoedema has been shown to produce a reduction in the excitability of peripheral nerves (Horsten & Boeles, 1949). This neuropathy may be due to structural changes in the nerves (Nickel et al, 1961). It has also been shown that the speed of contraction and relaxation of the muscle in the Achilles tendon reflex is increased in hyperthyroidism and reduced in hypothyroidism (Lambert et al, 1951), a fact consistent with the observed changes in the speeds of nervous conduction in both conditions. This change is, in fact, used as a clinical aid in the diagnosis of hyperthyroidism (Miles & Surveyor, 1965).

Muscular weakness is reported to occur in both hyper- and hypo-function of the gland. Whilst undoubtedly this is due largely to the metabolic effects of dysfunction, structural changes have been reported to occur in the muscle in myxoedema (Nickel et al, 1961).

It is the combination of both the metabolic changes and the more specific alterations in function which result in the behavioural changes seen in thyroid dysfunction. In myxoedema the patient shows a mental and

physical lethargy, and muscular weakness may leave the patient exhausted even after simple physical tasks. Mentally the patient is usually placid with a tendency to periods of depression, and occasional psychoses can arise.

The hyperthyroid patient may also suffer from muscular weakness, but the speed of muscular activity tends to be exaggerated. Mentally the patient is restless and irritable tending to be emotionally unstable; psychoses - though less commonly reported than in myxoedema - do occur.

Animal experiments have shown that excess or lack of thyroid hormones has little effect on the behaviour of the adult animal - as shown by maze learning or conditioning experiments (Eayrs, 1960). In immature animals, however, thyroid status has a significant effect, not only on the physical, but also on the behavioural development of the animal. Cretinous rats show a retardation in the development of various postural reflexes, and although they can learn to overcome simple problems quite readily, their behaviour when faced with a more complex situation suggests a reduced sensory input (Eayrs, 1959). Treatment of the immature animal with T4 leads to the earlier appearance of responses to external stimuli as shown by the EEG, (Schapiro & Norman, 1967).

b/THE EFFECTS OF THYROID STATUS ON THE PROPERTIES OF ENDOGENOUS SUBSTANCES.

In due course, it is intended to show within this thesis that changes in the level of thyroid hormone may induce changes in the responses of animals to certain centrally acting drugs; an understanding of these effects of thyroid hormone may depend in turn upon an adequate understanding of

the established modes of action of these centrally acting drugs, in particular their interference with neurohumoral transmission within central and peripheral nervous systems. Therefore, a necessary preliminary to the discussion of drug action is a consideration of the possible chemical transmitters within the CNS.

1. Possible central chemical transmitters

Because of the complexity of the CNS and the inaccessibility of its synapses, it has been impossible so far for workers to positively identify the chemical substances involved in central nervous humoral transmission. The available evidence is largely indirect, resulting from collection of substances released during nervous activity, iontophoretic application of various substances, studies of the localisation in the CNS of suspected transmitters, and the enzymes responsible for their synthesis and degradation. Such evidence indicates the possible involvement of a number of compounds as transmitters in the CNS, some of them having a similar function in the peripheral nervous system, (see symposium on neurotransmitters, 1968).

Before any substance can be considered as being a possible transmitter there are certain conditions which must be fulfilled:-

1. The compound must be present in the nervous tissue, particularly in the presynaptic fibres.

2. Suitable nervous activity must lead to a release of the compound.

3. Enzymes for its synthesis and degradation must be present in the tissue.

4. Application of the compound to the postsynaptic membrane must cause changes typical of nervous stimulation.

5. Compounds known to influence the actions of the suspected transmitter, or the enzymes responsible for its synthesis or degradation, should exert a predictable effect on nervous activity.

There are a number of compounds which present available evidence indicates may be involved in central nervous transmission, as follows:-

1. Actylcholine.

The peripheral role of acetylcholine (ACh) as a neurohumoral transmitter is now widely accepted (Crossland, 1965). It has been identified as the transmitter between motor nerves in the spinal cord and the inhibitory Renshaw cells in the anterior horns of the grey matter (Eccles, 1954 & 1956). Elsewhere in the CNS the distribution of ACh and choline acetylase provides strong though circumstantial evidence for a role of ACh as a transmitter at other central sites (Feldberg & Vogt, 1947 & 1948). Iontophoretic application of the ACh to cells in various areas of the brain has revealed sensitivity to the compound (Crossland, 1965). So far, however, ACh is the only compound which has been definitely shown to have a transmitter function in the CNS.

2. Noradrenaline.

Like ACh, noradrenaline (NA) is known to have a transmitter function in the peripheral nervous system (Crossland, 1965). In the CNS the amine is not evenly distributed (Garattini & Valzelli, 1965), and it appears to be concentrated, like ACh, in the presynaptic fibres (Carlsson, 1964). The highest concentrations of NA are found in the hypothalamus (Garattini & Valzelli, 1965), and in this area some reports

indicate that the amine has an alerting effect (Goldstein & Munzo, 1961). In other areas of the brain, however, NA appears to exert a depressant action, and on intracerebral administration it causes a condition resembling light anaesthesia (Feldberg & Sherwood, 1954). Unlike the peripheral nervous system adrenaline does not share the transmitter function of NA, since it is virtually absent from the CNS of mammals.

3. Dopamine

Dopamine (DA) is the metabolic precursor of noradrenaline and adrenaline. Its distribution in the CNS does not, however, parallel that of NA (Carlsson, 1959; Bertler, 1961), and there is ample evidence to suggest that DA possesses a central transmitter function in its own right. The distribution of DA is uneven, highest concentrations being found in the basal ganglia, thalamus and hypothalamus (Garattini & Valzelli, 1965). Some workers have reported the presence of anatomically discrete fibre tracts between the thalamus and the caudate nucleus (McLennan, 1964). Experiments involving the iontophoretic application of DA to certain neurones causes a hyperpolarisation of the postsynaptic membrane (McLennan, 1961; McGreer, 1961), suggesting an inhibitory role for this amine in the CNS. This suggestion is contradicted by the report that the administration of DOPA (the immediate precursor of DA), which freely passes the blood-brain barrier, causes an alerting reaction (Blaschko & Chrusciel, 1960; Wende & Spoerlein, 1962). This latter evidence is open to question, however, since DOPA increases levels of DA and also NA in all areas of the brain, not only those where the amines are normally localised.

4. 5-Hydroxytryptamine.

5-Hydroxytryptamine (5HT), like NA and DA, has an uneven distribution in the brain, highest concentrations being found in the hypothalamus, thalamus and midbrain (Garattini & Valzelli, 1965). The amine is found in the presynaptic terminals of some fibres (Whittaker, 1959; Michaelson & Whittaker, 1962). Although its distribution is similar to that of NA there is evidence that 5HT is contained in fibres different from those containing NA (Costa, 1960). Interest in the possibility that 5HT is concerned in central nervous transmission arose from the suggestion that the hallucinogenic properties of lysergic acid diethylamide (LSD) are due to a blockade of central 5HT receptors (Gaddum, 1953; Woolley & Shaw, 1954). This idea, coupled with the behavioural changes seen following increases in brain levels of the amine, forms the main basis for the hypothesis that 5HT has a transmitter function in the CNS.

Experimental evidence (Udenfriend, Weissbach & Bogdanski, 1957; Joyce & Mrosovsky, 1964), indicates that low doses of 5HT have a depressant action on the brain, whilst at higher doses excitation is seen. It has been suggested that 5HT in conjunction with NA is involved in the nervous control of thermoregulation in the hypothalamus (Feldberg & Meyrs, 1964; Cooper, Craston & Honour, 1965).

5. Other compounds with a possible transmitter function in the CNS.

A number of compounds occurring in the CNS have with more or less evidence - been suggested as possible transmitters in the CNS; γ-Aminobutyric acid (GAEA) is one such compound. In vertebrates it is found only in the CNS where it is evenly distributed, being a metabolic product from glutamic acid, (Tallen, Moore & Stein, 1954). The

general depressant action of GABA on the brain, (Elliot & Jasper, 1959; Roberts & Eidelberg, 1960) has led to the suggestion that the compound may have more than a simple metabolic function. Though it is present in inhibitory fibres in some species (Kravitz, Kuffler & Potter, 1963), the fact that it depresses both excitatory and inhibitory neurones casts some doubt on the idea that GABA is de facto a transmitter, although it seems certain that the compound exerts some direct influence on the general excitability of the CNS. It has been suggested that GABA in conjunction with its parent compound glutamic acid, which has a general stimulant action on the CNS, are responsible for the general control of central nervous excitability, (Crossland, 1965).

Ergothionine is another compound which has been suggested as a central transmitter. The compound was first reported by Crossland & Mitchell (1956) to be an excitatory material found in extracts of the cerebellum. It has since been found in several mammalian species and is in highest concentrations in the cerebellum and optic tracts (Crossland 1965). As yet no definite evidence for a transmitter function for the compound other than those mentioned here have been reported, and the precise function of the compound remains largely unknown.

Histamine which is found in highest concentrations in the hypothalamus and optic nerve (Werle,1949; Harris, 1952) has been shown to have an excitatory action on the electrical activity of the cerebellum (Crossland, 1952). Apart from this uneven distribution and activity there is little direct evidence that the substance has a transmitter function.

c/THE EFFECTS OF THYROID STATUS ON CHEMICAL TRANSMITTERS

Many workers have studied the relationship between hyperthyroidism and the effects of the catecholamines. It has been reported that the cardiovascular symptoms of thyrotoxicosis can be moderated by the administration of adrenergic β-receptor blocking agents, indicating that these symptoms may be mediated via the sympathetic nervous system (Gaffney, Braunwald & Kahler, 1961; Waldstein, 1964). More recent work, however, has shown that the cardiovascular potency of the catecholamines is not changed by the presence of high levels of T4 (Margolious, Gaffney & Werk, 1964). rather it would seem that the heart rate is increased by some direct action of T4 on cardiac metabolism (Hess & Shanfield, 1966; McDevitt et al, 1968). Although the cardiovascular effects of the catecholamines do not appear to be influenced by thyroid hormone levels, there are reports of the enhancement of the calorigenic responses to endogenous or exogenous catecholamines (Swanson, 1956 & 1957). This potentiation is of importance in the response of the animal to cold exposure; sensory input to central thermodetectors in the hypothalamus leads to a release of TRF and hence increased circulating levels of T4. In this situation the calorigenic potency of the catecholamines is increased and non-shivering thermogenesis facilitated (Stevens, et al, 1955; Woods & Carlson, 1956; Andersson et al, 1963; Andersson, Gale & Hökfelt, 1964).

In addition to the effects on catechol amine activity, there is also evidence that the actions of histamine and 5HT are modified in thyroid dys function. Sensitivity to both these amines is increased in hyperthyroidism and decreased in hypothyroidism in rats, (Parratt & West, 1957), and mice

(Spencer & West, 1961). Although quite large increases in tissue 5HT have been recorded (Parratt & West, 1957), these authors state that the sensitivity increase in hyperthyroidism is most likely due to altered metabolism of the amines.

CHAPTER III

THE EFFECTS OF THE THYROID HORMONES ON THE ACTIONS OF DRUGS

a/ Peripherally acting drugs.

Numerous observations have shown that T4 will significantly alter the toxic effects of a number of substances (Carrier & Buday, 1961). Thus, the toxic effects of bacterial endotoxin (Melby & Spink, 1959), alloxan (Houssay & Sara, 1945), anoxia (Smith, Emmens & Parkes, 1947; Smith 1947), 2, 4- dinitrophenol (Glaubach & Pick, 1934), adrenaline (Kroneberg, 1952), oxygen at normal and elevated pressures, (Smith et al, 1960), ergotamine-induced gangrene, (Wells, 1950), α-methyl meta tyrosine (Moore 1965), ephedrine (Halpern Drudi-Baraco & Bessirard, 1964) are all increased by T4. Some centrally acting drugs such as chloroform are more toxic in hyperthyroid animals, although this is thought to be due to a toxic action on the liver rather than a central effect (McIver, 1940). In contrast, a few substances such as acetonitrile (Hunt, 1923) and β-aminopropionitrile (Khogali, 1961) have been shown to be less toxic in animals treated with T4. Hypothyroidism induced by propylthiouracil (PTU) has a protective effect against oxygen toxicity (Grossman, 1949; Smith et al, 1960), but is without effect on gangrene induced by ergotamine (Wells, 1950).

It seems likely that many of these effects are the result of changes in the general metabolic systems of the animal produced by thyroid dysfunction.

b/Centrally acting drugs.

Conney and Garren (1960) reported that treatment of rats with T4 significantly increases the hypnotic potency of hexobarbitone, an effect due to inhibition of the metabolism of the drug in these animals. Later reports (Prange, Lipton 1962; Ellinwood and Prange, 1964) showed that the potency of pentobarbitone is increased in hyperthyroidism and also that treatment with adrenaline has the same potentiating effect. Since the effects of hyperthyroidism and adrenaline were not additive, the authors concluded that either the effects of one are mediated through the other, or that both were acting on the same mechanism to a similar degree. They thought the most likely mechanism was that both hormones caused potentiation by inhibiting the capacity of the liver to metabolise the barbiturate. This theory was supported by a later report (Prange et al, 1966) who found that in hyperthyroid mice the metabolism of both pentobarbitone and thiopentone is inhibited leading to a potentiation of the hypnotic effects of these drugs. In hypothyroidism induced by PTU or thyroidectomy, the anaesthetic potency of thiopentone, hexobarbitone and isopropyl-bromallyl barbituric acid is increased, (Holk, Hillard & Malone, 1951; 1954 a & b). In contrast other workers (Prange et al, 1966) reported that the hypnotic potency of pentobarbitone is decreased and that of thiopentone unaltered by hypothyroidism.

The potentiation of drug action does not seem to be limited to those barbiturates which have a hypnotic action, since Prange and Lipton (1965) have shown that the actions of the convulsant barbiturate 5-(1,3-dimethylbutyl)-5-ethyl barbituric acid (DMBEB) are potentiated by treatment of rats with T4. The same authors also reported that PTU treatment decreases the activity of this convulsant. However, this

latter result may be open to question since PTU is reported to have anticonvulsant action of its own (Woodbury et al, 1952).

The effects of various analgesic drugs are also reported to be influenced by thyroid status. Sung & Way (1953) found that thyroidectomy or treatment with antithyroid drugs caused tolerance to the actions of d, l-methadone (as shown by its depressant activity) and also that its analgesic potency was reduced too. Hyperthyroidism rendered the animals more susceptible to the toxic effects of the same drug but without effecting its analgesic potency. In vitro experiments showed that the metabolism of the drug was inhibited in both hyperthyroid and hypothyroid animals. It was suggested that in hypothyroidism it might be dangerous to use morphine or methadone. A later paper (Cochin & Sokoloff, 1960) confirmed that morphine metabolism is inhibited in vitro by prior treatment of rats with T4. In contradiction of the earlier report, however, Bhagat (1964) stated that the analgesic potency of morphine is significantly increased in hyperthyroidism produced in dogs with T4.

The potency of some antidepressant drugs is also reported to be altered by changes in thyroid status. Prange & Lipton (1962) showed that the toxicity of imipramine is increased in hyperthyroid mice, and later they showed that this is also true of the active desmethyl derivative (Prange, Lipton & Love, 1964). In the latter paper, however, they were unable to demonstrate any sparing effect in mice made hypothyroid by PTU treatment. More recent work (Anvi et al, 1967) has shown that although the potencies of imipramine and amitriptyline (as shown by their anti-tetrabenazine activities) are not altered in PTU-treated rats, their active derivatives desmethylimipramine and nortriptyline are significantly more

active in hypothyroid animals. Ashford & Ross (1968) have confirmed that in hyperthyroid mice, imipramine and nortriptyline are more toxic and the increased toxicity is not altered by treatment with α- or β-adrenergic blocking agents.

Alterations in the actions of certain tranquillisers have also been observed in hyperthyroid animals. Hoffman (1959) showed that treatment of the rat and ground squirrel with 3, 5, 3'-triiodothyropropionic acid will prevent chlorpromazine-induced hypothermia. More recently Ashford & Ross (1968) have shown that the toxicities of chlorpromazine, perphenazine and chlordiazepoxide are increased in hyperthyroid mice, but they could demonstrate no increase in reserpine or meprobamate toxicity. These authors suggested that the use of phenothiazines in uncontrolled hyperthyroidism might be dangerous.

In the field of stimulant drugs more work has been devoted to an investigation of the effects of amphetamine in animals with altered thyroid status. It is now well established that in thyroid hyperactivity the toxicity of amphetamine is increased (Askew, 1962; Halpern, Drudi-Baracco & Bessirard, 1964; More 1966; Dolfini & Kobayashi, 1967). Dolfini & Kobayashi (1967), studying tissue levels of the drug in hyperthyroid and euthyroid rats showed that the increased toxicity is not due to a reduced metabolism in the hyperthyroid animals. In hypothyroid animals, although there are fewer reports, it is known that the toxicity and the activity of amphetamine are decreased (Dolfini & Kobayashi, 1967; Mantegazza Niazada & Riva, 1968). The first named authors reported that both brain and liver levels of amphetamine in hypothyroid rats are increased even in the presence of reduced effects of the drug.

CHAPTER IV

RELATIONSHIP BETWEEN THE THYROID AND THE OTHER ENDOCRINES

a/The adrenal medulla.

This gland secretes both adrenaline and noradrenaline although the latter constitutes only about one quarter of the total catecholamine secretion. These agents are released in response to stimulation of the medulla by preganglionic sympathetic nerves with which it is richly supplied.

On release of the hormones a variety of effects are noted; a mild hyperglycaemia is produced, oxygen consumption of the tissues is increased, cardiac output increases and there is an increase in cardiac and muscular skeletal blood flow. (The possible relationships between catecholamines and the cardiac manifestations of hyperthyroidism have already been discussed in Chapter I f). Harrison (1964) has reviewed the relationships between the adrenal medulla and thyroid status; much work has been done but often, due to the use of more advanced techniques, modern work has disproved earlier theories. From the available evidence, however, it would seem that the glycogenolytic and respiratory effects of adrenaline are potentiated in hyperthyroidism (Abbot & Van Buskirk, 1931).

It is possible that in both hyper- and hypo- thyroidism, the adrenal medulla does not function efficiently. Thus the release of catecholamines in response to insulin-induced hypoglycaemia is inhibited in both hyper- and hypo-function of the gland (Harrison, 1961). In hyperthyroidism an increase in the total catecholamines secreted is reported (Hsi-Chiang & Zenker, 1968) and qualitative differences in the response to the amines have been reported (Marks, 1925).

b/The adrenal cortex.

A functional relationship between the adrenal cortex and the thyroid gland has been proposed for many years, following observations that cortical hyperplasia occurs in hyperthyroid animals (D'Angelo, 1964), a cortical atrophy results in hypothyroidism (Baumann & Marine, 1945). There are a number of possible mechanisms which may explain this phenomenon. It may be that cortical enlargement seen in hyperthyroidism is the result of an increased rate of degradation of corticosteroids (Hellman et al, 1961). Alternatively, in the presence of an experimental induced excess of T4, TSH secretion is inhibited and more ACTH secretion could result (Deane & Greep, 1947). Also, in hypofunction of the gland, TSH secretion should be maximal and ACTH secretion may be simultaneously reduced; this latter theory appears most likely since cortical hyperplasia or atrophy does not occur in hypophysectomised animals (Freedman & Gordon, 1950; Feldman, 1952). It is, of course possible that cortical hypertrophy seen in hyperthyroidism is the result of the primary condition acting as a nonspecific stress and thus stimulating the cortex to increased activity (Tepperman, Engel & Long, 1943).

The corticosteroids themselves, like the thyroid hormones, exert a profound effect on general metabolism, and also affect brain function. Some of the central effects of the corticosteroids are similar to those seen following T4 administration; for example convulsive disorders have been reported following the treatment of patients with cortisone (Lowell, 1951) and there are occasional reports of psychoses (Hellier, 1966). Conversely some reports indicate that corticosteroids have opposite effects to thyroid hormones; for example Bousquet (1965) reported that stress reduced the potency of hexobarbitone and pentobarbitone.

c/The sex hormones.

These hormones have an obvious effect on the behaviour of animals of all ages; in the mature animal the behavioural changes seen in the spring are due to increases in the circulating levels of sex hormones. In the immature animal administration of sex hormones will induce the precocious development of sexual behaviour. In the immature animal administration or deprivation of sex hormones can control the differentiation of the brain into a typically male or female structure. (Harris 1964).

The interrelationship between the thyroid and the gonads has not received as much attention as that between the thyroid and the adrenal glands. It is to be expected that dysfunction of one will lead to a change of function of the other, and it is known that both oestrogen and testosterone cause increased activity of the thyroid gland (Chu & You, 1945); Nathanson, 1940). The effects of thyroid dysfunction on gonadal activity is rather more vague and it may well be that any changes seen in these cases are the result of metabolic changes rather than to any specific action on gonadal function.

d/Insulin and glucagon.

That a relationship exists between the thyroid status of an animal and the secretions of the pancreatic islets is shown by the fact that T4 administration to diabetic animals results in an aggravation of the disease whereas some cases of diabetes lose all symptoms of the disease if they become myxoedematous (Means (1947), the symptoms returning when T4 is administered. Houssay (1960) has stated that the thyroid hormones appear to have no effect on diabetes other than those effects on general metabolism.

THE PROJECT: ITS BASIS AND AIMS

It has been the purpose of this introduction to outline the basic functions and actions of the thyroid hormones; in particular, to show first how they may play a part in normal and abnormal mammalian physiology, and secondly how they may also be implicated in changes in drug action. It can be seen that thyroid dysfunction in general, and hyperthyroidism in particular, may exert profound effects on the activities of certain drugs. Several of the workers cited have suggested that a number of drugs may be dangerous in uncontrolled hyperthyroidism due to the increased toxicity of these drugs in that condition. Therefore, it was postulated that sub-clinical changes in thyroid status might explain a number of unexpected drug actions in man.

The purpose of the following experimental work was to investigate how hyperthyroidism might alter the pharmacological responses of animals to a number of centrally acting drugs. The results of the work might then support or refute the original postulation.

SECTION 2.

METHODS

SECTION 2.

METHODS

1. Animals:

The majority of experiments reported in this thesis were carried out on male albino mice, although some preliminary work was carried out on female mice. The strains used were:-

i. Male and female Tuck No. 1;

ii. Male AS 1;

iii. Male TO mice (Scientific Products Farms, Ltd. Ash, Kent).

This was predominantly the strain used throughout the project, and exclusively during the latter two years.

No obvious differences were observed between the three strains. The need to change from one strain to another was determined largely by the quality and availability of animals. In this respect the TO mice have proved consistently satisfactory and it was for this reason that the majority of experiments were performed on this strain.

2. Animal husbandry:

The animals used were of an initial weight of 18 - 20 g. After receipt all animals were maintained in the animal house for a period of not less than 4 days prior to experiment. Animals were fed a conventional 41B cube diet (supplied by Pilsburys Ltd) and allowed tap water ad libitum to drink. The mice were kept in groups of 50 in large opaque polypropylene cages (40 cm. c 30 cm.) until commencement of the experiment, when they were randomly divided into groups of ten (sometimes five) for pretreatment and eventual experiment. At this stage the mice were kept in smaller cages measuring 10 cm. x 27 cm. Animal house conditions were:- relative humidity 50 - 55%, and temperature ranging from 21 - 23°C. There was a normal light/dark cycle determined

predominantly by natural lighting; as far as possible, all experiments were performed between 2.00p.m. and 8.00p.m. in an attempt to provide standard conditions of experiment. Laboratory temperatures were kept at 22° C unless otherwise stated; animals were removed from the animal house not less than 2 hr. before experiment, (and where possible left in the laboratory overnight before experiment).

3. Production and Detection of a Hyperthyroid State:

Hyperthyroidism was induced by daily subcutaneous injections of sodium l-thyroxine into the neck region at a dose of 2 mg/kg in a volume of 5 ml/kg. The hormone was dissolved in a minimal quantity of N/10 sodium hydroxide solution and diluted to volume with normal saline. This solution was found to produce no detectable local tissue necrosis during the course of the treatment. In all experiments control euthyroid animals were treated with the vehicle alone, such treatment being without effect on the metabolic rates of these animals.

The degree of hyperthyroidism produced by this treatment was assessed by measuring the change in oxygen consumption produced in the test animals compared to that of the controls throughout the treatment. The method of assessment was an anoxia method essentially similar to that reported by Spencer and West (1961). The animal was placed in a glass jar of known volume and the lid screwed down so as to make the jar airtight. The time taken for the animal to die from anoxia was observed and the volume of the animal measured by total immersion in water in a 100 ml. glass measuring cylinder. Knowing the volume of air utilised and therefore the

approximate volume of oxygen, the rate of oxygen utilisation by the mouse per gramme of tissue was calculated.

The results of experiments to produce hyperthyroidism in the three strains of mice are shown in Figs. 2 - 9 and in tables 1 - 8.

In the experiments with the male and female Tuck No. 1 mice, the graphs are produced from data from individual animals, since this experiment was carried out to obtain a broad indication of the time necessary to obtain a maximal degree of hyperthyroidism. As can be seen from the graphs (Figs. 2, 3, 4, and 5), the female animals were slightly more sensitive to the hormone, but with both sexes and with all doses used, a peak level of hyperthyroidism occurred after 6 to 8 days of treatment.

In subsequent experiments using male A. S. 1. mice the results shown in Figs. 6, 7, and 8 indicate that treatment with T3 or T4 results in a maximal level of hyperthyroidism on the eighth day (after 7 days of pretreatment).

Following this last reported series of experiments, it was decided to use male animals only in the experimental work since the cyclic changes in ovarian hormones may disguise the effects of the thyroid hormones in the female mice. Accordingly for experimental purposes male A. S. 1. mice were used for experiment after seven days pretreatment with T4 @ 2 mg/kg.

In the experiments with male TO mice, a maximal level of hyperthyroidism was obtained by 10 days pretreatment of the animals with T4 @ 2 mg/kg.

4. Neuropharmacological Techniques.

a/Duration of Hypnosis.

In experiments involving the measurement of sleeping times, one of two techniques was used:-

i. If the sleep time was relatively short then the time taken for an animal to regain its righting reflex following the administration of an hypnotic agent was simply assessed by watching the animal lying on its back and noting the time at which it turned over onto its feet.

ii. In experiments involving the measurement of longer periods of hypnosis an apparatus similar to that shown in Fig. 10 was employed. The apparatus consisted of a horizontal glass jar, attached along its length to, and suspended from, a horizontal glass rod pivoted at both ends. A pointer was attached at right angles to the jar so that it could be used to write on a smoked drum. The sleeping animal was placed on its back inside the jar and the pointer placed against a slowly rotating kymograph drum (which was also being marked by a time marker). Whilst the animal slept, the pointer traced a straight line on the drum. However, when the animal regained its righting reflex and turned over, it caused the jar to rock on the pivoted glass rod and the pointer moved vertically against the drum. A typical trace of this type is shown in Fig. 11; by measuring the length of the trace and relating this to the time marker trace, the sleep time was measured. This method was found to be more convenient for the measurement of long periods of hypnosis than simple observation.

FIGURE 10 a.

Apparatus employed in the measurement of sleep time.

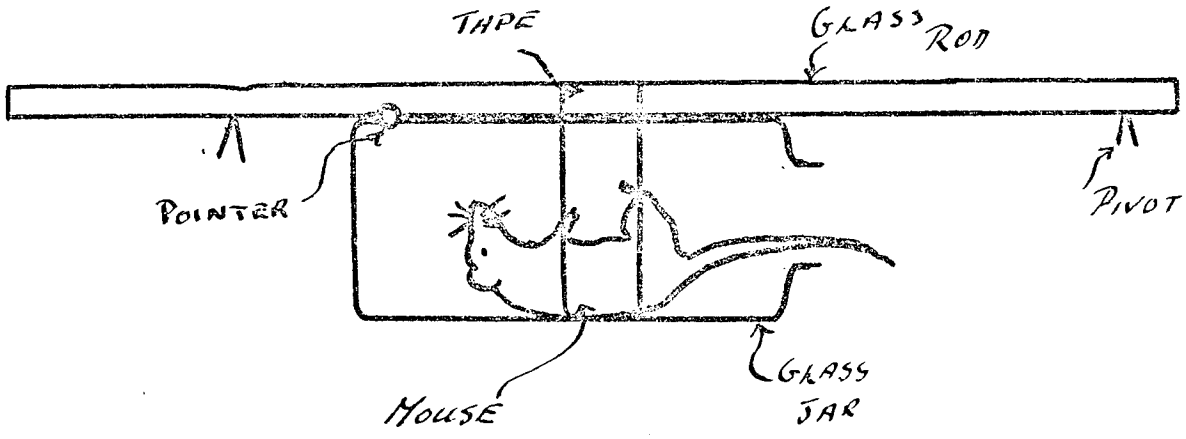
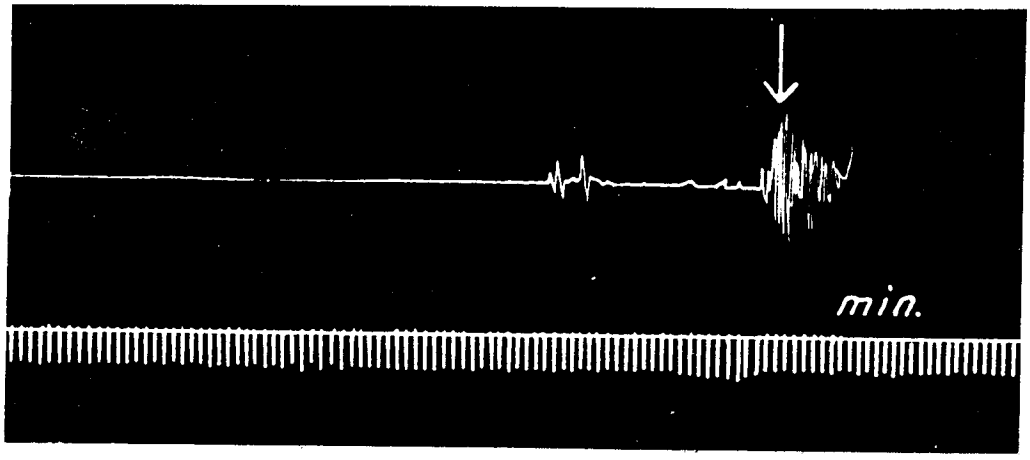


FIGURE 11a.

Typical trace obtained in experiments measuring barbiturate sleep time.



b/Analgesic testing.

The testing of analgesics in laboratory animals is rendered difficult by the problem of assessing the response of the animal to pain. In order to minimise the error, 3 different methods of testing, each relying on different end points, were used:

i. The "hot-plate" Method.

This method is essentially similar to that of Eddy & Leimbach (1953). The apparatus employed consisted of a metal dish the base of which was placed just beneath the surface of a water bath at 55 degrees centigrade. When the animal was placed on the floor of the dish, the discomfort of the heat caused it to respond characteristically by licking its paws. Two measurements of this response were made:-

1. The time taken before the animal responded to the pain stimulus.

2. The number of times the animal showed the response in a 30 second period. This added a degree of quantification to an otherwise qualitative assessment.

The animals were exposed to the stimulus before treatment with the analgesic drug and any animal not responding within a 30 second time period of exposure was rejected. After a three hour rest period the animals received the analgesic under test or received saline (controls). The responses of the drug treated mice were compared 45 min. later.

ii. The "tail-clip" Method.

This method was similar to that described for the assessment of analgesics by Bianchi & Franceschini (1954). A clip was placed at the base of the tail about 5 mm. from the body; the response to the pain

was taken as the time when the animal turned and bit the clip. As with the hot plate method, the experimental animals were exposed to the stimulus before drug treatment and any not responding within a 15 second exposure period were rejected. After a 3 hr. rest period the animals were given the analgesic or normal saline and then re-exposed to the pain stimulus a further 45 min. later. Analgesia was assessed by comparison of the responses of the drug treated with the saline treated mice.

iii. The phenylquinone writhing test.

It was reported by Seigmund et al (1957) that intraperitoneal injection of an aqueous solution of phenyl-p-quinone causes writhing in mice. It is now believed that this writhing is due to the action of endogenous substances released in the gut by the compound since it is antagonised by both analgesics and by anti-inflammatory agents. The writhing is in many ways similar to that seen following the administration of reserpine.

In using phenylquinone for analgesic testing, the ability of an analgesic to supress the writhing is compared with the intensity of writhing seen in control mice pretreated with the vehicle alone. The test was carried out using groups of 5 drug-treated or control mice, and counting the number of writhes occurring in four consequtive 5 minute periods (for 20 minutes) induced by phenylquinone given 1 hr. after the analgesics under test.

In each of the three tests of analgesic activity, untreated euthyroid and hyperthyroid animals were compared. This ensured that the results did record differences in sensitivity to the analgesic drugs used, as well as any difference in the inherent sensitivity of the two groups

of animals to the pain stimuli applied. Analgesia was assessed by the absence or delay of the response of the animals to the pain stimulus and/or reduction in the number of responses seen in the observation period.

c/Measurement of body temperature.

In all experiments in which body temperature was measured, oesophageal temperature was measured, since this provides a simple and accurate measurement and has been reported to provide a more accurate assessment of true body temperature than does measurement of rectal temperature. The method employed was that reported by Brittain & Spencer (1964). A thermocouple attached to an electric thermometer was inserted into the oesophagus to a depth of 2 cm. so that it lay just above the cardia of the stomach. With the thermocouple in this position the temperature was noted as soon as it became constant. In some experiments skin temperature was measured by placing a specially shaped thermocouple between the digits of the hind foot of the animal, and the temperature recorded when it had become constant.

d/Assessment of Tremor.

This was achieved by the use of a simple scoring method. Unrestrained animals were observed individually and tremor assessed as follows:- O = no tremor, 1 = moderate or intermittent tremor, and 2 = severe or continuous tremor.

e/Spontaneous Locomotor Activity.

The apparatus used in the assessment of SLA consisted of 2 opaque polypropylene cages measuring 10 cm by 27 cm. Each cage had 2 capacitor plates attached vertically to the outsides and at right

angles to one another. Any movement inside the cage changed the capacitance of the system and activated an integrating amplifier and digital counter. The cages were balanced electronically against each other so that the digital records were the same for similar degrees of movement. Activity records were made using groups of 5 mice, 2 groups being compared at one time. In experiments assessing the effects of tranquillizers, naive animals were used so that the reduction in the normal initial exploratory activity in a new environment could be seen.

f/Convulsant Activity

Convulsions were induced chemically by intraperitoneal injection of various convulsant agents. Following the injection the animals were caged individually in opaque cages measuring 10 cm. by 27 cm, and observed continuously for 15 or 30 minutes, according to the drug used. The time at which the first convulsion lasting for 3 or more seconds, the number of convulsions, and the time and number of animals dying were noted.

In some cases the temperature before the convulsant and at death (or the end of the observation)were also noted.

g/Intracerebral injection.

The method used for the intracerebral injection of drugs into mice was similar to that described by Haley & McCormick, (1957) as modified by Brittain & Handley (1967). The injection needle was a specially prepared 20 gauge 1/8 in. (0.32 cm.) long. The site of injection was, however, more rostral to that used by these latter two

authors, the needle being inserted perpendicularly into the brain in the region of the bregma on the midline. Trial experiments with indian ink diluted 1 in 10 with normal saline indicated that this site produced adequate penetration of the cerebral ventricles, and injection of normal saline at pH 7 produced no behavioural changes suggestive of severe brain damage. This site was chosen since, whilst it provides adequate penetration of the ventricular system, it is easy to find and, in this strain of mice, the needle can be easily introduced through the skull at this point. Most injections were made in a dose volume of 10 microlitres, although some animals received volumes ranging from 5 - 20 microlitres as stated in the results section. All injections were made using a Hamilton 50 microlitre syringe.

h/ Measurement of oxygen consumption in living animals.

The measurement of oxygen consumption in living animals was achieved by the use of an oxygen consumption chamber similar to that described by Maclagan & Sheahan (1950) supplied by Scientific Research Instruments, Ltd. The chamber consisted of a closed, water-jacketed compartment, with orifices for injecting known volumes of air, for the measurement of temperature, for the measurement of internal pressure, and for flushing the air within the chamber. The air within the chamber was circulated through soda lime by means of an electric fan, ensuring a rapid and adequate absorption of carbon dioxide. The animal under experiment was placed in the chamber and after a period of acclimatization the air within the chamber was flushed out by means of an electric fan. The chamber was then sealed and a known volume of air injected into the closed compartment. The internal pressure in the

chamber was now raised and monitored with a low pressure statham transducer attached to a Devices DC 2 amplifier pen recorder. When the pressure returned to atmospheric, the animal had consumed a volume of oxygen equivalent to the volume of air originally injected. Thus, knowing the time taken to consume this volume of oxygen the oxygen consumption rate per gramme of tissue could be calculated.

5. Determination of acute toxicity.

In experiments involving the determination of acute toxicity the experimental animals were randomly divided into groups of ten and caged in opaque polypropylene cages measuring 10 cm x 27 cm. The drug under investigation was administered and the animals left undisturbed in the laboratory at a constant environmental temperature of 22^{o}C. After a period of 24 hr. the number of dead animals in each group was counted and the LD50s calculated by the method of Litchfield & Wilcoxon (1949).

6. Injections, vehicles and drugs used.

All drugs injected peripherally were administered in a dose volume of 5 mls/kg (i.e. 0.1 ml/20 g). Drugs administered by intracerebral injection were injected in a dose volume of 10 microlitres (10 ul) except in a few stated cases where volumes ranging from 5 - 20 ul were injected. The following drugs (and their sources) were used.

Normal saline- 0.9% sodium chloride (BDH) dissolved in glass distilled water.

Thyroxine - dissolved in alkaline solvent. Sodium -l-thyroxine (BDH) used throughout, dose calculated as the sodium salt.

Triiodothyronine - dissolved in alkaline solvent. 3, 5, 3'triiodothyronine (BDH) used throughout.

Pentobarbitone sodium - dissolved in normal saline, dose calculated as the sodium salt (May & Baker).

Thiopentone sodium - dissolved in normal saline, dose calculated as the sodium salt (Abbot Laboratories).

Tremorine-hydrochloride - dissolved in normal saline, dose calculated as the hydrochloride. (supplied by Allen & Hanburys)

Oxotremorine oxalate - dissolved in normal saline, dose calculated as the oxalate. (supplied by Allen & Hanburys)

SKF 525 A - dissolved in normal saline. (Smith, Kline & French)

Chlorpromazine hydrochloride - dissolved in normal saline, dose calculated as the hydrochloride. (May & Baker)

Leptazol - dissolved in normal saline. (BDH)

Picrotoxin - dissolved in normal saline. (Sigma)

Nicotine - dissolved in normal saline. (BDH)

Strychnine hydrochloride - dissolved in normal saline, dose calculated as the hydrochloride. (BDH)

Chlordiizepoxide - dissolved in normal saline. (Roche)

Morphine sulphate - dissolved in normal saline, dose calculated as the base. (BDH)

Nialamide - dissolved in normal saline. (Pfizer)

Amphetamine sulphate - dextro isomer used, dissolved in distilled water, dose calculated as the base. (Ward Blenkinsop)

2,4-dinitrophenol - dissolved in 1.4% sodium bicarbonate. (BDH)

Reserpine - dissolved in a minimal amount of lactic acid with one drop of ethyl alcohol added. When the drug was dissolved the flask was gently warmed to remove the alcohol. The pH of the diluted solution was adjusted to 5.5 by the addition of sodium bicarbonate solution.

In all experiments involving intracerebral injection the vehicle used was made with water for injection.

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TABLE 1.

Oxygen consumptions (ml. O_2/g./min) of male and female Tuck No. 1. mice following treatment with sodium 1-thyroxine 0.5 mg/kg (HT) or normal saline (ET) by daily subcutaneous injection. Each result is derived fron one animal.

	MALES		FEMALES	
DAY OF TREATMENT	HT	ET	HT	ET
3	0.12	0.09	0.10	0.06
4	0.12	0.07	0.08	0.05
5	0.1	0.08	0.13	0.09
6	0.13	0.08	0.14	0.08
7	0.12	0.08	0.12	0.08
8	0.11	0.08	0.10	0.06
9	0.12	0.07	0.12	0.07
10	0.10	0.09	0.12	0.07

FIGURE 3.

Oxygen consumptions of male Tuck No 1 mice following treatment with sodium l-thyroxine 0.5 mg/kg by daily subcutaneous injection (HT). Control animals (ET) received an equivalent volume of normal saline by the same route.

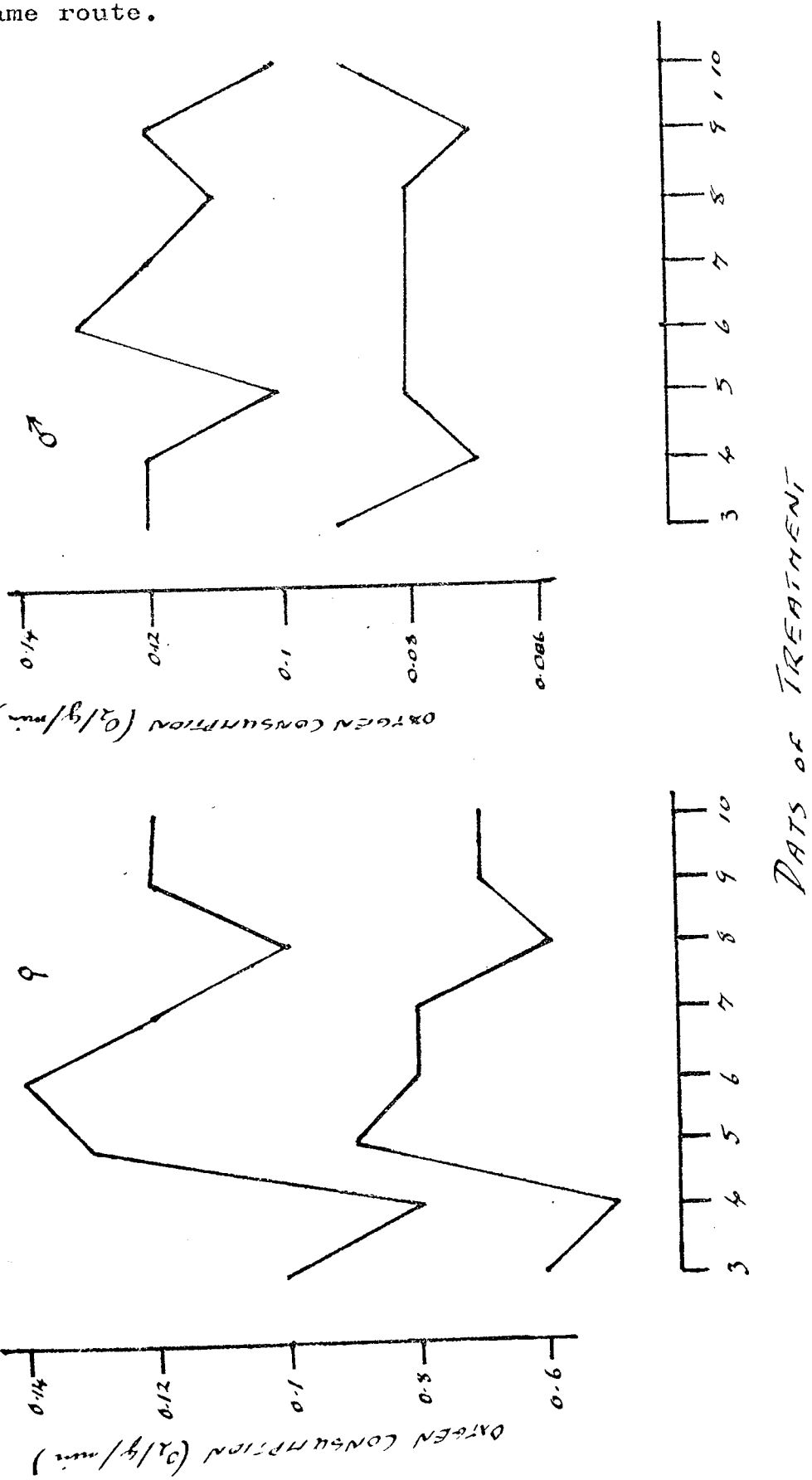


TABLE 2.

Oxygen consumption (ml. O_2/g./min) of male and female Tuck No. 1. mice following treatment with sodium 1-thyroxine 1 mg/kg (HT) or normal saline (ET) by daily subcutaneous injection. Each result is derived from one animal.

	MALES		FEMALES	
DAY OF TREATMENT	HT	ET	HT	ET
3	0.1	0.09	0.10	0.06
4	0.12	0.07	0.12	0.05
5	0.10	0.08	0.10	0.09
6	0.16	0.08	0.11	0.08
7	0.13	0.08	0.14	0.08
8	0.17	0.08	0.13	0.06
9	0.16	0.07	0.13	0.07
10	0.16	0.09	0.13	0.07

FIGURE 4.

Oxygen consumptions of male Tuck No 1 mice following treatment with sodium l-thyroxine 1 mg/kg by daily subcutaneous injection(HT). Control animals (ET) received an equivalent volume of normal saline by the same route.

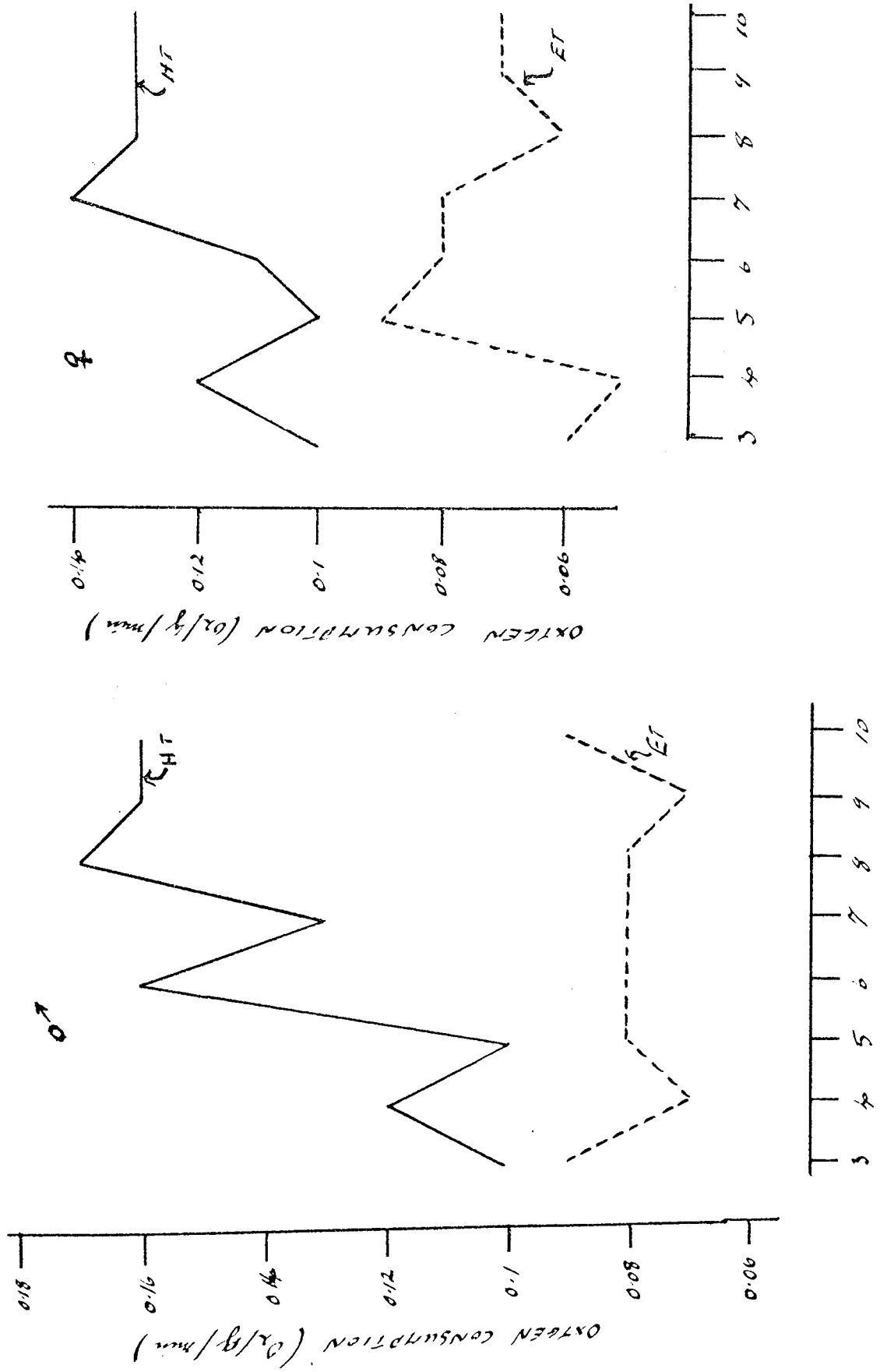


TABLE 3.

Oxygen consumptions (ml. O_2/g./min) of male and female Tuck No. 1. mice following treatment with sodium l-thyroxine 2 mg/kg (HT) or normal saline (ET) by daily subcutaneous injection. Each result is derived from one animal.

	MALES		FEMALES	
DAY OF TREATMENT	HT	ET	HT	ET
3	0.10	0.09	0.10	0.06
4	0.12	0.07	0.09	0.05
5	0.14	0.08	0.11	0.09
6	0.13	0.08	0.13	0.08
7	0.16	0.08	0.15	0.08
8	0.17	0.08	0.13	0.06
9	0.17	0.07	0.11	0.07
10	0.14	0.09	0.14	0.07

FIGURE 5.

Oxygen consumptions of male and female Tuck No 1 mice after treatment with sodium l- thyroxine 2 mg/kg by daily subcutaneous injection (HT). Control animals (ET) received an equivalent volume of normal saline by the same route. (see Table 3).

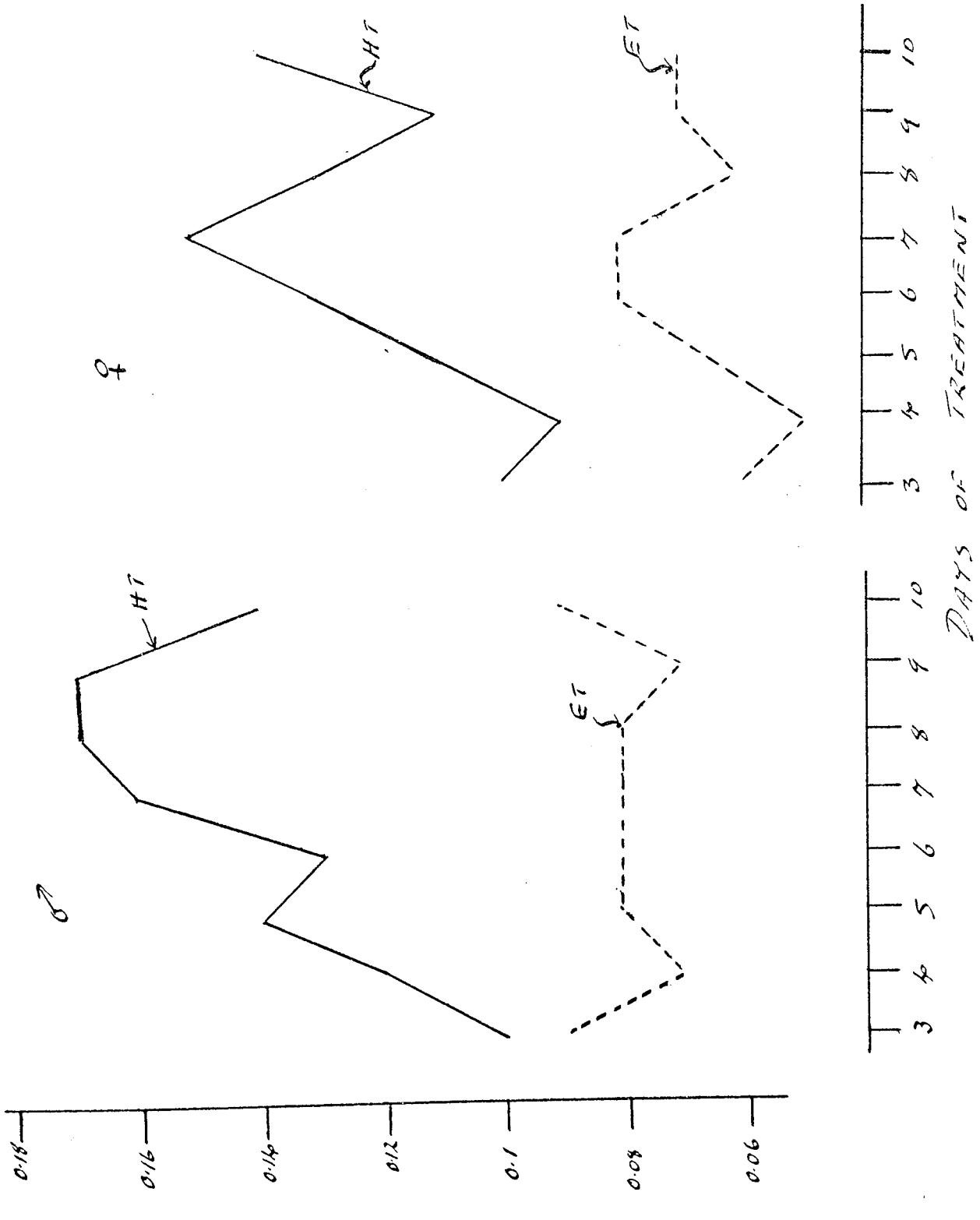


TABLE 4.

Oxygen consumptions (ml. O_2/g./min) of male and female Tuck No. 1. mice treated with sodium l-thyroxine at a starting dose of 0.4 mg/kg increasing daily by a factor of 1.2 (HT) or with normal saline (ET). Each result is derived from one animal.

	MALES		FEMALES	
DAY OF TREATMENT	HT	ET	HT	ET
3	0.10	0.09	0.08	0.06
4	0.12	0.07	0.11	0.05
5	0.10	0.08	0.12	0.09
6	0.11	0.08	0.14	0.08
7	0.14	0.08	0.14	0.08
8	0.13	0.08	0.10	0.06
9	0.13	0.07	0.11	0.07
10	0.13	0.09	0.13	0.07

FIGURE 6.

Oxygen consumptions of male and female Tuck No 1 mice after treatment with sodium l-thyroxine by daily subcutaneous injection at a dose of 0.4 mg/kg increasing daily by a factor of 1.2(HT). Control animals (ET) received an equivalent volume of normal saline by the same route. (see Table 4).

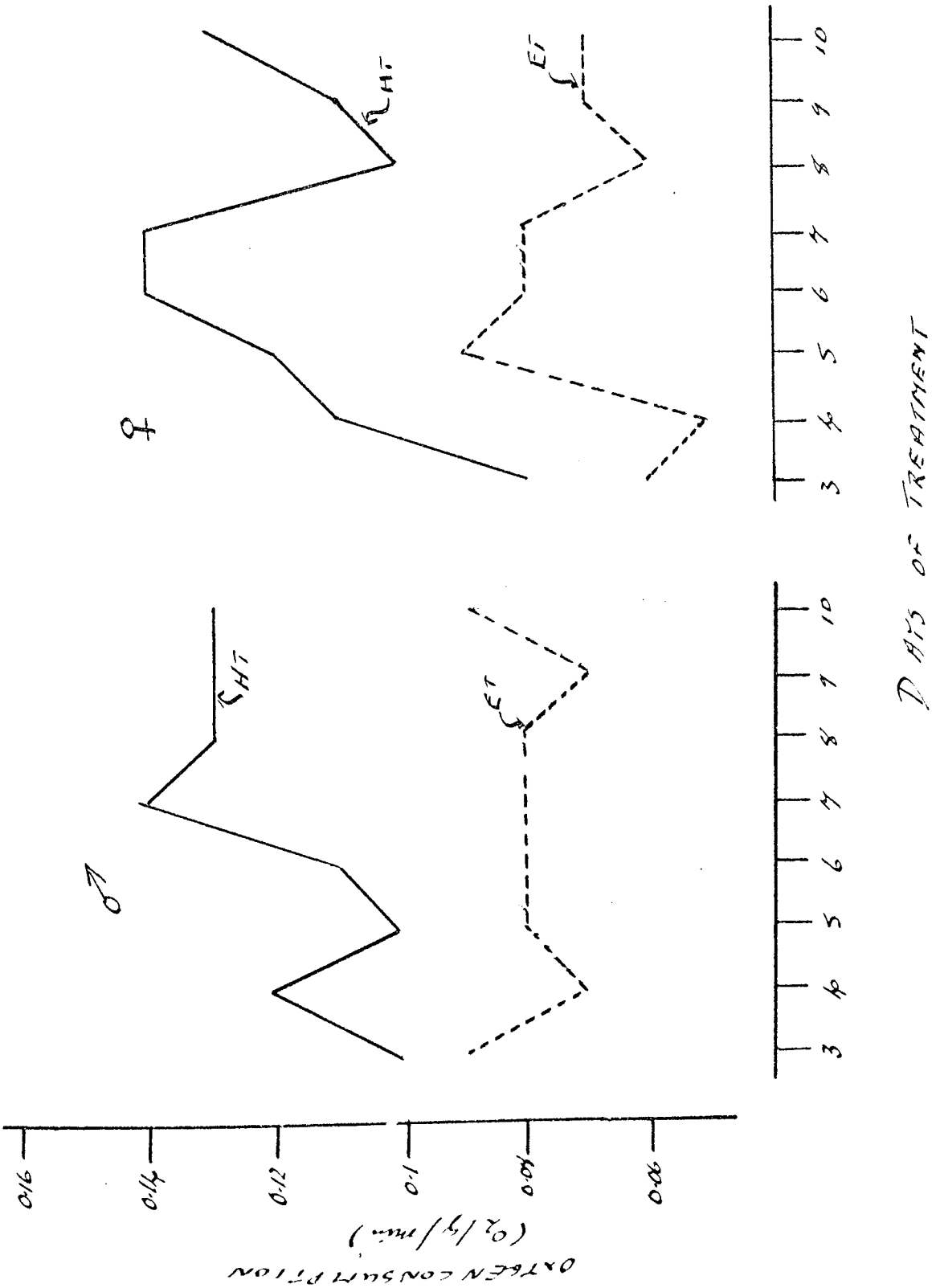


TABLE 5.

Oxygen consumptions (ml. O_2/g./min) of male AS 1 mice following treatment with sodium 1-thyroxine 2 mg/kg (HT) or with normal saline (ET). Each result is derived from 5 animals and expressed as the mean $\pm$ S.E.

DAY OF TREATMENT	HT	ET
5	0.17 $\pm$ 0.012	0.09 $\pm$ 0.007
7	0.2 $\pm$ 0.002	0.08 $\pm$ 0.005
8	0.22 $\pm$ 0.027	0.1 $\pm$ 0.005
9	0.17 $\pm$ 0.019	0.07 $\pm$ 0.006
10	0.19 $\pm$ 0.010	0.08 $\pm$ 0.004

FIGURE 7.

Oxygen consumptions of male AS1 mice after treatment with sodium l-thyroxine 2 mg/kg by daily subcutaneous injection (HT). Control animals (ET) received an equivalent volume of normal saline by the same route. (see Table 5)

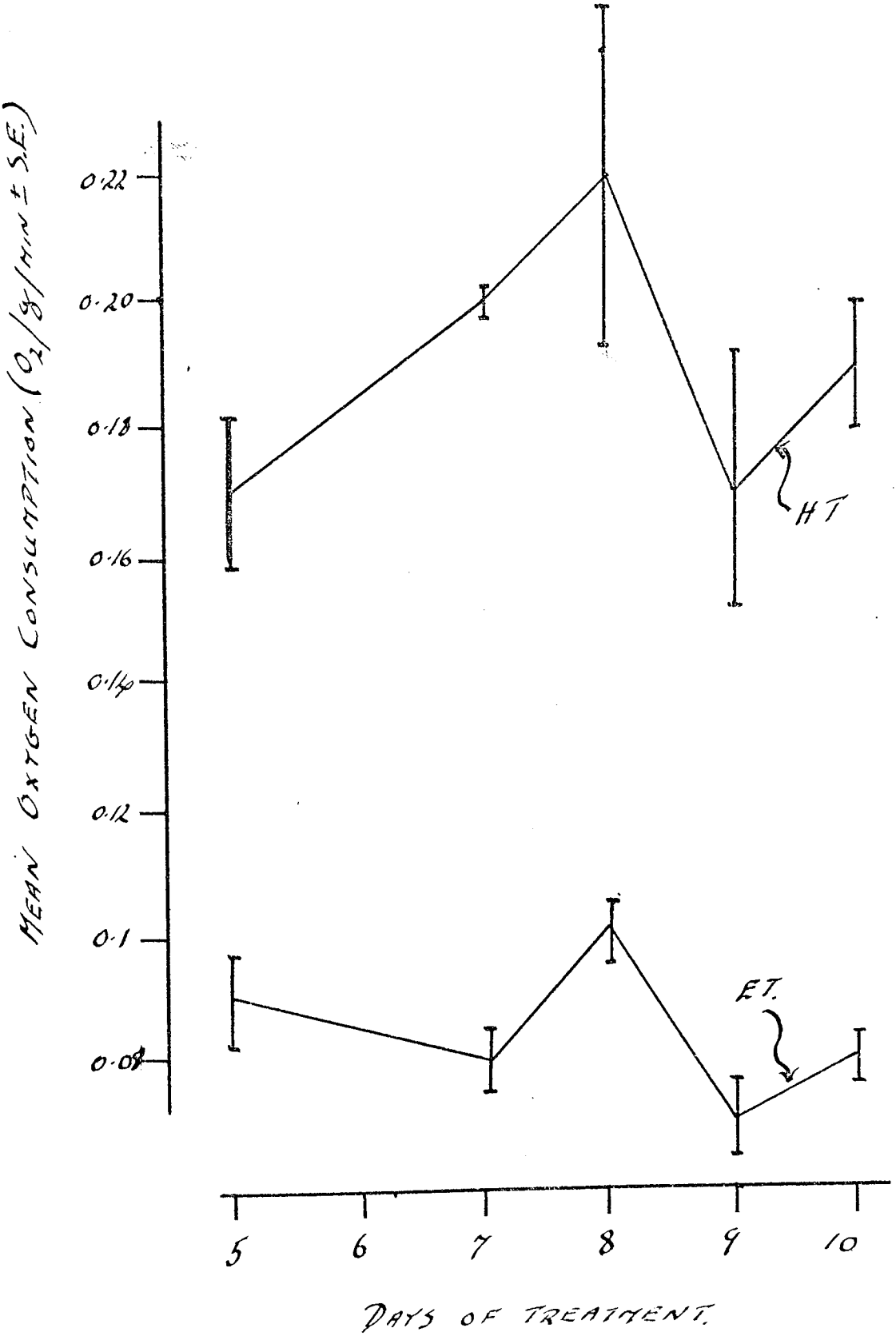


TABLE 6.

Oxygen consumptions (ml. O_2/g./min) of male AS 1 mice following treatment with triiodothyronine 0.5 mg/kg or with normal saline (ET). Each result is derived from 5 animals and expressed as the mean $\pm$ S.E.

DAY OF TREATMENT	HT	ET
5	0.14 $\pm$ 0.007	0.09 $\pm$ 0.007
7	0.16 $\pm$ 0.016	0.08 $\pm$ 0.005
8	0.17 $\pm$ 0.005	0.1 $\pm$ 0.005
9	0.17 $\pm$ 0.010	0.07 $\pm$ 0.006
10	0.18 $\pm$ 0.006	0.08 $\pm$ 0.004

FIGURE 8.

Oxygen consumptions fo male AS1 mice after treatment with 3,5,3'triiodothyronine o.5 mg/kg by daily subcutaneous injection (HT). Control animals (ET) received an equivalent volume of normal saline by the same route . (see Table 6).

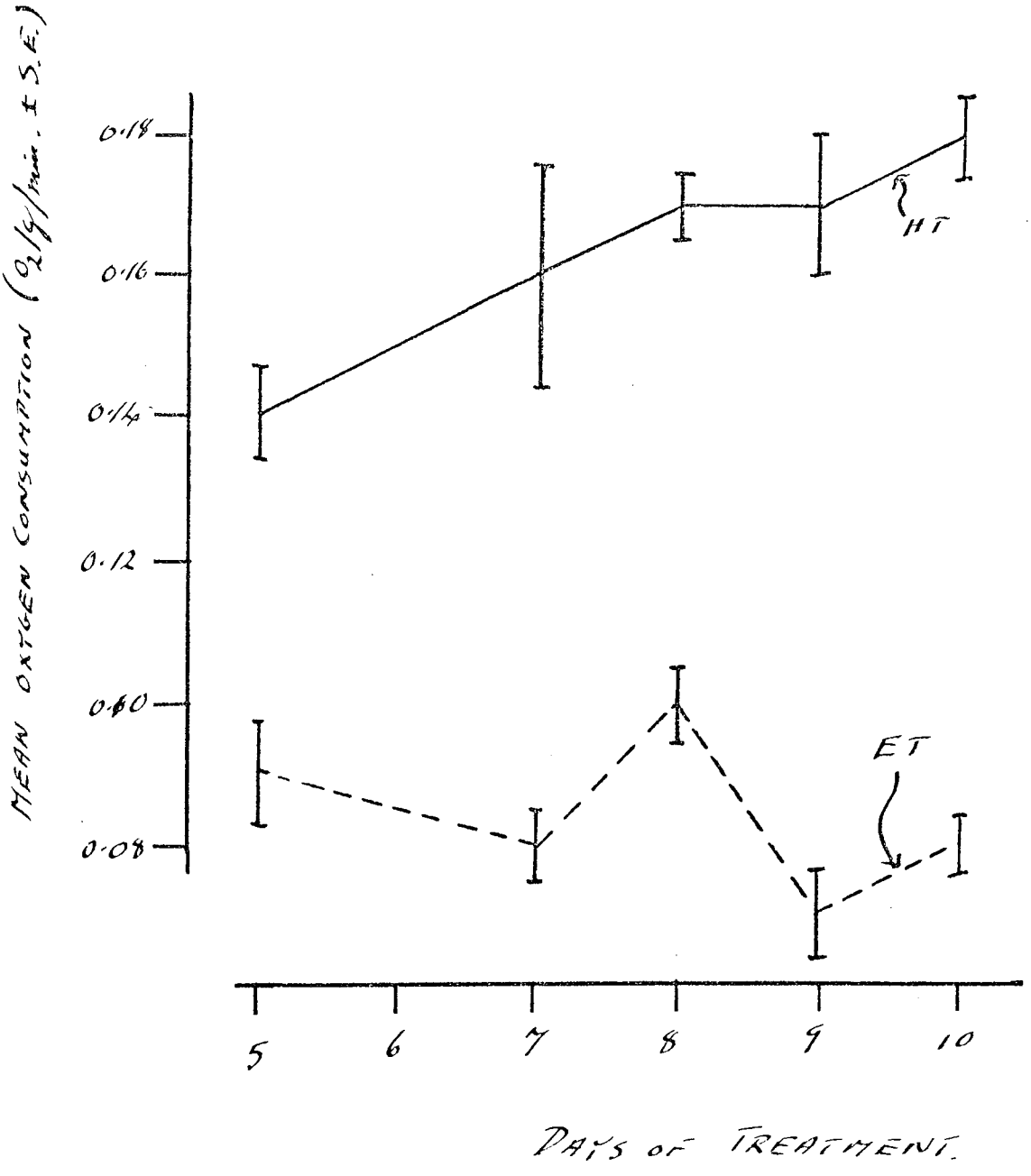


TABLE 7.

Oxygen consumptions (ml. O_2/g./min) of male AS 1 mice following treatment with triiodothyronine 0.125 mg/kg or with normal saline (ET). Each result is derived from 5 animals and expressed as the mean $\pm$ S.E.

DAY OF TREATMENT	HT	ET
5	0.15 ± 0.018	0.09 ± 0.007
7	0.17 ± 0.015	0.08 ± 0.005
8	0.18 ± 0.016	0.1 ± 0.005
9	0.15 ± 0.012	0.07 ± 0.006
10	0.17 ± 0.012	0.08 ± 0.004

FIGURE 9.

Oxygen consumptions of male AS1 mice after treatment with 3,5,3'triiodothyronine 0.125 mg/kg by daily subcutaneous injection (HT). Control animals (ET) received an equivalent volume of normal saline by the same route. (see Table 7).

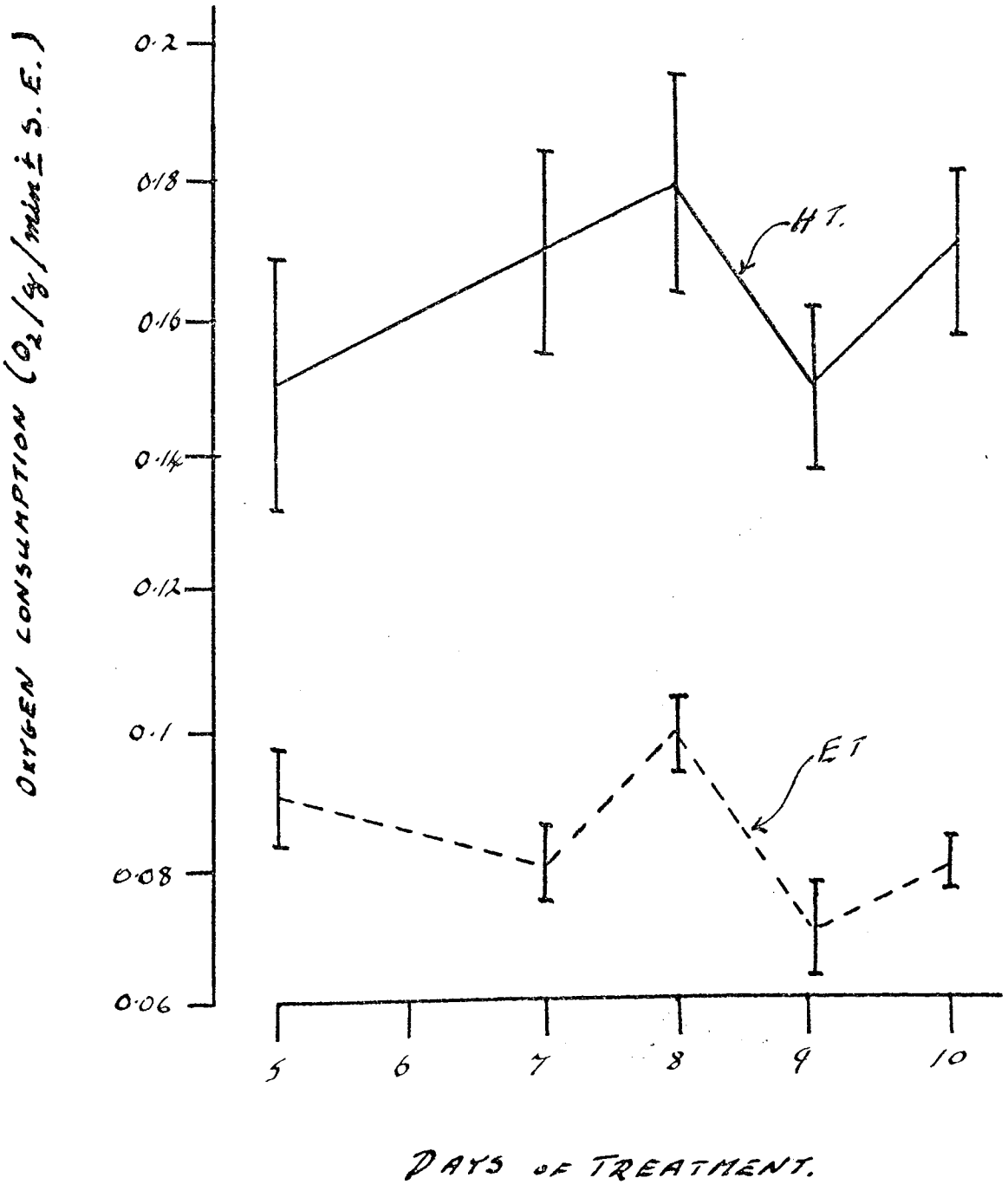


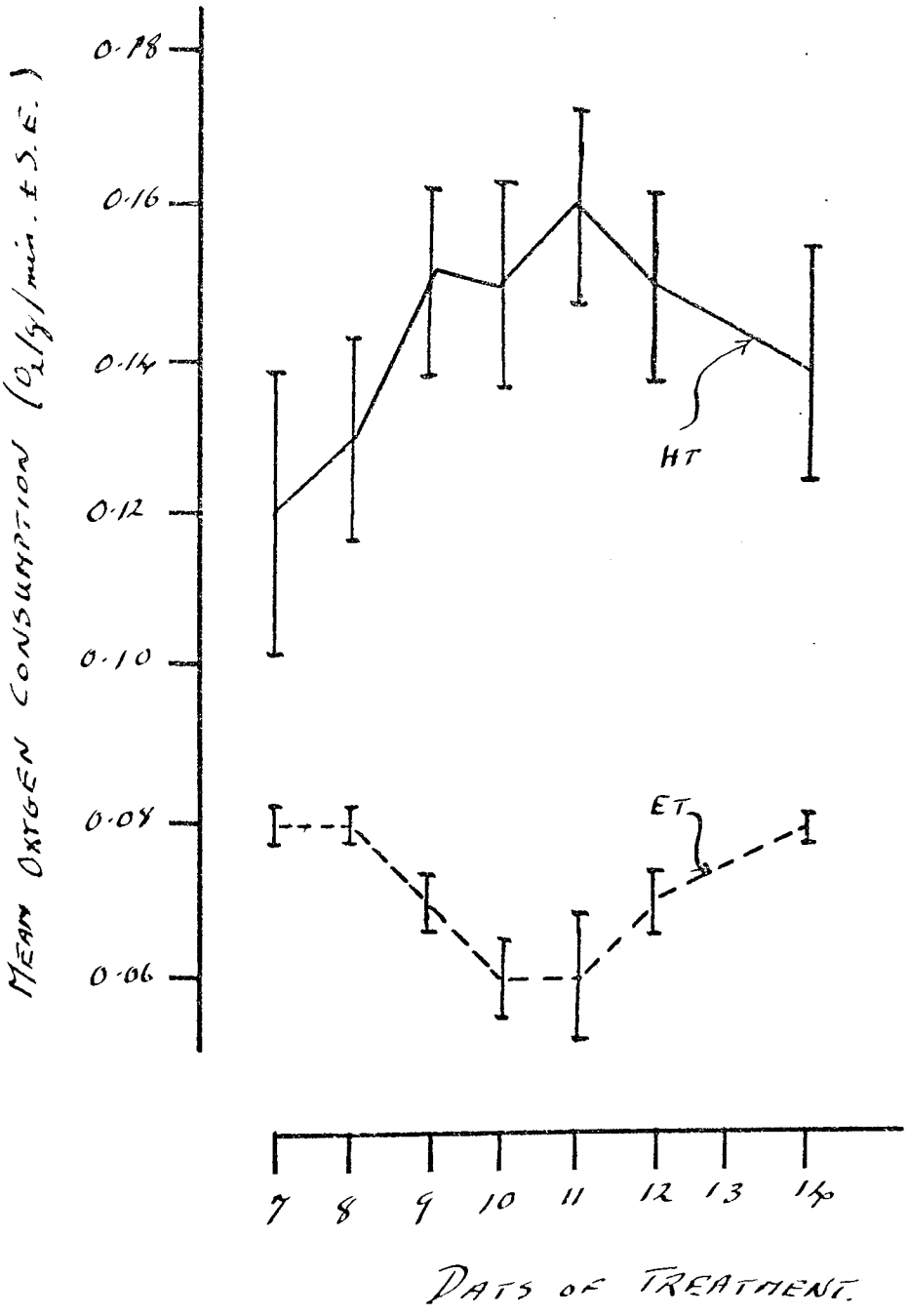
TABLE 8

Oxygen consumptions (ml O_2/g/min) of male TO mice following treatment with sodium l-thyroxine 2 mg/kg (HT) or with normal saline (ET). Each result is derived from 10 animals and expressed as the mean(°C $\pm$ S. E.)

Day of Treatment	HT	ET
7	0.12 $\pm$ 0.018	0.08 $\pm$ 0.002
8	0.13 $\pm$ 0.013	0.08 $\pm$ 0.002
9	0.15 $\pm$ 0.012	0.07 $\pm$ 0.002
10	0.15 $\pm$ 0.013	0.06 $\pm$ 0.005
11	0.16 $\pm$ 0.012	0.06 $\pm$ 0.008
12	0.15 $\pm$ 0.012	0.07 $\pm$ 0.004
14	0.14 $\pm$ 0.015	0.08 $\pm$ 0.002

FIGURE 10.

Oxygen consumptions of male TO mice after treatment with sodium l-thyroxine 2 mg/kg by daily subcutaneous injection (HT) . Control animals (ET) received an equivalent volume of normal saline b the same route. (see Table 8).



SECTION 3.

RESULTS

A

CHAPTER 1.

THE EFFECTS OF BARBITURATES IN HYPERTHYROID AND EUTHYROID MICE

CHAPTER 1.

THE EFFECTS OF BARBITURATES IN HYPERTHYROID AND EUTHYROID MICE.

1. Introduction.

There have been several reports in the literature that the hypnotic and toxic effects of various barbiturates are potentiated in hyperthyroidism, (see for example : Conney & Garren, 1960). It was decided to investigate the effects of pentobarbitone (of moderate duration of action) and thiopentone (ultra-short duration of action) in hyperthyroid and euthyroid mice. Two parameters of drug activity were determined : the duration of hypnosis as shown by the time taken to regain the righting reflex, and the acute toxicity of each drug. All experiments reported in this chapter were carried out at 22° C and the drugs were administered by intravenous injection.

2. Hypnotic activity of pentobarbitone and thiopentone in hyperthyroid and euthyroid mice

i. Pentobarbitone sodium.

The hypnotic potency of this drug at doses of 40, 50, & 60 mg/kg was determined in hyperthyroid, euthyroid (saline-treated) controls, and euthyroid-untreated mice. Two strains of mice were used in these experiments, namely male AS 1 and male TO animals.

In the AS 1 mice pentobarbitone sodium at doses of 40 and 50 mg/kg were administered to the three groups of animals as detailed above. The results, shown in Fig. // (Table?), show that the hyperthyroid mice were significantly more sensitive to the hypnotic effects of this drug than

were the euthyroid animals. It was also noted that the euthyroid-untreated animals consistently slept for a longer period than did the euthyroid-control mice, although this effect was not statistically significant at either dose level.

Since the only difference between the two groups of euthyroid mice was that one group had received daily injections of saline, it seemed possible that this stress might have been responsible for the difference in response. In order to investigate this possibility the adrenal glands of all three groups of mice were removed and weighed to reveal any hyperplasia, which would be suggestive of hyperactivity of the gland. The results (see Table 10) show that, as expected the glands of the hyperthyroid animals were significantly heavier than those of the two groups of euthyroid mice. There was, however, no consistent or significant difference between the weights of glands from control and untreated euthyroid mice.

These observations were confirmed in male TO mice. Doses of pentobarbitone sodium, 40, 50, & 60 mg/kg were administered to the three groups of mice as before. The results, shown in Fig. 12. (Table 11) reveal that the hyperthyroid mice again were more sensitive to the hypnotic effects of pentobarbitone than their euthyroid counterparts. This increase in sensitivity was not statistically significant at the lowest dose but was significant at the two higher doses. Once again, the untreated euthyroid animals showed a tendency to sleep longer than their saline treated counterparts, but this was not statistically significant.

ii . Thiopentone sodium.

This drug was administered in doses of 30, 40 & 50 mg/kg to hyperthyroid and euthyroid (saline treated) male TO mice. The results are illustrated in Fig. 13 (Table 12). Although the hyperthyroid mice slept for a significantly longer time than the euthyroid animals at the highest dose, in contrast the hyperthyroid animals were significantly less sensitive to thiopentone at the lower doses.

3. Estimation of acute toxicity of pentobarbitone and thiopentone in hyperthyroid and euthyroid mice.

In these experiments male TO mice were used throughout; in the case of pentobarbitone, groups of hyperthyroid, euthyroid-control and euthyroid-untreated mice were tested, whilst with the thiopentone hyperthyroid and euthyroid-control groups only were used.

1. Pentobarbitone sodium

The results of this experiment are shown in Fig. 14. (Table 13), and reveal that the hyperthyroid mice were significantly more susceptible to the toxic effects of this drug than the euthyroid animals. There was no significant difference between the responses of the two groups of euthyroid animals.

ii. Thiopentone sodium.

The results of this experiment are illustrated in Fig. 15. (Table 14)) and show, as with pentobarbitone, that the hyperthyroid mice were significantly more sensitive to the toxic effects of this drug than were their euthyroid counterparts.

DISCUSSION

The barbiturates, with a few exceptions, have a general depressant action on the CNS. The drugs are most frequently used as sedatives, hypnotics, anaesthetics and, in some cases, as anticonvulsants, (Doran, 1959). When administered in hypnotic doses the drugs produce a state resembling natural sleep, though the eye movements and characteristic EEG patterns of natural sleep are reduced (Sharpless, 1965). The reticular activating system seems to be particularly sensitive to the depressant action of these drugs, responding by an increase in the threshold for electrical stimulation, and a decrease in response to a variety of sensory inputs (Killam, 1962).

Following administration, providing they stay long enough in the plasma, the drugs are evenly distributed throughout the body. In the plasma, the drugs are reversibly bound to the albumin fraction of the plasma protein (Goldbaum & Smith, 1954). The localisation of the drugs in the tissues or fat depots is controlled by the physical properties of the molecule such as oil/water partition coeficient, and extent of ionisation at body pH. Barbiturates with a high lipid solubility are more rapidly absorbed into the nervous tissue and hence have a more rapid onset of action. The ionised molecule is not lipid soluble and increases in plasma pH can delay the onset of anaesthesia by increasing the proportion of ionised molecules (Waddel & Butler, 1957). Conversly decreases in pH inhibit ionisation and increase the rate of penetration of the drug into the nervous tissues (Brodie et al, 1950). Several workers have reported that the hypnotic effect of a barbiturate is related to the brain concentration of the drug but not to the plasma levels (Hollister, 1968; Kane 1959;

Butler 1950; Mark et al 1958). Within the brain there does not appear to be any uneven distribution of the drugs (Maynert & Van Dyke, 1950); however, it is reported that the grey matter is penetrated more quickly than the white, probably due to structural differences (Domek, 1960).

Of the two drugs used in this work pentobarbitone has a moderate duration of action, whilst thiopentone has an ultra-short duration of action. Although the two drugs are metabolised by different catalytic mechanisms (Cooper & Brodie, 1957), there is little difference in the rates at which they are degraded and the differences in duration of action are thought to be due more to differences in tissue localisation. Pentobarbitone owes its longer duration of action to the fact that it is not localised to any great extent in the tissues or fat depots, but remains in the blood and is therefore available to the brain, (Brodie 1953). Thiopentone is rapidly absorbed into nervous tissue, but its action is transient since from the nervous tissue it is rapidly redistributed to the fat depots (Brodie et al 1950; Brodie et al 1953), or lean tissues (Price, 1960).

The literature contains several reports that the hypnotic action of various barbiturates is potentiated by thryoxine treatment (Conney & Garren, 1960; Prange, Lipton & Love, 1962; Prange et al, 1966). Of these reports that of Prange et al (1966) includes a study of the metabolism of pentobarbitone and thiopentone in hyperthyroid and euthyroid mice and rats, These authors found that the hyperthyroid state was accompanied by a reduction in the rate of metabolism of the drugs. They

found no evidence to suggest that the hyperthyroid animals were any more sensitive to the drugs since waking brain concentrations of the drugs were similar in the hyperthyroid and euthyroid animals. The possibility of differences in fat distribution in the two groups of animals was also ruled out since the potentiation was similar for both drugs and they are known to have different degrees of fat localisation, (Goldstein & Arnow, 1960). In the light of these findings the authors explain the differences in sleep times on the basis of the different rates of metabolism of the drugs in the hyperthyroid and euthyroid animals. This hypothesis receives support from the earlier work of Conney & Garren, (1960), who showed that thyroxine treatment had a similar effect on the hexobarbitone metabolising system in the rat.

On the basis of these conclusions the results obtained here can be explained. Pentobarbitone hypnosis may be potentiated in the hyperthyroid mice due to a thyroxine-induced reduction in the rate of degradation of the drug, a factor predisposing also to the observed increase in toxicity. With thiopentone, as Brodie (1953) noted, small doses are ultra-short acting due to redistribution of the drug from the brain to the tissues whereas larger or repeated doses saturate the tissues, which then act as a mobile pool for the drug, thereby prolonging hypnosis.

Thus, in the hyperthyroid mice it could be postulated that, although the rate of thiopentone metabolism may be reduced, the hypnosis is of shorter duration than that seen in the euthyroid animals due to a more rapid distribution of the drug. This hypothesis receives support from the reports of Sensenbach et al (1956) and Sokoloff et al (1956)

that cerebral blood flow is increased in hyperthyroidism, and the report of Price (1960) that the duration of hypnosis is controlled by the rates of perfusion of the tissues with blood. This more rapid removal of the drug from the brain to the tissues, (which are not saturated with drug) explains why low doses of thiopentone in hyperthyroid animals produce a shorter sleep.

With the higher dose of thiopentone, the tissues are more likely to be saturated with the drug; a slower metabolism of the drug in the hyperthyroid mice will have a greater influence on the duration of effect than will the postulated increased cerebral blood flow. Consequently, with larger doses, the hyperthyroid animals sleep for a longer period than the euthyroid mice. For the same reason, the toxicity of thiopentone is increased.

SUMMARY

1. In male mice of the AS 1 and TO strains, thyroxine-induced hyperthyroidism significantly increases the hypnotic potency of pentobarbitone sodium. The acute toxicity of pentobarbitone sodium is also increased in hyperthyroid mice of the TO strains.

2. In male mice of the TO strain thyroxine-induced hyperthyroidism significantly reduced the hypnotic potency of small doses of thiopentone sodium, but increases the potency of larger doses. The acute toxicity of thiopentone sodium also is increased in hyperthyroid animals of this strain.

TABLE 9.

Sleep times of hyperthyroid (HT), euthyroid control (ET) and euthyroid untreated (UT) male AS 1 mice following intravenous injection of pentobarbitone sodium. The values given are the mean sleep times (min), ± S.E.

Dose of pentobarbitone mg/kg	No.of Mice	HT	ET	UT
40	30	99.3 ± 11.2	10.3 ± 4.6	51.2 ± 4.1
50	20	165.0 ± 23.5	77.4 ± 6.0	83.7 ± 7.5

TABLE 10.

Weights of adrenal glands (mg.) removed from hyperthyroid (HT) euthyroid control (ET) and euthyroid untreated male TO mice. Thirty pairs of glands of each type were weighed and the results are expressed as group means ± S.E.

HT	ET	UT
5.26 ± 0.2	3.9 ± 0.1	3.72 ± 0.1

FIGURE 11.

Sleep times of hyperthyroid (HT) euthyroid control(ET) and euthyroid untreated (UT) male AS1 mice after intravenous injection of pentobarbitone sodium (see Table 9).

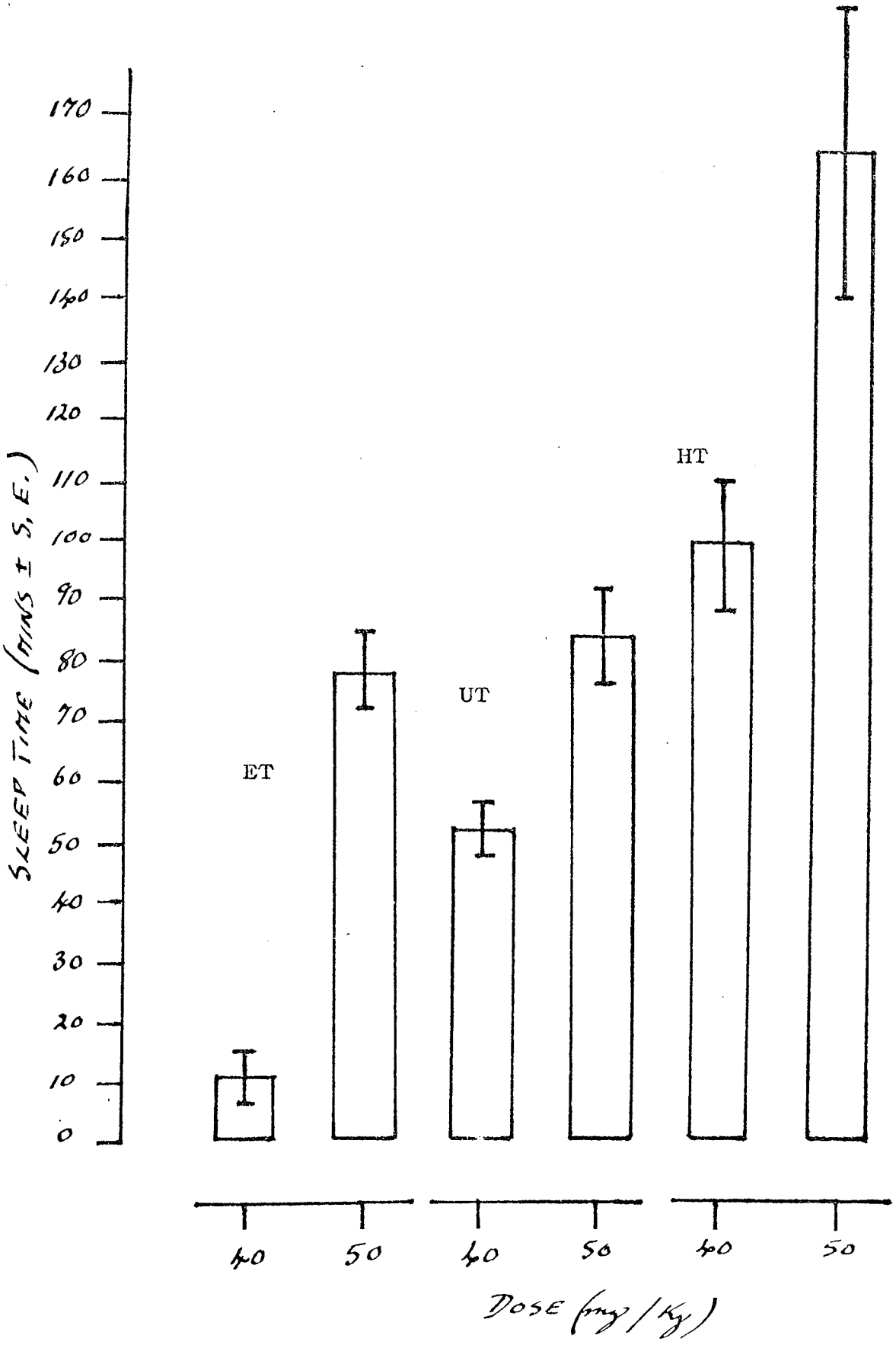


TABLE 11.

Sleep times of hyperthyroid (HT), euthyroid control (ET) and euthyroid untreated (UT), male TO mice following intravenous injection of pentobarbitone sodium. The values given are the mean sleep times (min), ± S.E.

Dose of Pentobarbitone mg/kg	No. of Mice	HT	ET	UT
40	30	67.9 ± 7.1	40.9 ± 4.9	49.9 ± 4.5
50	30	107.2 ± 9.2	66.0 ± 6.1	81.2 ± 8.2
60	20	200.0 ± 15.0	119.7 ± 7.9	156.6 ± 12.1

TABLE 12.

Sleep times of hyperthyroid (HT) and euthyroid control (ET) male TO mice following intravenous thiopentone sodium. The results are expressed as group means (min), ± S.E.

Dose of thiopentone mg/kg	No. of Mice	HT	ET
30	30	4.0 ± 0.2	5.8 ± 0.3
40	20	10.1 ± 1.3	15.7 ± 1.4
50	10	138.7 ± 12.5	75.7 ± 13.0

FIGURE 12.

Sleep times of hyperthyroid(HT) euthyroid control (ET) and euthyroid untreated (UT) male TO mice after intravenous injection of pentobarbitone sodium (see Table 11).

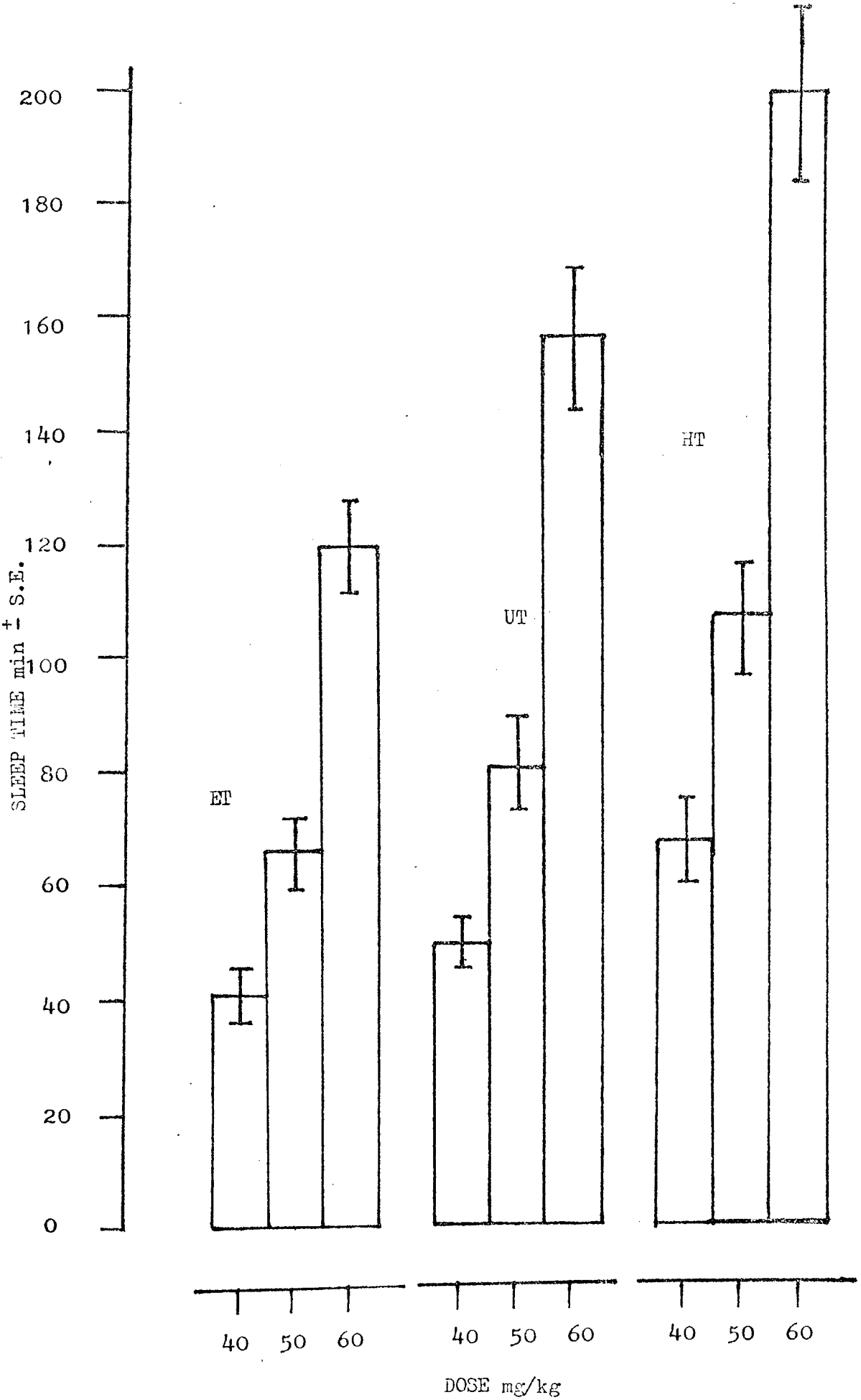


FIGURE 13.

Sleep times of hyperthyroid (HT) and euthyroid (ET) male TO mice after intravenous injection of thiopentone sodium (see Table 12).

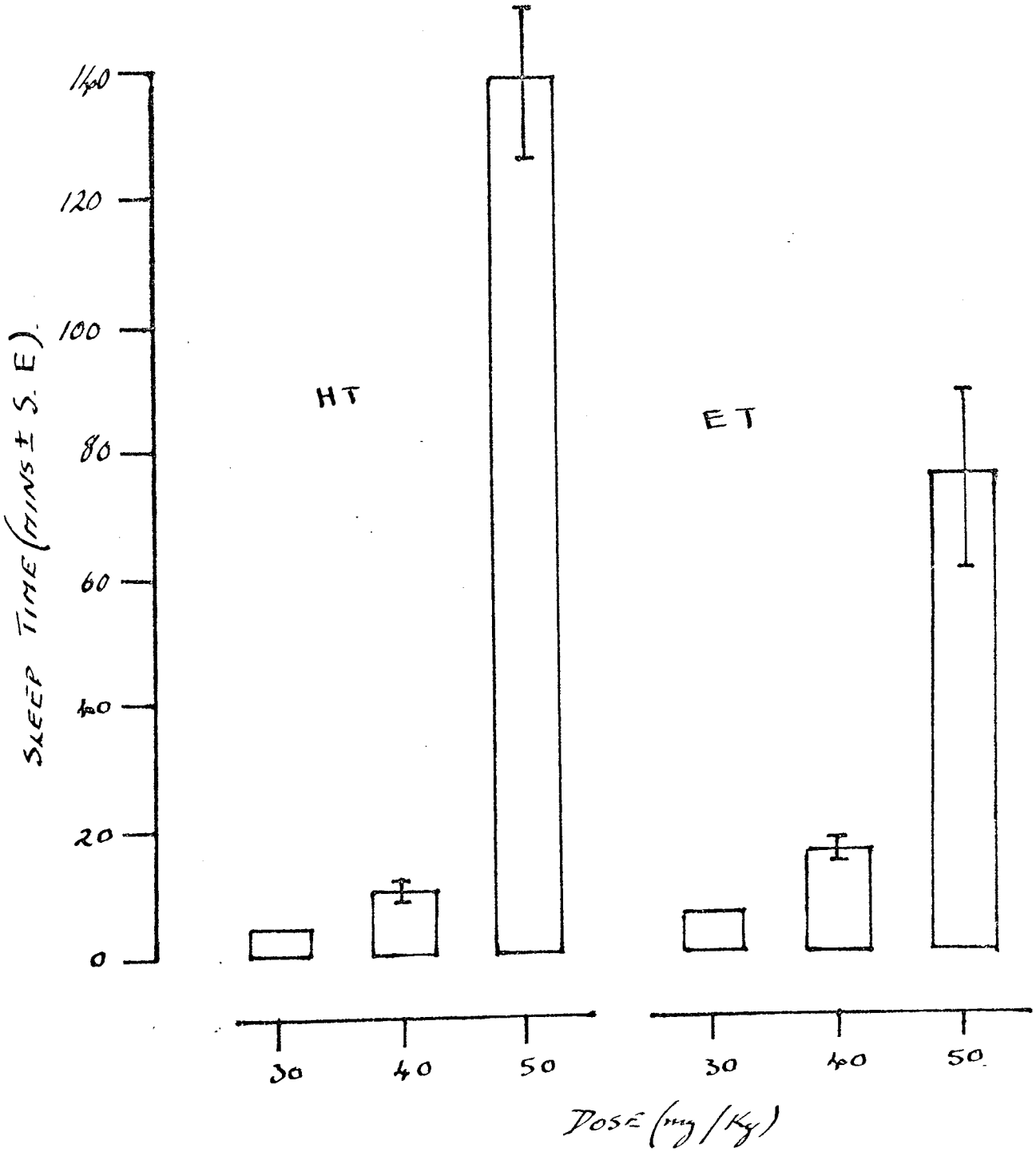


TABLE 13.

Mortality of hyperthyroid (HT) euthyroid control (ET) and euthyroid untreated (UT) male AS1 mice after intravenous injection of pentobarbitone sodium at an ambient temperature of 22°C. The table shows the doses of pentobarbitone (mg/kg), the number of animals tested (No), and the resulting percentage mortality in each group (%).

DOSE	No.	HT %	ET %	UT %
82	20	33	0	0
90	20	50	11	17
99	20	61	44	17
109	20	94	39	50
120	20	100	67	67
132	20	100	100	78

The sensitivities of the two euthyroid groups were not significantly different.

LD_{50} HT= 86 mg/kg

LD_{50} ET = 108 mg/kg

These results are significantly different : potency ratio = 1.26 with an upper limit of 1.39 and a lower limit of 1.14 for 19/20 probability

FIGURE 14.

Mortality of hyperthyroid (HT) euthyroid control (ET) and euthyroid untreated (UT) male AS1 mice after intravenous injection of pentobarbitone sodium (see Table 13).

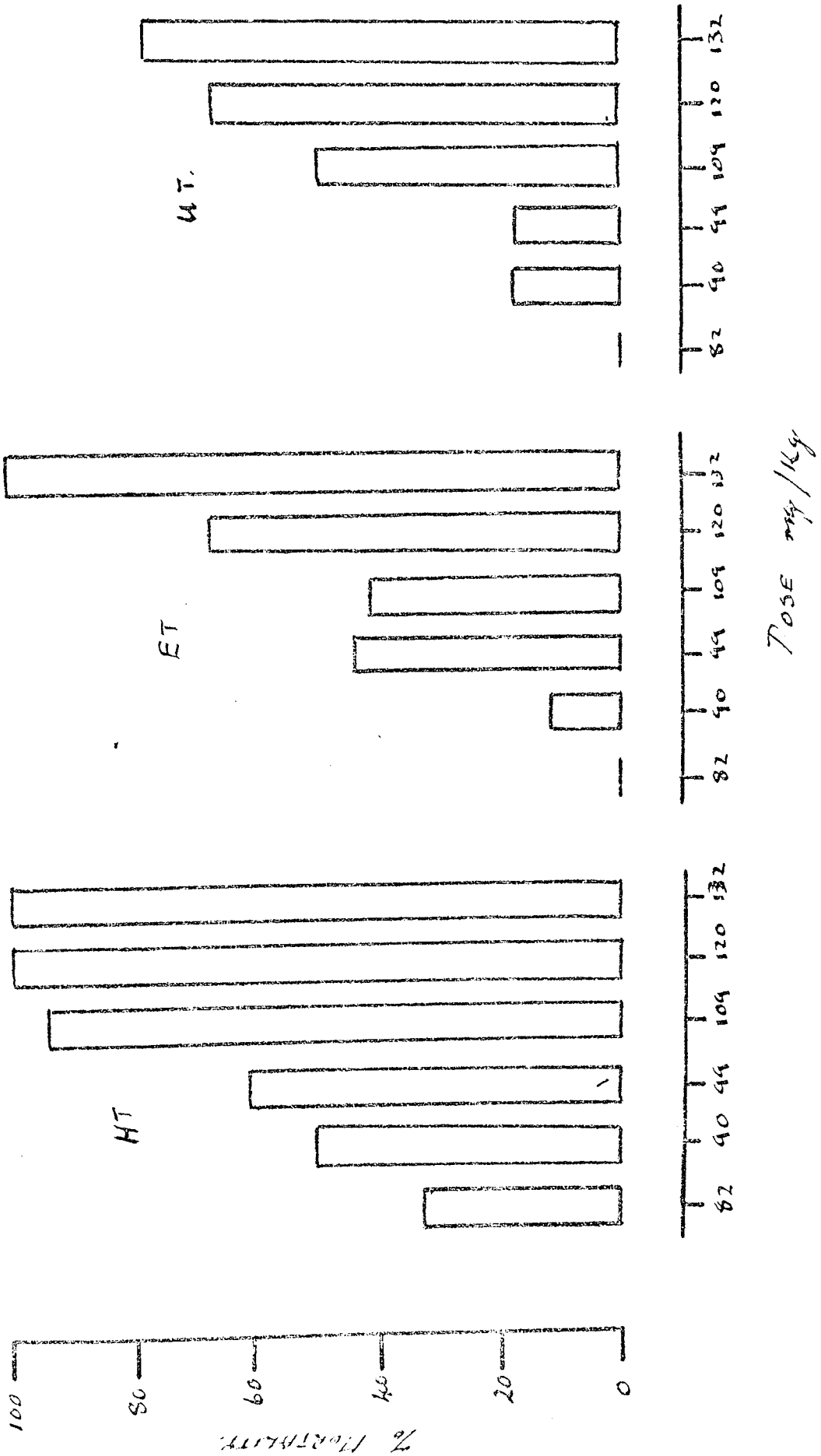


TABLE 14.

Mortality of hyperthyroid (HT) and euthyroid (ET) male TO mice after intravenous administration of thiopentone sodium at an ambient temperature of 22°C. The table shows doses of thiopentone (mg/kg), the number of animals tested (No) and the resulting percentage mortality in each group (%).

DOSE	No.	HT %	ET %
75	10	60	0
100	10	60	10
125	10	90	20
150	10	100	30
175	10	100	65
200	10	100	80

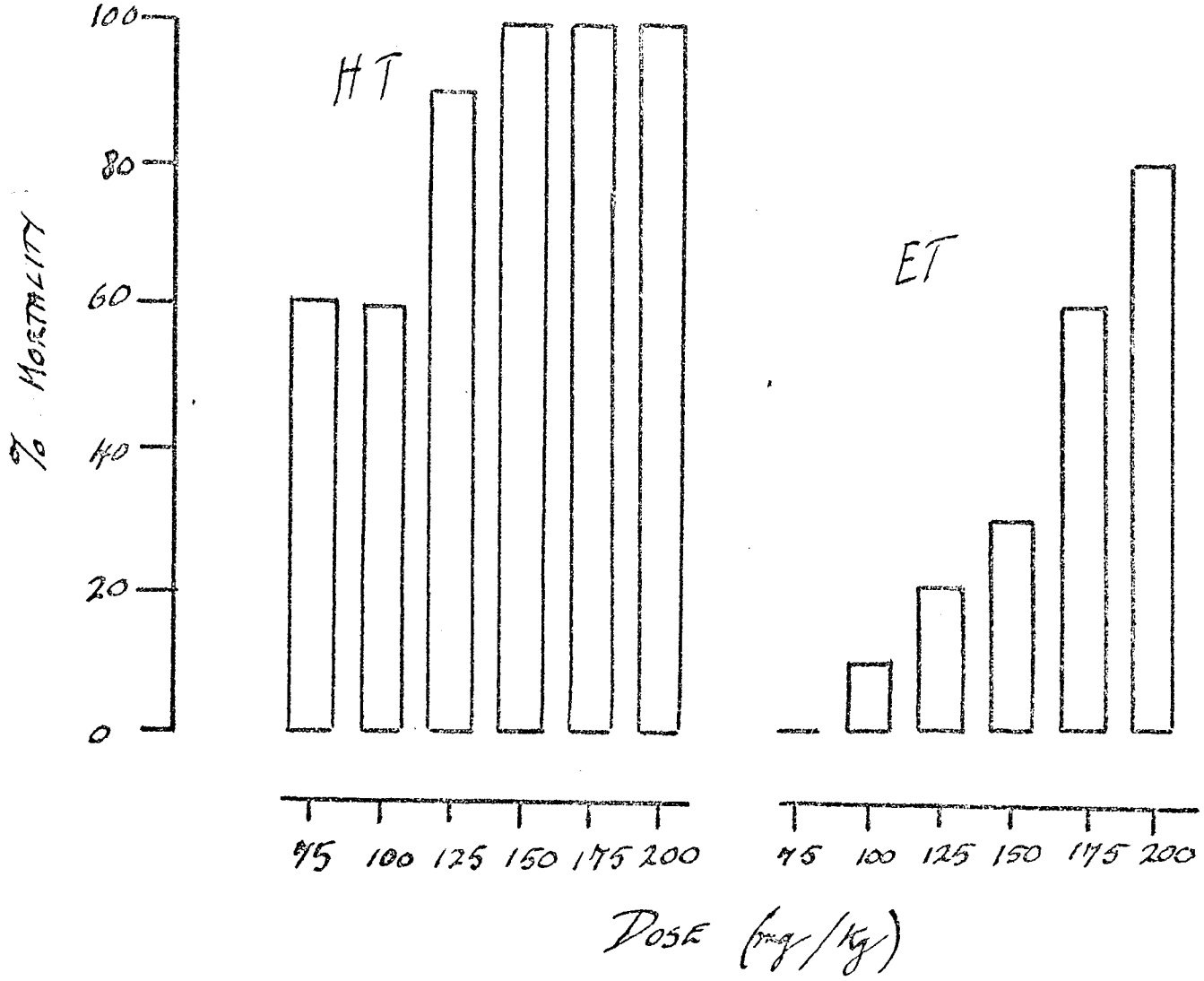
LD_{50} HT = 90 mg/kg

LD_{50} ET = 160 mg/kg

These results are significantly different : potency ratio = 1.78 with an upper limit of 2.22 and a lower limit of 1.42 for 19/20 probability.

FIGURE 15.

Mortality of hypertnyroid (HT) and euthyroid (ET) male TO mice after intravenous injection of thiopentone sodium (see Table 14).



CHAPTER II

THE EFFECTS OF INTRACEREBRALLY ADMINISTERED 2, 4-DINITROPHENOL AND PENTOBARBITONE IN HYPERTHYROID AND EUTHYROID MICE.

CHAPTER II

THE EFFECTS OF INTRACEREBRALLY ADMINISTERED 2,4-DINITROPHENOL AND PENTOBARBITONE IN HYPERTHYROID AND EUTHYROID MICE.

1. Introduction.

For reasons detailed in the discussion of this chapter it was decided to investigate the effects of intracerebrally administered 2,4-dinitrophenol (DNP) and pentobarbitone in groups of hyperthyroid and euthyroid mice.

In an initial experiment doses of 50, 100, 150, and 200 µg of DNP from a solution of 10 mg/ml were given i.c. to groups of euthyroid mice and the effects noted. As had been hoped, DNP by this route produced a dose-dependent period of hypnosis in the mice, whilst treatment with the vehicle alone at the same pH and osmotic pressure was without any obvious effect. Typically the hypnosis was preceeded by a short period of intense excitement during which the animals ran wildly about their cages, becoming increasingly disorientated and finally losing their righting reflexes. Following the hypnosis the animals regained consciousness, appeared normal, and 24 hr. later were behaviourally indistinguishable from untreated mice. The results of this experiment, which was conducted at an environmental temperature of 22° C are illustrated in Fig. 16 (Table 15). Since the initial excitement was of short duration the sleep times quoted were measured from the time of injection of the DNP until the return of the righting reflex; as can be seen from Fig. 16 the doses used produced a hypnosis varying in duration of 8 - 16 min. In Fig. 16 the graph of duration of hypnosis v. log dose

of DNP tails off at the higher dose levels. It was thought that this was probably due to the "leakage" of DNP from the brain along its concentration gradient. In an attempt to overcome or at least reduce this proposed leakage, an experiment was performed to investigate the effects of pretreatment with DNP intravenously just before intracerebral administration of the compound. The results shown in Table 16 show that the sleep time produced by 60µg DNP i.c. was significantly increased by peripheral pretreatment with DNP at a dose of 10 mg/kg iv. This suggests that leakage along a concentration gradient may be a factor in the observed cut off of the dose-response relationship.

2. Comparison of the effects of 2, 4-dinitrophenol and pentobarbitone in euthyroid mice.

In order to investigate further the hypnotic action of DNP administered by this route it was decided to compare its effects with those of pentobarbitone sodium administered by the same route. Three parameters of activity were compared, namely: hypnotic potency, effects on core temperature and effects on oxygen consumption.

a/Comparison of hypnotic effects.

The intracerebral injection of pentobarbitone sodium at pH 9 in doses of 100, 150, 200, 250 and 300µg (using a constant dose-volume of 10µl) produced period of hypnosis ranging from 5.5 to 12.7 min. duration, (see Fig. 17 Table 17). As with DNP, the hypnosis was preceeded by a short period of intense excitement. Treatment with the vehicle alone at the same osmotic pressure and pH was without obvious

effect on the animals. The effects of DNP at doses of 40, 60, 80 and 100 µg (using a constant dose volume of 10 µl) at pH 6.7 were also measured (Fig. 17). It can be seen from the figure that doses of 60 µg DNP and 200 ug pentobarbitone were approximately equipotent since both produced periods of hypnosis of 9.1 min, making DNP approximately 3 times more potent than pentobarbitone. However, since the DNP was administered at pH 6.7 and the pentobarbitone at pH9, this difference might have been due to the different pHs. Therefore, the sleeping times of a group of animals treated with DNP at pH9 were determined. Table 18 reveals that this change in pH produced no statistically significant change in the period of hypnosis produced by 60 ug of DNP. Consequently, there appears to be a true difference in potency between these two agents by this route of administration.

b/Comparison of effects on core temperature.

In these experiments the effects of equipotent (hypnotic) doses of 60 ug DNP and 200 ug pentobarbitone on the core temperatures of mice were measured. The results of this experiment conducted at an ambient temperature of 22°C are shown in Fig. 18,19 (Table 19), and reveal that both agents following intracerebral injection produce a hypothermia. The hypothermia was of a similar magnitude and duration with both compounds, the DNP producing a maximum hypothermia of 3°C after 10 min and the pentobarbitone a maximum hypothermia of 4.5°C after 15 min. Whilst this effect of pentobarbitone is similar to that seen following peripheral administration, in the case of DNP production of hypothermia is the exact opposite of its peripheral effect where it is known to give rise to hyperthermia

(Bianchetti, Pugliatti & Jori, 1967). Thus, this experiment showed that equipotent hypnotic doses of both agents by the intracerebral route gave rise to hypothermia similar in degree and duration.

c/ Comparison of effects on oxygen consumption.

As in the preceding experiment equipotent doses of 60 µg DNP and 200 µg pentobarbitone were administered intracerebrally to groups of euthyroid mice. As with the effects on oxygen consumption the results (Table 20 Figs 20+21) showed that both agents produced a similar reduction in the oxygen consumption of the animals whilst injection of saline at the same pH in both cases produced only a marginal effect. Thus, once again the effects of intracerebrally administered DNP were found to be the opposite of those resulting from peripheral administration.

/3. Comparison of the hypnotic effects of 2, 4-dinitrophenol and Pentobarbitone administered intracerebrally to hyperthyroid and euthyroid mice.

Since the effects of intravenously administered barbiturates are potentiated in hyperthyroid animals (see Chapter I) it was decided to investigate the effects of hyperthyroidism on the hypnosis induced by intracerebral administration of DNP and pentobarbitone. Accordingly, groups of hyperthyroid mice were treated with these agents by intracerebral injection and the resulting sleep times noted. The results (Table 21) showed that there was no potentiation of the hypnotic effects of DNP in the hyperthyroid mice compared to that seen in the euthyroid animals. Similarly with pentobarbitone no potentiation of the hypnotic effect was seen in the hyperthyroid mice.

Thus the results showed that, when administered by this route the sleep time resulting from treatment with either DNP or pentobarbitone was not significantly different in hyperthyroid or euthyroid mice.

DISCUSSION

Despite the time for which the barbiturates have been used clinically, their precise modes of action remain largely unknown. Several theories have been advanced relating the action of these drugs to their biochemical and physical properties. In the early 1900's Meyer and Oreton suggested that hypnotic drugs owed their action to the physical penetration of nerve cells and reported that the potency of the drugs is proportional to their lipid/water partition coefficient. This theory has been restated by Meyer (1937) who suggested that "depression appears if any chemically indifferent substance penetrates into the cell lipids to attain a definite concentration, which is a property of the cell and independent of the substance." Studies of partition coefficients and molar concentration attained have shown that this theory is acceptable, (Doran 1959). It is postulated that a barbiturate dissolved in the cell lipid reversible increases the surface tension of the water/lipid surface. This change in the state of the membrane is believed to lead to a loss of excitability, (Doran, 1959; Larsen, van Dyke & Chenoweth, 1968).

Attempts have also been made to establish a biochemical mechanism of barbiturate activity, correlating depression with enzyme inhibition. These latter theories are not widely accepted since present evidence is somewhat inconclusive. However, as early as 1930 Warburg suggested that anaesthesia is the result of depression of respiration. Brody and Bain (1951) reported that certain barbiturates in vitro cause an uncoupling of oxidation from phosphorylation. This effect is produced by pentobarbitone, thiopentone and amylobarbitone in

doses approximating to those producing anaesthesia. Consequently, the authors postulated that the uncoupling of oxidative phosphorylation might be one of the mechanisms whereby the barbiturates produce their hypnotic effect. In a later paper the same authors (Brody & Bain, 1954) compared the uncoupling effects of a number of barbiturates with those of 2,4-dinitrophenol (DNP) and concluded that their effects are qualitatively similar but that those of the phenolic agent are more potent. Later still, Hulme & Krantz (1955) compared the uncoupling potencies of a number of barbiturates and found that those compounds which are hypnotic uncouple, whereas those which are not hypnotic do not uncouple. Furthermore, these authors demonstrated that the hypnotic activity of five barbiturates tested correlated with their capacity to uncouple.

As Brody (1955) pointed out in a review on drugs which uncouple oxidative phosphorylation, one of the major arguments against the hypothesis is the fact that potent uncouplers like DNP had not been shown to have hypnotic activity. This could be due to the poor penetration of such a polar molecule into the brain. Deichman et al (1942) studied the fate of pentachlorophenol, a potent phenolic uncoupler, and found that after doses of 100 mg/kg in the rabbit less than 0.1% was recoverable from the CNS. It has been shown (Waddel & Butler, 1957) that raising the plasma pH will delay the onset of barbiturate anaesthesia due to an increase in the proportion of ionised barbiturate molecules which are not fat soluble, demonstrating the importance of tissue penetration in the production of hypnosis. Although DNP has not previously been shown to be a hypnotic agent, Brody & Killam (1952) showed that pretreatment with the compound will potentiate the hypnotic effects of certain barbiturates without influencing the rates at which they are detoxified.

Since these reports, a number of other studies have linked an uncoupling effect with hypnosis and anaesthesia. Thyroxine, which may also exert an uncoupling effect at experimental dose levels, potentiates significantly the hypnotic effects of a number of barbiturates (Conney & Garren, 1960; Prange, Lipton & Love, 1962; Prange et al, 1966). Also Holtz et al (1957) and Shore et al (1955) have shown that pretreatment with 5HT potentiates barbiturate-induced hypnosis, an effect which Mahler & Humoller (1968) ascribe to the uncoupling activity of one of the metabolites of 5HT. Finally, chlorpromazine, another agent reported to cause uncoupling (Abood, 1955) potentiates barbiturate induced hypnosis (Richards, Forney & Hughes, 1965).

This potentiation of barbiturate induced hypnosis, produced by a number of uncoupling agents with widely different pharmacological properties, adds much weight to the original concept that barbiturates owe at least some of their action to a capacity to uncouple oxidation from phosphorylation.

In the experiments reported here the effects of DNP have been observed following the intracerebral administration of the compound. This gives brain levels of the substance which could not be achieved by conventional administration because DNP is not readily absorbed by the brain and toxic doses would have to be administered by peripheral injection to obtain similar brain concentrations.

The results shown in this chapter demonstrate that following the IC administration of DNP a dose-related period of hypnosis is produced. Afterwards the mice awake and 24 hours later are indistinguishable behaviourally from normal animals. Injection of the vehicle alone at the same osmolarity and pH as the drug solution is

without any effect. When the hypnotic action of DNP was compared with that of pentobarbitone, DNP was about three times more potent. That this difference in potency is not the result of differences in pH of the two solutions was shown by the fact that DNP administered at pH 9 caused a period of hypnosis which was not significantly different from that produced by the same agent administered at pH 7. This result strengthens the above hypothesis since DNP is reported to be a more potent uncoupler of oxidative phosphorylation than is pentobarbitone.

The hypothesis produced by the two agents was further compared and it can be seen that equivalent doses of each agent produce similar reductions in core temperature and in oxygen consumption. This last effect of DNP is the reverse of its peripheral action, where it is known to produce an increase in core temperature with a simultaneous increase in oxygen consumption. This perhaps shows the difference between central and peripheral uncoupling; uncoupling in peripheral tissues leads to an increase in core temperature and oxygen consumption without any overt behavioural symptoms. Conversely uncoupling within the central nervous system results in hypothermia, decreased oxygen consumption and hypnosis.

The fact that the effect of DNP was not potentiated in the hyperthyroid animals does not undermine the strength of this hypothesis since the pentobarbitone also was not potentiated. One explanation might be that increase in cerebral blood flow in hyperthyroidism facilitates the removal of the drugs from the brain along their concentration gradients thus overriding any potentiating effect of T4 treatment. This explanation is supported by the fact that the dose response curves for both agents fall off at high doses; in fact, almost lethal doses of both

agents produce only a relatively short period of hypnosis - suggesting that removal from the brain is the limiting factor in the duration of hypnosis in euthyroid animals. Also it has been shown that IV administration of DNP before IC administration caused potentiation of the hypnosis. This could be explained by the fact that DNP in the blood would effectively reduce the concentration gradient between blood and brain and thereby prolong the presence of DNP in the brain with a resultant increase in the period of hypnosis.

In conclusion, it is postulated that the experimental evidence presented here is in agreement with the hypothesis that the barbiturate hypnotics owe at least some of their activity to their ability to uncouple oxidation from phosphorylation. Since T4 is reported to cause uncoupling of oxidative phosphorylation, it may be that this property is related to its potentiating action, although this has not been proved.

SUMMARY

1. Intracerebral injection of 2,4-dinitrophenol (DNP) in male TO mice produces a dose dependant period of hypnosis.

2. By this route DNP is about three times as potent as pentobarbitone as a hypnotic agent.

3. Equipotent hypnotic doses of both DNP and pentobarbitone produce similar decreases in core temperatures and oxygen consumption following intracerebral injection in mice.

3. Thyroxine-induced hyperthyroidism in male TO mice does not render these animals any more susceptible to the hypnotic effects of either DNP or pentobarbitone administered by intracerebral injection.

TABLE 15.

Sleep times produced by 2, 4-dinitrophenol administered by intracerebral injection (pH 6.7) to euthyroid male TO mice. The results are expressed as the group means of five animals (min), ± S.E.

Dose of 2, 4-dinitrophenol μg/mouse	DOSE VOLUME μl/mouse	SLEEP TIME
50	5	8.3 ± 0.8
100	10	12.6 ± 1.1
150	15	15.6 ± 0.6
200	20	16.3 ± 1.6

TABLE 16.

Sleep times produced by intracerebral administration of 2, 4-dinitrophenol in a dose volume of 10 μl. into untreated male AS 1 mice (A) and similar animals (B) pretreated with 2, 4-dinitrophenol 10 mg/kg by intravenous injection. The results are expressed as the means of 10 animals (min), ± S.E.

Dose of 2, 4-dinitrophenol μg/mouse	A.	B.
40	5.0 ± 0.57	10 ± 1.2
60	9.1 ± 0.7	11 ± 0.8

FIGURE 16.

Sleep times of euthyroid male TO mice after intracerebral injection of 2,4-dinitrophenol at pH 6.7 from a solution of 1 µg/µL (see Table 15).

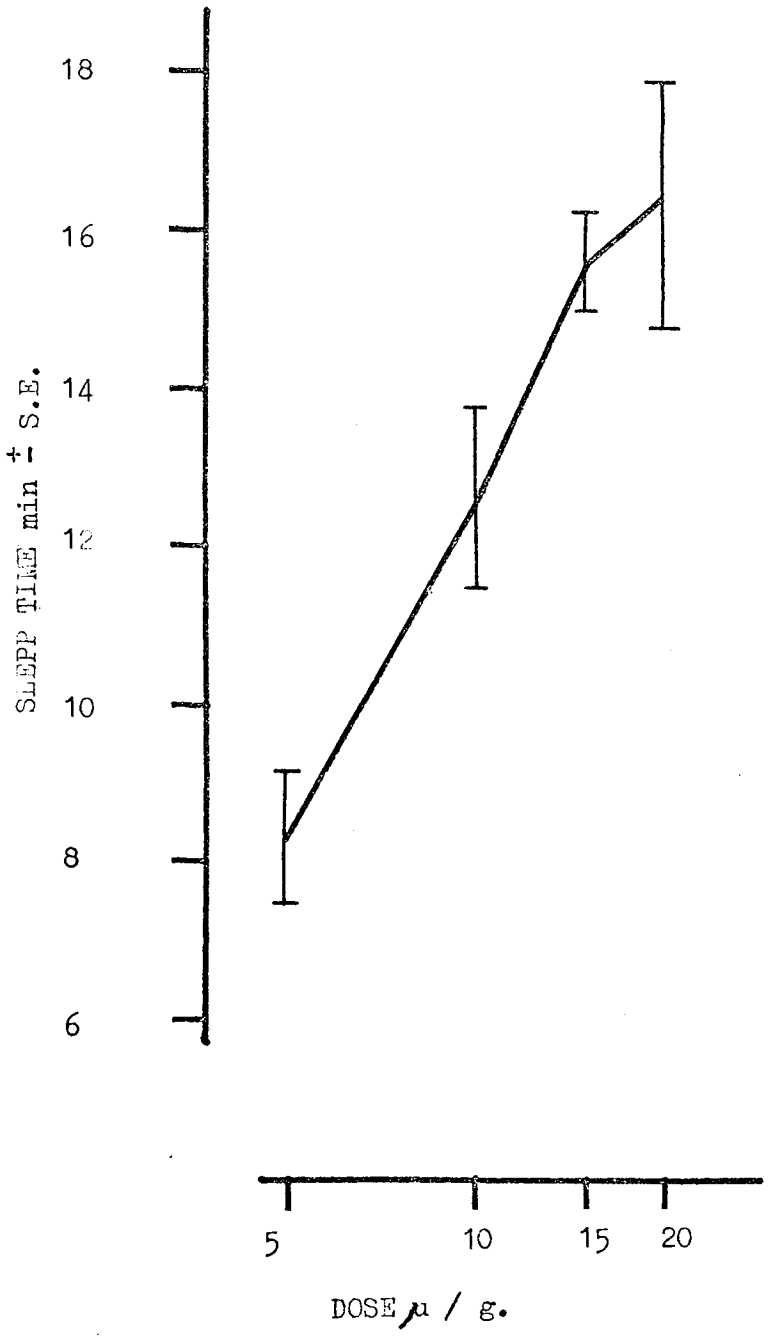


TABLE 17.

Sleep times produced by intracerebral administration of pentobarbitone sodium (B5) at pH 9 and 2, 4-dinitrophenol (DNP) at pH 6.7 in a dose volume of 10 µl. The results are expressed as the means of groups of 10 animals (min), ± S.E.

B5		DNP	
DOSE µg/Mouse	SLEEP TIME	DOSE µg/mouse	SLEEP TIME
100	5.5 ± 0.5	40	5.0 ± 0.6
150	6.2 ± 0.5	60	9.1 ± 0.7
200	9.1 ± 1.0	80	13.1 ± 1.2
250	11.2 ± 1.2	100	14.5 ± 1.3

TABLE 18.

Sleep times produced by 2, 4-dinitrophenol 60 µg/mouse at pH 6.7 (A) and pH 9 (B); also, by pentobarbitone soidum 200 µg/mouse at pH 9 (C) following intracerebral injection in a dose volume of 10 µl. The results are expressed as the means of groups of 10 animals (min) ± S.E.

A	B	C
9.1 ± 0.7	9.3 ± 0.6	9.1 ± 1.0

FIGURE 17.

Sleep times of euthyroid male TO mice after intracerebral injection of 2,4-dinitrophenol at pH6.7 (DNP) or pentobarbitone sodium at pH 9 (B5) in a dose volume of 10 uL (see Table 17)

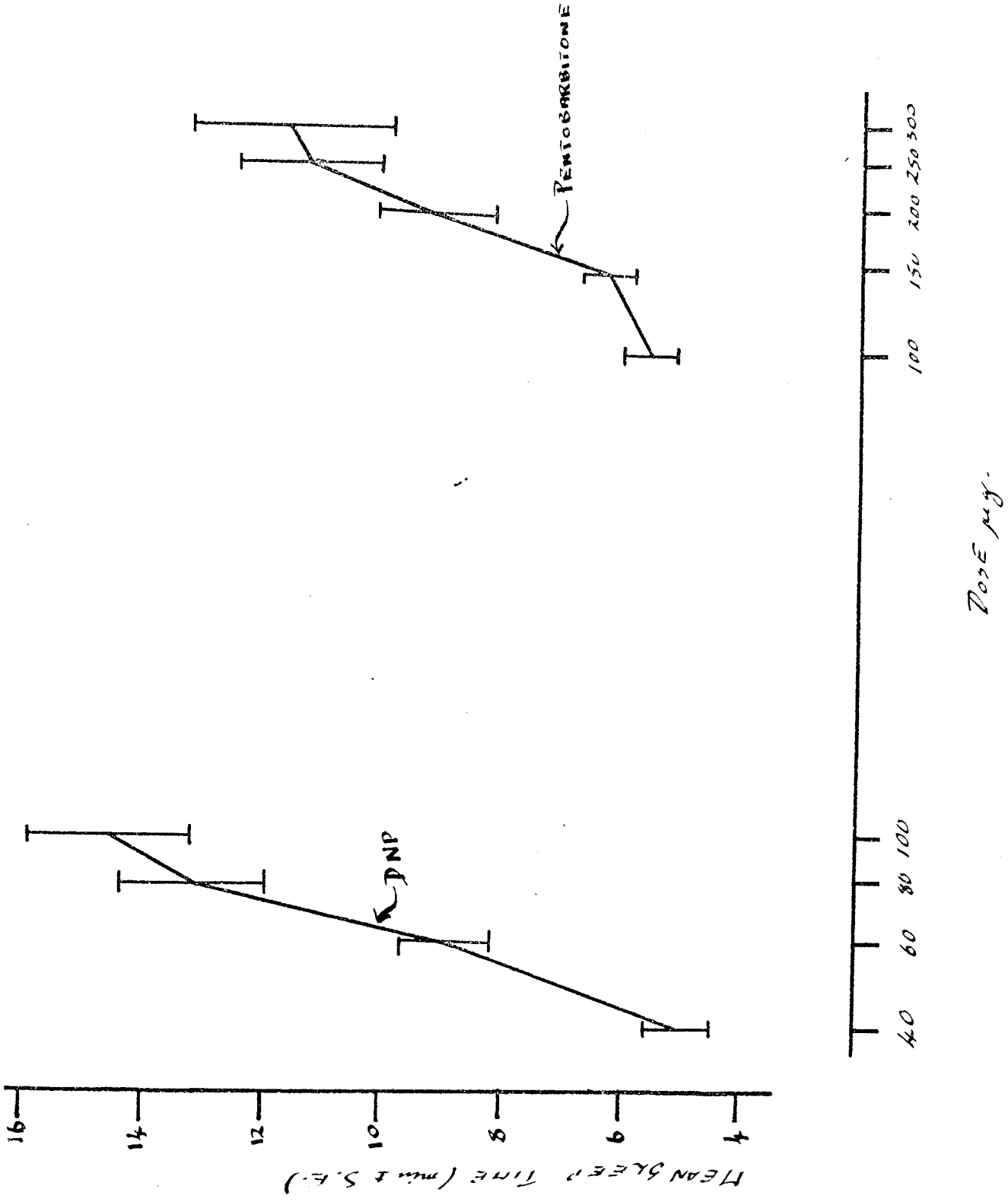


TABLE 19.

Effect of pentobarbitone sodium 200 ug/mouse at pH 9, 2,4-dinitrophenol, 60 ug/mouse at pH 6.7 (DNP) or normal saline at pH 9 (B5-C) or pH 6.7 (DNP-C) on core temperatures of euthyroid male TO mice following intracerebral administration. Results are expressed as mean oesophageal temperatures of groups of 5 animals (°C ± S.E.)

Time (min)	B5	B5-C	DNP	DNP-C
0	36.0 ± 0.1		36.1 ± 0.1	
5	33.7 ± 0.3	35.5 ± 0.2	33.2 ± 0.2	35.6 ± 0.3
10	32.5 ± 0.5	35.5 ± 0.4	32.0 ± 0.1	35.6 ± 0.3
15	33.0 ± 0.8	35.7 ± 0.4	31.5 ± 0.6	36.1 ± 0.2
20	34.0 ± 0.8	35.9 ± 0.3	32.0 ± 1.0	36.4 ± 0.1
25	34.8 ± 0.7	35.9 ± 0.3	33.3 ± 0.8	36.7 ± 0.1
30	34.9 ± 0.7	36.1 ± 0.2	34.1 ± 0.7	36.8 ± 0.1
35	35.3 ± 0.6	36.1 ± 0.2	35.0 ± 0.7	36.7 ± 0.1

Dose volume = 10 µl.

FIGURE 18.

Core temperatures of euthyroid male TO mice after intracerebral injection of 2,4-dinitrophenol 60 µg in 10 µL at pH 6.7. (see Table 19).

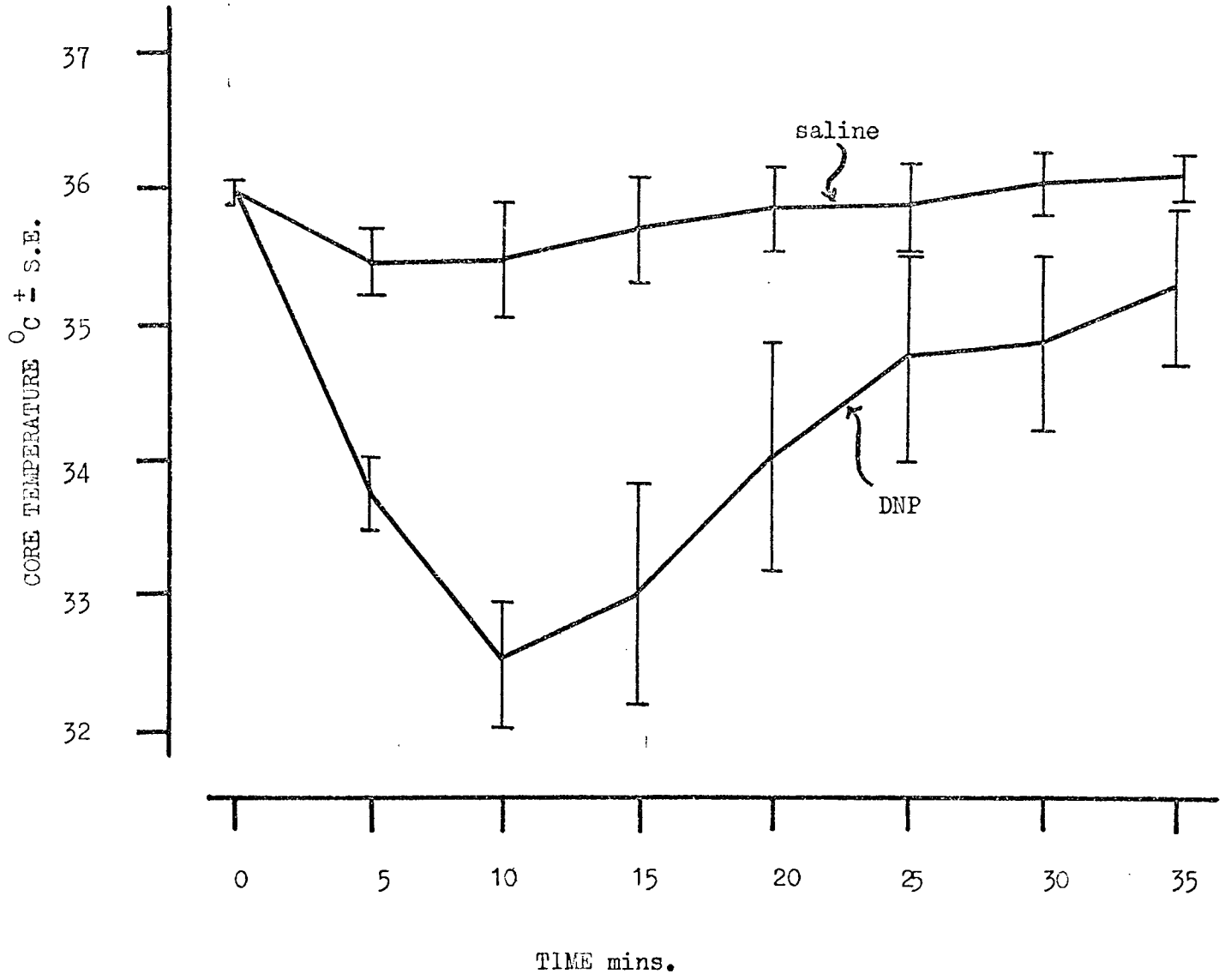


FIGURE 19.

Core temperatures of euthyroid male TO mice after intracerebral injection of Pentobarbitone sodium 200 µg in 10 µL at pH 9 (see Table 19).

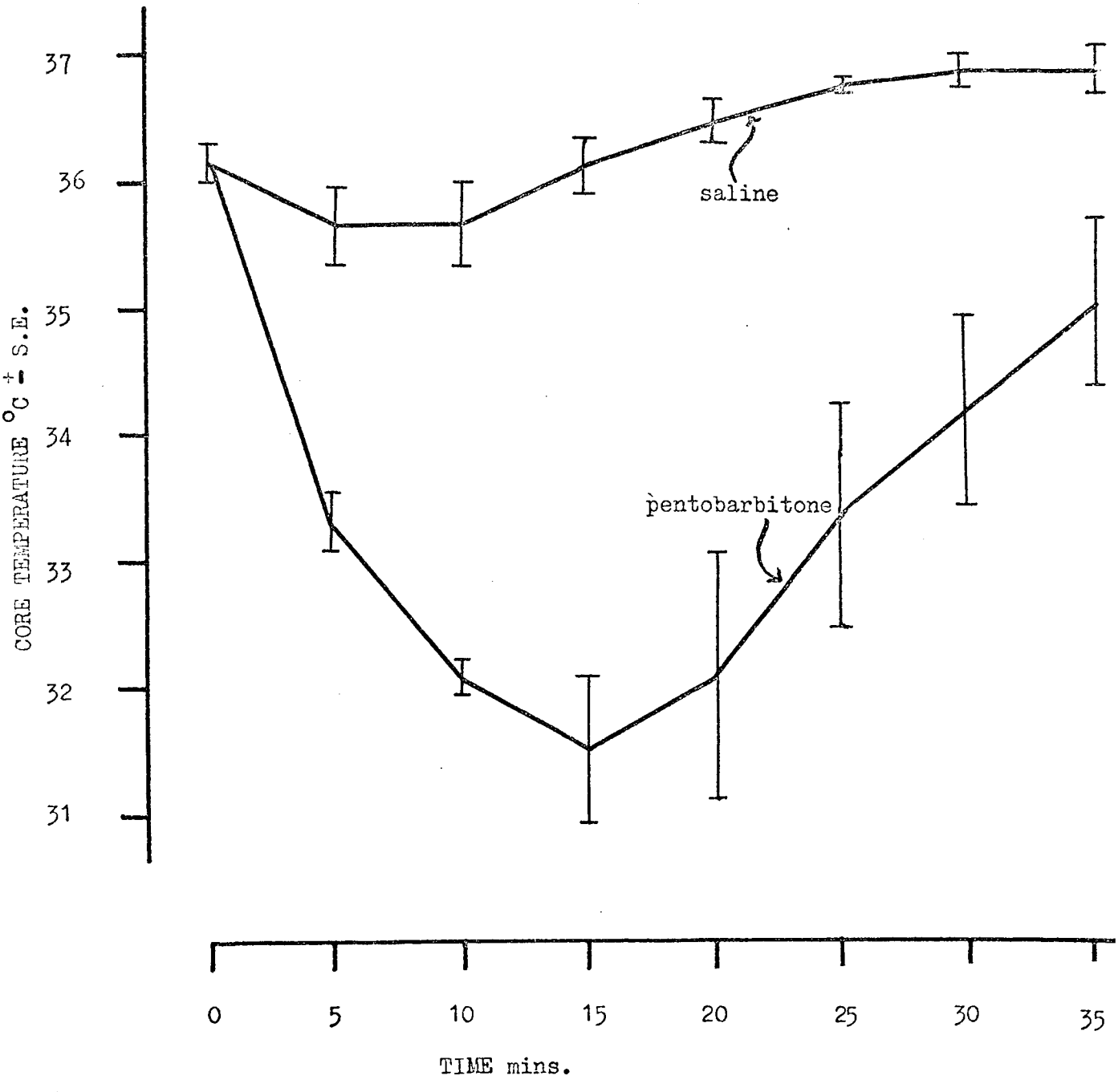


TABLE 21.

Sleep times (min) of hyperthyroid and euthyroid male TO mice following treatment with pentobarbitone sodium, 200 µg, at pH 9, or 2,4-dinitrophenol, 60 µg, at pH 6.7 following intracerebral administration. Results are expressed as the mean sleep time of groups of 10 animals, min ± S.E.

	PENTOBARBITONE		DNP	
HYPERTHYROID	8.8	1.4	8.9	0.5
EUTHYROID	9.1	1.0	9.1	0.7

Dose volume = 10 ul.

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FIGURE 20.

Oxygen consumptions of euthyroid male TO mice after intracerebral injection of 2,4-dinitrophenol 60 µg in 10 µL at pH 6.7 (see Table 20).

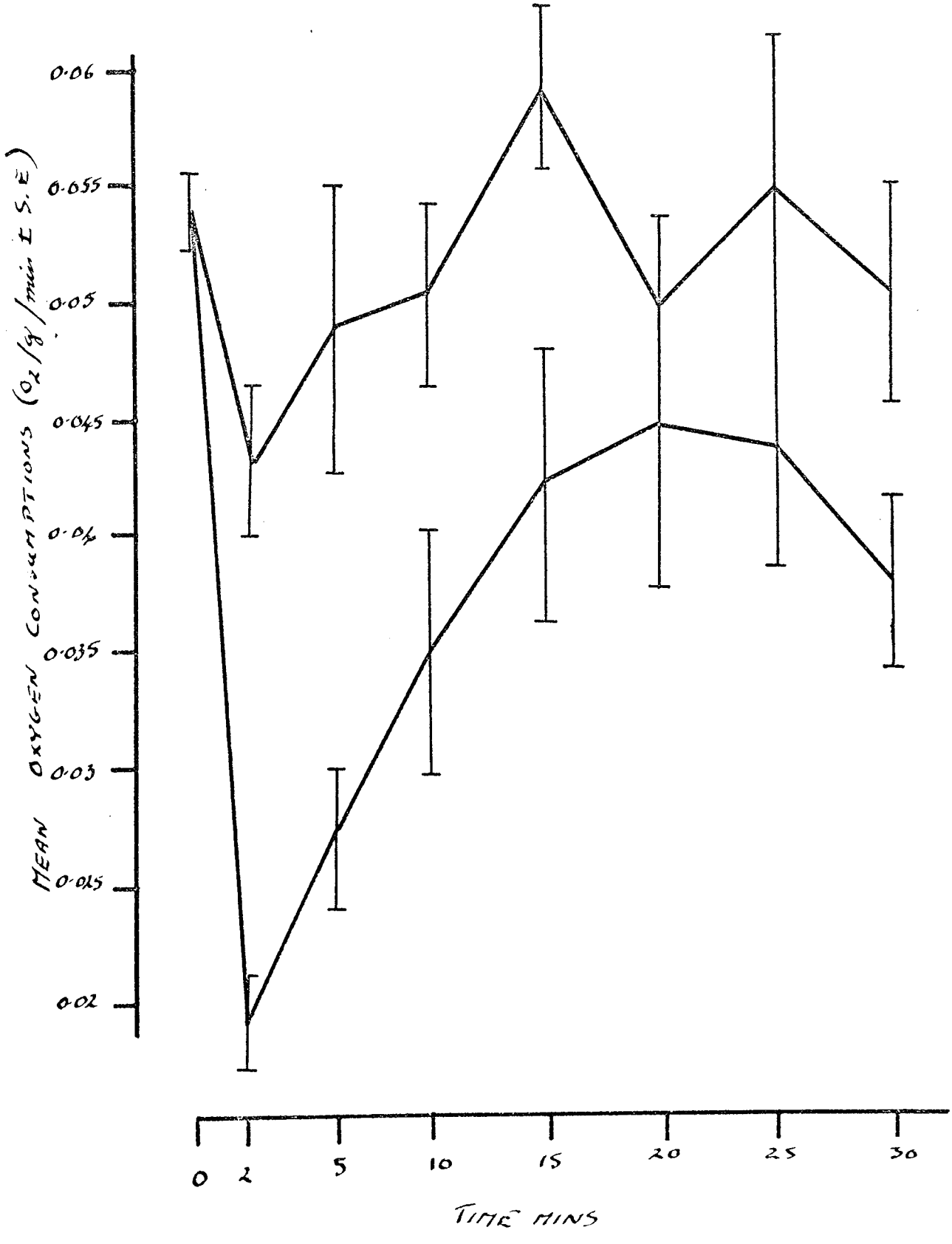
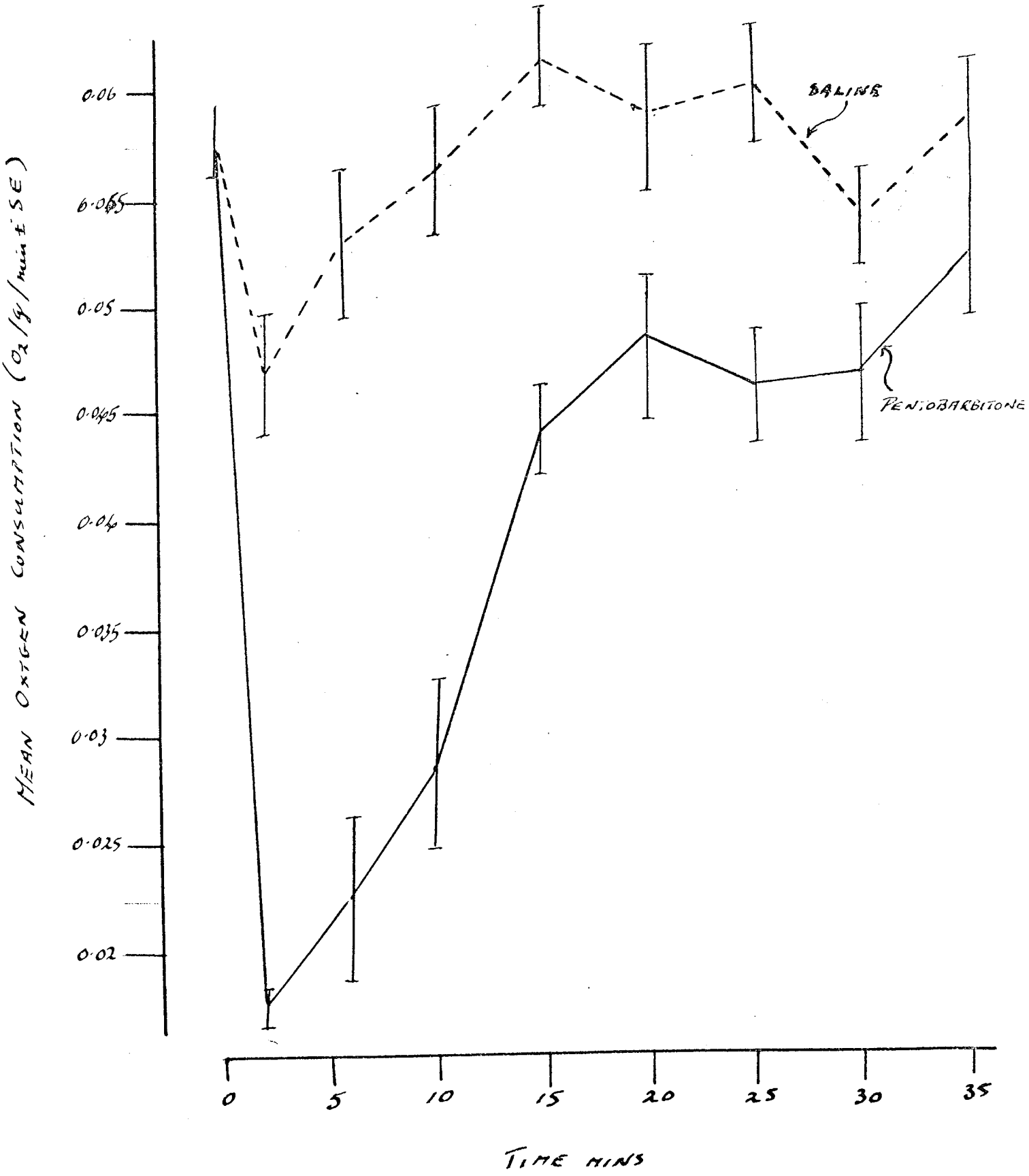


FIGURE 21.

Oxygen consumptions of euthyroid male TO mice after intracerebral injection of pentobarbitone sodium 200 µg in 10 µL at pH9 (see Table 20).



B

CHAPTER III

THE EFFECTS OF CHLORPROMAZINE IN HYPERTHYROID AND EUTHYROID MICE

CHAPTER III

THE EFFECTS OF CHLORPROMAZINE IN HYPERTHYROID AND EUTHYROID MICE

1. Introduction

Hoffman (1959) reported that pretreatment of the rat or the ground squirrel with the synthetic thyroxine analogue 3, 5, 3'triiodothyropropionic acid inhibited the hypothermic action of chlorpromazine. Also it has been reported (Lettau, Sellers & Schönbaum, 1964) that pretreatment of rats with l-triiodothyronine protected the animals from the lethal effects of CPZ in a cold environment. It therefore seemed of interest to investigate the effects of CPZ in hyperthyroid mice.

An initial experiment was carried out at a laboratory temperature of 22° C. Groups of 10 hyperthyroid and euthyroid mice receiving either chlorpromazine hydrochloride (CPZ) 10 mg/kg intraperitoneally, or an equivalent volume of normal saline (controls). It was observed that at this temperature CPZ produced a distinct hyperthermia of about 1.3° C in the hyperthyroid animals, whilst in the euthyroid mice it elicited the expected well-marked hypothermia of about 5° C. Control animals of both the hyperthyroid and the euthyroid groups maintained a fairly constant temperature throughout the experiment, although the hyperthyroid mice had a temperature consistently about one degree higher than the euthyroid animals. The results of this experiment are illustrated in Fig. 22 (Table 22)

2. The effect of dose and of ambient temperature on the response to CPZ.

The above experiment seemed to suggest that a qualitative difference existed between the responses of hyperthyroid and euthyroid mice to CPZ.

Accordingly, these preliminary experiments were undertaken to investigate the effects of ambient temperature and dose of CPZ on the response of the two groups of animals. The effects of two doses of CPZ (5 and 10 mg/kg), at laboratory temperatures of 20 & 26.5° C were investigated.

i. CPZ 5 mg/kg at 20° C.

The results of this experiment are shown in Fig.23 (Table24). At this dose level the hyperthyroid animals showed a hypothermia of only 1.5° C reaching a peak after $1\frac{1}{2}$ hr. and declining rapidly thereafter. In contrast the euthyroid mice showed a marked hypothermia of 5.5° C reaching a maximum after $2\frac{1}{2}$ hr.

ii. CPZ 10 mg/kg at 20° C.

Fig.24 (Table23), shows the results of this experiment. The hyperthyroid mice showed a marked hypothermia of 5° C reaching a peak 2 hrs. after the administration of the drug after which the temperatures of the animals began to return slowly to normal. In contrast the euthyroid animals responded to this dose with a much greater hypothermia of 8° C which reached a maximum after $2\frac{1}{2}$ hr.

Thus at 20° C hypothermia was shown by both the hyperthyroid and the euthyroid mice, although at both dose levels the euthyroid animals responded to the drug with a much greater degree of hypothermia than did the hyperthyroid mice.

iii. CPZ 5 mg/kg at 26.5° C.

As can be seen from Fig.25 (Table25) at this temperature the hyperthyroid mice responded to the drug with a mild hyperthermia of

1.5° C reaching a peak after $1\frac{1}{2}$ hr. In contrast the euthyroid animals continued to show a hypothermia at this elevated temperature though it was only of 2° C reaching a maximum after 2 hr.

iv. CPZ 10 mg/kg at 26.5° C.

At this dose level the effect of the CPZ on the hyperthyroid mice was even more marked, as shown in Fig. 26 (Table 26) they again showed a hyperthermia this time of 2° C after 2 hr. After this time the experiment was discontinued due to the deaths of several of the animals in this group from heat stroke. The euthyroid animals showed a hypothermia which was of 1.5° C after 2 hr.

COMMENT

As can be seen from Figs. 25 & 26, at 26.5° C the response of the euthyroid mice to a dose of 5 mg/kg CPZ was greater than that to 10 mg/kg. Since all other conditions were the same (the experiments were carried out at the same time in the same laboratory), this apparent discrepancy in the results could be attributed to a difference in the ventilation of the cages due to their positions in the laboratory. As the animals approach their critical temperature heat loss becomes more difficult, thus the group exposed to the greatest air flow would be expected to show the greatest degree of hypothermia providing the doses of CPZ are not too dissimilar. At the lower environmental temperature heat loss is more easily accomplished (gradient between animal and environment being greater) and the effect of ventilation becomes less significant. Therefore, the response of the animal is more closely related to the dose of drug administered.

3. Effects of CPZ on core and skin temperature and on spontaneous locomotor activity (SLA) of hyperthyroid and euthyroid mice.

The previous experiments demonstrated that a dose of 5 mg/kg of CPZ was sufficient to exert a profound effect on the thermoregulatory mechanisms of the mouse. Further they showed that a qualitative difference existed between the responses of hyperthyroid and euthyroid animals to the drug. It appeared that the responses, especially those of the hyperthyroid mice, were particularly dependant on the ambient temperature at which the experiments were conducted. Accordingly, a further series of experiments was undertaken to more fully investigate the influence of environmental temperatures on the responses of both types of animal to CPZ. Two parameters of drug activity, core temperature and SLA, were noted at a variety of ambient temperatures, and a third parameter namely skin temperature was recorded at one temperature only. In all these experiments groups of hyperthyroid and euthyroid animals were treated with a constant dose of CPZ (5 mg/kg) or with saline (controls).

A/Effects of CPZ on the temperature of hyperthyroid and euthyroid mice.

The ambient temperatures at which these experiments were carried out were 20°, 22°, 26°, & 30°C.

i. CPZ 5 mg/kg at 20°C.

The results of this experiment are illustrated in Fig.27 (Table27). As seen before both groups of animals, at this temperature, showed hypothermia after CPZ, and again the euthyroid mice showed a more profound response than did the hyperthyroid animals. Whereas the hyperthyroid mice had a maximum hypothermia of 1.5°C after 2 hr, the

euthyroid animals, in contrast, showed a greater hypothermia of 3° C reaching a peak after $1\frac{1}{2}$ hr.

ii CPZ 5 mg/kg at 22° C.

Fig 28 (Table 28) shows the effects of CPZ on core temperature at this environmental temperature. Hyperthyroid animals responded with a mild hyperthermia of 1° C after 2 hr. returning to a slightly subnormal level at 3 hr. In contrast the euthyroid mice showed a marked hypothermia increasing to 5° C at 3 hr.

Fig 29 (Table 29) shows the effects of the drug on skin temperature at 22° C. This experiment was designed to investigate further the effects of CPZ on thermoregulatory functions in mice. This ambient temperature was chosen because, although it is a normal, laboratory temperature, previous work had shown that at this temperature the drug produced its usual hypothermia in euthyroid animals, whilst causing a mild hyperthermia in hyperthyroid mice. Groups of 5 drug treated or control hyperthyroid or euthyroid animals were used and the effects of CPZ on the skin temperature of the hind foot measured. The figure, which shows the combined results from two such experiments, shows that inspite of opposite effects on the core temperatures both the hyperthyroid and the euthyroid animals responded to the drug with an increase in skin temperature. In both the hyperthyroid and the euthyroid mice the rise in skin temperature was maximal at 1 hr ; that of the hyperthyroid mice being greater than that of the euthyroid group. The former reached 6.5° C above control values whilst the latter reached 5.3° C above the control level. (This difference is significant $P = < 0.05$). The rise in skin temperature of

the hyperthyroid mice was also maintained for longer than that of the euthyroid animals, their respective values at 3 hr. being 2.8° and 1.6°C above the controls. As with the core temperature it was noted that the skin temperature of the hyperthyroid mice was consistently higher than that of the euthyroid animals, the difference in this case being 1.5° - 2°C.

iii. CPZ 5 mg/kg at 26°C.

The results of this experiment are shown in Fig.30 (Table30). At this relatively high temperature, the hyperthyroid mice showed a marked hyperthermia rising to 3°C after 1 hr. In fact, the results shown for 2 & 3 hr. are probably misleadingly low since a number of the animals died of heat stroke between 2 & 3 hr. and therefore are not included in the results. The euthyroid animals showed a mild but significant hypothermia which reached a peak of 2°C at 1 hr.

iv. CPZ 5 mg/kg at 30°C.

The extreme sensitivity of the hyperthyroid animals to the hyperthermia caused by CPZ at this temperature rendered the use of such animals impossible, since treatment with the drug at this temperature invariably led to the death of all the hyperthyroid mice. Fig.31 (Table31). contrasts the effects of CPZ in euthyroid mice at 22°C & 30°C and demonstrates the profound influence which the ambient temperature has on the response of these animals to the drug. At the lower temperature the normal hypothermic effects are seen, reaching a maximum of 3°C at 1 hr. At 30°C however, there was only a slight non-significant tendency to hypothermia ; CPZ producing a mean core temperature at 2 hr. only 0.4°C lower than the controls.

B/Effects of CPZ on spontaneous locomotor activity of hyperthyroid and euthyroid mice.

To investigate the relationships between the behavioural and thermoregulatory effects of the drug, its effects on SLA of hyperthyroid and euthyroid mice were recorded concurrently with the above experiments : the SLA of groups of 5 hyperthyroid or euthyroid drug-treated mice or control mice were recorded at 22°, 26°, & 30°.

The experiments revealed, as can be seen from the control results at each ambient temperature, that the normal SLA of hyperthyroid mice was consistently less than that of the euthyroid animals.

i SLA following CPZ 5 mg/kg at 22° C.

Fig. 32 (Table 32) shows graphically the results obtained in this experiment from drug-treated and control hyperthyroid mice. Inspite of the CPZ-induced hyperthermia at this ambient temperature the drug produced a marked reduction in the SLA first detectable within 10 min. of the administration. The euthyroid animals, (see Fig 12, Table 32), also showed a reduction in SLA first detectable within 10 min., and which, as with the hyperthyroid mice, persisted throughout the period of the experiments. The proportional decrease in the SLA was greater in the euthyroid than in the hyperthyroid mice, but the former animals had a greater normal level of activity.

ii. SLA following CPZ 5 mg/kg at 26° C.

At this temperature CPZ reduced the SLA of the hyperthyroid mice (Fig. 33 Table 33); again the effects persisted throughout the experiment and were detectable within 10 min. of drug administration. The euthyroid mice

(Fig. 35 Table 33) also suffered a marked decrease in SLA which was detectable within 10 min. and persisted throughout the experiment. As before, the effects of CPZ on the SLA of the euthyroid animals was greater than that on the hyperthyroid mice.

iii. SLA following CPZ 5mg/kg at 30° C.

As indicated before, no results could be obtained from hyperthyroid mice at this temperature, and the results illustrated in Fig. 36 (Table 34) are derived from euthyroid animals only. As at the lower environmental temperatures, the CPZ produced a marked reduction in the SLA which was detectable within ten min. and persisted throughout the experiment. It should be noted that this reduction in SLA occurred in the absence of concomitant hypothermia.

DISCUSSION

Chlorpromazine is a neuroleptic drug which has achieved much clinical importance as a tranquillising agent in psychiatric medicine. Pharmacologically, the drug produced a wide spectrum of effects of which sedation is the most important; after receiving the drug, animals become quiet and sleepy but are easily roused although catalepsy is seen at higher doses. Low doses cause a depression of the reticular activating system whilst at higher doses extrapyramidal symptoms, resembling Parkinsonism in man are produced (Gordon, 1967). The drug is also known to produce hypothermia, which is marked in the smaller species.

The sedative and hypothermic properties of the drug have been investigated in this work. Though the mechanisms of action of CPZ are not fully understood there are several studies which shed light on this problem. Maichel (1967) reported that the behavioural effects of small doses (e.g. 4 mg/kg) of CPZ in rats can be reversed by treatment with amphetamine, whilst higher (16 mg/kg) doses can be counteracted by treatment with amphetamine and anticholinergic agents such as atropine, but not by amphetamine alone. This author postulated that the central actions of the drug are not mediated solely through its central α-adrenergic blocking activity (Brodie, 1959; Phillips & Bradley, 1969) but also, at high doses by a concomitant cholinergic action. The latter action is postulated to occur as a result of the reported anticholinesterase activity of the drug (Johannesson & Lausen, 1961). Another possibility

is that the drug exerts its actions by the uncoupling of oxidative phosphorylation, but it is not clear if this action is a cause or a product of sedation (see Chapter II discussion).

The drug is thought to produce its behavioural effects by inhibition of the collateral afferent inputs to the reticular formation (Bradley & Key, 1958; Bradley 1965).

The hypothermia produced by CPZ is thought to be mediated via central and peripheral components. Centrally the centres of thermoregulation are depressed (Skobba & Miya, 1969), whilst behavioural (Weiss & Laties, 1963) and postural (Le Blanc, 1958) changes predispose the animal to hypothermia. Peripherally the α-adrenergic blocking activity of the drug leads to vasodilation with consequent loss (Dandiya et al 1960). Bonaccorsi, Garattini and Jori (1964) reported that the hypothermia is accompanied by a concomitant hyperglycaemia which is proportional to the hypothermia. Mueller and McDonald (1968) interpreted this as indicative of an impairment in the ability of CPZ treated animals to utilise calorigenic substances.

The body temperature of an animal is controlled by the ratio of heat loss : heat production. In the homeotherm the effect of ambient temperature is minimised by the presence of specific mechanisms for control of heat loss and heat conservation. By the depression of central thermoregulation (Kollias & Bullard, 1964) CPZ tends to convert the homeotherm into a poikilothermic animal. Thus, as reported by Shemano and Nickerson (1958), the response of the animal is influenced by the prevailing ambient temperature and hyperthermia can result from

CPZ treatment if the environmental temperature is high enough. The deciding factor in determining the response of an animal to a given dose of drug at a given ambient temperature is the critical temperature of the animal. The critical temperature is the environmental temperature at which, in the absence of adequate thermoregulation, the heat production from metabolic processes is equal to the heat loss, and the core temperature of the animal remains constant. At ambient temperatures above the critical temperature hyperthermia will result from drug treatment and at temperatures below the critical temperature hypothermia will result.

In the results shown here, at 20° C both the hyperthyroid and the euthyroid mice responded to CPZ with hypothermia. However, the hyperthyroid animals were not so greatly affected as their euthyroid counterparts. This indicates that the ratio of heat production: heat loss is greater in the hyperthyroid mice than in the euthyroid mice. That the hyperthyroid animals were losing heat is hown by the results of the experiment carried out to measure skin temperature which indicate that these animals were, in fact, more affected than their euthyroid counterparts.

At 22° C whilst the euthyroid mice still showed a marked hypothermia, the hyperthyroid mice responded to the drug with a mild hyperthermia. This indicates that the hyperthyroid animals were in an environment above their critical temperature which must therefore fall between 20 - 22° C for this dose of CPZ.

At 26° C the hyperthyroid mice suffered such severe hyperthermia that several of them died from heat stroke. This result is in agreement

with that of Skobba & Miya (1969) who suggested that the increased toxicity of CPZ in hyperthyroid rats is a result of a hyperthermia produced by the drug in such animals. This high ambient temperature was not, however, above the critical temperature of the euthyroid animals; these latter continued to show hypothermia after CPZ, though this was not so severe as that seen at lower temperatures.

At 30° C the critical temperature of the euthyroid mice was reached and treatment with CPZ at this ambient temperature produced no significant hypothermia.

Thus, the results obtained here can be explained on the basis of critical temperature. The hyperthyroid mice have a higher BMR than the euthyroid animals so that their heat production is higher and their critical temperature lower than that of their euthyroid counterparts.

Lessin and Parkes (1957) suggested that any agent producing hypothermia would predispose an animal to sedation. They claimed that unless hypothermia occurred CPZ or reserpine would not depress the SLA of mice. The results presented here are not in agreement with theirs since the SLA of the euthyroid mice was reduced by the drug in the absence of hypothermia at 30° C. That of the hyperthyroid mice was reduced in the presence of a concomitant hyperthermia at 22 and 26° C. These results indicate that the phenomena of hypothermia and sedation are separable. Rather than sedation depending on the presence of hypothermia, these results suggest that hypothermia (under proper conditions) is caused by loss of temperature control under circumstances when heat production is reduced.

SUMMARY

1. At an environmental temperature of 20° C thyroxine-induced hyperthyroidism in male TO mice renders the animals significantly less sensitive to the hypothermic effects of chlorpromazine than euthyroid animals.

2. At ambient temperatures of 22° C and 26° C, whilst the euthyroid mice respond to chlorpromazine with a significant hypothermia, the hyperthyroid mice show hyperthermia.

3. At an ambient temperature of 30° C, chlorpromazine is without significant effect on the core temperature of euthyroid mice.

5. Inspite of the differences in response to chlorpromazine at 22° C both the hyperthyroid and the euthyroid mice show an increase in skin temperature following treatment with this drug.

6. Chlorpromazine reduces the SLA of both hyperthyroid and euthyroid mice. This effect is largely independent of the temperature response of the animals.

TABLE 22.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCI 10 mg/kg, at an ambient temperature of 22° C. Control animals (HT. C and ET. C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means of groups of 10 animals, ° C ± S. E.

TIME (min)	HT	HT-C	ET	ET-C
0	38.0 ± 0.2		36.4 ± 0.3	
30	38.8 ± 0.3		34.0 ± 0.4	
60	39.2 ± 0.3	38.3 ± 0.2	32.4 ± 0.4	37.4 ± 0.2
120	39.5 ± 0.4	38.1 ± 0.1	33.0 ± 0.7	36.8 ± 0.3
180	39.3 ± 0.4	38.2 ± 0.2	32.4 ± 1.1	37.1 ± 0.3

FIGURE 22.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 10 mg/kg at an ambient temperature of 22°C . Control animals (HT-C and ET-C respectively) received an euqivalent volume of normal saline by the same route (see Table 22).

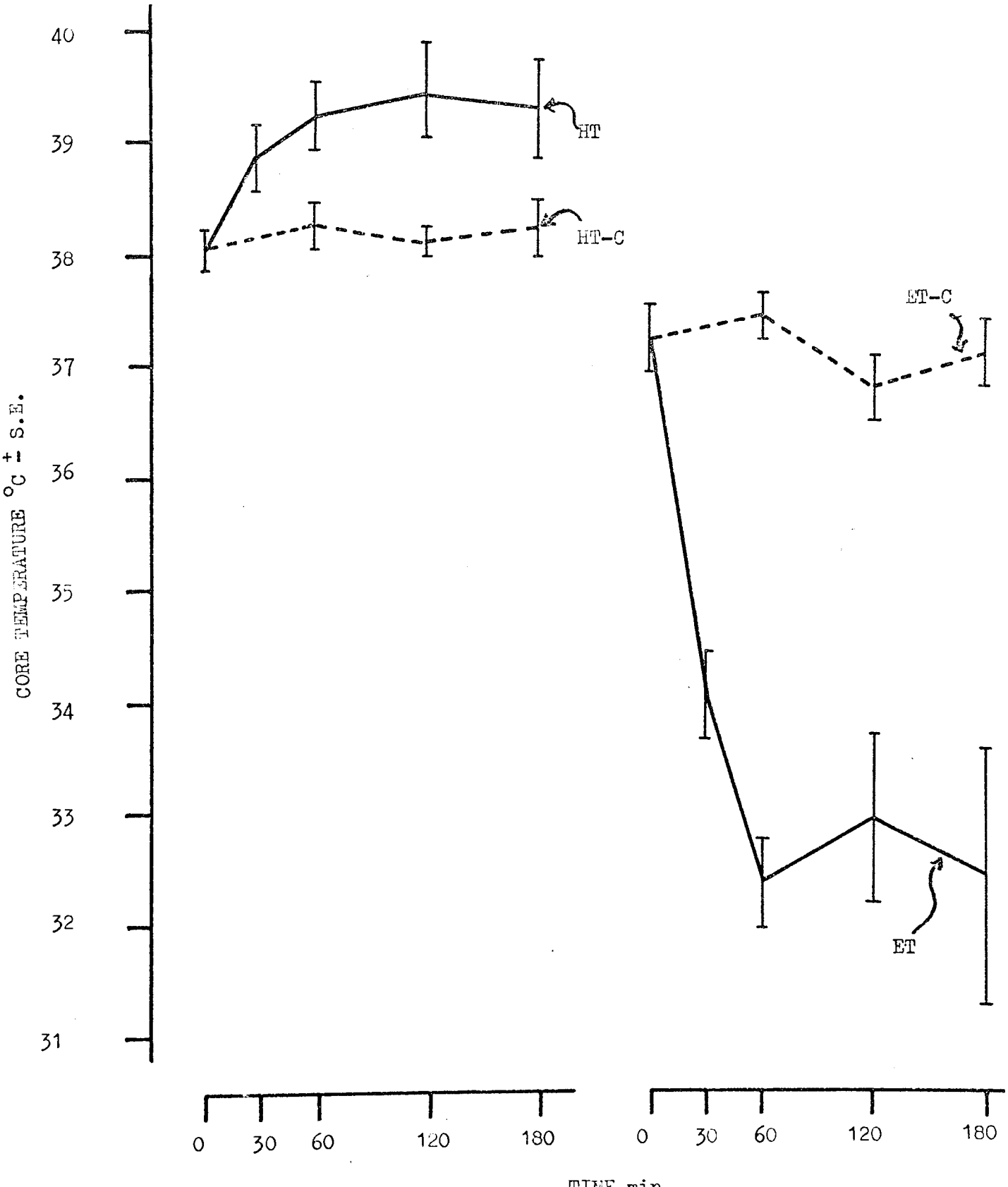


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TABLE 23

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCL 10 mg/kg at an ambient temperature of 20° C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as means of groups of 5 animals (°C ± S. E.)

Time (min)	HT	HT-C	ET	ET-C
0	37.6 ± 0.4		37.0 ± 0.2	
60	34.5 ± 0.9	37.4 ± 0.2	30.4 ± 1.2	36.9 ± 0.3
120	33.2 ± 0.9	37.8 ± 0.1	27.9 ± 1.5	37.1 ± 0.2
150	34.0 ± 1.6		26.9 ± 1.5	
210	34.3 ± 2.0	37.9 ± 0.3	27.1 ± 1.5	36.7 ± 0.2
300	34.1 ± 1.4	37.6 ± 0.1	27.5 ± 1.7	36.8 ± 0.3

FIGURE 24.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 10 mg/kg at an ambient temperature of 20°C. Control animals (HT-C and ET-C respectively)received an equivalent volume of normal saline by the same route (see Table 23).

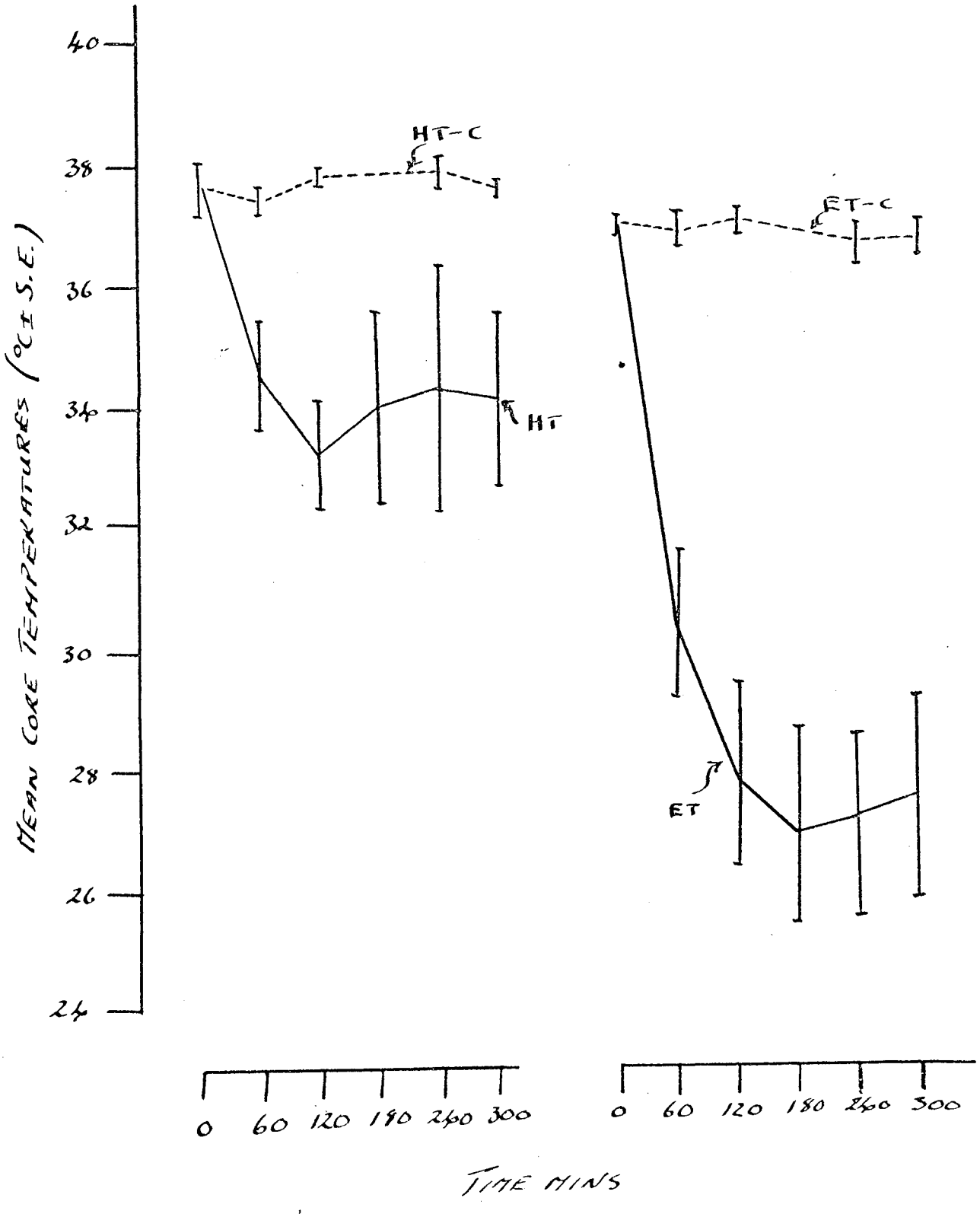


TABLE 24

Core temperatures in hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCI 5 mg/kg, at an ambient temperature of 20° C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means of groups of 5 animals, °C ± S. E.

TIME (min)	HT	HT-C	ET	ET-C
0	37.5 ± 0.1		36.4 ± 0.3	
30	37.0 ± 0.6	37.6 ± 0.1	34.4 ± 0.3	37.4 ± 0.5
60	35.6 ± 1.0	37.7 ± 0.3	32.8 ± 0.5	37.3 ± 0.3
90	36.1 ± 1.1		32.3 ± 0.9	
120	36.7 ± 0.9	38.1 ± 0.3	32.0 ± 1.1	37.6 ± 0.4
150	37.7 ± 0.6		31.7 - 1.0	
180	37.5 ± 0.2	38.1 ± 0.1	31.9 ± 1.1	37.3 ± 0.3
240	37.5 ± 0.3	37.8 ± 0.2	32.3 ± 1.3	37.5 - 0.5

FIGURE 23.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 5 mg/kg at an ambient temperature of 20°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route (see Table 24)

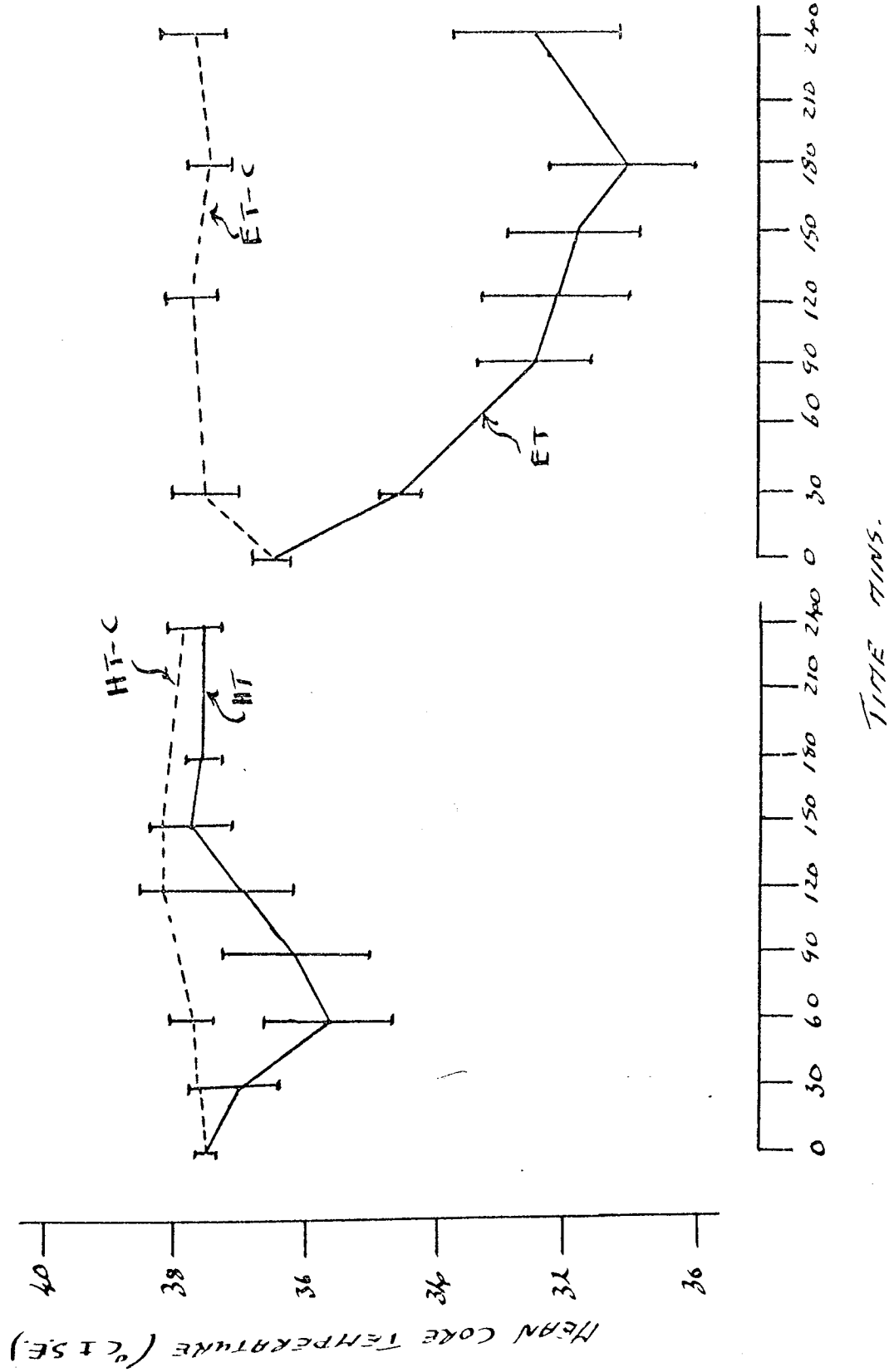


TABLE 25.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCl, 5 mg/kg, at an ambient temperature of 26.5°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means of groups of 5 animals, °C ± S.E.

TIME (min)	HT	HC	ET	EC
0	39.4 ± 0.1		38.7 ± 0.2	
30	41.0 ± 0.2	39.7 ± 0.2	38.6 ± 0.3	39.3 ± 0.1
60	41.3 ± 0.3	39.8 ± 0.2	38.0 ± 0.2	39.2 ± 0.2
90	41.5 ± 0.3	39.9 ± 0.2	37.3 ± 0.2	38.6 ± 0.2
120	41.3 ± 0.3	39.9 ± 0.2	37.0 ± 0.2	38.9 ± 0.2

FIGURE 25.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 5 mg/kg at an ambient temperature of 26.5°C Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route (see Table25).

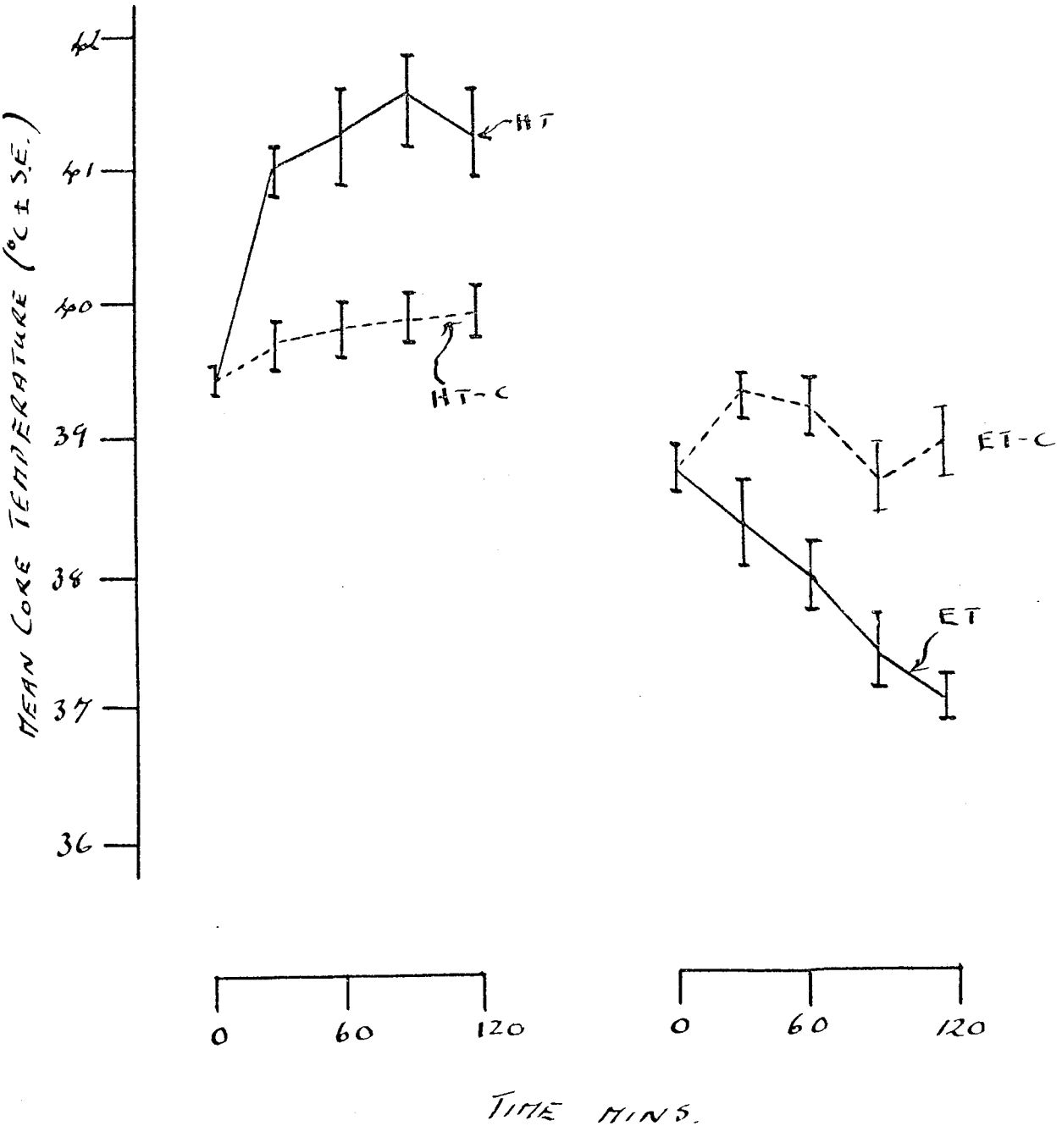


TABLE 26.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine (HCI, 10 mg/kg at an ambient temperature of 26.5° C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means of groups of 5 animals, °C ± S.E.

TIME (min)	HT	HT-C	ET	ET-C
0	39.4 ± 0.1		38.7 ± 0.2	
30	41.3 ± 0.2	39.7 ± 0.2	38.3 ± 0.3	39.3 ± 0.1
60	41.7 ± 0.2	39.8 ± 0.2	38.0 ± 0.3	39.2 ± 0.2
90	41.9 ± 0.3	39.9 ± 0.2	37.6 ± 0.4	38.6 ± 0.2
120	41.9 ± 0.6	39.9 ± 0.2	37.3 ± 1.0	38.9 ± 0.2

FIGURE 26.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 10 mg/kg at an ambient temperature of 26.5°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route (see Table 26).

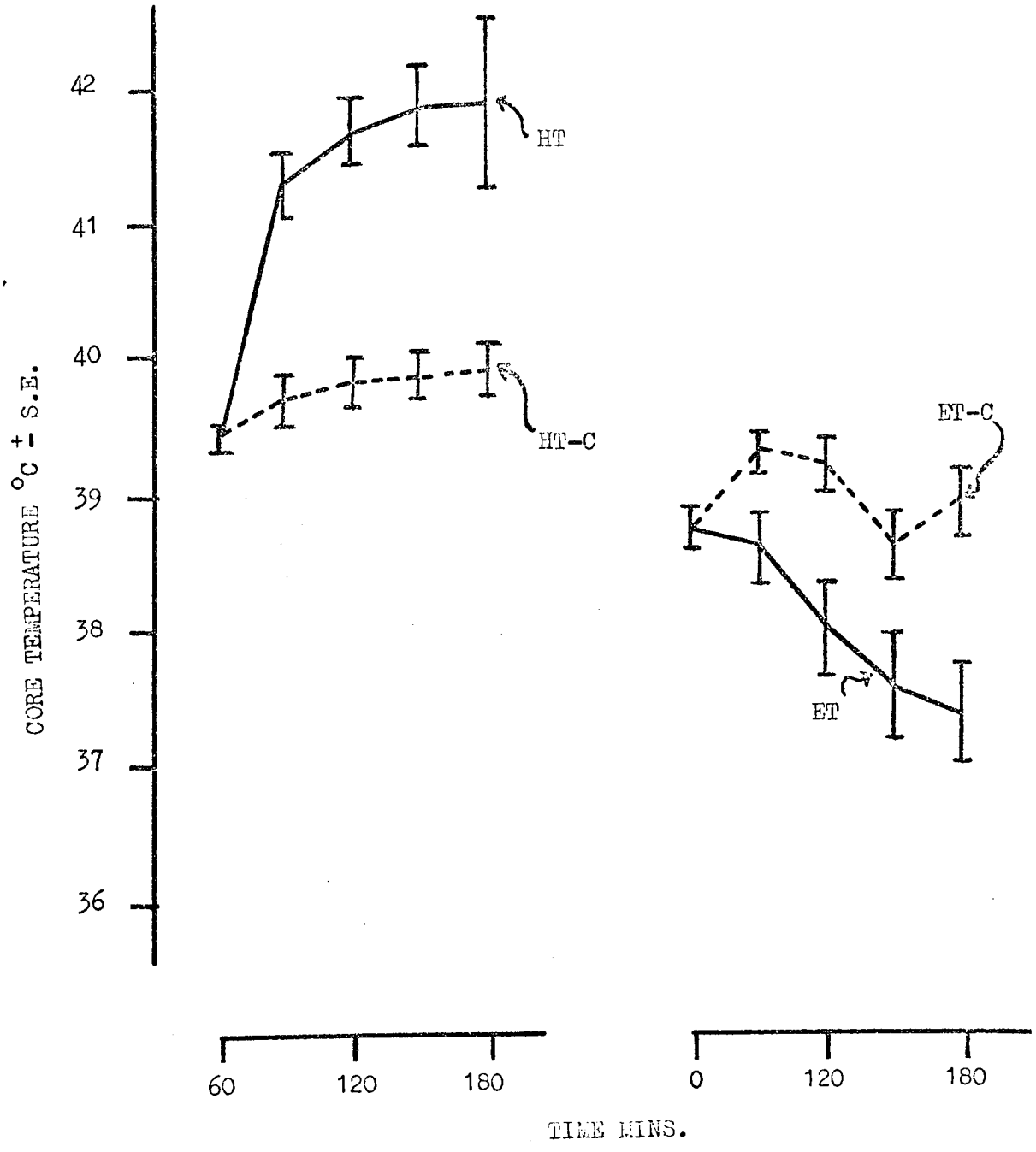


TABLE 27.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCl, 5 mg/kg at an ambient temperature of 20° C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means of 5 animals, °C ± S.E.

TIME (min)	HT	HT-C	ET	ET-C
0	38.4 ± 0.1		38.1 ± 0.2	
30	38.0 ± 0.3	38.4 ± 0.2	35.7 ± 0.7	37.7 ± 0.3
60	37.7 ± 0.4	38.2 ± 0.3	34.7 ± 0.7	37.6 ± 0.2
90	37.3 ± 0.4	38.3 ± 0.2	34.4 ± 0.8	37.4 ± 0.3
120	37.3 ± 0.5	39.1 ± 0.3	34.5 ± 1.0	37.8 ± 0.2
150	37.4 ± 0.5	39.1 ± 0.2	35.1 ± 0.6	37.5 ± 0.2
180	37.3 ± 0.5	39.0 ± 0.3	35.3 ± 0.9	37.7 ± 0.2
210	37.2 ± 0.3	38.9 ± 0.3	35.8 ± 0.8	37.4 ± 0.2
240	37.4 ± 0.4	38.6 ± 0.4	35.9 ± 0.9	37.5 ± 0.4

FIGURE 27.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 5 mg/kg at an ambient temperature of 20°C. Control animals (HT-Cand ET-C respectively) received an equivalent volume of normal saline by the same route (see Table 27)

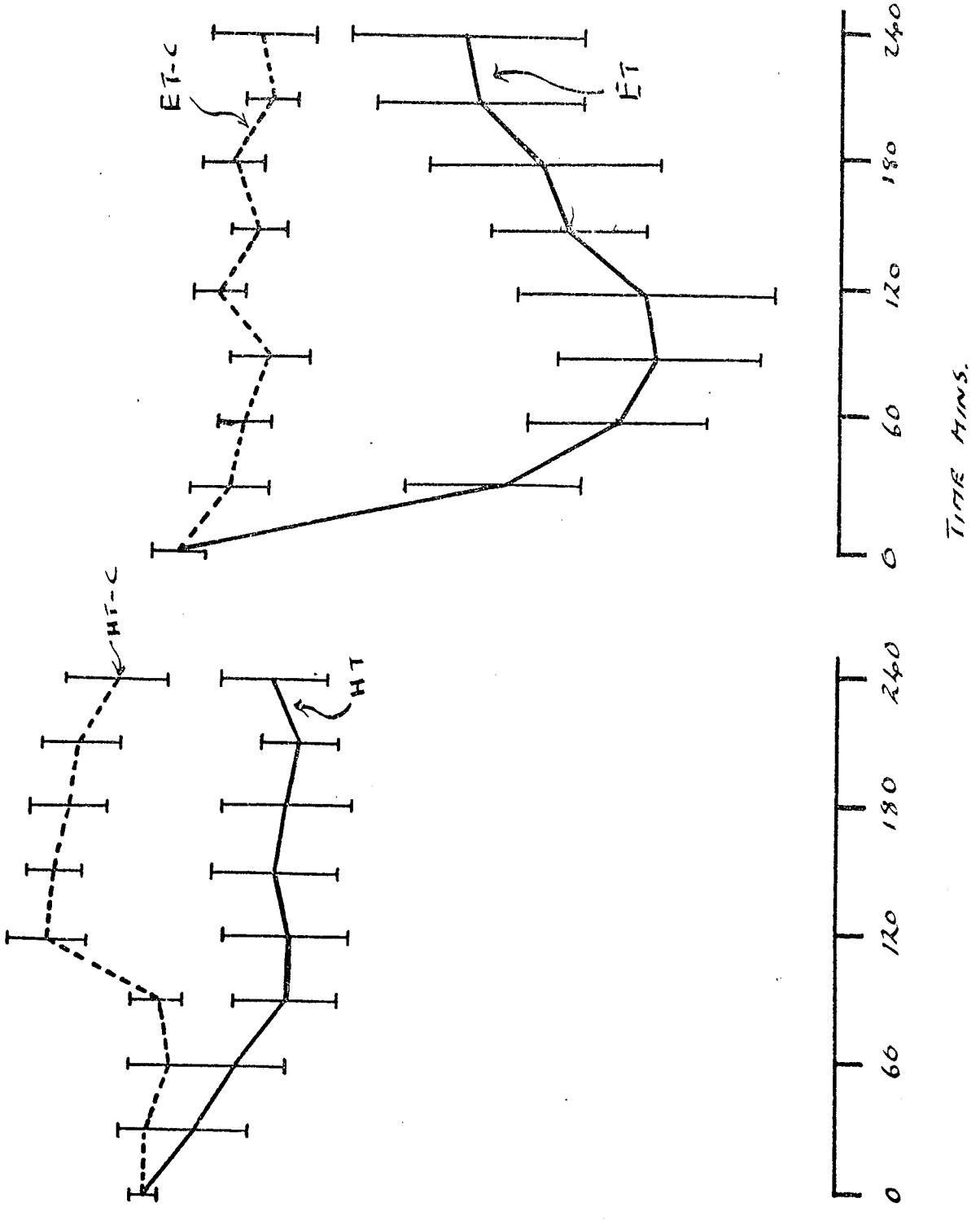


TABLE 28.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCl, 5 mg/kg, at an ambient temperature of 22°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means of groups of 5 animals, °C ± S.E.

TIME (min)	HT	HT-C	ET	ET-C
0	38.4 ± 0.1		37.1 ± 0.3	
30	38.5 ± 0.2	37.9 ± 0.1	34.1 ± 0.4	37.3 ± 0.4
60	38.4 ± 0.1	37.9 ± 0.1	33.3 ± 0.6	37.0 ± 0.1
120	38.9 ± 0.4	38.0 ± 0.1	32.9 ± 0.9	37.2 ± 0.1
180	37.7 ± 0.2	38.1 ± 0.1	32.7 ± 1.2	37.5 ± 0.3

FIGURE 28.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 5 mg /kg at an ambient temperature of 22°C, Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route (see Table 28)

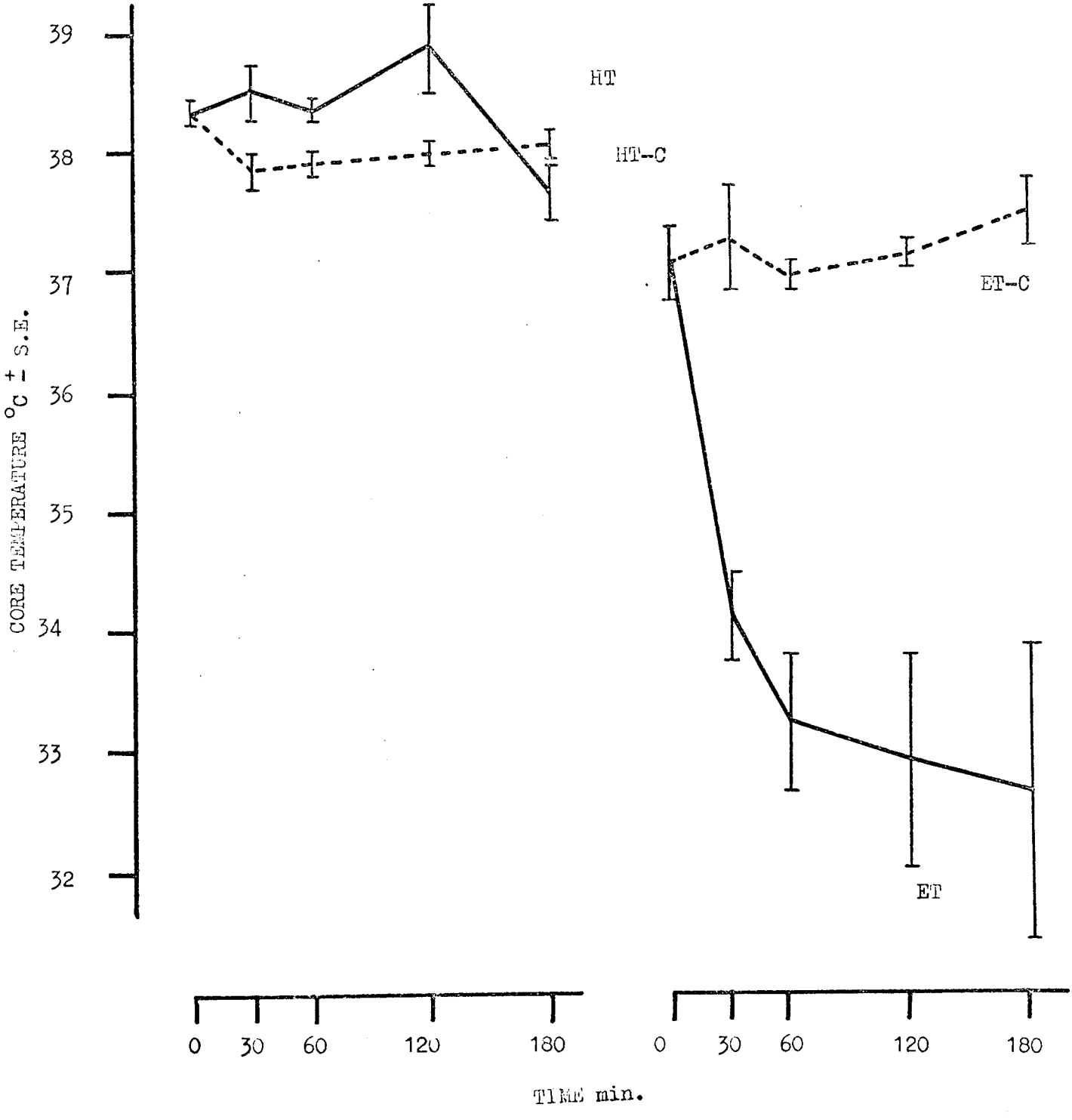


TABLE 29

Skin temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCL 5 mg/kg at an ambient temperature of 22°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means of groups of 10 animals (°C ± S.E.).

Time (min)	HT	HT-C	ET	ET-C
0	28.0 ± 0.4		26.1 ± 0.2	
30	33.5 ± 0.5	27.1 ± 0.3	30.7 ± 0.2	26.7 ± 0.2
60	33.0 ± 0.6	28.8 ± 0.2	29.7 ± 0.3	26.7 ± 0.2
120	32.9 ± 0.5	27.9 ± 0.3	29.1 ± 0.5	26.8 ± 0.3
180	30.6 ± 0.5	27.8 ± 0.3	28.1 ± 0.5	26.9 ± 0.3

FIGURE 29.

Skin temperatures of hyperthyroid (HT) and euthyroid(ET) male TO mice after intraperitoneal injection of chlorpromazine 5 mg/kg at an ambient temperature of 22°C. Control animals (HT-Cand ET-C respectively) received an equivalent volume of normal saline by the same route. (see Table 29).

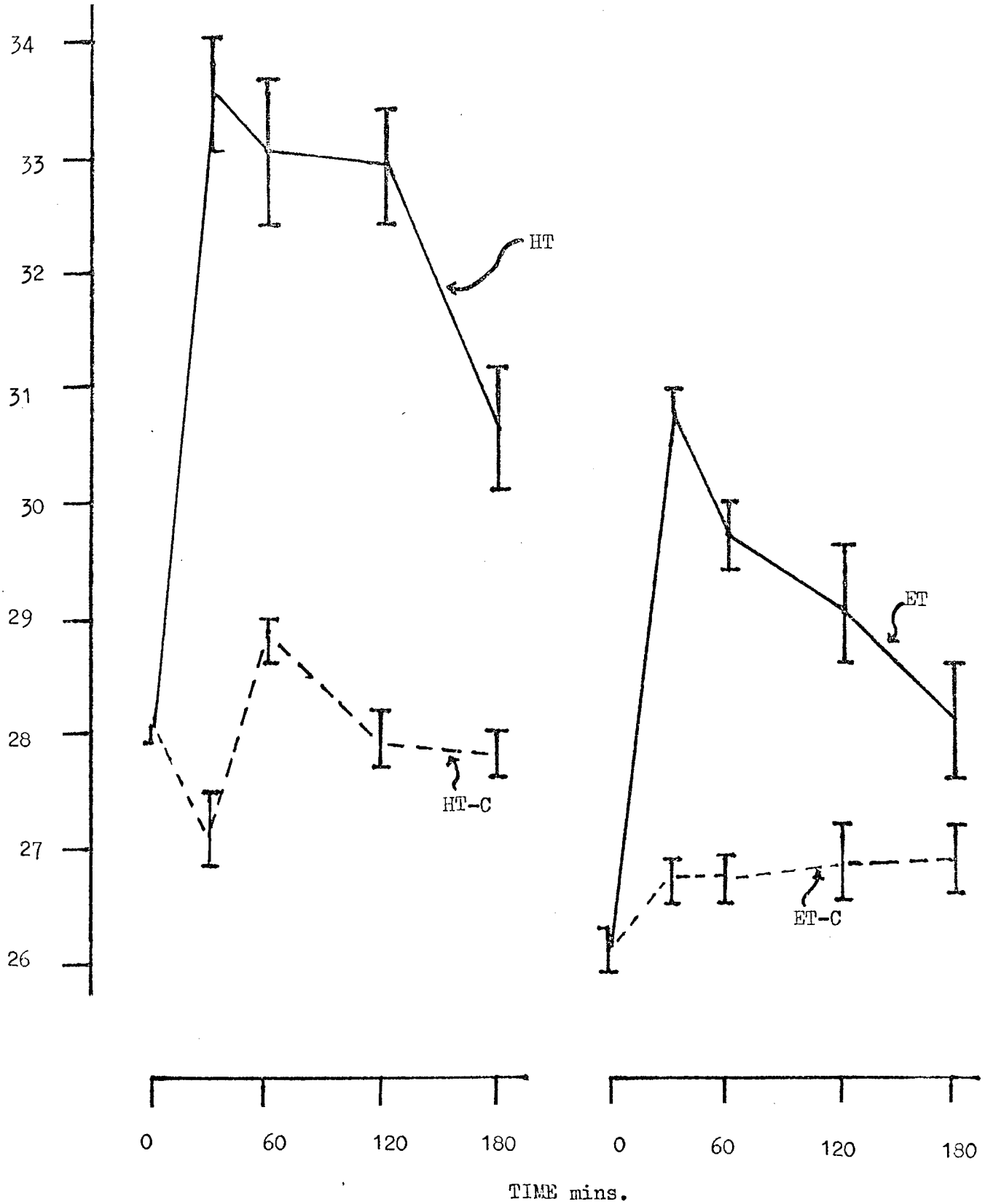


TABLE 30

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCL 5 mg/kg at an ambient temperature of 26°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means of groups of 10 animals (°C ± S.E.)

Time (min)	HT	HT-C	ET	ET-C
0		38.4 ± 0.1		37.4 ± 0.2
60	41.2 ± 0.2	38.1 ± 0.1	37.8 ± 0.2	36.0 ± 0.2
120	40.1 ± 0.7	38.1 ± 0.1	37.8 ± 0.2	35.2 ± 0.3
180	38.4	37.8 ± 0.2	37.6 ± 0.2	34.7 ± 0.2

FIGURE 30.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 5 mg/kg at an ambient temperature of 26°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route (see Table 30)

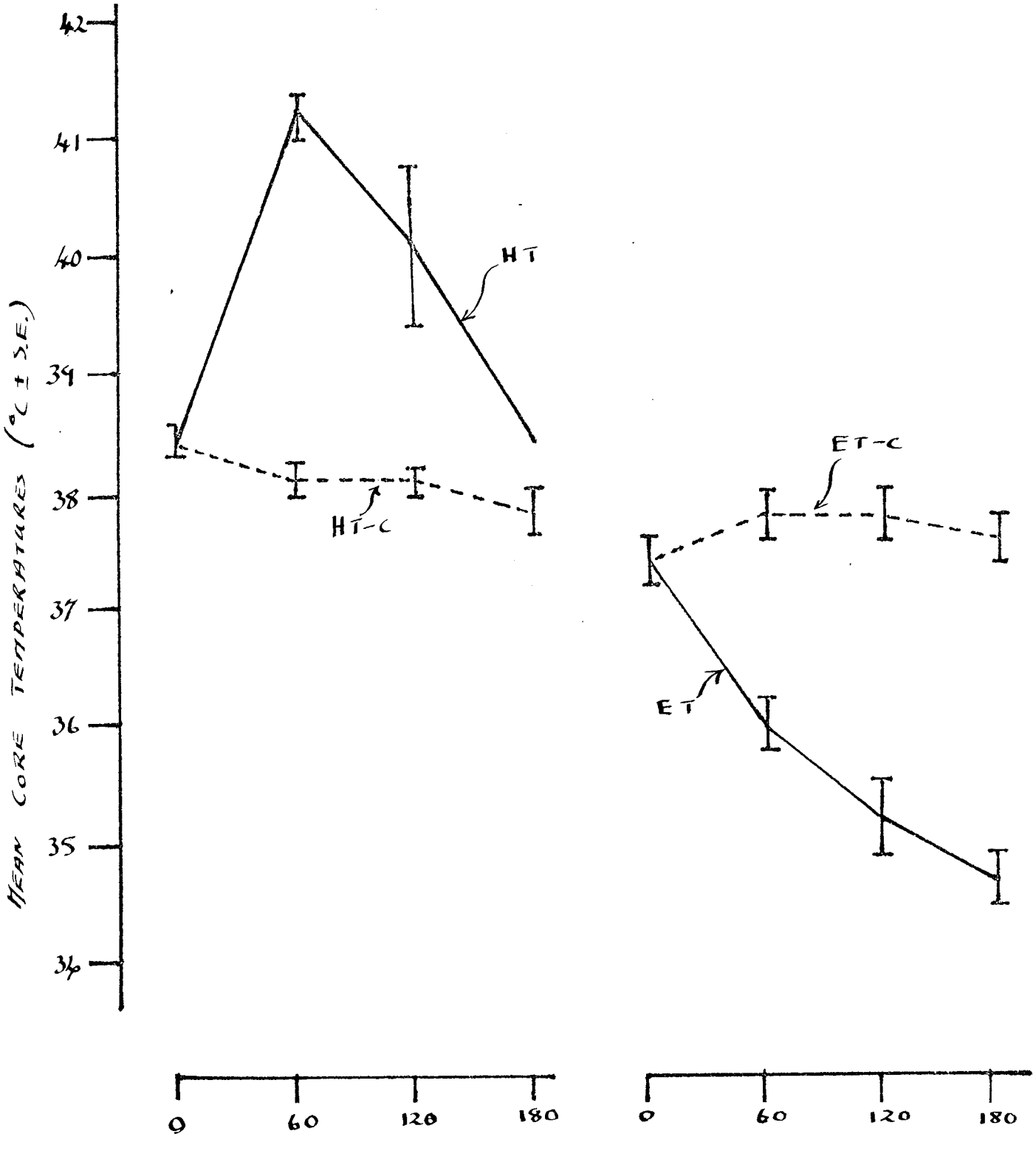


TABLE 31

Core temperatures of euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCL 5 mg/kg at an ambient temperature of 30°C. Control animals (ET-C) received an equivalent volume of normal saline by the same route. The results are expressed as the means of groups of 10 animals(°C S.E.)

Time (min)	ET	ET-C
0	38.1 ± 0.1	
60	37.6 ± 0.3	37.6 ± 0.1
120	37.3 ± 0.2	37.7 ± 0.1
180	37.6 ± 0.1	37.9 ± 0.1

FIGURE 31.

Core temperatures of euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 5 mg/kg at an ambient temperature of 22° or 30°C. Control animals (ET-C) received an equivalent volume of normal saline b the same route (see Table 31).

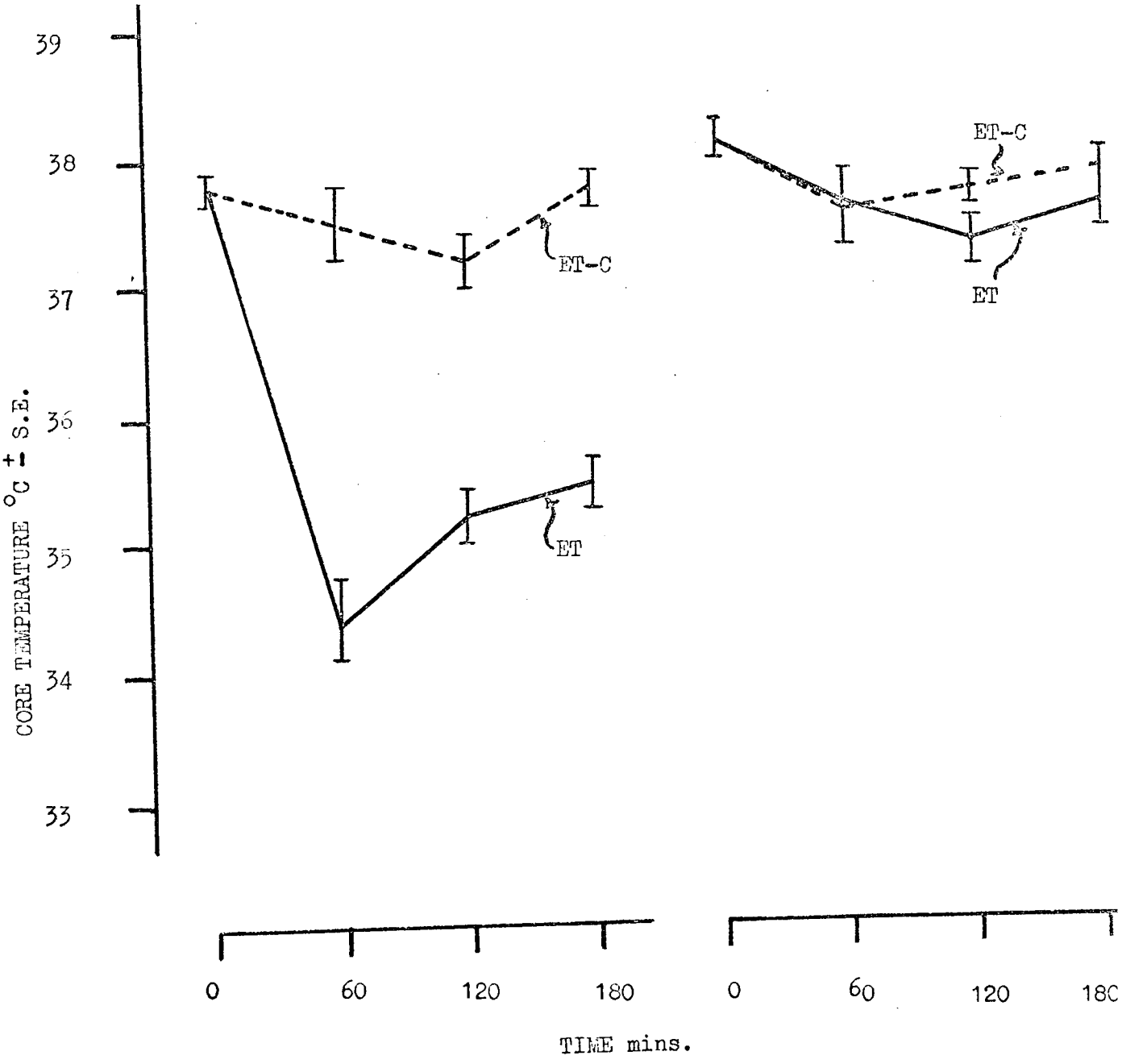


TABLE 32.

Spontaneous locomotor activity of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCl, 5 mg/kg, at an ambient temperature of 22° C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results represent 10 minute counts obtained from groups of 5 animals.

TIME (min)	HT	HT-C	ET	ET-C
10	318	441	644	444
20	34	309	123	433
30	39	483	13	813
40	69	333	24	599
50	50	255	0	593
60	35	123	8	451
70	35	136	0	644
80	25	143	0	322
90	20	140	0	324
100	25	132	0	324
110	14	97	0	128
120	0	100	0	311
130	12	70	2	164
140	11	45	0	55
150	49	63	0	12
160	53	66	0	5
170	2	23	20	20
180	72	65	74	23

FIGURE 32.

Spontaneous locomotor activity of hyperthyroid (HT) male TO mice after intraperitoneal injection of chlorpromazine 5 mg/kg at an ambient temperature of 22°C. Control animals (HT-C) received an equivalent volume of normal saline by the same route(see Table 32).

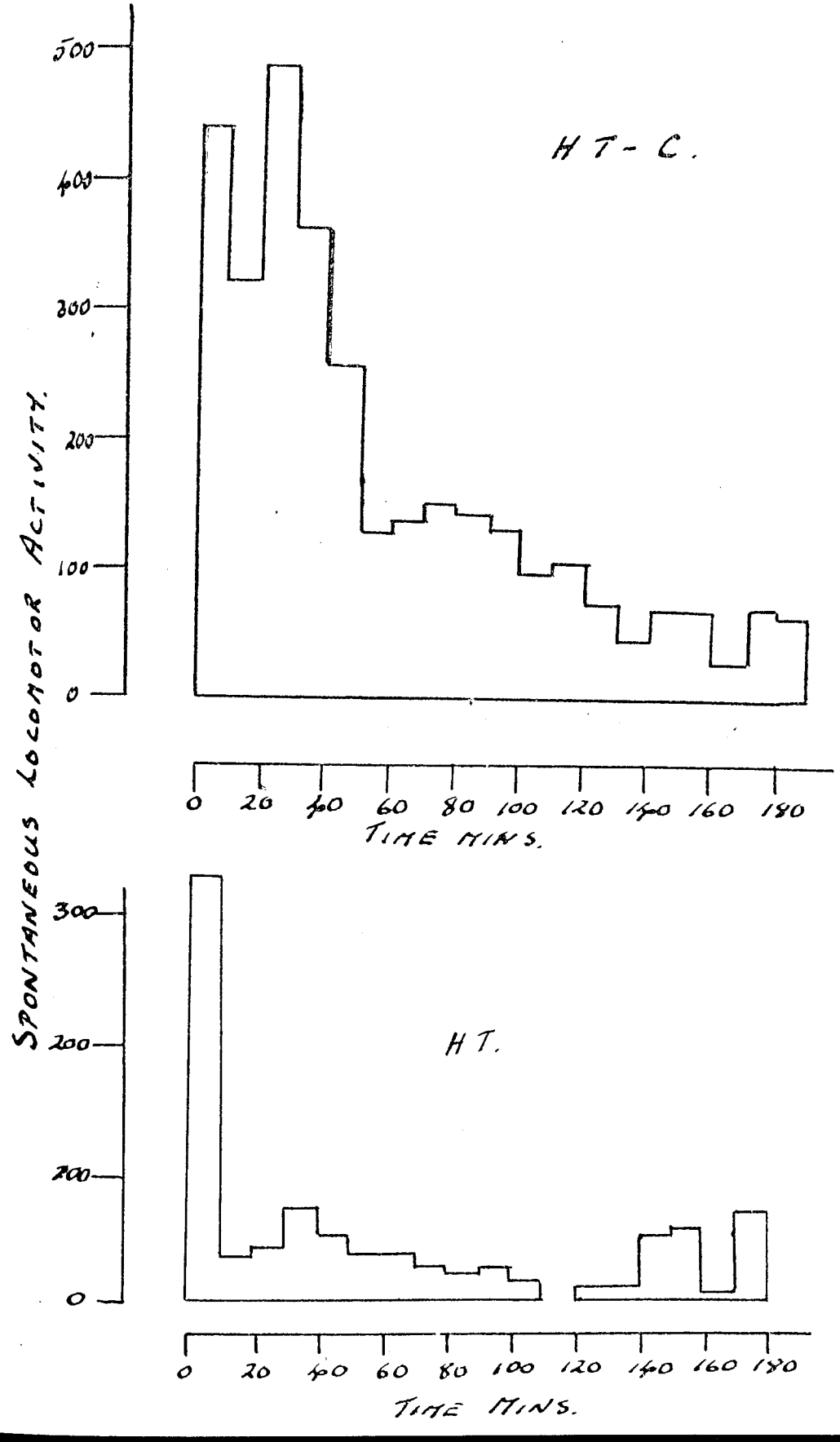


FIGURE 33.

Spontaneous locomotor activity of euthyroid (ET) male TO mice after intravenous injection of chlorpromazine 5 mg/kg at an ambient temperature of 22 °C. Control animals (ET-C) received an equivalent volume of normal saline by the same route (see Table 42).

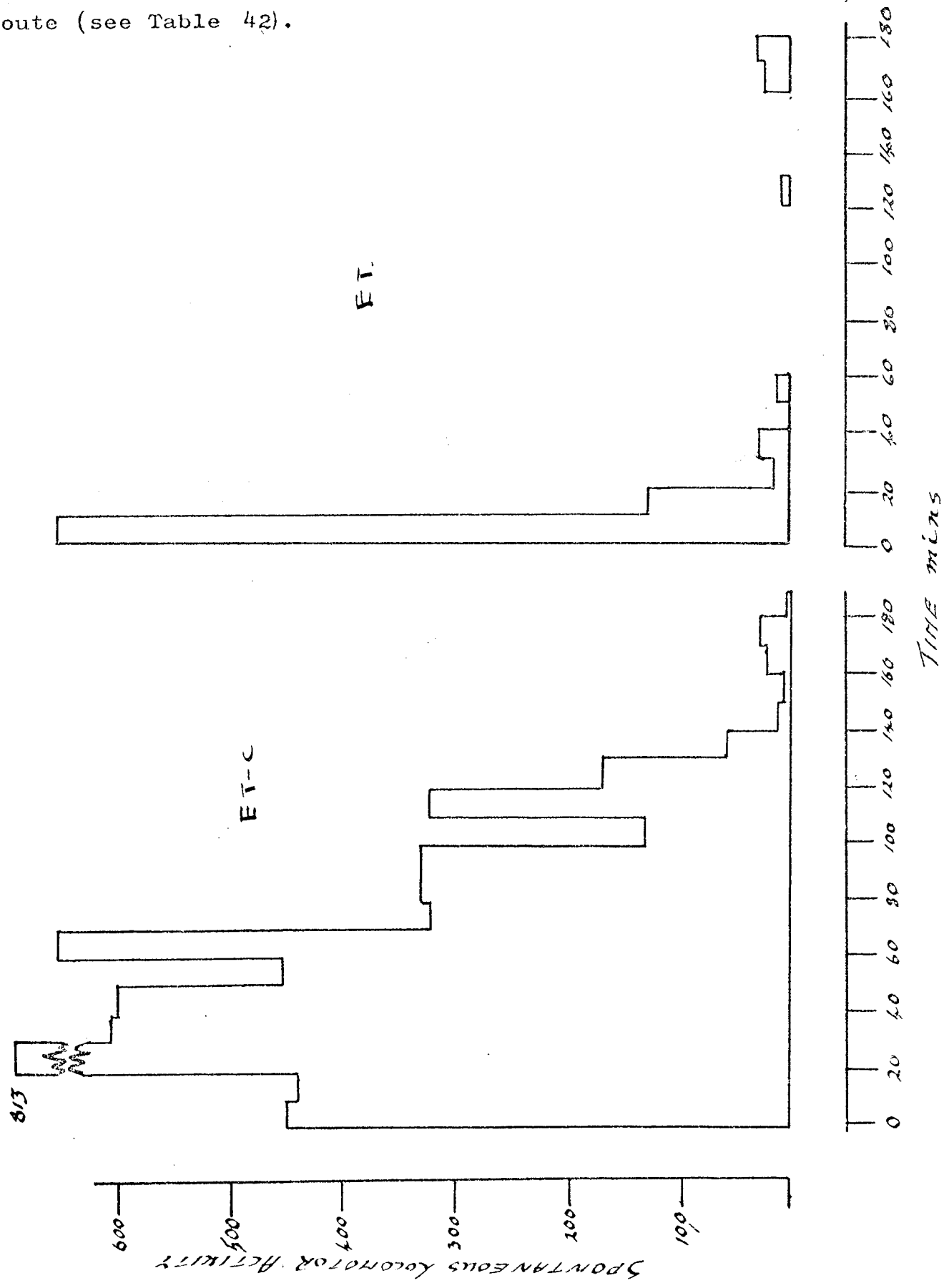


TABLE 33.

Spontaneous locomotor activity of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCl, 5 mg/kg, at an ambient temperature of 26° C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results represent 10 minute counts obtained from groups of 5 animals.

TIME (min)	HT	HT-C	ET	ET-C
10	109	358	149	597
20	32	200	32	420
30	14	330	14	330
40	11	290	11	380
50	1	272	0	364
60	4	87	0	324
70	8	150	0	307
80	8	75	0	96
90	5	292	0	90
100	9	84	10	24
110	4	122	8	55
120	5	97	0	12
130	27	87	0	24
140	17	46	2	14
150	7	113	9	65
160	5	27	0	11
170	57	84	2	3
180	73	113	61	3

FIGURE 34.

Spontaneous locomotor activity of hyperthyroid (HT) male TO mice after intraperitoneal injection of chlorpromazine 5 mg/kg at an ambient temperature of 26°C. Control animals (HT-C) received an equivalent volume of normal saline by the same route (see Table 33).

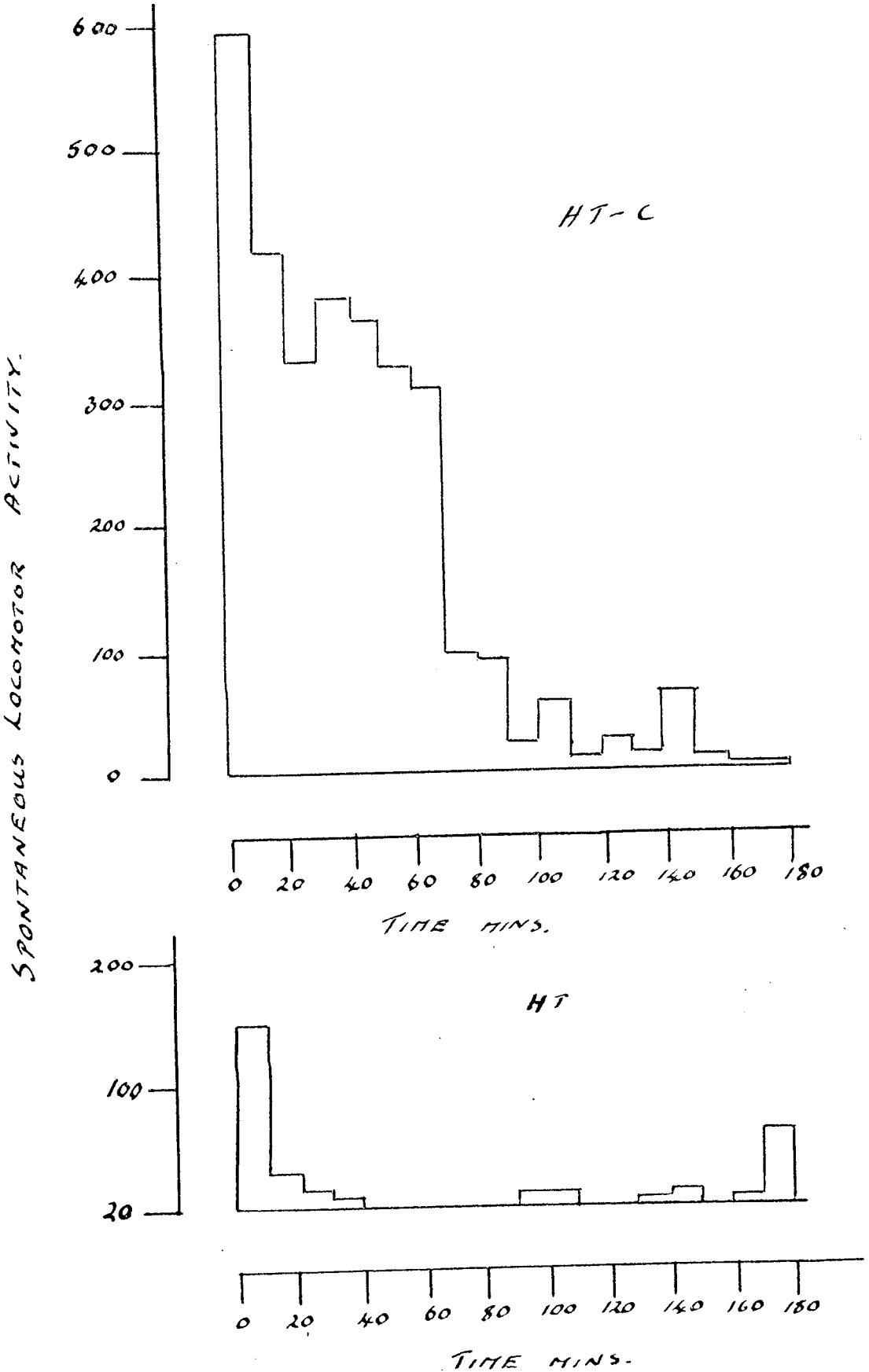


FIGURE 35.

Spontaneous locomotor activity of euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 5 mg/kg at an ambient temperature of 26°C. Control animals (ET-C) received an equivalent volume of normal saline by the same route (see Table 33).

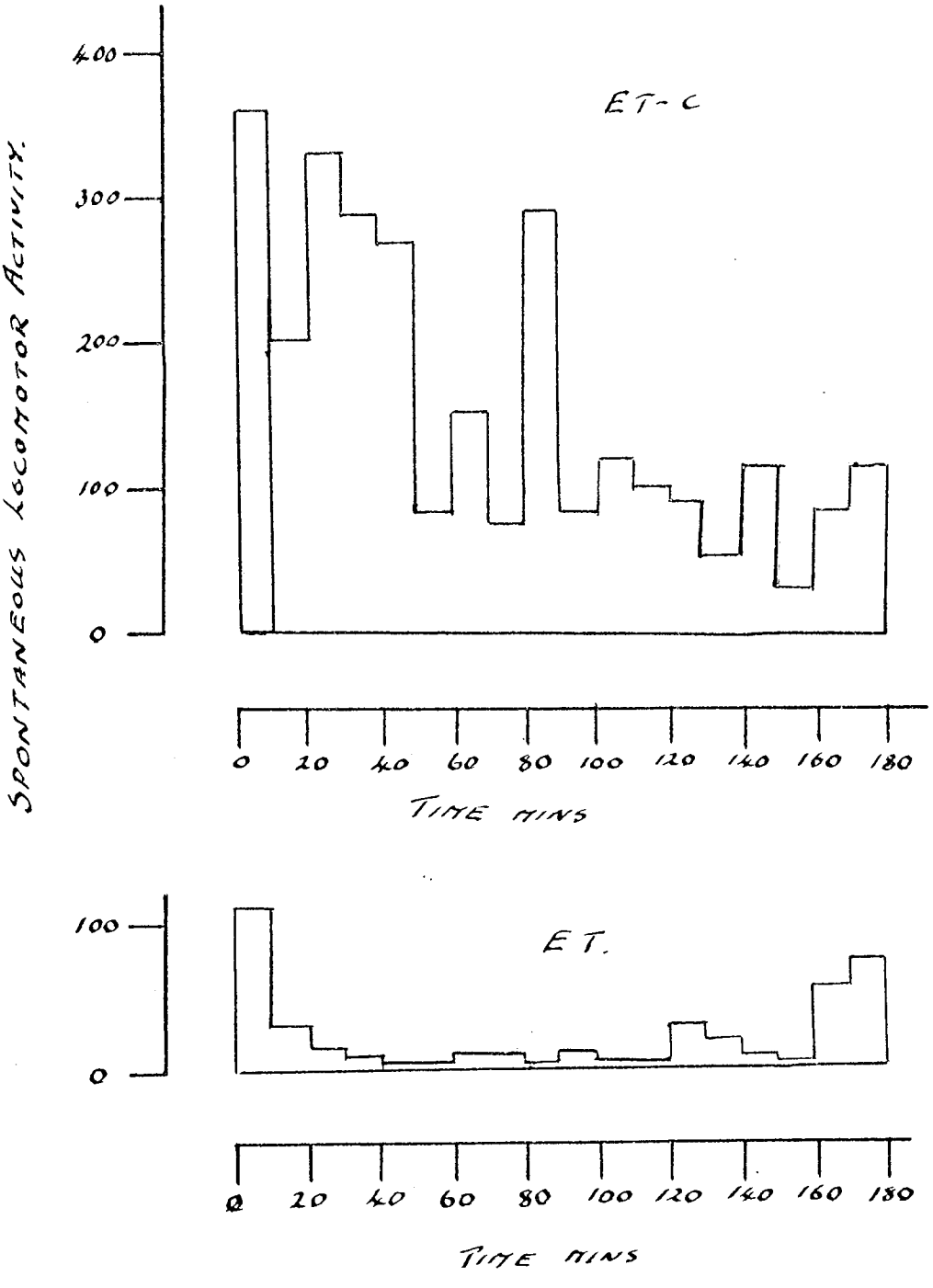


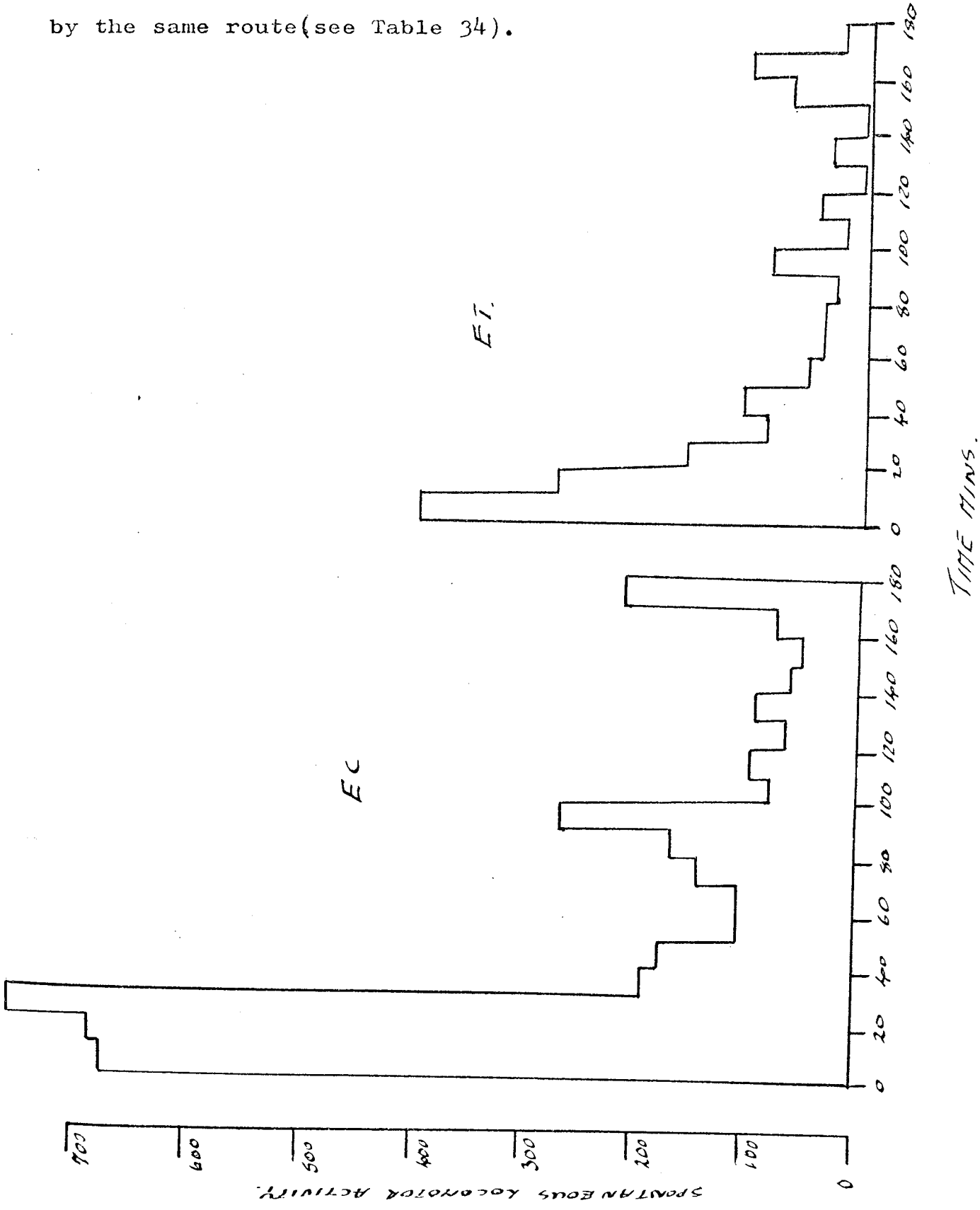
TABLE 34.

Spontaneous locomotor activity of euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine HCl, 5 mg/kg, at an ambient temperature of 30° C. Control animals (ET-C) received an equivalent volume of normal saline by the same route. The results represent 10 minute counts obtained from groups of 5 animals.

TIME (min)	ET	EC
10	397	669
20	275	682
30	161	756
40	88	192
50	110	176
60	51	107
70	39	107
80	42	140
90	28	155
100	85	266
110	21	79
120	44	93
130	4	63
140	35	89
150	2	58
160	68	50
170	106	74
180	25	207

FIGURE 36.

Spontaneous locomotor activity of euthyroid (ET) male TO mice after intraperitoneal injection of chlorpromazine 5 mg/kg at an ambient temperature of 30°C. Control animals (ET-C) received an equivalent volume of normal saline by the same route(see Table 34).



CHAPTER IV

THE EFFECTS OF RESERPINE IN HYPERTHYROID AND EUTHYROID MICE

CHAPTER IV.

THE EFFECTS OF RESERPINE IN HYPERTHYROID AND EUTHYROID MICE

1. Introduction

Reserpine, like chlorpromazine, is known to produce marked central depressive effects, including reduced motor activity and hypothermia in the mouse (Mueller & McDonald, 1968). It was decided to investigate the properties of this drug in hyperthyroid and euthyroid mice to reveal any differences in the responses of the two types of animals to the hypothermic effects of the drug, similar to those seen with chlorpromazine (reported in Chapter III).

In a preliminary experiment mice of both groups were treated with reserpine 1 mg/kg intravenously or with an equivalent volume of vehicle (controls) and oesophageal temperatures were recorded at 1 hr. intervals. The results of this experiment, which was carried out at a laboratory temperature of 22° C, are illustrated in Fig. 37 (Table 35). The graph shows that, at this temperature, both groups responded to the drug with a hypothermia, but, as with chlorpromazine, the response of the euthyroid mice was much more profound than that of the hyperthyroid animals. The latter showed a mild hypothermia which reached a maximum of 1.5° C after 4 hr. whilst their euthyroid counterparts showed a much more marked hypothermia of 4.3° C after 5 hr.

Thus, although both groups of animals showed a hypothermia after reserpine administration, it seemed possible that a qualitative difference in the response, similar to that seen after chlorpromazine, was present.

In order to investigate further this phenomenon, a series of experiments were undertaken to observe the effects of the reserpine on core and skin temperatures and spontaneous locomotor activity (SLA) in hyperthyroid and euthyroid mice at a variety of ambient temperatures.

2. Effect of environmental temperatures on the response of hyperthyroid and euthyroid mice to reserpine.

In all these experiments a dose of 0.5 mg/kg of reserpine was administered, and the effects on core temperatures & SLA determined at 22° C, 26° C and 30° C. The effects of the drug at this dose on the skin temperatures of hyperthyroid and euthyroid mice was also determined at an environmental temperature of 22° C.

a/Effects on body temperature.

i. Reserpine 0.5 mg/kg at 22° C.

The effects of reserpine on core temperature at this ambient temperature are shown in Fig 38 (Table 36). At this temperature there was little difference in the hypothermic response from that produced by 1 mg/kg at the same environmental temperature. The hyperthyroid mice suffered a mild hypothermia of 1° C reaching a peak at 3 hr., thereafter the temperatures returned towards the control level. In contrast the euthyroid animals showed a much more profound hypothermia of 4.6° C at 5 hr.

As another parameter of reserpine's activity, its effects on skin temperature were also recorded at 22° C (see Fig. 39; Table 37). Both groups of mice responded to the drug with a rise in skin temperature, and, as with chlorpromazine earlier, in spite of a smaller hypothermia the

hyperthyroid mice underwent a larger rise in skin temperature than did the euthyroid animals. The skin temperatures of the reserpine treated hyperthyroid mice reached a peak level of 2.4° C above control values after 2 hrs, whilst the euthyroid mice treated with the drug reached a peak skin temperature of 2° C above control values after 3 hrs. The high skin temperature of the hyperthyroid mice was also better maintained than that of the euthyroid animals, the former being 1.5° C above control values at 5 hrs and the latter being 0.7° C above control values at that time.

ii. Reserpine 0.5 mg/kg at 26° C.

The results of this experiment are shown in Fig 40 (Table 38). At this higher environmental temperature the effect of the drug on the core temperatures of hyperthyroid mice was reversed, the animals showing a mild hyperthermia of 1.5° C after 3 hr. The extreme sensitivity of hyperthyroid mice to hyperthermia was again demonstrated in this experiment. By the fifth hour after administration of reserpine, 60% of these animals had died from heat stroke; thus the results shown for 4 hr. and 5 hr. are probably not a true reflection of the intense hyperthermia which these mice suffered. The euthyroid mice continued to respond to the drug with a marked hypothermia at this temperature, in this case reaching 5.8° C after 5 hr.

iii. Reserpine 0.5 mg/kg at 30° C.

As with chlorpromazine, the increased toxicity of reserpine in hyperthyroid mice at this temperature precluded the use of such animals in this experiment. (Fig. 41 (Table 39), contrasts the effects of the drug on the core temperatures of euthyroid mice at 22° and 30° C, showing, as with chlorpromazine, the dependance of this response on environmental

temperature. At the lower temperature the hypothermia was of 4°C. after 5 hr. whereas exposures to 30°C largely inhibited the hypothermia (1.5°C after 5 hr.).

b/Effects on spontaneous locomotor activity (SLA).

These experiments, carried out in conjunction with those reported above, were designed to investigate the relationship between the behavioural and thermoregulatory responses of mice to this drug.

i SLA after reserpine 0.5 mg/kg at 22°C.

Inspite of the difference in magnitude of thermal response shown by the two groups the SLA of both was markedly reduced by treatment with reserpine at this temperature. The hyperthyroid animals (see Fig.42 Table40) were more affected than the euthyroid mice (see Fig.43 Table40) eventhough, as noted before (Chapter 4), their normal activity was less than that of their euthyroid counterparts.

ii SLA after reserpine 0.5 mg/kg at 26°C.

Fig.44 &45(Table 41) illustrate the results of these experiments. Once again the SLA of both groups of animals was markedly reduced by the drug, inspite of the fact that it had a completely opposite effect on the core temperature of the two groups at this high ambient temperature.

iii SLA after reserpine 0.5 mg/kg at 30°C

At 30°C (Fig.46Table42) the SLA of the euthyroid mice was again markedly reduced by the drug, this occurring with only a very mild degree of concomitant hypothermia. It should be noted that, despite differences in the normal locomotor activities of the animals of both groups at different temperatures, the proportional reduction of SLA drug treated: untreated was of the same order throughout for the hyperthyroid and euthyroid animals.

3. Effect of pretreatment with a monoamine oxidase inhibitor on the response of hyperthyroid and euthyroid mice to reserpine.

Pretreatment of mice with monoamine oxidase inhibitors is known to antagonise or reverse the behavioural and thermoregulatory effects of reserpine (Chessin et al, 1959). In view of the temperature response of hyperthyroid mice to reserpine, it was decided that an investigation of this phenomenon in hyperthyroid animals might prove of interest. Groups of hyperthyroid and euthyroid mice were treated with normal saline or with the MAO inhibitor nialamide, 15 mg/kg intraperitoneally, 3 hr. before treatment with reserpine, 1 mg/kg intravenously. The results obtained with hyperthyroid mice in this experiment, conducted at 22° C, are illustrated in Fig.47(Table43). The untreated hyperthyroid mice responded to the reserpine with a mild hypothermia of 0.75° C after 1 hr. whilst the nialamide treated animals showed a marked hyperthermia of 2° C 15 min. after the reserpine, falling to almost control levels after 2 hr. In marked contrast the untreated euthyroid animals responded to reserpine with their usual marked hypothermia reaching 3° C after 2 hr. Nialamide treatment did antagonise the hypothermia in the euthyroid mice, which maintained temperatures similar to the control animals for 45 min. and then fell 1.5° C below after 2 hrs. The drug did not, however, produce any hyperthermia in these animals.

DISCUSSION.

Reserpine, like CPZ, has neuroleptic properties and has been used since ancient times as a tranquillising agent. The drug is also useful clinically as an antihypertensive agent. Among its other properties, which include production of blepharospasm and miosis, the drug also gives rise to hypothermia in experimental animals, (Lewis 1963; Schittler & Plummer, 1964).

The precise mechanisms responsible for the central actions of reserpine are not fully understood, although the release of 5HT and NA from central stores is probably involved. There have been many attempts to relate the actions of the drug to the depletion of one of these amines. Brodie and his co-workers have suggested that 5HT is the chemical transmitter of a central trophotrophic (depressant) system. These workers postulate that the sedative properties of reserpine are due to the continuous release of 5HT from its storage sites onto receptors, thereby producing a continuous stimulation of the trophotrophic system with resulting sedation. This theory is attractive and has received some experimental support (see Brodie & Reid, 1968). Some doubt is cast on the theory by the report of Koe & Weissman (1968) that an almost total depletion of brain 5HT is without any significant behavioural effects. These latter results are in some question since the mode of action of p-chlorophenylalanine (the depleting agent they used) is not understood. Nevertheless, the Brodie hypothesis is far from proven (see Carlsson, 1968).

It is generally accepted that reserpine owes its antihypertensive effect to its ability to deplete catecholamines from peripheral stores (Schittler & Plummer, 1963).

The hypothermic action of reserpine has been ascribed to central thermoregulatory changes (Jori, Paglialunga & Garattini, 1967) resulting from depletion of brain 5HT and NA (Demming et al, 1956), two amines which are implicated as transmitters in hypothalamic thermoregulatory centres (Feldberg & Myers, 1963; Cooper, 1966; Reid et al, 1968).

The results presented in this chapter can be explained in a similar manner to those obtained with CPZ (Chapter III). At an ambient temperature of 22° C reserpine treatment causes hypothermia in both the hyperthyroid and the euthyroid mice. The response of the hyperthyroid animals is, however, significantly smaller than that of their euthyroid counterparts, since the former animals have a lower critical temperature.

At an environmental temperature of 26° C the hyperthyroid mice responded to the drug with a severe hyperthermia which caused the death of 60% of these animals. This increase in toxicity of reserpine is not in agreement with the results of Ashford & Ross (1968) who could demonstrate no increase in reserpine toxicity in hyperthyroid mice. This discrepancy can be explained, however, since the above authors appear to have conducted their experiments at an environmental temperature of 21.5° C, which as the present results demonstrate would not give rise to hyperthermia in the hyperthyroid animals. The hyperthermia present at 26° C is probably responsible for the increased toxicity noted here. This conclusion is in agreement with the findings of Skobba & Miya (1969) that hyperthermia is also the cause of an increase in the toxicity of CPZ in hyperthyroid animals. The response of the

hyperthyroid animals indicates that at this temperature they are in an environment above their critical temperature, which must therefore lie between 22 - 26° C for this dose of reserpine. The euthyroid mice at this environmental temperature still responded to reserpine with an intense hypothermia, indicating that at 26° C they are well below their critical temperature. It will be noted from the results that the hypothermia of the euthyroid mice at 26° C is greater than that noted at 22° C. The most likely explanation for this is that the ventilation of the cages, and therefore the ease of heat loss was different for these two experiments.

At an ambient temperature of 30° C the euthyroid mice, although still responding to reserpine with a hypothermia, are obviously closer to their critical temperature since the hypothermia is smaller than that seen at the lower environmental temperatures.

The effects of reserpine after pretreatment with the monoamine oxidase inhibitor nialamide emphasise further the effect of hyperthyroidism on the critical temperatures of mice. Nialamide pretreatment prevented the reserpine-induced hypothermia in euthyroid animals at 22° C. In contrast, due to their lower critical temperature, hypothermia in the hyperthyroid mice was reversed by pretreatment and hyperthermia ensued even at 22° C.

It is thought that MAO inhibitors reverse the effects of reserpine by prolonging the presence of released catecholamines in the body, by preventing their metabolism. According to the theory of Brodie et al (see Brodie & Reid, 1968) the result of MAO inhibition is that large amounts of free 5 HT are continuously available at receptor sites so that the amine

blocks its own action. This allows the opposing ergotrophic (stimulant) system to exert its influence.

The results presented here with reserpine, as with CPZ previously, are not in agreement with those of Lessin & Parkes (1957 a & b). They found that a concomitant hypothermia was necessary for reserpine to exert its sedative actions. The present results show that reserpine causes a marked reduction in SLA in euthyroid mice at an ambient temperature of 30° C, when the animals show only a mild degree of hypothermia. The results obtained with hyperthyroid mice at 26° C, where a concomitant hyperthermia occurred, must be suspect since the animals were suffering from the toxic effects of the drug. However, none of the hyperthyroid mice used for the SLA experiment died, possibly because these animals were not handled.

SUMMARY

1. At an ambient temperature of 22° C thyroxine induced hyperthyroidism in male TO mice renders the animals significantly less sensitive to the hypothermic effects of reserpine than euthyroid animals.

2. At an ambient temperature of 26° C whilst euthyroid mice respond to reserpine with a significant hypothermia the hyperthyroid animals show hyperthermia.

3. At an ambient temperature of 30° C the hypothermic effects of reserpine in euthyroid mice are significantly reduced compared with those seen at 22° C.

4. In spite of the difference in magnitude of core temperature response to reserpine at 22° C both the hyperthyroid and the euthyroid mice show an increase in skin temperature following treatment with the drug.

5. Reserpine reduces the spontaneous locomotor activity of both hyperthyroid and euthyroid mice. This effect is largely independant of the temperature response of the animals.

TABLE 35

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following treatment with reserpine 1 mg/kg by intravenous injection at an ambient temperature of 22°C. Control animals (HC and EC respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means (°C) of groups of 5 animals ± S.E.

Time (min)	HT	HT-C	ET	ET-C
0	38.0 ± 10.2		37.4 ± 0.3	
120	37.7 ± 0.3	38.2 ± 0.3	36.5 ± 0.3	37.2 ± 0.1
180	37.2 ± 0.3	38.1 ± 0.3	35.8 ± 0.4	37.2 ± 0.2
240	36.6 ± 0.6	38.2 ± 0.1	33.7 ± 0.9	37.1 ± 0.2
300	37.2 ± 0.4	38.2 ± 0.2	33.0 ± 1.3	37.4 ± 0.1

FIGURE 37.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intravenous injection of reserpine 1 mg/kg at an ambient temperature of 22°C. Control animals (HT-C and ET-C received an equivalent volume of normal saline by the same route (see Table 35).

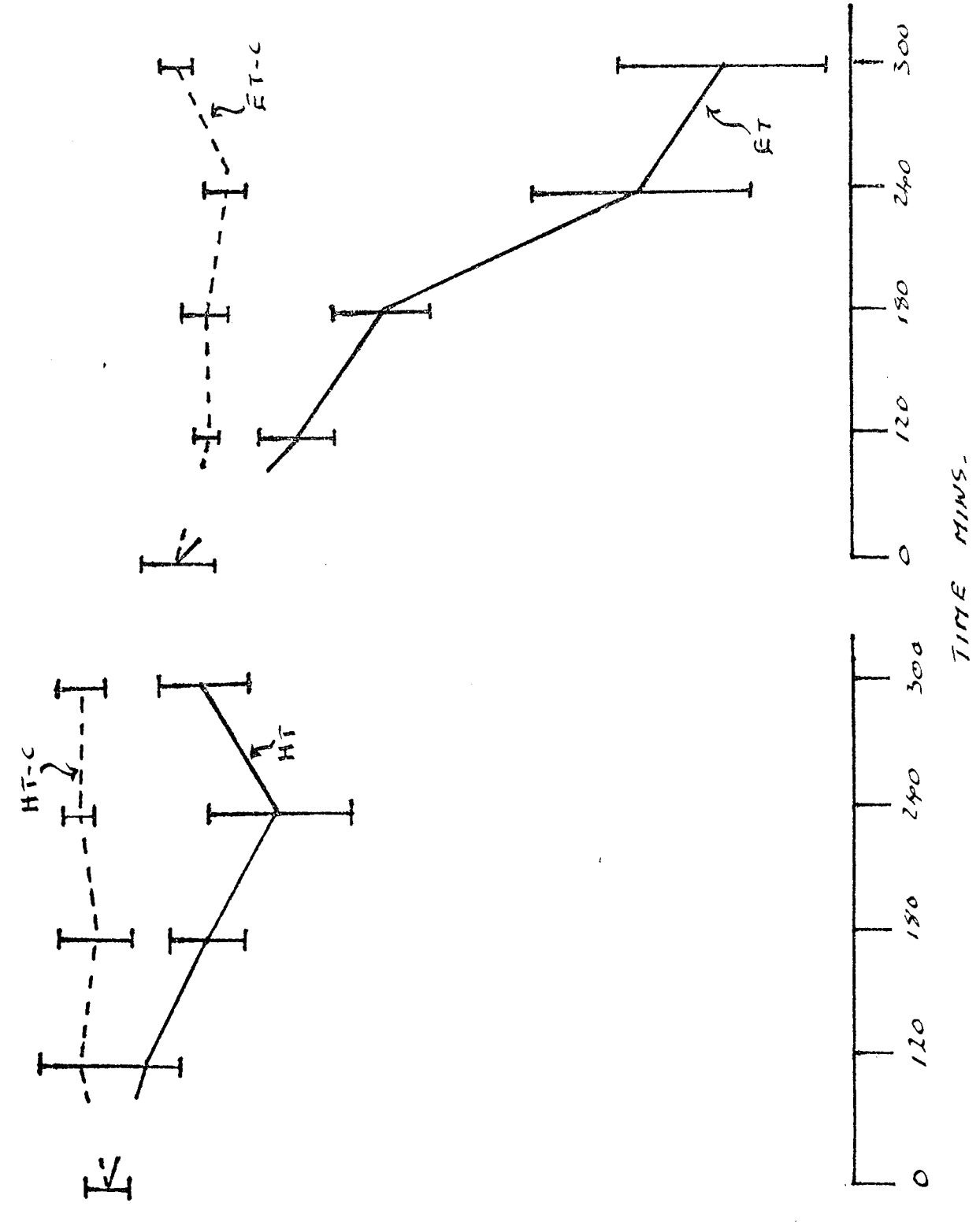


TABLE 36

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following treatment with reserpine 0.5 mg/kg by intravenous injection at an ambient temperature of 22°C. Control animals (HC and EC respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means (°C) of groups of 5 animals ± S.E.

Time (min)	HT	HT-C	ET	ET-C
0	38.5 ± 0.1		38.3 ± 0.1	
180	37.7 ± 0.3	38.5 ± 0.1	34.6 ± 0.3	37.4 ± 0.2
240	37.4 ± 0.4	38.5 ± 0.1	33.5 ± 0.3	38.0 ± 0.1
300	38.0 ± 0.5	38.3 ± 0.1	33.4 ± 0.4	38.1 ± 0.1

FIGURE 38.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intravenous injection of reserpine 0.5 mg/kg at an ambient temperature of 22°C. . Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route (see Table 36).

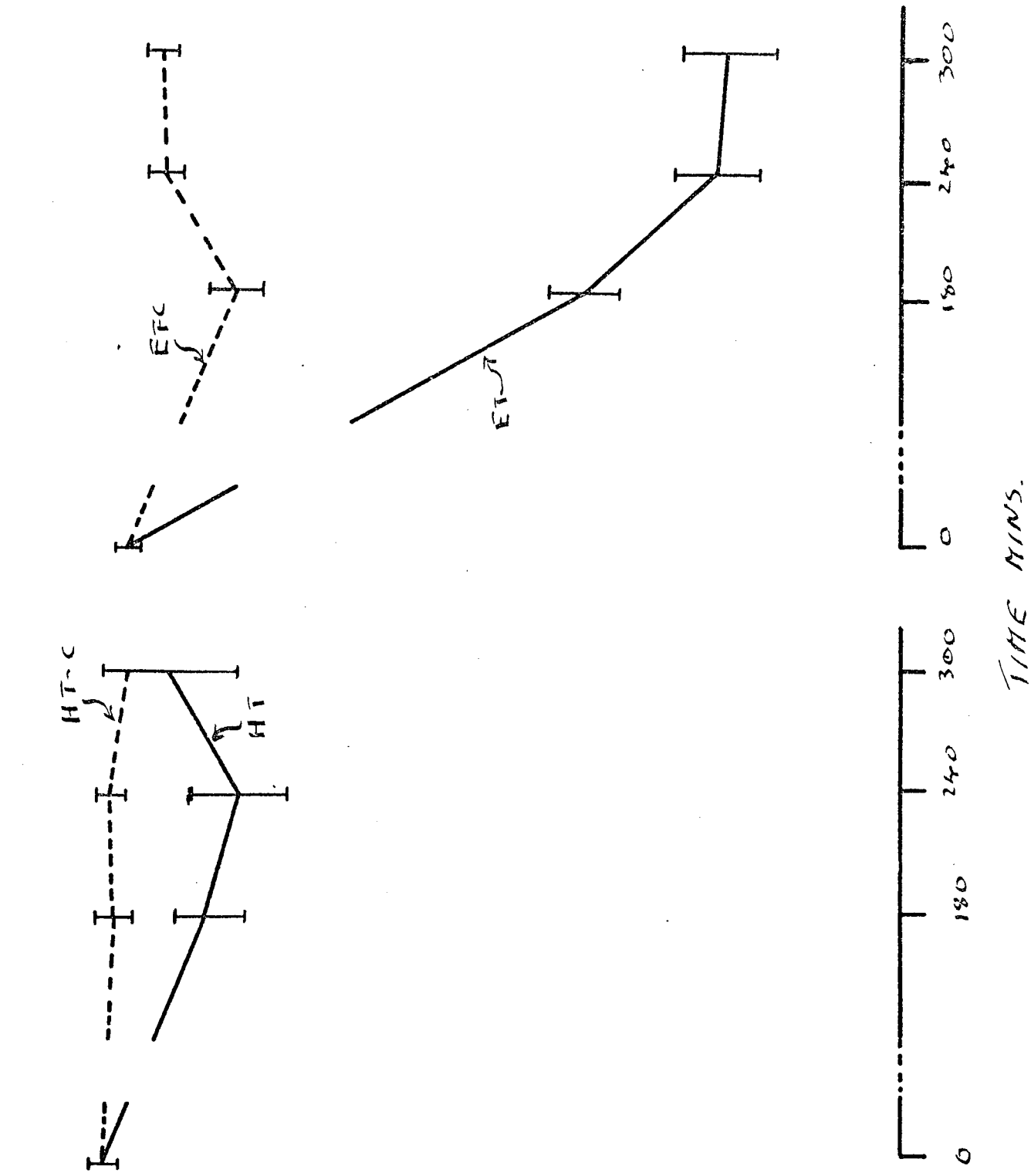


TABLE 37

Skin temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following treatment with reserpine 0.5 mg/kg by intravenous injection at an ambient temperature of 22°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as means (°C ± S.E.) of groups of 10 animals.

Time (min)	HT	HT-C	ET	ET-C
0	25.7 ± 0.2		25.7 ± 0.2	
60	26.4 ± 0.2	26.7 ± 0.3	25.9 ± 0.1	26.3 ± 0.2
120	29.2 ± 0.3	26.8 ± 0.3	28.3 ± 0.3	26.9 ± 0.4
180	28.1 ± 0.3	25.7 ± 0.2	27.6 ± 0.3	25.7 ± 0.3
240	27.5 ± 0.2	27.0 ± 0.1	26.5 ± 0.2	25.6 ± 0.3
300	28.1 ± 0.1	26.6 ± 0.3	27.2 ± 0.2	26.1 ± 0.3

FIGURE 39.

Skin temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intravenous injection of reserpine 0.5 mg/kg at an ambient temperature of 22°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route (see Table 37).

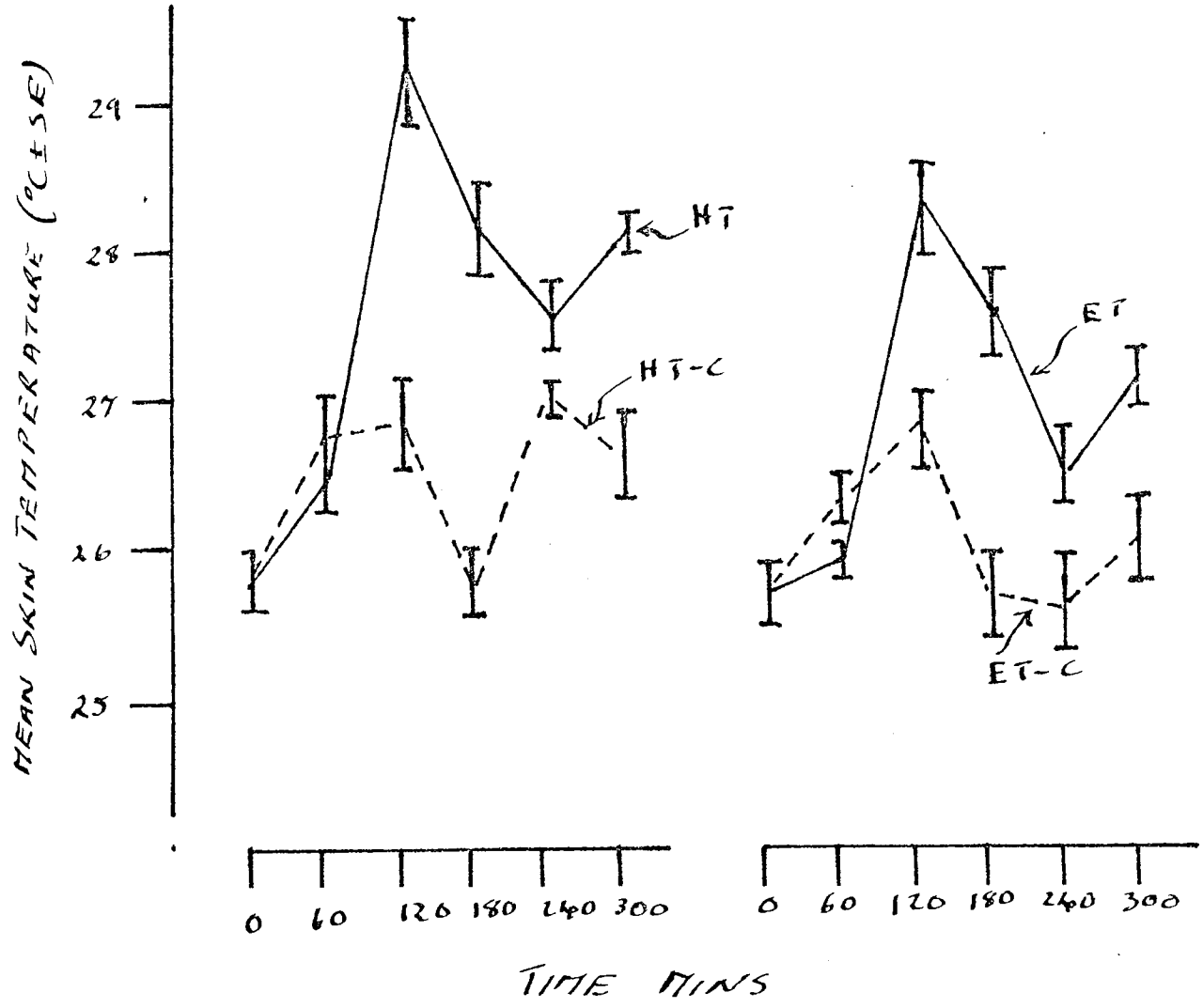


TABLE 38

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following treatment with reserpine 0.5 mg/kg by intravenous injection at an ambient temperature of 26°C. Control animals (HC and EC respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the means (°C) of groups of 10 animals ± S.E.

Time (min)	HT	HC	ET	EC
0	38.7 ± 0.1		37.8 ± 0.1	
180	40.1 ± 0.5	38.6 ± 0.1	34.7 ± 0.4	37.8 ± 0.1
240	39.7 ± 1.0	38.7 ± 0.2	33.6 ± 0.5	38.3 ± 0.1
300	36.6 ± 0.6	38.5 ± 0.1	32.4 ± 0.4	38.2 ± 0.1

FIGURE 40.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after intravenous injection of reserpine 0.5 mg/kg at an ambient temperature of 26°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route (see Table 38).

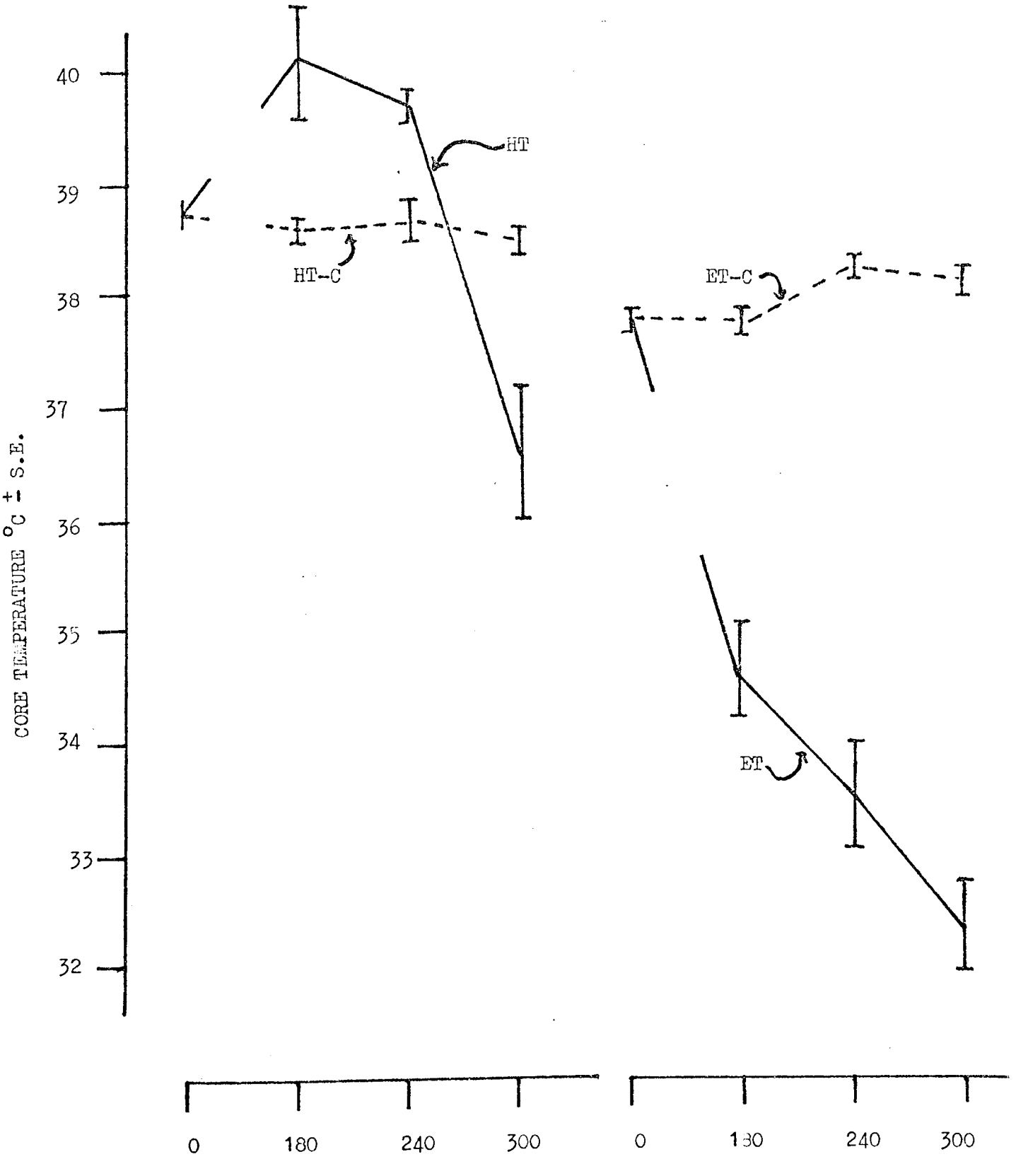


TABLE 39

Core temperatures of euthyroid (ET) male TO mice following treatment with reserpine 0.5 mg/kg by intravenous injection at an ambient temperature of 30° C. Control animals (EC) received an equivalent volume of normal saline by the same route. The results are expressed as the mean (° C) of groups of 10 animals ± S.E.

Time (min)	ET	EC
0	38.4 ± 0.1	
180	36.8 ± 0.1	37.6 ± 0.1
240	36.5 ± 0.1	37.9 ± 0.1
300	36.2 ± 0.2	37.9 ± 0.1

FIGURE 41.

Comparison of the effects of intravenous injection of reserpine 0.5 mg/kg in euthyroid (ET) male TO mice at ambient temperatures of 22^0 and 30^oC. Control animals (ET-C) received equivalent volumes of normal saline by the same route (see Table39).

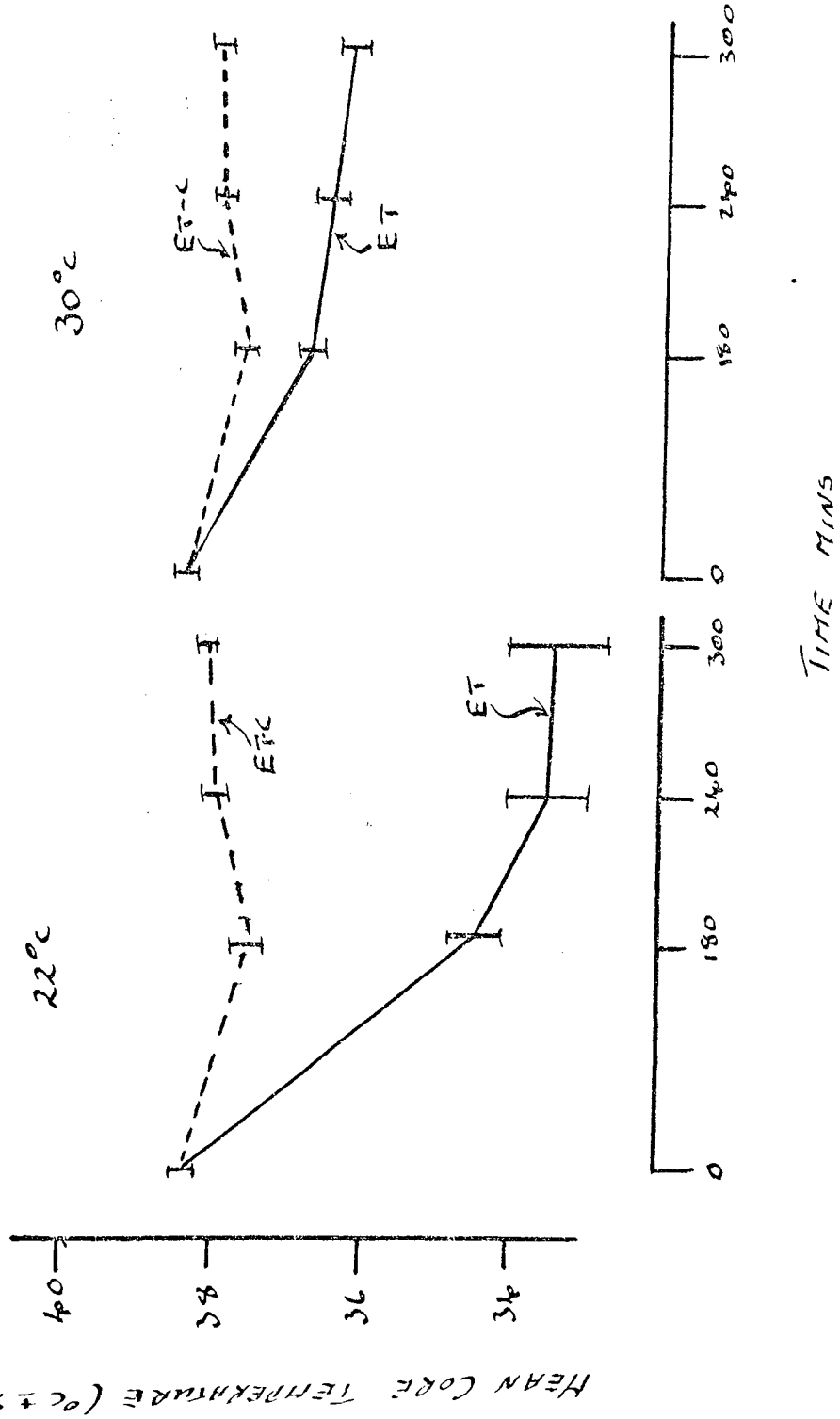


TABLE 40.

Spontaneous locomotor activity of hyperthyroid and euthyroid (ET) male TO mice following treatment with reserpine 0.5 mg/kg by intravenous injection at an ambient temperature of 22°C. Control animals (HC and EC respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the counts obtained from groups of five animals.

TIME (min)	HT	HT-C	ET	ET-C
20	495	1229	1928	1370
40	273	765	991	1688
60	49	353	282	968
80	28	413	57	537
100	25	204	78	167
120	23	189	71	244
140	15	66	74	91
160	16	120	23	60
180	28	371	16	642
200	21	213	5	64
220	2	93	8	39
240	3	90	68	57
260	4	92	17	160
280	50	102	10	51
300	71	86	72	63

FIGURE 43.

Spontaneous locomotor activity of euthyroid male TO mice after intravenous injection of reserpine 0.5 mg/kg at an ambient temperature of 22°C. Control animals (ET-C) received an equivalent volume of normal saline by the same route (see Table 40).

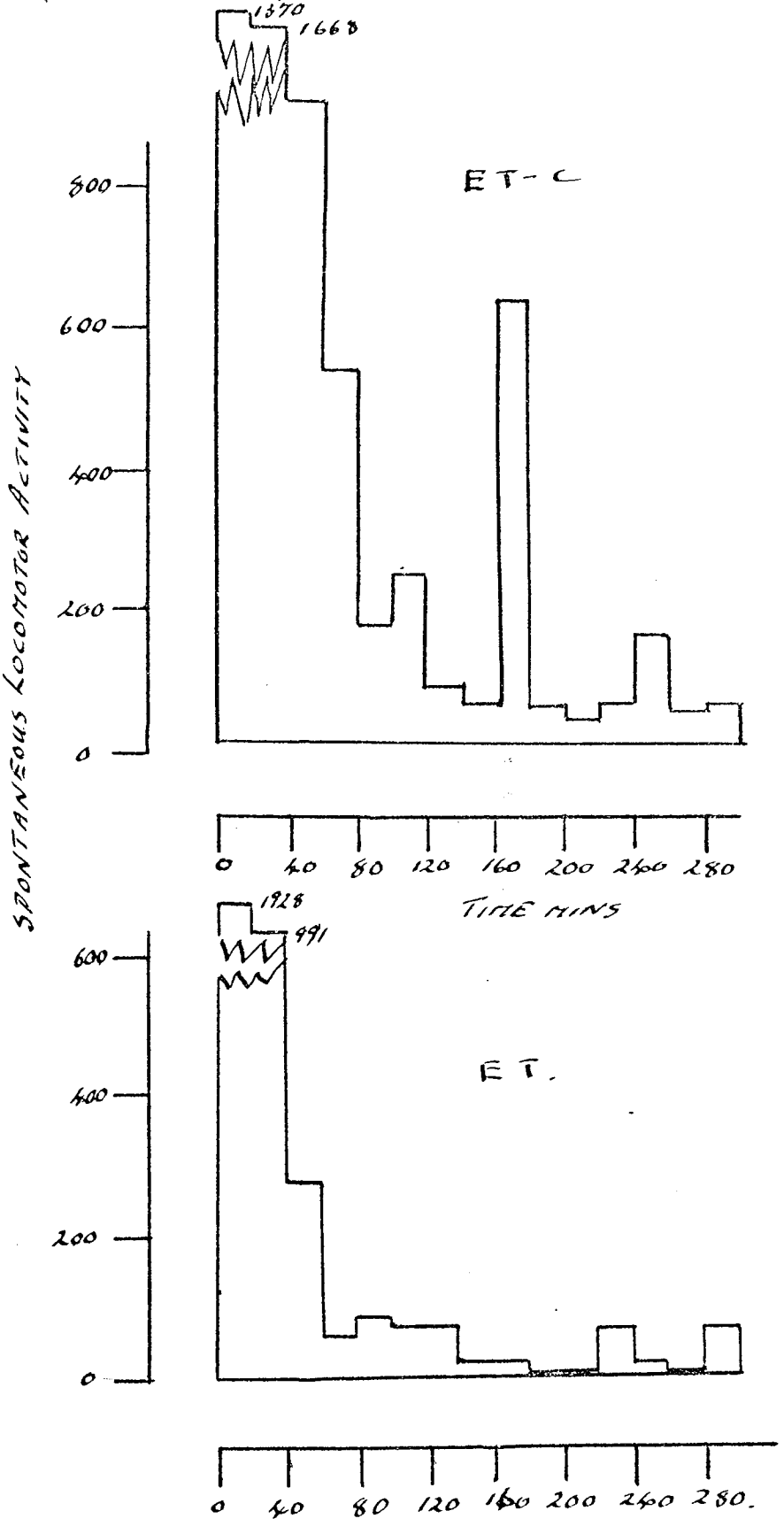


FIGURE 42.

Spontaneous locomotor activity of hyperthyroid (HT) male TO mice after intravenous injection of reserpine 0.5 mg/kg at anambient temperature of 22°C. Control animals (HT-C) received an equivalent volume of normal saline by the same route (see Table 40).

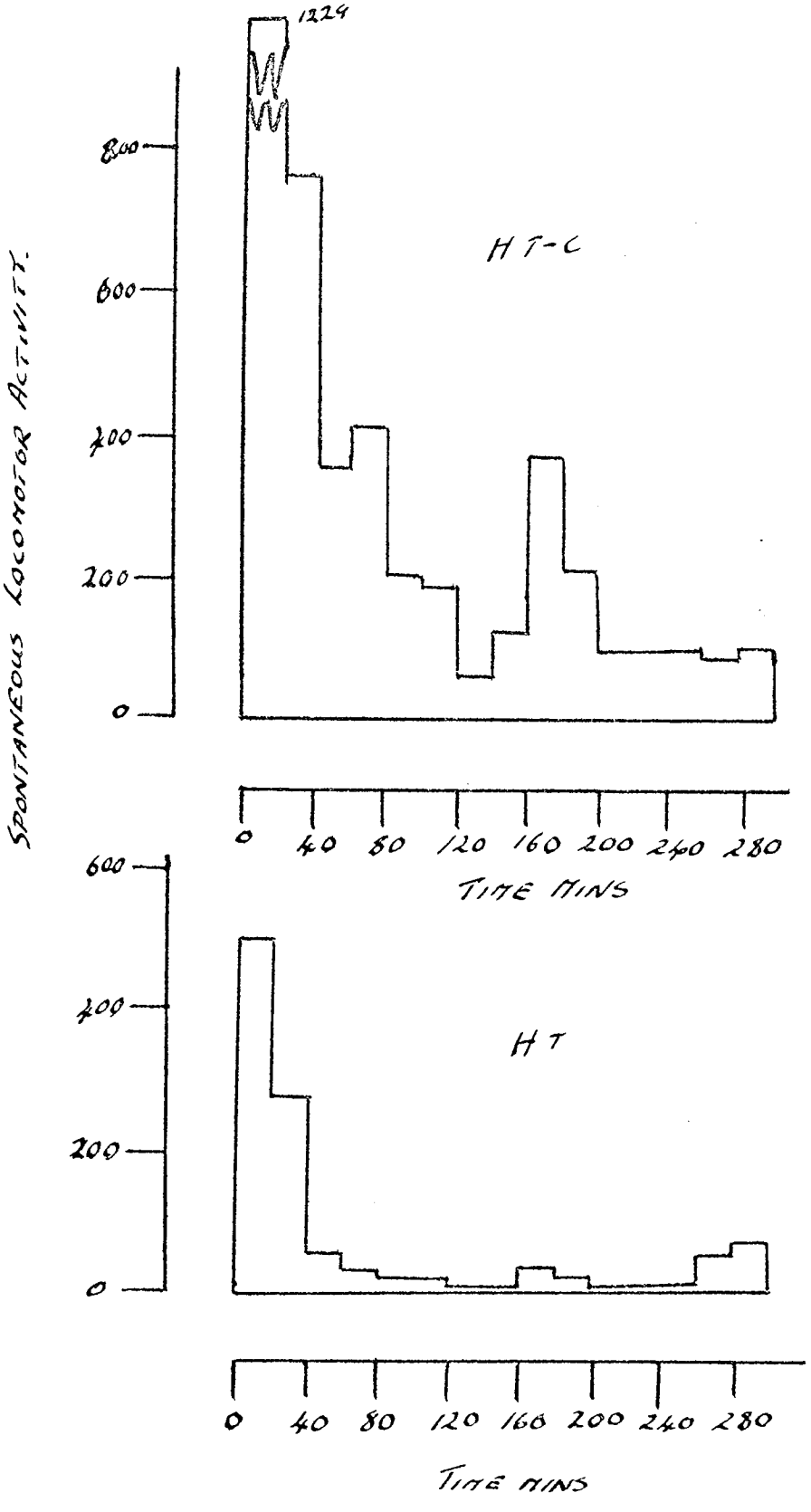


TABLE 41

Spontaneous locomotor activity of hyperthyroid and euthyroid male TO mice after intravenous injection of reserpine 0.5 mg/kg at an ambient temperature of 26 C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the 20 min counts from groups of 5 animals.

Time (min)	HT	HT-C	ET	ET-C
20	736	598	698	1070
40	277	610	63	710
60	40	359	29	688
80	57	125	20	403
100	40	376	30	114
120	20	219	35	67
140	22	133	37	38
160	57	130	24	76
180	49	197	111	60
200	15	500	0	47
220	52	70	1	27
240	73	65	4	33
260	35	53	20	85
280	45	18	15	30
300	13	57	10	54

FIGURE 44.

Spontaneous locomotor activity of hyperthyroid (HT) male TO mice after intravenous injection of reserpine 0.5 mg/kg at an ambient temperature of 26°C. Control animals (HT-C) received an equivalent volume of normal saline by the same route (see Table 41).

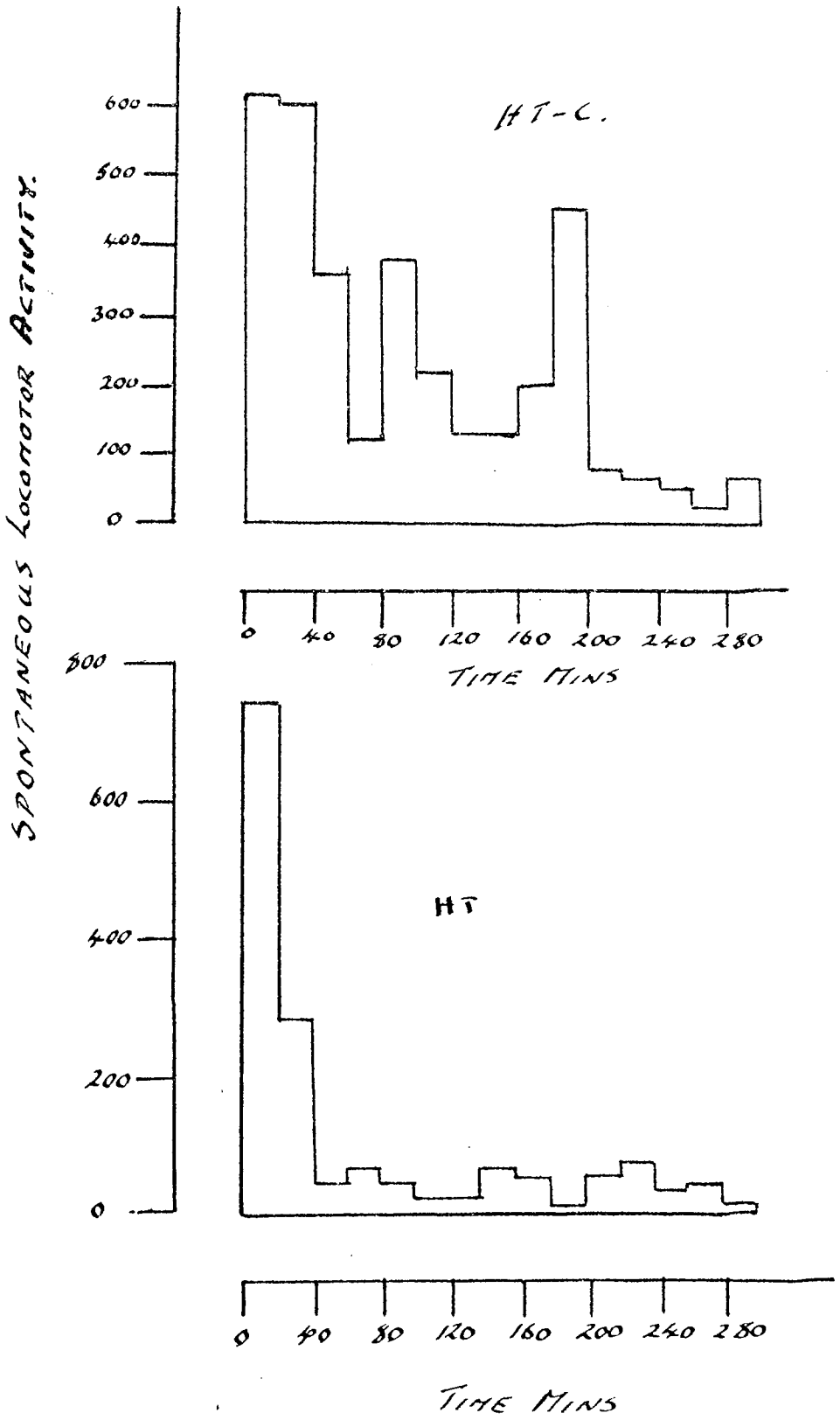


FIGURE 45.

Spontaneous locomotor activity of euthyroid male TO mice (ET) after intravenous injection of reserpine 0.5 mg/kg at an ambient temperature of 26°C. Control animals (HT-C) received an equivalent volume of normal saline by the same route (see Table 41).

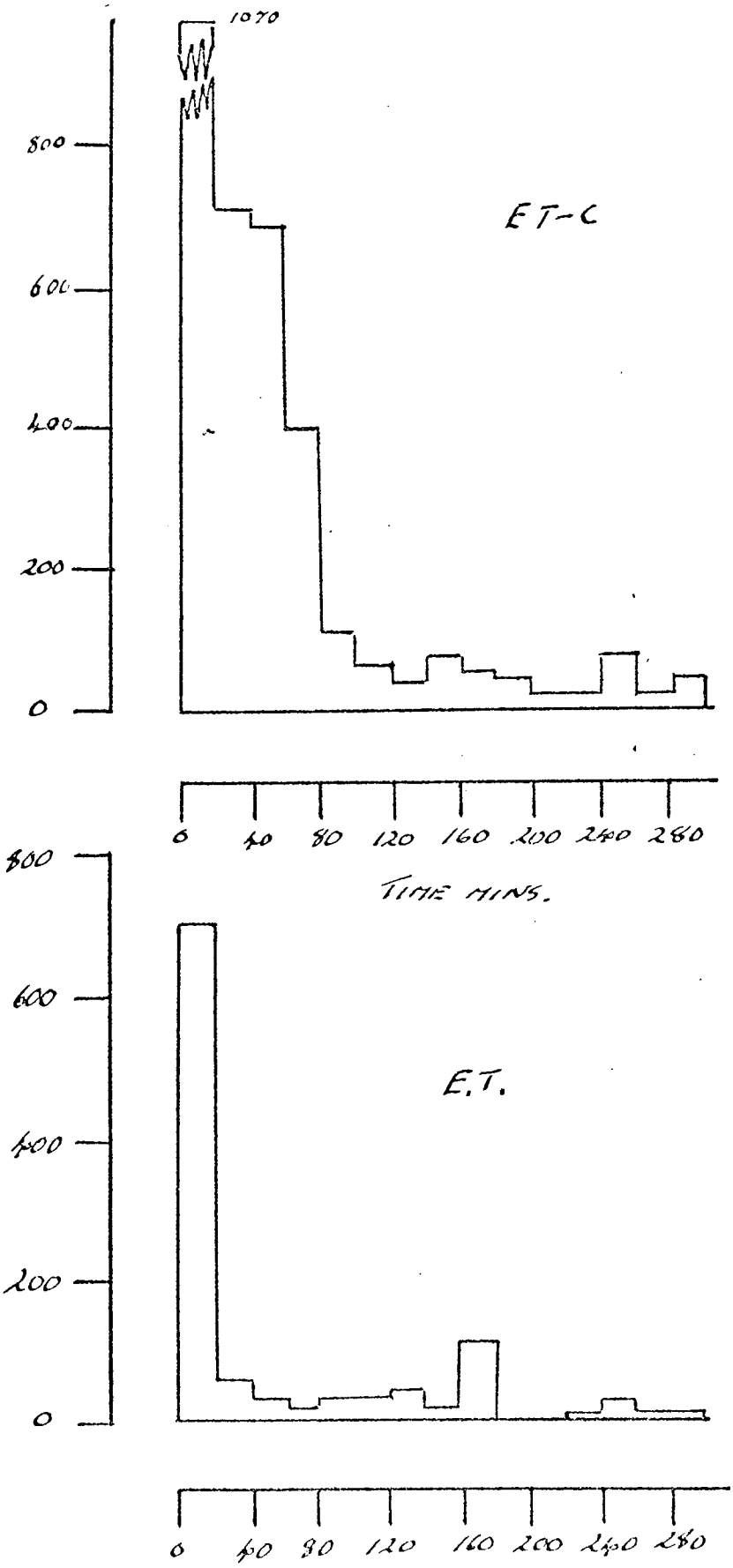


TABLE 42.

Spontaneous locomotor activity of euthyroid (ET) male mice after intravenous injection of reserpine 0.5 mg/kg at an ambient temperature of 30°C. Control animals (ET-C) received an equivalent volume of normal saline by the same route. The results are expressed as the 20 min counts from groups of 5 animals.

TIME (min)	ET	ET-C
20	750	1531
40	172	304
60	427	213
80	65	129
100	88	109
120	3	103
140	46	116
160	16	52
180	89	150
200	3	105
220	13	57
240	36	56
260	24	303
280	15	177
300	8	58

FIGURE 46.

Spontaneous locomotor activity of euthyroid (ET) male TO mice after intravenous injection of reserpine 0.5 mg/kg at an ambient temperature of 30°C. Control animals (ET-C) received an equivalent volume of normal saline by the same route (see Table 42).

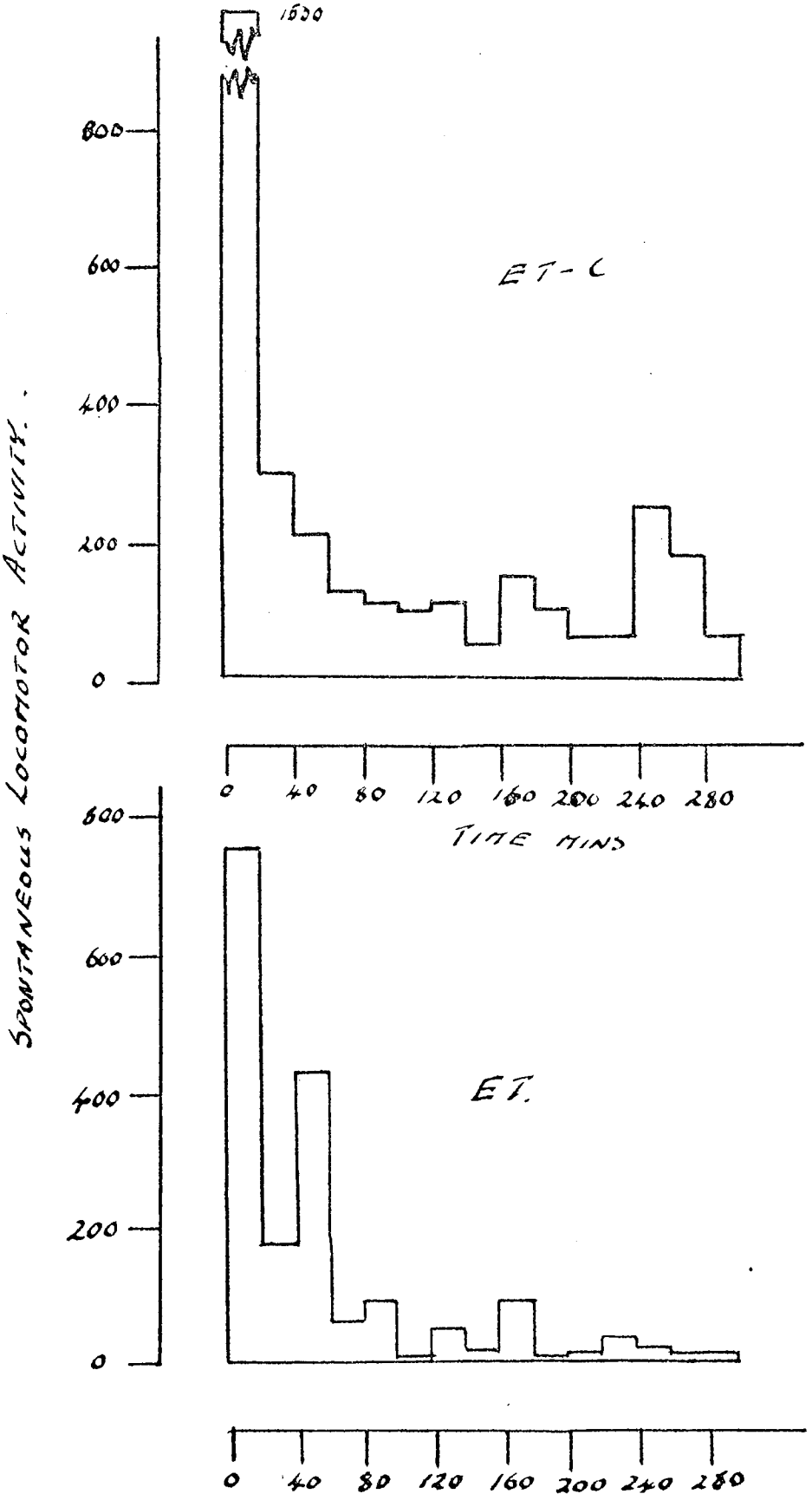


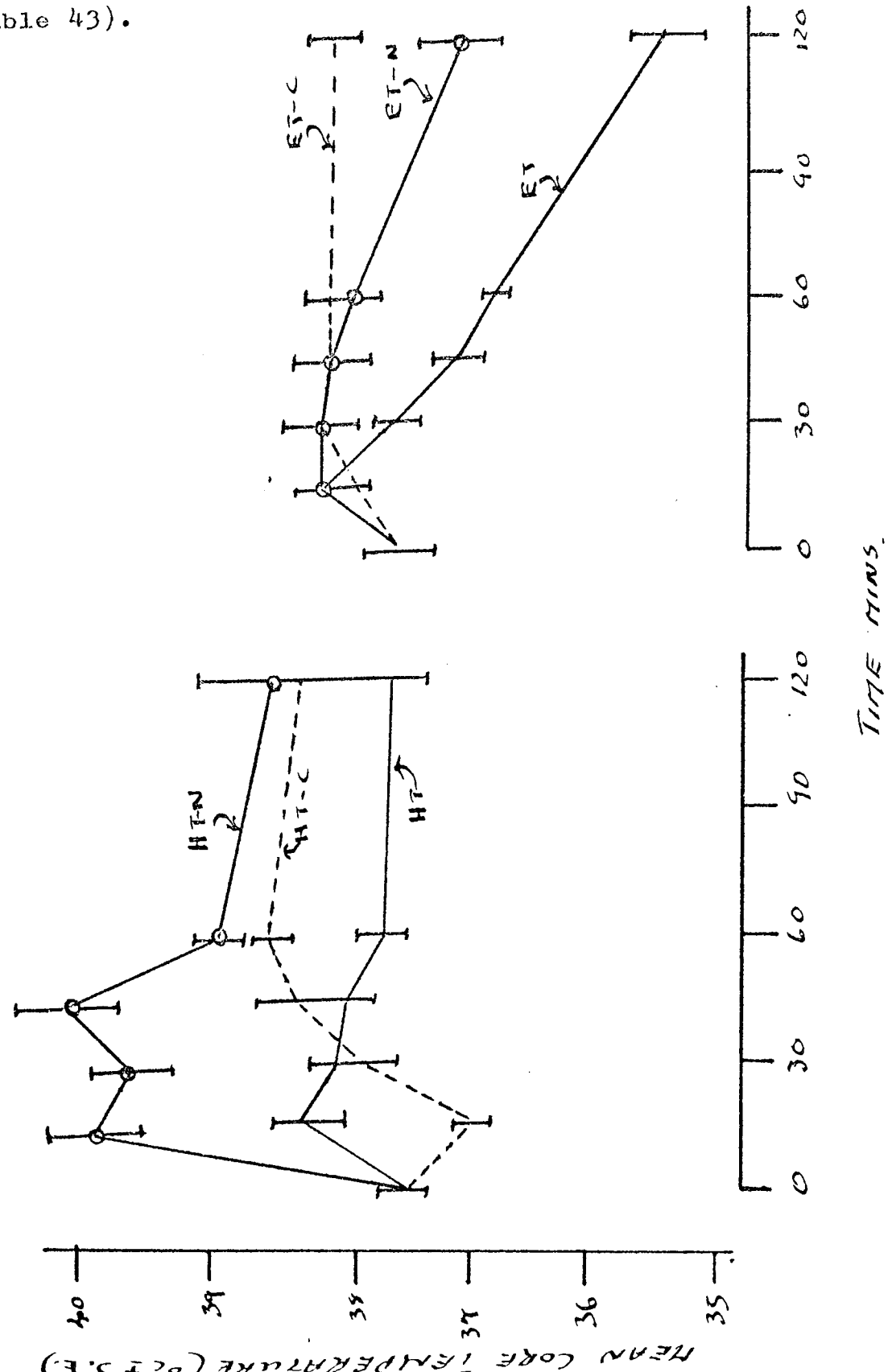
TABLE 43

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following intravenous injection with reserpine 0.5 mg/kg and after 3 hrs pretreatment with nialamide 15 mg/kg intraperitoneally (HT-N and ET-N respectively) at an ambient temperature of 22°C. Control animals (HT-C and ET-C respectively) received equivalent volumes of normal saline by the same routes. The results are expressed as the mean core temperatures (°C S.E.) of groups of 5 animals.

Time (min)	HT	HT-N	HT-C
0		37.6 ± 0.2	
15	38.3 ± 0.3	40.0 ± 0.4	37.0 ± 0.2
30	37.9 ± 0.3	39.7 ± 0.3	38.1 ± 0.2
45	38.0 ± 0.2	40.2 ± 0.4	38.4 ± 0.3
60	37.7 ± 0.2	39.5 ± 0.2	38.4 ± 0.2
120	37.6 ± 0.3	38.5 ± 0.6	38.3 ± 0.3
	ET	ET-N	ET-C
0		37.5 ± 0.3	
15	38.1 ± 0.2	38.1 ± 0.2	37.8 ± 0.1
30	37.5 ± 0.2	38.1 ± 0.3	38.1 ± 0.2
45	37.0 ± 0.2	38.0 ± 0.3	38.0 ± 0.1
60	36.7 ± 0.1	37.8 ± 0.2	38.1 ± 0.1
120	35.3 ± 0.3	36.9 ± 0.3	37.9 ± 0.2

FIGURE 47.

Core temperatures of hyperthyroid (HT-N) and euthyroid (ET-N) male TO mice treated with nialamide 15 mg/kg intraperitoneally or with normal saline (HT and ET respectively) 3 hr before reserpine 1 mg/kg intravenously at an ambient temperature of 22°C. Control animals (HT-C and ET-C respectively) received equivalent volumes of normal saline throughout(see Table 43).



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CHAPTER V.

THE EFFECTS OF AMPHETAMINE IN HYPERTHYROID AND EUTHYROID MICE

CHAPTER V.

THE EFFECTS OF AMPHETAMINE IN HYPERTHYROID AND EUTHYROID MICE

1. Introduction

There are numerous reports that the toxicity of amphetamine is increased in hyperthyroid animals (Askew, 1962; Halpern, Drudi-Baracco & Bessirard, 1964; Moore, 1966; Dolfini & Kobayashi, 1967). Since in the previous two chapters it was found that hyperthyroidism caused changes in the temperature responses to both CPZ and reserpine, it was decided to investigate the effects of d-amphetamine on the body temperature of hyperthyroid and euthyroid mice. The experiments reported in this chapter were performed using a dose of d-amphetamine 2.5 mg/kg orally at ambient temperatures of 22° and 26° C.

2. Effects of amphetamine 2.5 mg/kg at 22° C.

The results of this experiment are shown in Fig. 48 Table 44), and reveal that at this ambient temperature the drug produced a statistically significant hyperthermia in hyperthyroid animals reaching a maximum of 1.75° C after 20 min; amphetamine did not kill any of the mice at this temperature. In contrast the same dose of amphetamine was without significant effect on the core temperatures of euthyroid mice.

3. Effects of amphetamine 2.5 mg/kg at 26° C.

At this higher ambient temperature the drug produced a marked hyperthermia of 2.7° C in the hyperthyroid mice (see Fig. 49 Table 45). The hyperthermia was so severe (some animals reaching 43° C)that

amphetamine killed 90% of the hyperthyroid mice within 2 hr. of its administration. The effect on euthyroid mice was less marked; they responded with a mild (though significant) hyperthermia of 0.7° C reaching a maximum after 20 min.

Amphetamine under these conditions was not lethal in the euthyroid mice. The hyperthermia in hyperthyroid mice was better maintained than that in euthyroid animals. Thus, at 80 min. the euthyroid mice were no longer significantly hyperthermic yet the hyperthyroid animals still had a mean core temperature 1° C above control values.

DISCUSSION

Amphetamine is a psychomotor stimulant drug which produces increased motor activity, hyperthermia, increased blood pressure and anorexia in experimental animals (see Holtz & Westermann, 1965). The drug is believed to exert its central stimulant effects through a stimulant action on the reticular formation (Killam 1962).

The mechanisms involved in the production of these actions are still a matter of conjecture some workers favour a direct sympathomimetic action of the drug at central receptor sites (Brodie, Spector & Shore, 1959; Smith, 1965). This theory receives support from the recent report of Bradley (1969) that iontophoretic application of noradrenaline or of amphetamine to single brain neurones produces the same electrical response. Other workers (Glowinski & Baldessarini 1966) have implicated the release of noradrenaline from central nerve endings, coupled with the inhibition of breakdown and re-uptake of the amine as being an indirect pathway whereby the drug exerts its effects.

The hyperthermic effect of the drug is thought to be due cheifly to the calorigenic effects of peripherally released noradrenaline (Mantegazza, Niamzada & Riva, 1968) since this effect can be abolished by treatment with adrenergic-receptor blocking agents (Gessa, et al. 1969).

Much work has been devoted to the study of the toxic effects of amphetamine in experimental animals. It has been reported that the toxicity of the drug is increased by rises in ambient temperature

(Askew, 1962). Aggregation of animals is also known to increase the toxic effects of the drug (Chance, 1947) an effect accompanied by increased motor activity and hyperthermia in response to the drug (Greenblatt & Osterberg 1961). Hardinge and Peterson (1964) reported that forced exercise caused a rise in body temperature and resulted in increased toxic effects of amphetamine similar to those seen in aggregated animals. In hyperthyroid animals the toxic effects of the drug are significantly greater than in euthyroid animals (Moore 1966; Halpern, Drudi-Barraco & Bessirard 1964). It has been reported (Clark et al. 1967) that the toxicity of amphetamine is related to the degree of hyperthermia produced, the increased toxicity being due to CNS damage resulting from the extreme hyperthermia.

The results presented here show that the hyperthyroid mice are more susceptible to the hyperthermic effects of non-lethal doses of amphetamine. This is thought to be due to an increase in the calorigenic potency of noradrenaline in these animals (Swanson, 1956 & 1957) and aggravated by the high normal metabolic rate of the animals. At higher ambient temperatures the hyperthermia produced by the drug is so severe as to cause a very high degree of mortality in the hyperthyroid mice. The euthyroid animals, however, show only a mild hyperthermia at 26°C and none of these animals die. The results support the suggestion of Clark (1967) that the hyperthermia seen in the hyperthyroid animals is the major cause of death.

SUMMARY

1. At an ambient temperature of 22° C thyroxine-induced hyperthyroidism in male TO mice renders these animals significantly more susceptible to the hyperthermic effects of amphetamine than euthyroid mice.

2. At an ambient temperature of 24° C the hyperthermic and lethal effects of amphetamine in hyperthyroid mice are significantly increased compared to those seen at 22° C.

TABLE 44

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following oral administration of d-amphetamine 2.5 mg/kg at an ambient temperature of 22°C. Control animals (HT-C and ET-C respectively) recieved an equivalent volume of water by the same route. The results are expressed as the mean temperatures (°C ± S.E.) of groups of 5 animals.

Time (min)	HT	HT-C	ET	ET-C
0		38.1 ± 0.2		37.0 ± 0.5
20	40.4 ± 0.1	38.5 ± 0.2	37.2 ± 0.2	36.8 ± 0.1
40	40.3 ± 0.2	37.9 ± 0.1	37.1 ± 0.5	36.8 ± 0.2
60	39.8 ± 0.2	37.9 ± 0.1	37.3 ± 0.4	37.0 ± 0.2
80	39.3 ± 0.9	38.3 ± 0.2	37.4 ± 0.4	37.0 ± 0.3

FIGURE 48.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after oral administration of d-amphetamine 2.5 mg/kg at an ambient temperature of 22°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of water by the same route (see Table 44)

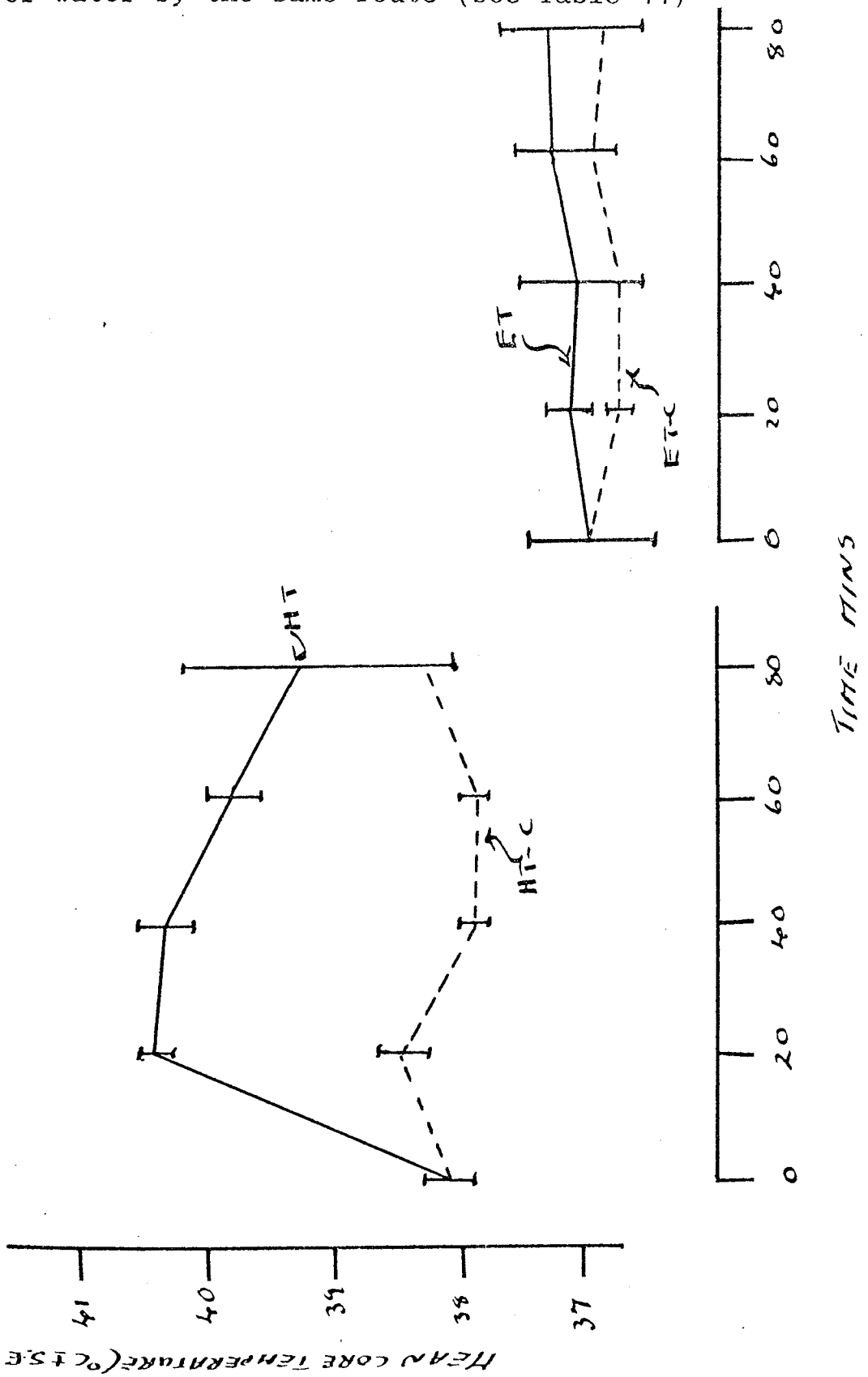


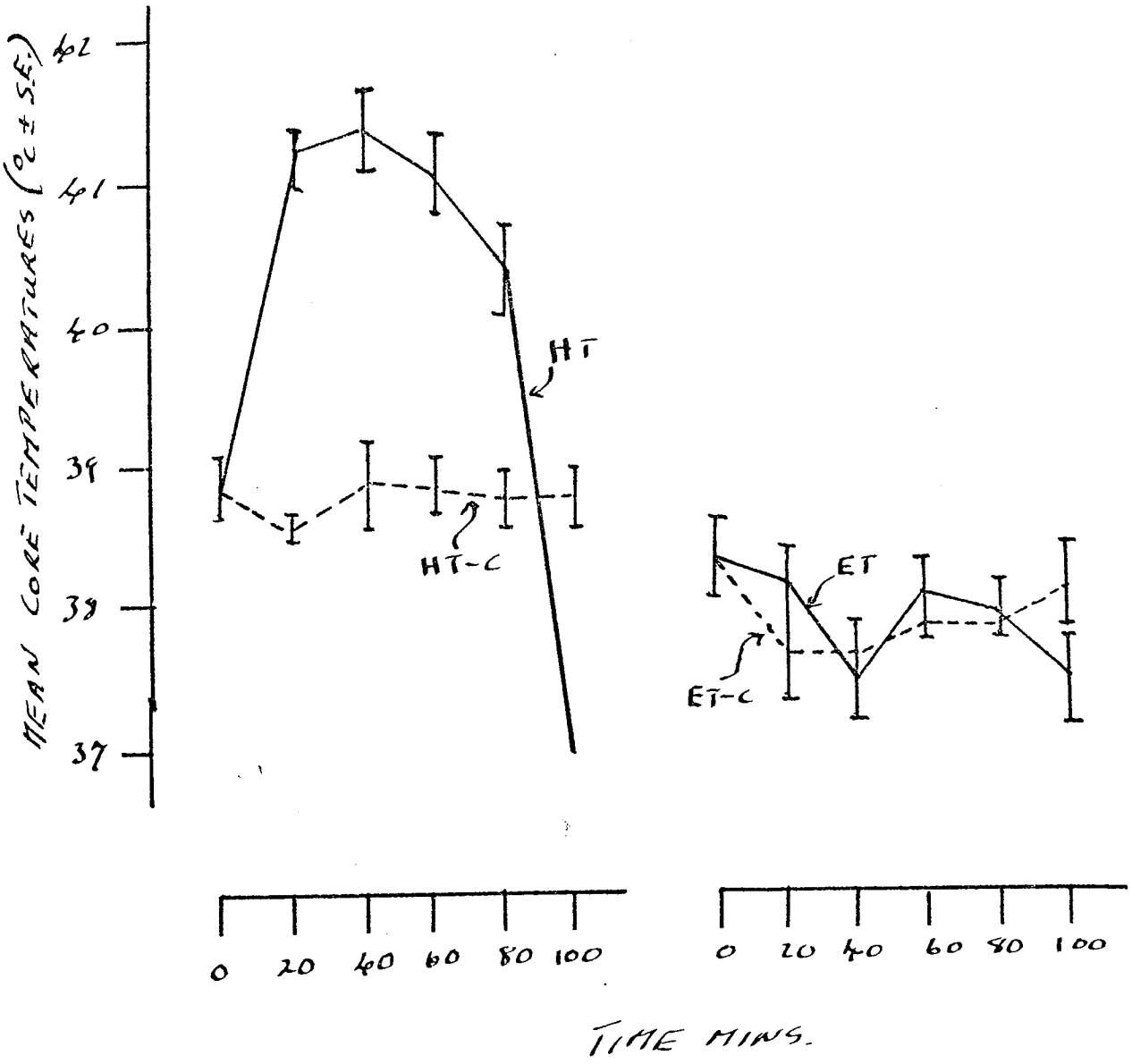
TABLE 45

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following oral administration of d-amphetamine 2.5 mg/kg at an ambient temperature of 26°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of water by the same route. The results are expressed as the mean core temperatures (°C ± S.E.) of groups of 5 animals.

Time (min)	HT	HT-C	ET	ET-C
0	38.9 ± 0.2		38.4 ± 0.3	
20	41.2 ± 0.2	38.6 ± 0.1	38.2 ± 0.2	37.7 ± 0.3
40	41.4 ± 0.3	38.9 ± 0.3	37.5 ± 0.3	37.7 ± 0.2
60	41.1 ± 0.3	38.9 ± 0.2	38.1 ± 0.2	37.9 ± 0.1
80	40.4 ± 3.0	38.8 ± 0.2	37.9 ± 0.1	38.0 ± 0.2
100	37.0	38.8 ± 0.2	37.5 ± 0.3	38.2 ± 0.3

FIGURE 49.

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice after oral administration of d-amphetamine 2.5 mg/Kg orally at an ambient temperature of 26°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of water by the same route (see Table 45).



CHAPTER VI.

THE EFFECTS OF TREMORINE AND OXOTREMORINE IN HYPERTHYROID AND EUTHYROID MICE

CHAPTER VI

THE EFFECTS OF TREMORINE AND OXOTREMORINE IN HYPERTHYROID AND EUTHYROID MICE

1. Introduction

Tremorine (TMN) produces both central and peripheral muscarinic effects (Everett, 1956), which are mediated through its active metabolite oxotremorine (OTMN) (Kocsis & Welch, 1960; George, Haslett & Jenden, 1962). In the mouse, administration of either compound causes sustained tremor, miosis, salivation, diarrhoea and hypothermia, all of which can be prevented by centrally acting anticholinergic drugs (Blockus & Everett, 1957). For several reasons, some interaction might be expected between a hyperthyroid state and the effects of TMN and OTMN. For example, a frequent feature of hyperthyroidism is a tremor of the hands or of the whole body (Morgans, 1964), which suggests that the effects of these compounds might be enhanced in hyperthyroid animals. The well documented hypothermic effects of TMN and OTMN also made them a subject of interest because of the effects of hyperthyroidism on thermoregulation, as discussed in Section 2. Finally, since TMN has to be metabolised to OTMN before it exerts its effects, there might be differences in the onset and/or duration of symptoms in the hyperthyroid mice due to thyroxine-induced changes in drug metabolism.

A preliminary experiment was conducted in which hyperthyroid and euthyroid mice were treated with TMN, 20 mg/kg or with an equivalent volume of normal saline (controls). The injection was made

subcutaneously into the flank region since T4 (or saline) had been injected repeatedly subcutaneously into the neck to produce the hyperthyroid state. Following the administration of TMN, oesophageal temperature was recorded and tremor assessed at 30 min. intervals. The results of this experiment, which was conducted at a laboratory temperature of 20° C, are illustrated in Fig. 50. (Table 46). TMN produced a mild degree of hypothermia in the hyperthyroid animals reaching a peak of 2° C after 30 min. These animals showed only a mild tremor for the first hour and none thereafter. In contrast the euthyroid mice responded with a very marked hypothermia of 8° C at 1 hr. and also suffered a intense tremor with a peak after 1 - $1\frac{1}{2}$ hr. and which persisted throughout the 2 hr. of the experiment.

This first experiment indicated that hyperthyroid animals were relatively insensitive to the hypothermic and tremorigenic effects of TMN. However, it was observed that the peripheral muscarinic activity of TMN was equally well marked in the hyperthyroid and the euthyroid mice. There are four possible explanations for this apparent lack of sensitivity in hyperthyroid mice to the tremorigenic and hypothermic properties of TMN:-

i. Lack of response is due to inhibited biotransformation of TMN to OTMN in hyperthyroid mice;

ii. There is an absence of concomitant hypothermia in the hyperthyroid mice which might otherwise induce shivering (tremor);

iii. The active metabolite fails to enter the CNS of hyperthyroid mice;

iv. There is a changed sensitivity of the CNS in hyperthyroid mice.

Experiments were designed therefore to examine the validity of each of these four hypotheses.

2. Effect of hyperthyroidism on the metabolism of Tremorine.

The presence of peripheral muscarinic activity in hyperthyroid mice following treatment of TMN suggested that either the peripheral activity of TMN was in fact due to the parent compound and not to its metabolite OTMN, or that the metabolism of the compound was not inhibited in the hyperthyroid animal and absence of central effects (tremor and hypothermia) was due to some other cause. To prove the validity of the latter hypothesis, two groups of euthyroid mice were treated with either saline or with the metabolic inhibitor SKF525A, at 12.5 mg/kg orally 2 hr. before administration of TMN. Core temperature, tremor and peripheral muscarinic activity, were noted at 30 min. intervals. Fig. 51. (Table 47) illustrate that the SKF525A not only inhibited the hypothermia and tremor caused by TMN, but also completely abolished the peripheral signs of muscarinic hyperactivity. It appears that the peripheral muscarinic symptoms of TMN are indeed mediated via OTMN. Since the hyperthyroid animals showed signs of peripheral muscarinic stimulation, it suggests that the lack of a central response of these mice to TMN cannot be due to the faulty biotransformation of TMN.

In order to show conclusively that the insensitivity of the hyperthyroid mice was not due to faulty metabolism of the TMN, groups of hyperthyroid and euthyroid mice were treated with OTMN (which it is believed is not metabolised before it acts). The results illustrated in Fig. 52 (Table 48) show that the euthyroid mice responded to OTMN with their normal intense hypothermia (9.7° C at 1 hr.) and tremor (peak

at 30 min,), which persisted throughout the 2 hr. of the experiment. In contrast, the hyperthyroid animals showed only a mild hypothermia of 1.8° C at 30 min. and a very low level of tremor which had ceased within 1 hr. of the administration of the OTMN. It appears that the lack of response of hyperthyroid animals to TMN is not due to any faulty transformation of the compound to its active metabolite, but must be due to some other cause.

3. Effect of environmental temperature on the response to tremorine and oxotremorine.

These experiments investigated the possibility that the low level of tremor seen in hyperthyroid mice following TMN or OTMN was due to a lack of concomitant hypothermia in these animals. The experiments were carried out using groups of euthyroid animals only at elevated laboratory temperatures in an attempt to abolish the hypothermia produced, and note what effect this abolition had on the tremor, Fig. 53 (Table 48) shows the results of an experiment carried out at 30° C. The hypothermia produced by TMN 20 mg/kg, was much reduced compared with that seen at 20° C. The tremor was intense but more transient than that produced at 20° C. This result was confirmed in an experiment in which OTMN 0.5 mg/kg was administered to euthyroid mice at an ambient temperature of 36° C, (see Fig. 54. and Table 50). The hypothermia seen with this compound was abolished. The tremor under these circumstances was again transient, reaching a peak level during the first 15 min. after administration.

Fig. 55 (Table 51) shows the results of a further experiment conducted at 37° C in which euthyroid mice were treated with OTMN, 0.75 mg/kg. The results show that at this high ambient temperature the mice responded to treatment with a mild hyperthermia of 0.6° C at 30 min. The tremor seen at 15 min. was comparable to that seen with the lower dose at 30 min. and 20° C; thereafter the tremor was considerably less.

The results indicated that, although the degree of tremor appears to some extent to be dependant on the presence of a concomitant hypothermia, the two phenomena are separable. Although the results also suggest that the previously observed lack of tremor in hyperthyroid mice might be due to a lack of hypothermia, nevertheless because the tremor in euthyroid mice at higher temperatures was more transient, then the tremor in hyperthyroid mice might have been of sudden onset and short duration and missed when the tremor was first looked for, i.e. at 30 min. In order to rule out this latter possibility a group of hyperthyroid mice treated with OTMN were closely observed for signs of tremor continuously for a period of 1 hr. following administration. The tremor seen was as slight as that reported earlier and no early peak was observed.

Since it had been shown that, in euthyroid mice tremor could be produced without hypothermia by a dose of OTMN 0.75 mg/kg, this experiment was repeated at 22° C using groups of hyperthyroid mice. The results are shown in Fig. 56 (Table 52), as can be seen even with this high dose of OTMN the hyperthyroid animals showed only a slight hypothermia of 2° C after whilst the tremor produced was as slight as

that shown by the lower dose of the drug at this temperature.

4. Intracerebral administration of oxotremorine.

Since the lack of response of the hyperthyroid mice may have been due to a failure of OTMN to penetrate the CNS, it was decided to compare the effects of intracerebrally administered OTMN in hyperthyroid and euthyroid mice. The results of this experiment conducted at 22° C are illustrated in Fig. 57 (Table 53). It can be seen that the administration of 1.5 g of OTMN by this route to hyperthyroid mice produced a mild hypothermia of 1.3° C after 20 min. and only a transient low level of tremor which had subsided after 15 min. In contrast, euthyroid animals showed a severe hypothermia of 4.7° C after 30 min. and a high level of tremor which persisted for 30 min.

Therefore, the lack of response of hyperthyroid mice to TMN or OTMN can not be ascribed to any failure of the active metabolite to penetrate the CNS of these animals.

DISCUSSION

Treatment with tremorine (TMN) produces characteristic symptoms including, tremor, hypothermia, and signs of increased peripheral parasympathetic activity. These effects are due to the actions of oxotremorine (OTMN) a metabolite of the parent compound (Cho et al, 1961 & 1964). The tremor and hypothermia are thought to be the result of the central muscarinic activity of the compound, since they can be reversed by treatment with centrally acting anticholinergic and sympathomimetic agents and also by thymoleptics (Spencer, 1965 & 1966). Peripherally acting anticholinergic drugs will abolish the diarrhoea, miosis and salivation caused by OTMN but like peripherally acting sympathomimetics they have little effect on the hypothermia and no effect on the tremor produced (Spencer 1965).

It has been suggested that OTMN produces its central effects as a result of increasing brain levels of ACh (Holmstedt & Lundgren, 1965). However, this hypothesis has been questioned by Cox and Potkonjak (1969 a & b) who ascribe the effects of the drug to a direct stimulation of some central receptor site.

The results presented in this chapter show that hyperthyroid mice are considerably less sensitive to the hypothermic and tremorigenic effects of TMN and OTMN than are euthyroid animals. This lack of sensitivity in hyperthyroid mice could be due to a number of reasons: faulty biotransformation of TMN; changes in the blood brain barrier; lack of severe hypothermia; or changes in central nervous activity. Investigation revealed that, in spite of the lack of hypothermia and tremor, hyperthyroid mice show signs of increased peripheral muscarinic

activity with TMN. Since this symptom is reduced or abolished by treatment with the metabolic inhibitor SKF525A (Welch & Kocsis, 1961) this finding shows that conversion of TMN to OTMN does occur in the hyperthyroid animals. This result, combined with the lack of sensitivity of the hyperthyroid mice to the active metabolite OTMN, rules out the possibility that there is any failure or biotransformation of TMN to OTMN in these animals.

The hyperthyroid animals also failed to show a normal response to OTMN following intracerebral administration. This suggests that the lack of tremor in the hyperthyroid mice is not due to any T4-induced changes in the blood/brain barrier which it was previously thought could prevent access of OTMN to the CNS of these animals.

This last observation may be open to question in view of the reported increase in cerebral blood flow in hyperthyroidism (Sensenbach et al, 1965; Sokoloff et al, 1953) which would facilitate removal of any agent administered intracerebrally. However, by the same argument, the increased blood flow would also be expected to facilitate entry of peripherally administered drugs to the brain, thereby causing an early onset of action - and this does not occur in these animals.

An investigation of the relationship between the hypothermia and tremor produced by OTMN in euthyroid animals showed that at high ambient temperatures the drug causes a detectable tremor even in the presence of a hyperthermia. However, the tremor produced under such conditions is somewhat reduced compared with that seen at lower environmental temperatures when it is accompanied by hypothermia;

a larger dose of OTMN will produce comparable effects. This finding is in agreement with Hammer, Karlen and Sjöqvist (1968) who found that agents which reduce OTMN-induced hypothermia also cause some inhibition of the tremor because of an increased rate of metabolism of OTMN. Consequently, the lack of response of the hyperthyroid animals in the present study may be due to the observed absence of hypothermia with OTMN or TMN. Nevertheless, treatment of hyperthyroid mice at normal temperatures with the larger dose of OTMN, (0.75 mg/kg which produced tremor and hyperthermia in the euthyroid mice at high ambient temperatures), still does not cause a high level of tremor. Thus the lack of hypothermia in hyperthyroid animals is not the main cause of their insensitivity to the tremorigenic effects of TMN and OTMN.

Therefore, since there are arguments against the other suggestions, it is possible that the insensitivity of hyperthyroid mice to TMN and OTMN may be due to a change in the sensitivity of the CNS to the active agent OTMN. There is some evidence to support this last suggestion. It is known that drugs with central sympathomimetic activity are capable of antagonising the central effects of TMN and OTMN (Spencer, 1965; 1966). Also, a large number of papers have implicated enhanced activity in the central adrenergic nervous system of hyperthyroid animals (for review, see: Harrison, 1961). Finally, Ross (Personal communication) has found that brain DA levels are increased in hyperthyroid animals. Consequently, it is suggested that the observed insensitivity of hyperthyroid mice to OTMN (and TMN) is due to increased activity in central adrenergic synapses. Admittedly, there is as yet no evidence to support or refute the possibility that there is also a change in the level of activity of central cholinergic synapses.

SUMMARY

1. In male TO mice, thyroxine-induced hyperthyroidism inhibits the hypothermic and tremorigenic effects of tremorine and oxotremorine.

2. The lack of tremor in hyperthyroid animals is not due to faulty biotransformation of tremorine, nor to failure of oxotremorine to penetrate the CNS; nor does it appear to be associated with the absence of concomitant hypothermia.

3. It is postulated that there is a change in the sensitivity of the CNS of the hyperthyroid mice, possibly due to an enhancement of central adrenergic mechanisms in these animals. A reduction in central cholinergic activity is also a possibility.

TABLE 46

Core temperatures and tremor of hyperthyroid (HT) and euthyroid (ET) male TO mice following subcutaneous injection of tremorine hydrochloride 20 mg/kg at an ambient temperature of 20° C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the mean core temperature (°C ± S.E.) and the total tremor score (T) of groups of 10 animals.

Time (min)	HT	T	HT-C	ET	T	ET-C
0		38.6 ± 0.2			38.4 ± 0.2	
30	36.6 ± 0.4	11	38.5 ± 0.1	31.5 ± 0.3	12	38.4 ± 0.2
60	37.1 ± 0.4	1	38.6 ± 0.2	30.1 ± 0.5	16	38.3 ± 0.1
90	37.2 ± 0.3	0	38.8 ± 0.2	30.2 ± 0.7	16	38.3 ± 0.2
120	37.5 ± 0.6	0	38.9 ± 0.2	31.4 ± 0.7	7	38.3 ± 0.5

FIGURE 50.

Mean core temperatures and total tremor scores of groups of hyperthyroid (HT) and euthyroid (ET) male TO mice after subcutaneous injection of tremorine hydrochloride 20 mg/kg at an ambient temperature of 20°C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route (see Table 46).

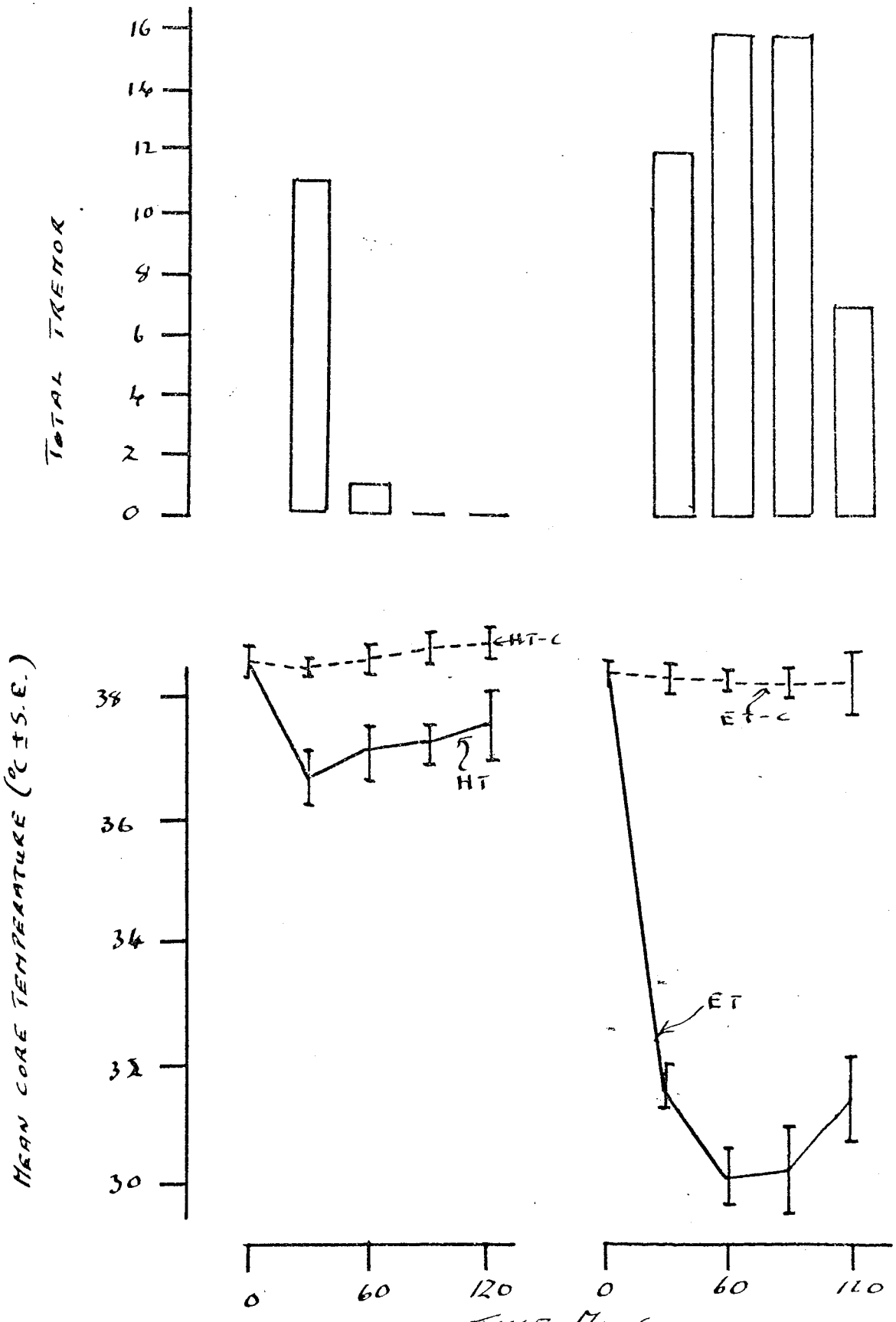


TABLE 47

Core temperatures of euthyroid (ET) male TO mice following subcutaneous injection of tremorine hydrochloride 20 mg/kg. One group (ET-S) were pretreated with SKF 525A 2 hrs. before tremorine administration. Control animals were treated with equivalent volumes of water orally and normal saline subcutaneously. (ET-C). The results are expressed as the mean core temperature (°C ± S.E.) and the total tremor scores (T) of groups of 10 animals.

Time (min)	ET	T	ET-S	T	ET-C
0			38.57 ± 0.2		
30	32.66 ± 0.4	8	35.3 ± 0.4	0	38.3 ± 0.2
60	31.5 ± 0.6	13	34.3 ± 0.5	0	38.3 ± 0.2
120	31.9 ± 0.8	11	36.5 ± 0.5	0	37.9 ± 0.3

FIGURE 51.

Mean core temperatures and total tremor scores of euthyroid mice (ET) after subcutaneous injection of tremorine 20 mg / kg at an ambient temperature of 22°C. One group ET-S had been pretreated with SKF 525A (12.5 mg/kg orally) 2 hrs before administration of tremorine. Control animals (ET-C) were treated with equivalent volumes of water orally and saline subcutaneously .

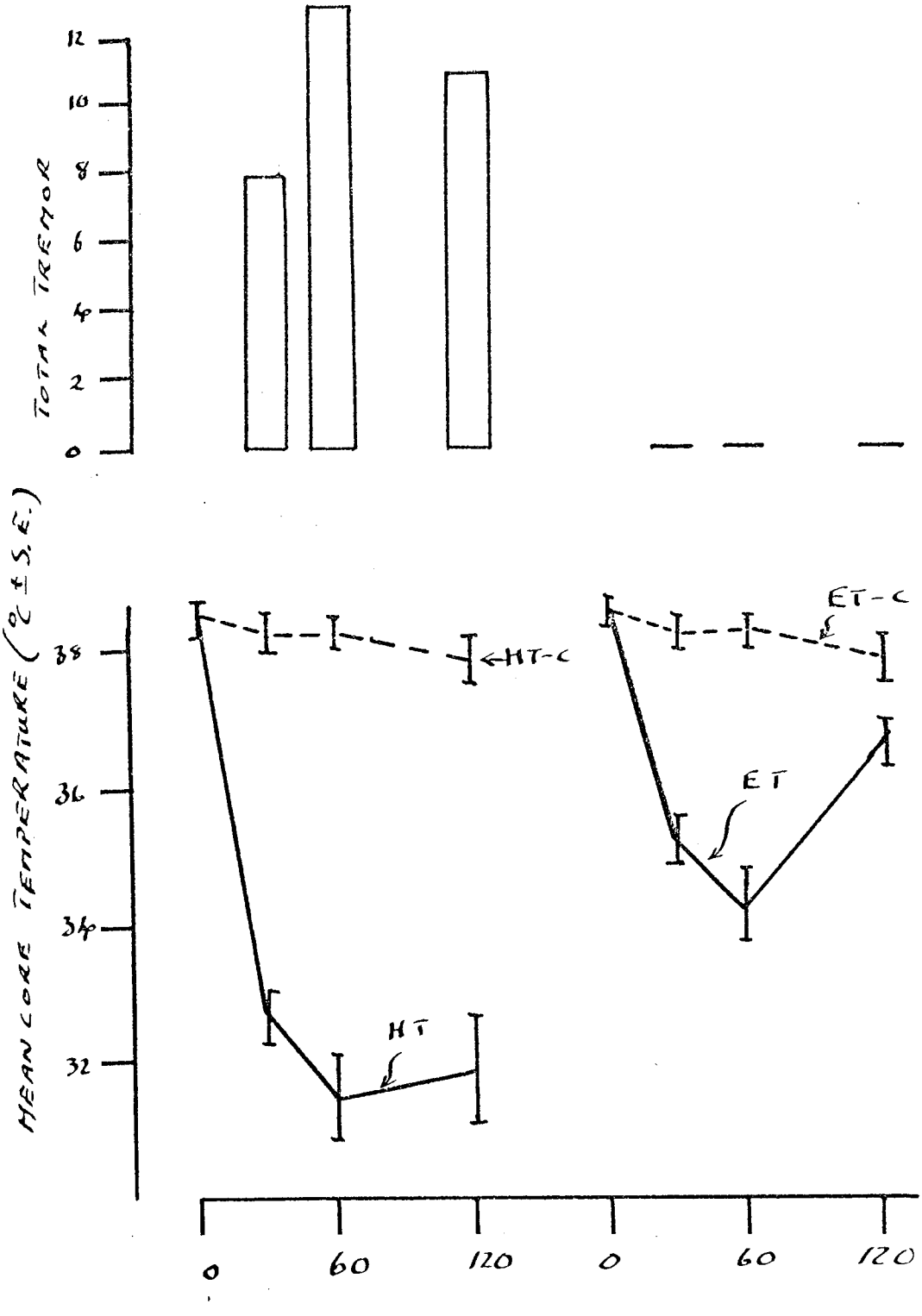


TABLE 48

Core temperatures and tremor of hyperthyroid (HT) and euthyroid (ET) male TO mice following subcutaneous injection of oxotremorine oxalate 0.5 mg/kg at an ambient temperature of 20° C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. The results are expressed as the mean core temperatures (° C ± S.E.) and the total tremor scores (T) of groups of 10 animals.

Time (min)	HT	T	HT-C	ET	T	ET-C
0		38.8 ± 0.1			38.4 ± 0.1	
30	36.9 ± 0.4	1	38.6 ± 0.3	30.0 ± 0.3	19	38.3 ± 0.1
60	37.2 ± 0.4	0	38.5 ± 0.2	28.4 ± 0.5	14	38.3 ± 0.1
90	38.0 ± 0.2	0	38.4 ± 0.1	29.9 ± 0.8	10	38.1 ± 0.2
120	38.4 ± 0.1	0	38.4 ± 0.1	33.8 ± 0.8	1	38.0 ± 0.1

FIGURE 52.

Mean core temperatures and total tremor scores of groups of hyperthyroid(HT) and euthyroid (ET) male TO mice after subcutaneous injection of oxotremorine oxalate 0.5 mg/kg at an ambient temperature of 20°C . Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route (see Table 48)

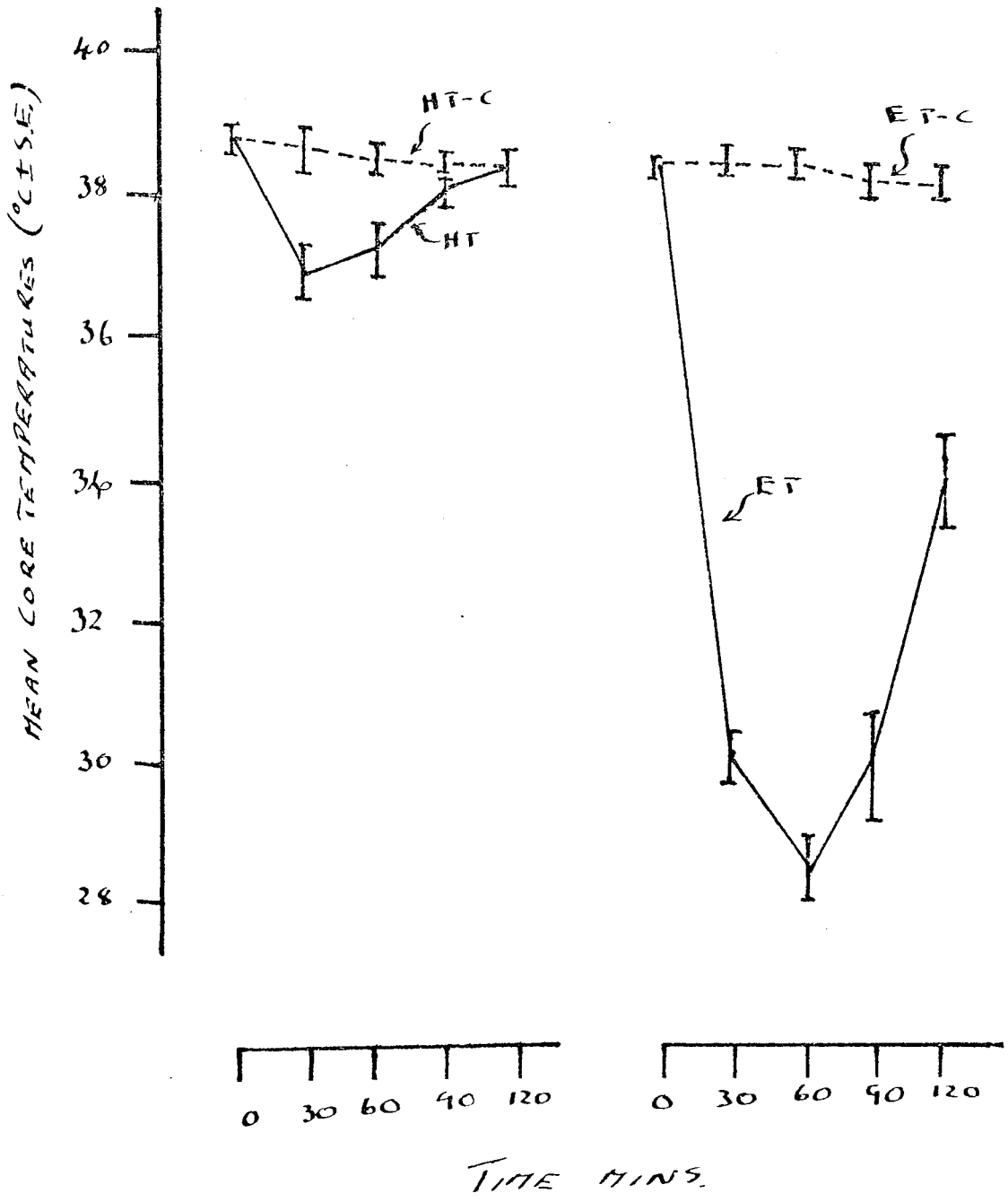


TABLE 49

Core temperatures and tremor of euthyroid (ET) male TO mice following subcutaneous injection of tremorine hydrochloride 20 mg/kg at an ambient temperature of 30° C. Control animals (ET-C) received an equivalent volume of normal saline by the same route. The results are expressed as the mean core temperatures (°C ± S.E.) and total tremor scores (T) of groups of 5 animals.

Time (min)	ET	T	ET-C
0		38.0 ± 0.1	
15	37.2 ± 0.3	0	38.6 ± 0.1
30	36.7 ± 0.6	2	38.4 ± 0.2
60	36.9 ± 0.7	2	38.2 ± 0.1

FIGURE 53.

Mean core temperatures and total tremor scores of euthyroid (ET) male TO mice after subcutaneous injection of tremorine hydrochloride 20 mg/kg at an ambient temperature of 30°C. Control animals (ET-C) received an equivalent volume of normal saline by the same route (see Table 49)

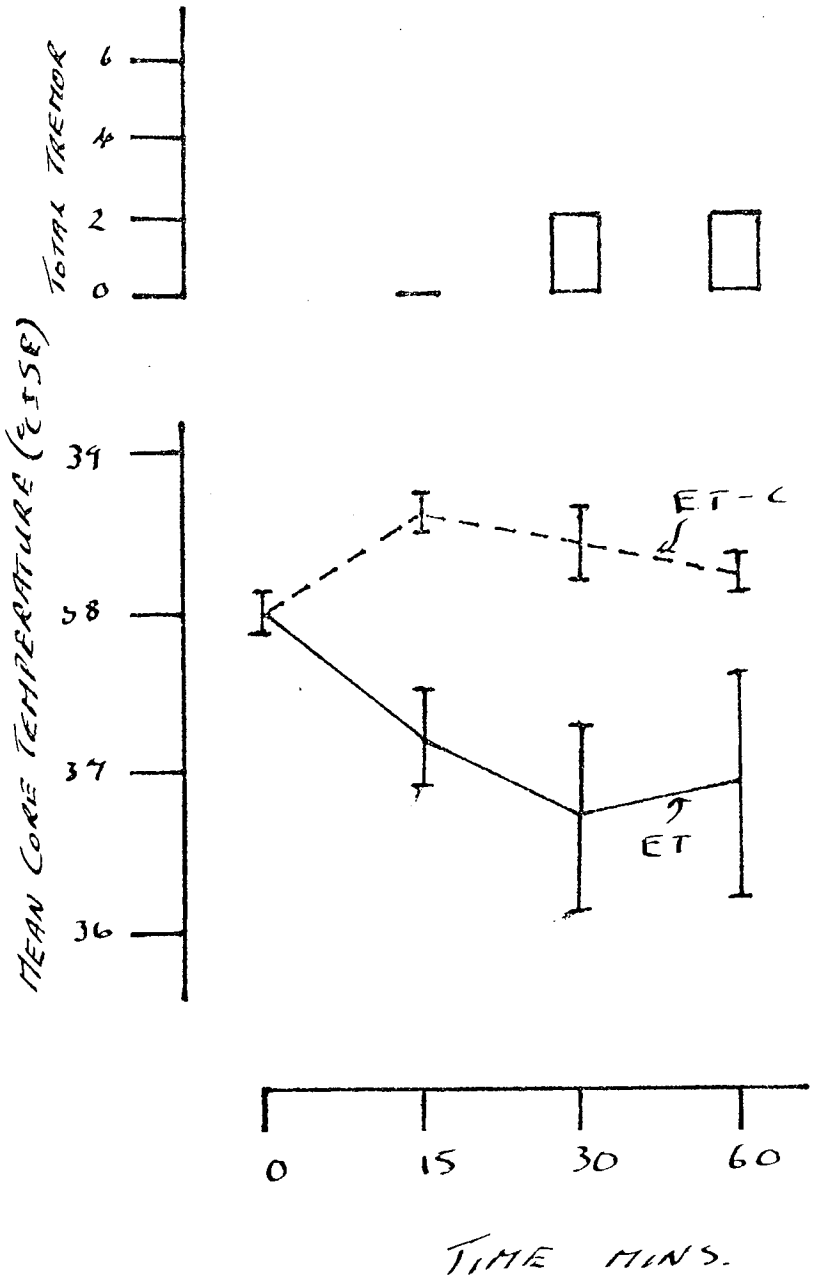


TABLE 50

Core temperatures and tremor of euthyroid (ET) male TO mice following subcutaneous injection of oxotremorine oxalate 0.5 mg/kg at an ambient temperature of 36° C. Control animals (ET-C) received an equivalent volumej of normal saline by the same route. The results are expressed as the mean core temperatures (° C ± S.E.) and total tremor scores (T) of groups of 5 animals.

Time (min)	ET	T	ET-C
0		39.4 ± 0.1	
15	38.8 ± 0.4	5	38.8 ± 0.3
30	39.0 ± 0.4	3	38.8 ± 0.3
60	38.7 ± 0.3	0	38.8 ± 0.3

FIGURE 54.

Mean core temperatures and total tremor scores of euthyroid (ET) male TO mice after subcutaneous injection of oxotremorine oxalate 0.5 mg/kg at an ambient temperature of 36°C. Control animals(ET-C) received an equivalent volume of normal saline by the same route(see Table 50).

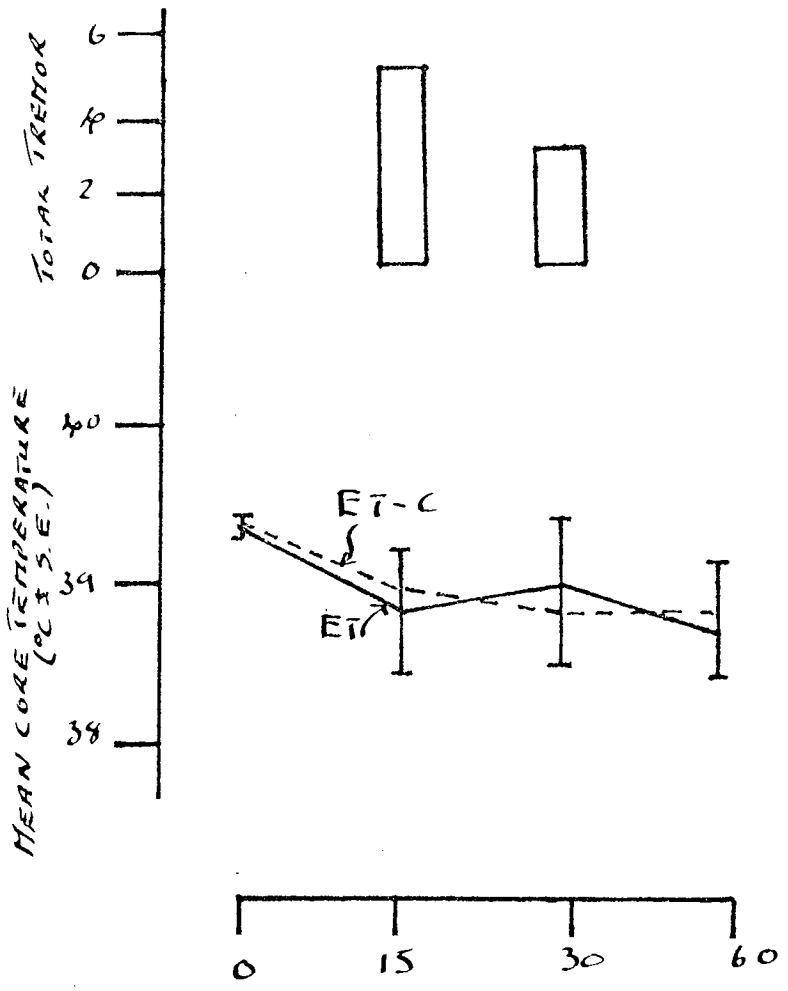


TABLE 51

Core temperatures and tremor of euthyroid (ET) male TO mice following subcutaneous injection of oxotremorine oxalate 0.75 mg/kg at an ambient temperature of 37°C. Control animals (ET-C) received an equivalent volume of normal saline by the same route. The results are expressed as the mean core temperatures (°C ± S.E.) and total tremor scores (T) of groups of 5 animals.

Time (min)	ET	T	ET-C
0		39.9 ± 0.1	
15	39.7 ± 0.4	8	40.0 ± 0.2
30	40.3 ± 0.4	5	39.6 ± 0.1
60	40.5 ± 0.2	3	39.8 ± 0.1

Figure 55.

Mean core temperatures and total tremor scores of euthyroid (ET) male TO mice after subcutaneous injection of oxotremorine oxalate 0.75 mg/kg at an ambient temperature of 37°C. Control animals (ET-C) received an equivalent volume of normal saline by the same route (see Table 51).

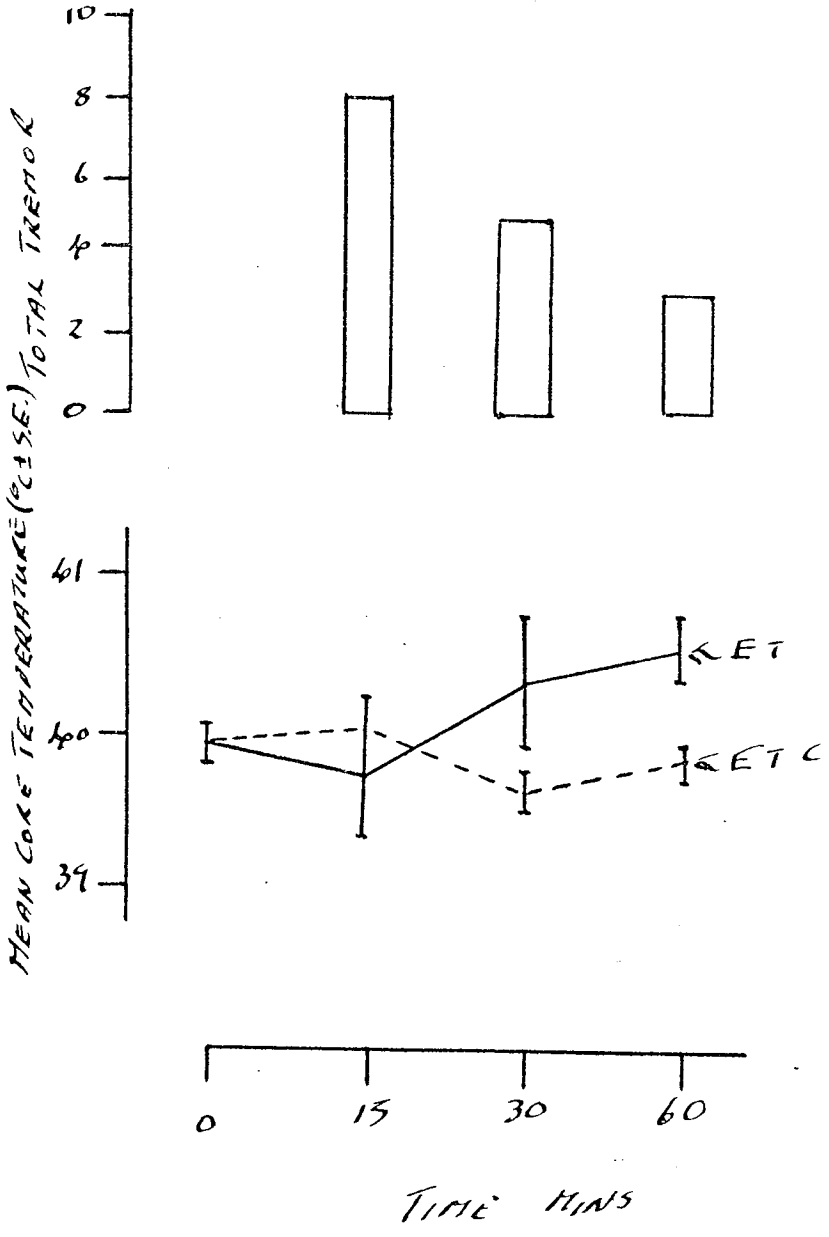


TABLE 52

Core temperatures and tremor of hyperthyroid (HT) male TO mice following subcutaneous injection of oxotremorine oxalate 0.75 mg/kg at an ambient temperature of 22° C. Control animals (HT-C) received an equivalent volume of normal saline by the same route. The results are expressed as the mean core temperatures (° C ± S.E.) and total tremor scores (T) of groups of 5 animals.

Time (min)	HT	T	HT-C
0		37.8 ± 0.2	
30	35.6 ± 1.3	4	37.9 ± 0.1
60	35.5 ± 0.9	1	38.0 ± 0.1
90	37.6 ± 0.7	0	38.0 ± 0.1

FIGURE 56.

Mean core temperatures and total tremor scores of hyperthyroid (HT) male TO mice after subcutaneous injection of oxotremorine oxalate 0.75 mg/kg at an ambient temperature of 22°C. Control animals (ET-C) received an equivalent volume of normal saline by the same route (see Table 52).

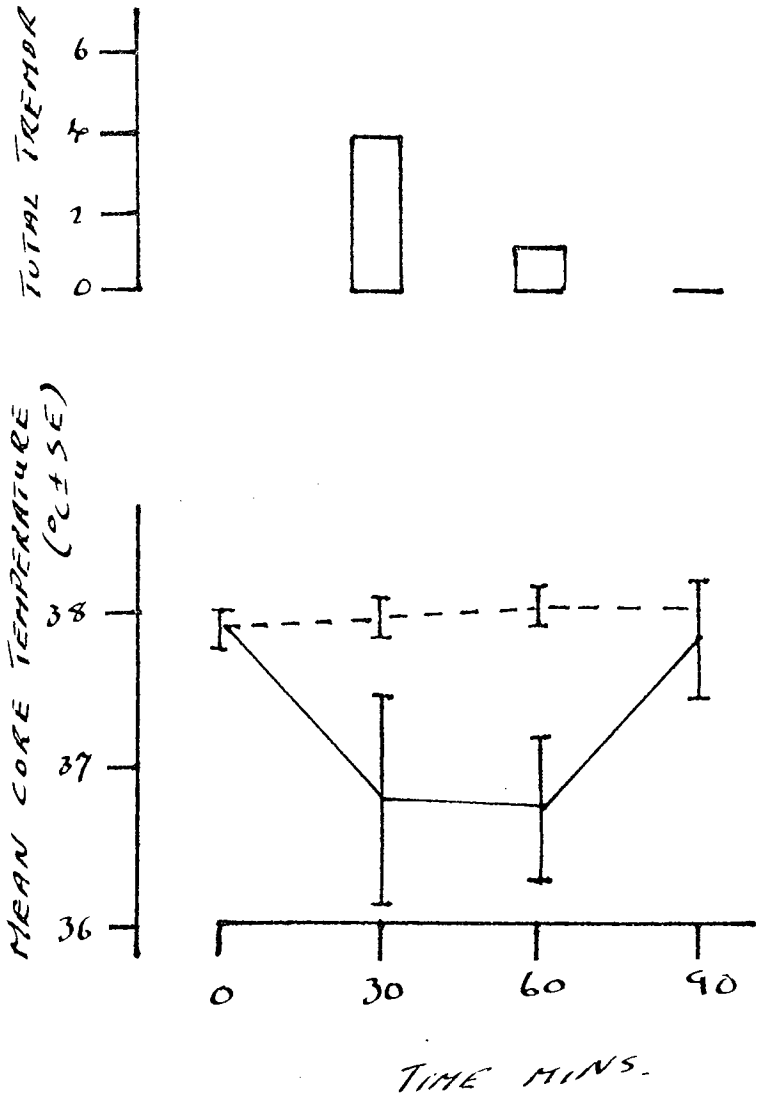


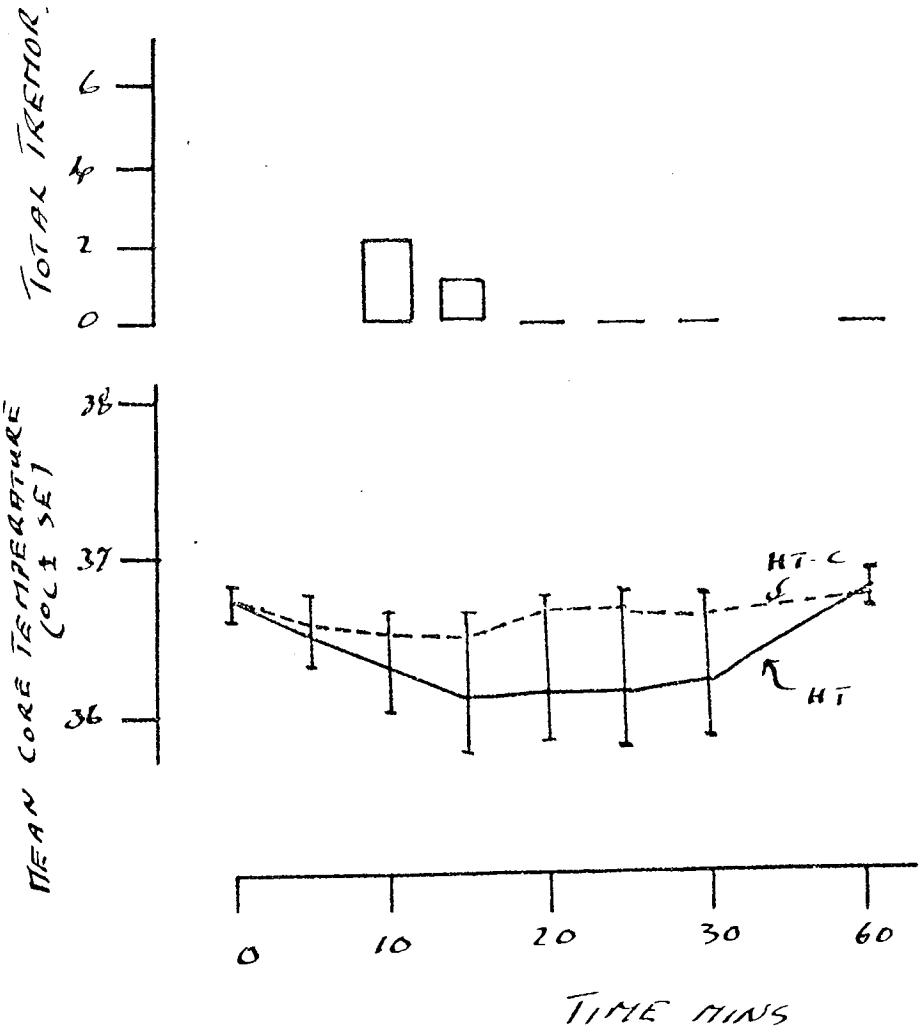
TABLE 53

Core temperatures and tremor of hyperthyroid (HT) and euthyroid (ET) male TO mice following intracerebral injection of oxotremorine oxalate 1.5 ug at an ambient temperature of 22° C. Control animals (HT-C and ET-C respectively) received an equivalent volume of normal saline by the same route. Results are expressed as the mean core temperatures (°C ± S.E.) and total tremor scores (T) of groups of 5 animals.

Time (min)	HT	T	HT-C	ET	T	ET-C
0		37.4±0.2			36.0±0.3	
5	37.0±0.4	0	37.1±0.4	36.0±0.3	7	35.8±0.3
10	36.6±0.6	2	37.0±0.3	32.5±0.2	9	35.9±0.3
15	36.2±0.7	1	37.0±0.3	31.9±0.2	6	36.0±0.3
20	36.3±0.7	0	37.1±0.4	31.3±0.5	5	36.1±0.2
25	36.3±0.7	0	37.3±0.3	30.9±0.3	3	36.1±0.1
30	36.4±0.7	0	37.3±0.2	30.9±0.4	1	36.0±0.1
60	37.6±0.2	0	37.5±0.1	32.6±0.8	0	36.2±0.2

FIGURE 57.

Mean core temperatures and totaltremor scores of hyperthyroid (HT) male TO mice after intracerebral injection of oxotremorine oxalate 1.5 ug at an ambient temperature of 22°C. Control animals (HT-C)received an equivalent volume of normal saline by the same route (see Table 53)



CHAPTER VII.

THE EFFECTS OF CONVULSANT COMPOUNDS IN HYPERTHYROID AND EUTHYROID MICE

CHAPTER VII

THE EFFECTS OF CONVULSANT COMPOUNDS IN HYPERTHYROID AND EUTHYROID MICE.

1. Introduction.

It is known that in hyperthyroidism the EEG shows changes suggestive of a state of hyperexcitability similar to that seen in epilepsy. Also, it has been reported that the susceptibility of hyperthyroid animals to convulsions induced by electroshock is increased compared to that of euthyroid animals (Timiras & Woodbury, 1956). In view of these observations, it was decided to investigate the activities of a number of convulsant drugs in hyperthyroid and euthyroid mice. In these experiments, three parameters of drug activity were determined: (a) the time at which the mice showed the first convulsion of more than 3 sec. duration; (b) the number of animals dying and the times at which they died; (c) in some cases, the effects of the convulsant on body temperature were also recorded. All the drugs used were administered intraperitoneally and the mice caged individually for observation as detailed in the methods.

2. Effects of leptazol in hyperthyroid and euthyroid mice.

The effects of this drug administered at doses of 60, 80, 90, and 100 mg/kg to mice of both groups were determined at ambient temperatures of 22° and 24° C.

i. Effects of leptazol at 22° C.

Fig. 58 (Table 55) shows the dose-mortality relationship obtained with 60, 80 & 100 mg/kg of this drug in hyperthyroid and euthyroid mice.

As the histogram shows, at the two lower doses the hyperthyroid animals were significantly more susceptible to the lethal effects of the drug than the euthyroid animals. At the highest dose, however, 100% mortality was obtained with both groups. Table 55 shows the mean times of onset of leptazol induced convulsions; at each of the dose levels used, the onset of convulsions occurred significantly more quickly in the hyperthyroid mice than in the euthyroid. It was also observed that the time of onset of convulsions was dose dependant. In contrast consistent or significant difference was observed in the times at which death occurred in the two groups of animals (see Table 55).

In a preliminary experiment it had been noted that hyperthyroid mice became hyperthermic after injection of leptazol. Measurements of core temperature before treatment with the drug and at 15 min. (or at death as applicable), revealed that the drug produced a mild hyperthermia in the hyperthyroid mice whilst, in contrast, the euthyroid animals showed a more distinct hypothermia. The hyperthermia seen in the hyperthyroid mice was of 0.8° and 1.0°C following doses of 60 and 80 mg/kg respectively, whilst the hypothermia in the euthyroid animals was of 1.1 and 3°C following the same doses of leptazol (see Table 54). (These values are statistically significant $P = <0.001$). Thus this initial experiment showed not only that the hyperthyroid animals were more susceptible to the convulsant and lethal effects of this drug, but also that there was a qualitative difference in the temperature responses of the two groups of animals following treatment with leptazol. It seemed reasonable to assume that the increased sensitivity to convulsion in hyperthyroid mice might be, to some extent, attributable to the hyperthermia produced by

leptazol. To investigate this possibility further, the effects of leptazol were compared in the two groups of mice at a higher environmental temperature, 24° C.

ii. Effects of leptazol at 24° C.

Fig. 59 (Table 56) shows the dose-mortality relationship for leptazol at 80 & 90 mg/kg at an ambient temperature of 24° C in hyperthyroid and euthyroid mice. As seen at the lower temperature the drug was significantly more toxic in the hyperthyroid animals than in their euthyroid counterparts, and further there was a significant increase in the toxicity of the drug in both groups of mice compared with that seen at 22° C. The time of onset of convulsions was significantly reduced in the hyperthyroid mice (Table 56) and was again dose dependant. As at the lower temperature there was no significant or consistent difference in the times at which death occurred in the two groups of mice (see Table 56).

At this environmental temperature, treatment with leptazol again produced a mild but significant hyperthermia in the hyperthyroid mice whilst causing a significant hypothermia in the euthyroid animals. The values in this case are shown in Table , the hyperthyroid mice responded to treatment with a hyperthermia of 1.1° C following 80 mg/kg, whilst the euthyroid animals showed a hypothermia of 1.3° C with the same dose.

Thus this experiment showed that the toxicity of the drug was significantly influenced by the prevailing ambient temperature. It also added weight to the hypothesis that the increased toxicity seen in the hyperthyroid mice might, to some extent, be due to the hyperthermia produced by this drug in these animals.

3. Effects of picrotoxin in hyperthyroid and euthyroid mice.

The effects of this drug were investigated in hyperthyroid and euthyroid mice at doses of 4 & 5 mg/kg at an ambient temperature of 22° C. In addition to the other parameters of drug activity determined a fifth parameter, namely the number of convulsions shown by each mouse in the 30 min. period of the experiment, was also noted.

Fig. 60 (Table 58) shows the dose-mortality relationship for hyperthyroid mice treated with a dose of picrotoxin which was non-lethal in euthyroid animals. As seen with leptazol, the time of onset of the convulsions was dose-dependant and occurred sooner in hyperthyroid than in euthyroid animals (see Table 58). Table 58 shows the number of convulsions occurring during the experiment; hyperthyroid animals showed significantly more convulsions than did euthyroid mice. Whilst the former all convulsed with either dose the euthyroid mice showed a 70% response to the lower dose and a 90% response to the higher dose. Recordings of core temperature revealed that the drug produced a hyperthermia of 1.9° and 0.4° C in the hyperthyroid mice, whereas it caused a hypothermia of 3.8° and 3.4° C in the euthyroid animals at doses of 4 & 5 mg/kg respectively, (see Table 59).

Thus with this convulsant, as with letpazol, hyperthyroid mice were more susceptible to the convulsant and lethal effects than were their euthyroid counterparts. Once again this increase in sensitivity occurred in the presence of a mild hyperthermia in hyperthyroid mice in contrast to a hypothermia in euthyroid animals.

4. Effects of other convulsant compounds in hyperthyroid and euthyroid mice.

a/STRYCHNINE

The effects of 1.0 and 1.5 mg/kg of Strychnine given intraperitoneally were recorded at an ambient temperature of 22° C. Fig. 62 (Table 61) shows that, as with the other convulsants investigated, hyperthyroid mice were significantly more prone to the lethal effects of the drug than were euthyroid mice. Again, the onset of convulsions was dose-dependant and occurred significantly more quickly in hyperthyroid mice (see T able).

c/NICOTINE

In these experiments, doses of 10 & 20 mg/kg of nicotine were administered to groups of hyperthyroid and euthyroid mice at an ambient temperature of 22° C.

Once again the hyperthyroid mice were found to be significantly more sensitive to the toxic effects of the compound than were the euthyroid animals (see Fig. 61 Table 60). The time of onset of the convulsions was also reduced in the hyperthyroid animals compared to that seen in the euthyroid mice as shown in Table .

The results of these experiments carried out with four different convulsants showed that the hyperthyroid mice were consistently more prone to the toxic and convulsant effects of all the drugs tested. It was decided, therefore, to investigate the effects of drugs known to influence the activities of convulsant compounds in hyperthyroid and euthyroid mice, to reveal any differences in the activities of such compounds induced by hyperthyroidism.

5. Effects of pretreatment with amphetamine on the convulsant properties of leptazol in hyperthyroid and euthyroid mice.

Amphetamine and certain monoamine oxidase inhibitors which possess sympathomimetic activity have been shown to have a pro-convulsant effect in mice (Turner & Spencer, 1968). It was decided to investigate the effects of pretreatment with d-amphetamine on the convulsant action of leptazol in hyperthyroid and euthyroid mice.

In this experiment groups of hyperthyroid and euthyroid mice were pretreated with d-amphetamine sulphate 3 mg/kg orally, or with distilled water (controls), 30 min. before administration or leptazol 60 mg/kg intraperitoneally. The experiment was carried out at 22° C and the parameters of drug activity recorded were: %mortality, time of onset of convulsions, and changes in core temperature, as before.

It was found that, as noted before, the hyperthyroid animals were more susceptible to the lethal effects of the drug than were the euthyroid mice. Pretreatment of both groups of animals with amphetamine potentiated the lethal effects of the drug, as shown in Fig. 63 (Table 62). The onset of convulsions occurred sooner in the hyperthyroid than in the euthyroid mice, and was in both cases significantly shortened by the amphetamine, as shown in Table 62 The amphetamine caused an increase in the hyperthermia shown by the hyperthyroid mice in response to letpazol, and reduced the degree of hypothermia shown by the euthyroid animals (see Table 63)

The experiment showed that the proconvulsant properties of amphetamine were accompanied by an increase in the hyperthermic response of the hyperthyroid mice to leptazol and a decrease in the hypothermic

response of the euthyroid animals. This gives further weight to the theory that the difference in the responses of the hyperthyroid and euthyroid mice is related to the different susceptibilities of the two types of animal to changes in core temperature.

6. Effects of pretreatment with chlordiazepoxide on the convulsant properties of leptazol in hyperthyroid and euthyroid mice.

In these experiments groups of hyperthyroid and euthyroid mice were pretreated with chlordiazepoxide at doses of 2.5 & 5 mg/kg subcutaneously, or with saline (controls), 30 min. before administration of leptazol at ambient temperatures 22° and 24° C.

i. Effects of pretreatment with chlordiazepoxide at 22° C.

At 5 mg/kg and an ambient temperature of 22° C, the anticonvulsant prevented the lethal effects of the leptazol in both groups of mice (see Fig 64 Table 64) and also significantly delayed the time of onset of convulsions in both groups (see Table 64). The control animals of both groups showed the same differences in mortality and time of onset of convulsions as reported previously. The anticonvulsant also decreased the hyperthermia seen in the hyperthyroid mice and potentiated the hypothermia shown by the euthyroid animals in response to leptazol (see Table 65).

ii. Effects of pretreatment with 2.5 mg/kg at 22° C.

At the lower dose 2.5 mg/kg the anticonvulsant did not completely abolish the lethal effects of leptazol at this temperature. However the effects of leptazol were significantly reduced in both the hyperthyroid and euthyroid mice (Fig. 65 Table 66). Once again the times of onset were significantly prolonged by the anticonvulsant in both groups of animals (see Table 66)

iii. Effects of pretreatment with chlordiazepoxide 5 mg/kg at 24° C.

At this higher environmental temperature the anticonvulsant was no longer completely effective against the lethal effects of the drug and the treated mice of both groups suffered a 10% mortality as shown in Fig. 66 (Table 67). The times of onset of convulsions were again significantly prolonged as shown in Table 67

Thus, in these experiments, although the perceptage mortality was reduced to the same level in the hyperthyroid and the euthyroid animals, the anticonvulsant was more potent in the hyperthyroid mice since these animals were already more susceptible to the effects of leptazol. The experiments also showed that the anticonvulsant effect of chlordiazepoxide was accompanied by a reduced hyperthermia in hyperthyroid mice and an increase in the hypothermia of euthyroid animals in response to leptazol. This may be a basis for the antileptazol activity of chlordiazepoxide since the anticonvulsant activity was antagonised by increasing the laboratory temperature.

7. The effects of DNP on the convulsant action of leptazol.

The foregoing results indicate that the temperature response of the mice of both groups are intimately related to their susceptibility to convulsion. However, Timiras and Woodbury (1956) have reported that doses of DNP which raise the core temperature are not proconvulsant. It was, therefore, decided to investigate the effects of DNP on leptazol convulsions in euthyroid mice. The animals were pretreated with DNP 10 mg/kg I.P. 90 mins. before the administration of the leptazol since this dose had been found to produce a hyperthermia of about 1° C i.e. similar to that of the hyperthyroid mice. The results (see Fig. 67

Table 68) confirmed those of Timiras and Woodbury (1956) in that the compound did not exert any proconvulsant effect. However, temperature measurements revealed that although the DNP treated mice had initial core temperatures similar to those of the hyperthyroid animals these animals responded to leptazol with a hypothermia as did the euthyroid mice. Thus the results indicated that although the hyperthermia produced by DNP or T4 was quantitatively similar there were qualitative differences which resulted in different responses of the two groups of animals to leptazol.

DISCUSSION

There have been a number of reports that hyperthyroidism induced by T4 or T3 increases the susceptibility of experimental animals to electrically induced convulsions, (see for example Timiras & Woodbury, 1956). This increase in brain excitability has been ascribed by Woodbury and co workers (1952 & 1956) to changes in the distribution of brain electrolytes resulting from T4 treatment and associated changes in adrenal cortical function. These workers however were unable to demonstrate any relationship between T4-induced changes in body weight or body temperature and susceptibility to convulsion. Furthermore, treatment with DNP - whilst increasing body temperature and metabolic rate - had no effect on brain excitability.

The results presented in this chapter show that hyperthyroid mice are more sensitive than euthyroid mice to the lethal and convulsant effects of the four convulsant agents studied. It has also been shown that increases in environmental temperature have a proconvulsant effect in both hyperthyroid and euthyroid animals. Following administration of leptazol or picrotoxin, hyperthyroid mice undergo a mild hyperthermia whilst euthyroid animals respond to the drugs with a hypothermia. Also increases in environmental temperature increase the hyperthermia of the hyperthyroid mice, inhibit or reduce the hypothermic response of the euthyroid animals, and simultaneously exert proconvulsant effect in both groups. Thus it is postulated that the temperature response of the hyperthyroid animals is one of the factors which predisposes these animals to convulsion. The report of Timiras and Woodbury (1956), that DNP treatment does not significantly alter the excitability of euthyroid animals,

has been confirmed. However it was found that, although this agent itself produced a hyperthermia, subsequent treatment of the animals with a leptazol still lead to the onset of a mild hypothermia similar to that seen in untreated euthyroid mice. Therefore, although both T4 and DNP induce quantitatively similar degrees of hyperthermia, qualitative differences induce mice to respond differently to leptazol. Since leptazol treatment is reported to result in increased peripheral sympathetic activity (Hahn 1960) it may well be that the hyperthermia seen in response to leptazol is the result of increased calorigenic potency of peripherally released catecholamines in these animals (Swanson 1956).

In the investigation of the effects of d-amphetamine on the actions of letpazol in hyperthyroid and euthyroid mice, the report of Turner and Spencer (1968) was confirmed; a definite proconvulsant activity of the drug was demonstrated in both groups of mice. This proconvulsant effect is accompanied by an increase in the hyperthermia of the hyperthyroid mice and an inhibition of the hypothermia of the euthyroid animals in response to the convulsant.

The anticonvulsant action of chlordiazepoxide (Swinyard & Castellion 1966) has also been investigated. The results show that the anticonvulsant action is accompanied by an inhibition of the hyperthermia of the hyperthyroid mice and a potentiation of the hypothermia of the euthyroid animals in response to leptazol. Thus the hyperthyroid mice show hyperthermia in response to convulsant compounds and are more susceptible to the lethal and convulsant effects of such compounds than are euthyroid animals. Further, increases in environmental temperature and treatment with d-amphetamine, (both of which potentiate the hyperthermia in hyperthyroid animals and reduce the hypothermia in euthyroid animals),

have a proconvulsant effect in both groups of mice. Finally, chlordiazepoxide has an anticonvulsant action in both the hyperthyroid and the euthyroid mice which is accompanied by an inhibition of the hyperthermia of the hyperthyroid mice and a potentiation of the hypothermia of the euthyroid animals. Considered together, these facts are strongly suggestive of a role of body temperature in the response of mice to convulsant compounds. It is postulated that the thermal response of hyperthyroid mice to convulsant agents is one of the factors which predisposes these mice to convulsion.

These results, however, only suggest a subsidiary mechanism whereby the convulsant actions of these drugs are potentiated by T4-induced hyperthyroidism; other work (Timiras & Woodbury, 1952 & 1956) suggesting other mechanisms for the proconvulsant activity of T4 cannot be ignored.

SUMMARY

1. Thyroxine induced hyperthyroidism in male TO mice renders these animals more susceptible to the lethal and convulsant effects of leptazol, picrotoxin, nicotine and strychnine than euthyroid animals.

2. At an ambient temperature of 22° C the hyperthyroid mice show a significant hyperthermia and the euthyroid animals a significant hypothermia in response to treatment with leptazol or picrotoxin.

3. At an ambient temperature of 24° C the hyperthermia of the hyperthyroid mice in response to leptazol was increased and the hypothermia of the euthyroid animals was reduced. This effect was accompanied by an increase in the convulsant and lethal potency of leptazol in both groups of mice.

4. Amphetamine has a proconvulsant action in both hyperthyroid and euthyroid mice. This effect is accompanied by an increase in the hyperthermic response of the hyperthyroid animals and a decrease in the hypothermic response of the euthyroid animals following administration of leptazol.

5. Chlordiazepoxide has an anti-convulsant action in both hyperthyroid and euthyroid mice. This effect is accompanied by a decrease in the hyperthermic response of the hyperthyroid mice and an increase in the hypothermic response of the euthyroid animals following administration of leptazol.

6. Treatment with 2,4-dinitrophenol does not influence the convulsant potency of leptazol in euthyroid mice, nor does it alter the temperature responses of these animals to the convulsant.

TABLE 54

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following intraperitoneal injection of leptazol at an ambient temperature of 22° C. The table shows the dose (mg/kg) of leptazol, the number (No) of animals tested, the mean core temperatures (° C S.E.) before (PRE) treatment with leptazol, at death or 15 min. after administration (POST) and the mean change in core temperature (DIFF ° C).

HT

DOSE	No	PRE	POST	DIFF	P
60	10	39.1 ± 0.2	39.9 ± 0.3	+0.7	<0.05
80	35	38.3 ± 0.1	39.3 ± 0.2	+1.0	<0.001

ET

60	10	38.6 ± 0.1	35.5 ± 0.3	-3.0	<0.001
80	35	37.6 ± 0.1	36.4 ± 0.2	-1.1	<0.001

TABLE 55

Effects of intraperitoneal injection of leptazol in hyperthyroid (HT) and euthyroid (ET), male TO mice at an ambient temperature of 22° C. The table shows; the dose of leptazol (mg/kg), the number of animals tested (No), the mean time of the onset of convulsions (F - secs ± S.E.) the mean time at which death occured (D - min ± S.E.) and the percentage mortality produced (%).

Dose	No	F sec	D min	%
		HT		
60	10	90.9 ± 8.7	1.2 ± 0.1	33
80	56	59.9 ± 2.1	5.7 ± 0.5	63
90	20	42.6 ± 1.6	3.1 ± 0.7	95
100	10	44.3 ± 3.3	2.7 ± 1.4	100
		ET		
60	10	120.0 ± 15.9	-	-
80	56	67.9 ± 2.8	4.3 ± 0.7	37
90	20	58.3 ± 5.6	3.5 ± 0.8	71
100	10	45.0 ± 1.6	3.4 ± 1.0	100

FIGURE 58

Mortality of hyperthyroid (HT) and euthyroid(ET) male TO mice after intraperitoneal injection of leptazol at an ambient temperature of 22°C.

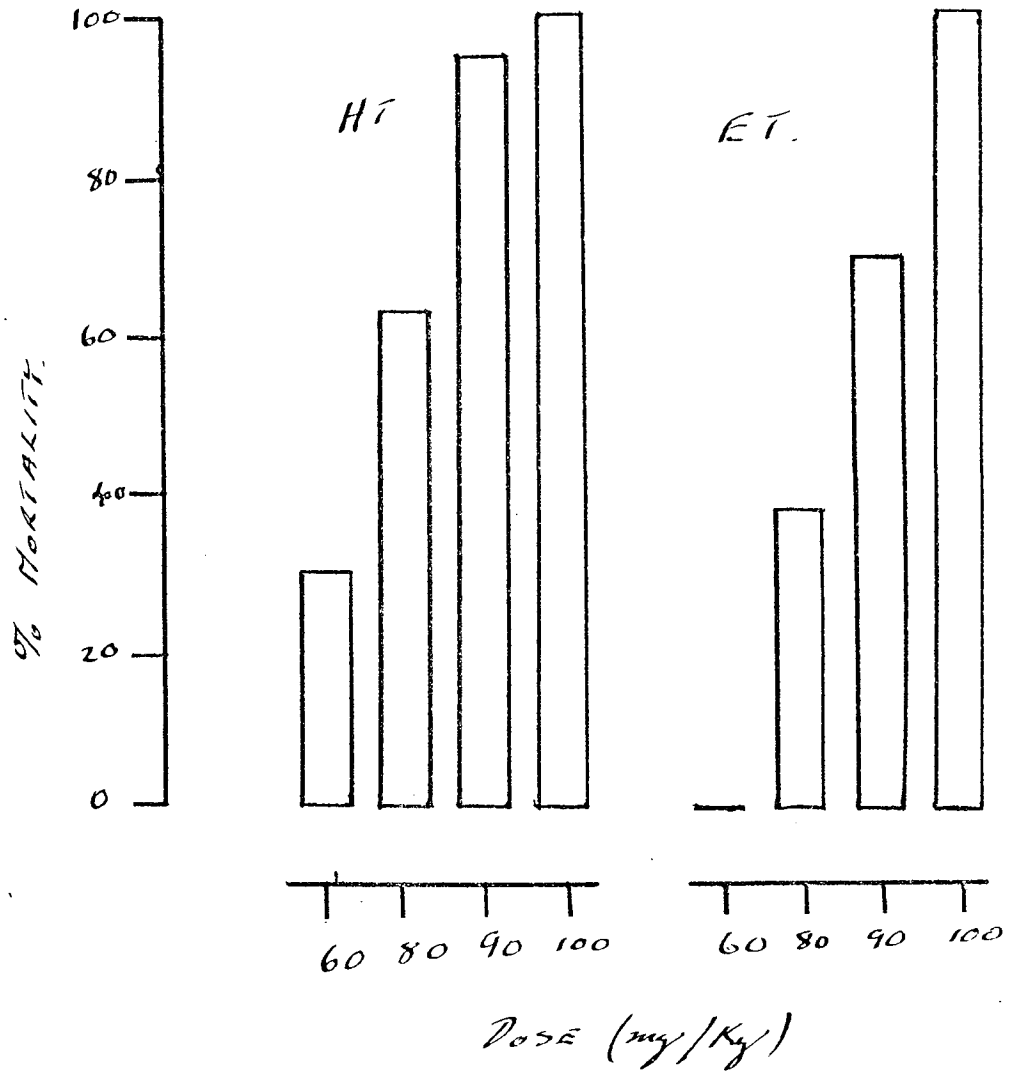


TABLE 56

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following intraperitoneal injection of leptazol at an ambient temperature of 24° C. The table shows the dose (mg/kg) of leptazol, the number (No) of animals tested, the mean core temperatures (° C ± S. E.) before (PRE) treatment with leptazol, at death or 15 min. after administration (POST) and the mean change in core temperature (DIFF ° C).

HT

DOSE	No	PRE	POST	DIFF	P
60	15	38.4 ± 0.1	40.1 ± 0.2	+1.7	<0.001
80	18	38.1 ± 0.1	39.3 ± 0.2	+1.1	<0.001

ET

60	15	37.9 ± 0.3	36.7 ± 0.3	-1.2	<0.01
80	18	36.9 ± 0.2	35.5 ± 0.2	-1.3	<0.001

TABLE 57

Effects of intraperitoneal injection of leptazol in hyperthyroid (HT) and euthyroid (ET) male TO mice at an ambient temperature of 24° C. The table shows; the dose of leptazol (mg/kg), the number of animals tested (No), the mean time of the onset of convulsions (F - secs $\pm$ S. E.), the mean time at which death occured (D - min $\pm$ S. E.) and the percentage mortality produced (%).

Dose	No	F sec	D min	%
		HT		
60	11	59.3 $\pm$ 0.4	6.9 $\pm$ 0.8	73
80	28	66.8 $\pm$ 5.6	4.7 $\pm$ 0.8	75
		ET		
60	11	62.7 $\pm$ 5.1	6.4 $\pm$ 0.5	47
80	28	72.7 $\pm$ 5.1	4.9 $\pm$ 0.8	50

FIGURE 59.

Mortality of hyperthyroid(HT) and euthyroid (ET) male TO mice after intraperitoneal injection of leptazol at an ambient temperature of 24°C.

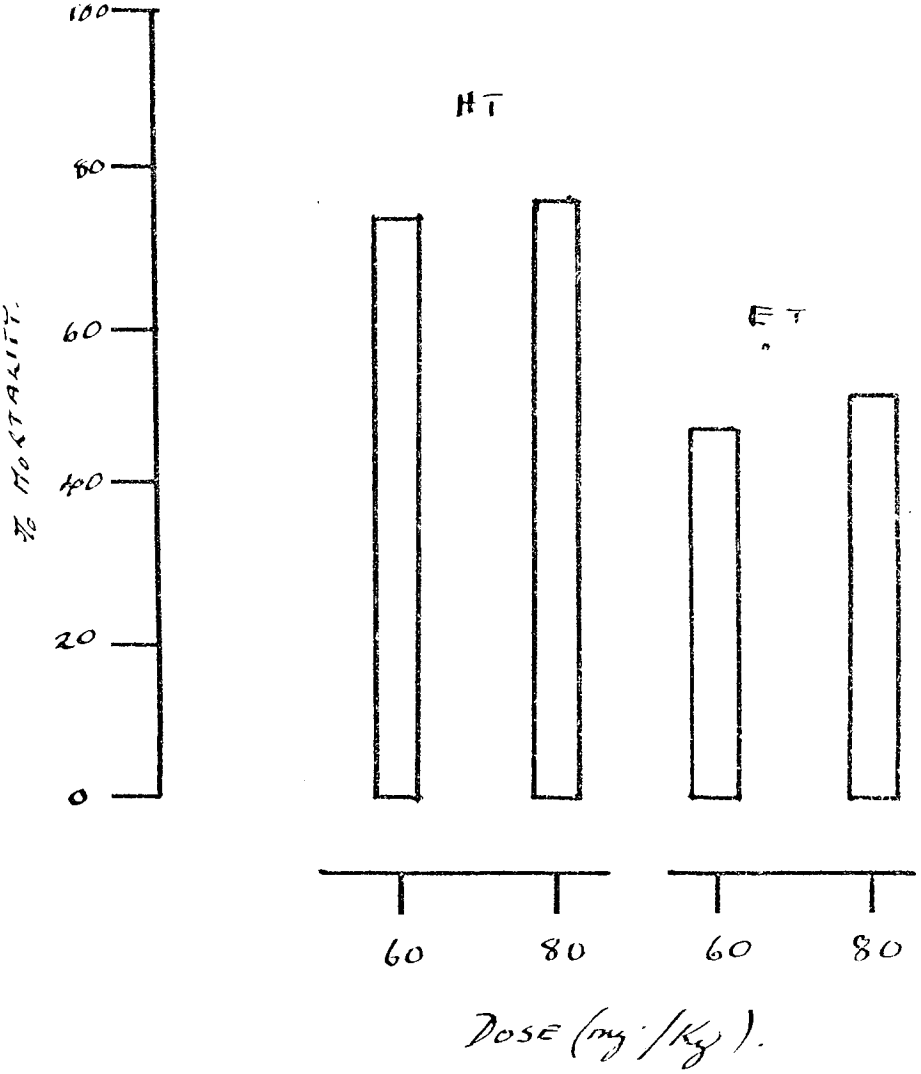


TABLE 58

Effects of intraperitoneal injection of picrotoxin in hyperthyroid (HT) and euthyroid (ET) male TO mice at an ambient temperature of 22°C. The table shows the dose of picrotoxin (mg/kg), the number of animals tested (No), the mean time of onset of convulsions (F min ± S.E.), the mean time at which death occured (D min. ± S.E.), the percentage mortality produced (%), and the total number of convulsions seen in the 30 min. period of the experiment (C).

DOSE	No	F min	D min	%	C
		HT			
4	10	14.5 ± 1.2	20.1 ± 3.2	40	21
5	10	8.3 ± 0.8	12.9 ± 1.7	70	55
		ET			
4	10	23.0 ± 0.8	-	-	7
5	10	10.3 ± 1.3	-	-	13

FIGURE 60.

Mortality of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of picrotoxin at an ambient temperature of 22°C (see Table 58).

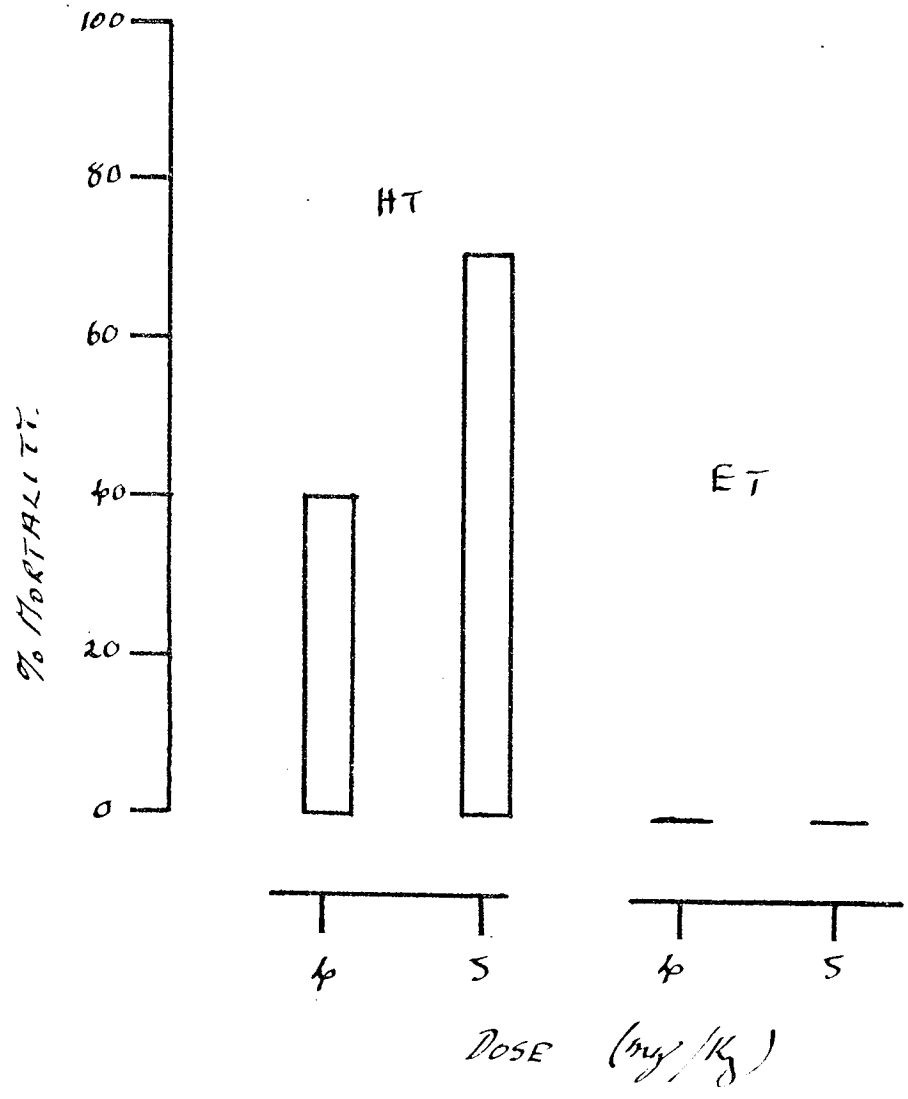


TABLE 59

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following intraperitoneal injection picrotoxin at an ambient temperature of 22° C. The table shows the dose of picrotoxin (mg/kg), the number of animals tested, the mean core temperatures (° C ± S. E.) before (PRE) treatment with picrotoxin, at death or at 15 min. after administration (POST) and the mean change in core temperature (DIFF ° C).

HT

DOSE	No	PRE	POST	DIFF	P
4	10	38.3 ± 0.1	39.2 ± 0.4	1.9	<0.05
5	10	38.3 ± 0.1	38.7 ± 0.2	0.4	<0.01

ET

4	10	38.2 ± 0.1	34.4 ± 0.2	3.8	<0.001
5	10	37.8 ± 0.1	34.4 ± 0.3	3.4	<0.001

TABLE 60

Effects of intraperitoneal injection of nicotine in hyperthyroid (HT) and euthyroid (ET) male TO mice at an ambient temperature of 22° C. The table shows the doses of nicotine, (mg/kg) the number of animals tested (No) the mean time of onset of convulsions (F min. ± SE) the mean time at which death occured (D min. ± SE) and the percentage mortality produced (%).

Dose	No	F sec	D min	(%)
		HT		
10	10	1.4±0.1	3.0±0.6	50
20	10	1.3±0.1	1.9±0.3	70
		ET		
10	10	1.9±0.2	2.5±0.4	30
20	10	1.6±0.1	4.1±1.2	60

FIGURE 61.

Mortality of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of nicotine at an ambient temperature of 22°C. (see Table 60).

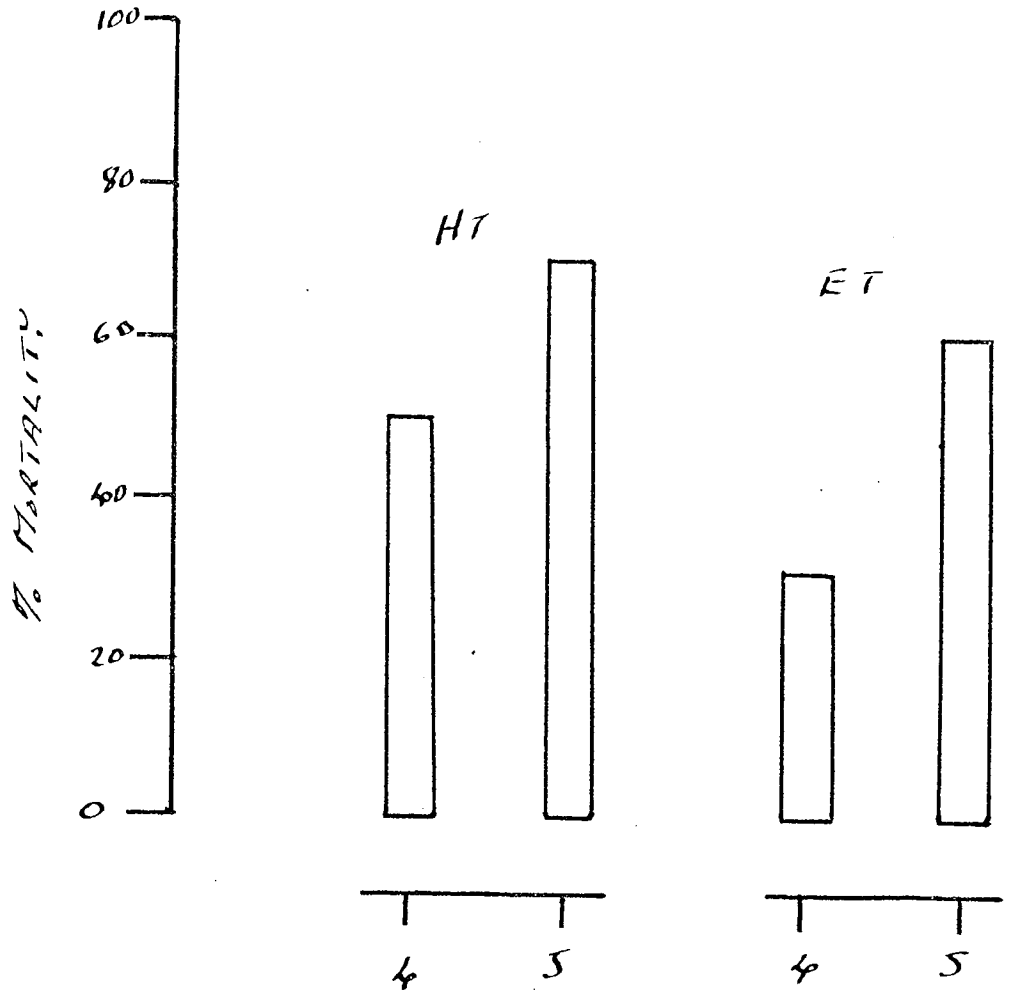


TABLE 61

Effects of intraperitoneal injection of strychnine HCl in hyperthyroid (HT) and euthyroid (ET) male TO mice at an ambient temperature of 22° C. The table shows the doses of strychnine (mg/kg), the number of animals tested (No), the mean time of onset of convulsions (F min ± S. E.) and the percentage mortality produced (%).

		HT			ET		
DOSE	No	F	D	%	F	D	%
1	20	5.0 ± 0.2	5.8 ± 0.6	50	8	8	10
1.5	20	3.7 ± 0.2	3.8 ± 0.2	90	6.8 ± 0.8	6.9 ± 0.6	60

FIGURE 62.

Mortality of groups of hyperthyroid (HT) and euthyroid (ET) male TO mice after intraperitoneal injection of strychnine at an ambient temperature of 22°C (see Table 61).

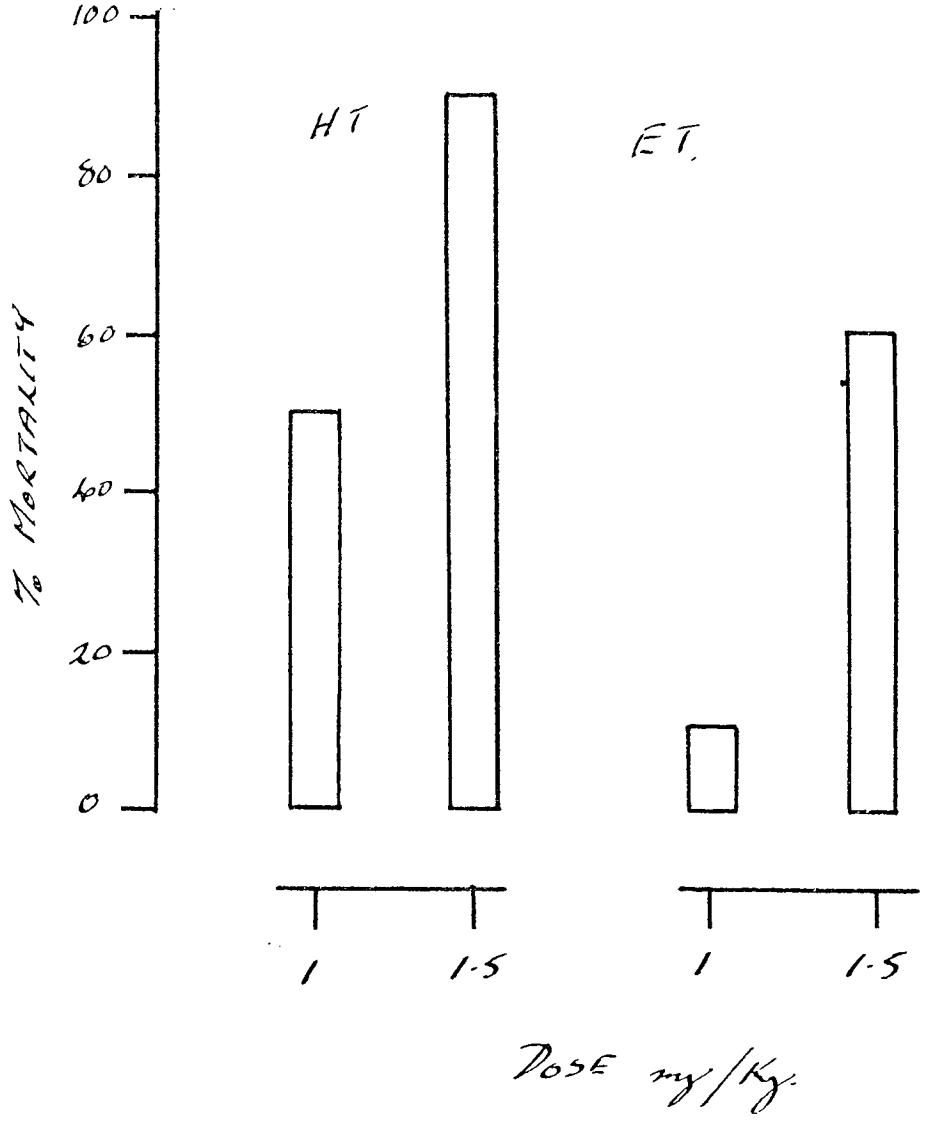


TABLE 62

Effects of intraperitoneal injection of leptazol (60 mg/kg) in hyperthyroid and euthyroid male TO mice after 30 min. pretreatment with d-amphetamine 3 mg/kg orally (HT and ET respectively) at an ambient temperature of 22°C. Controls (HT-C and ET-C respectively) were pretreated with an equivalent volume of water by the same route. The table shows the mean times of onset of convulsions (F sec ± S.E.), the mean times of death (D min. ± S.E.) and the percentage mortality of groups of 10 mice.

HT			HT-C		
F	D	%	F	D	%
64 ± 7.3	6.7 ± 1.1	40	85 ± 10.2	1.3 ± 0.7	30
ET			**ET-C**		
76 ± 12.0	1.1 ± 0.3	20	87 ± 13.5	-	-

FIGURE 63.

Mortality of hyperthyroid (HT-C) and euthyroid (ET-C) male TO mice after intraperitoneal injection of leptazol 60 mg/kg at an ambient temperature of 22°C. Two groups (HT and ET respectively) had been pretreated with d-amphetamine 3 mg/KG orally 30 min before administration of the convulsant(see Table 62).

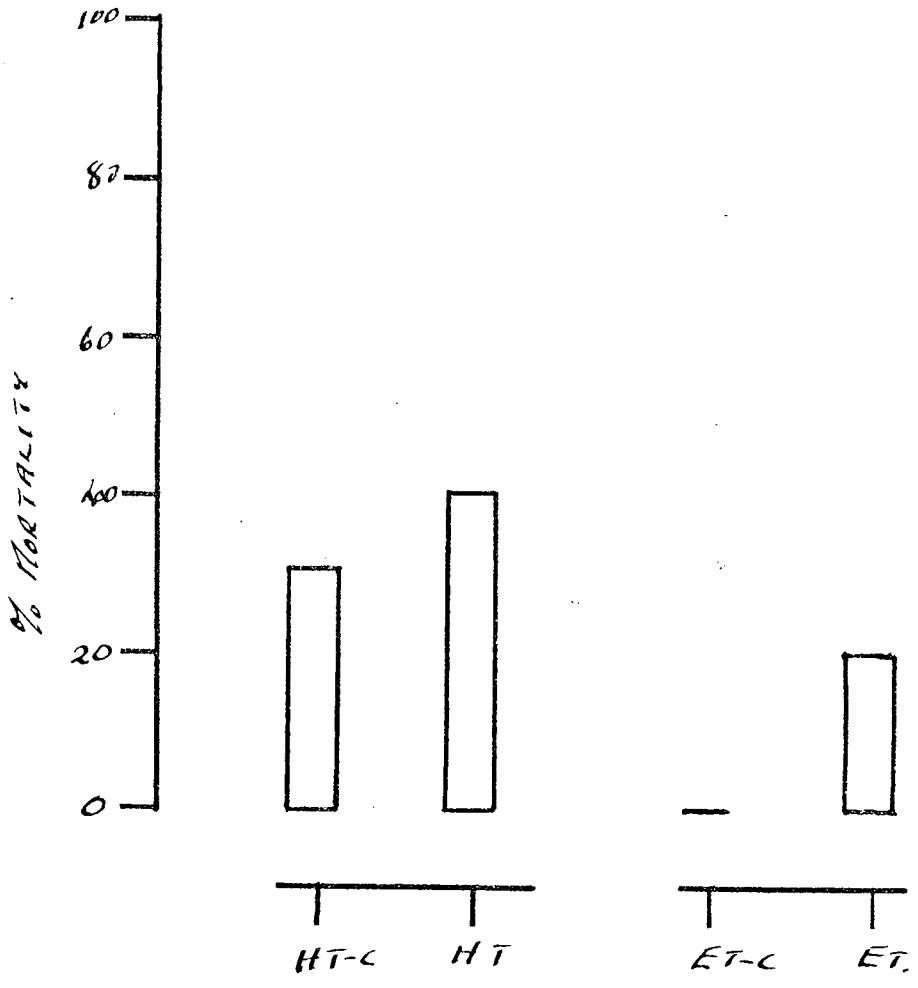


TABLE 63

Core temperatures of hyperthyroid and euthyroid male TO mice following intraperitoneal injection of leptazol (60 mg/kg) after 30 min. pretreatment with d-amphetamine 3 mg/kg orally (HT and ET respectively) at an ambient temperature of 22°C. Controls (HT-C and ET-C respectively) were pretreated with an equivalent volume of water by the same route. The table shows the mean core temperatures (°C ± S.E.) before (PRE), 15 min. after the administration of the convulsant or at death (POST), and the mean change in core temperature of groups of 10 animals.

HT				HT-C			
PRE	POST	DIFF	P	PRE	POST	DIFF	P
39.5 ± 0.3	40.9 ± 0.4	1.4	<0.02	39.1 ± 0.2	39.9 ± 0.3	0.8	<0.05
ET				**ET-C**			
37.2 ± 0.4	36.7 ± 0.5	0.5	<0.4	38.6 ± 0.1	35.5 ± 0.3	3.4	<0.001

TABLE 64

The effects of intraperitoneal injection of leptazol (08 mg/kg) in hyperthyroid (HT) and euthyroid (ET) male TO mice after 30 min. pretreatment with chlordiazepoxide 5 mg/kg subcutaneously at an ambient temperature of 22° C. Controls were pretreated (HT-C and ET-C respectively) were pretreated with an equivalent volume of water by the same route. The table shows the mean times of onset of convulsions (F sec ± S. E.), the mean times of death D min. ± S. E.) percentage response (R) and the percentage mortality (%) of groups of 10 mice.

HT				HT-C			
F	D	R	%	F	D	R	%
100. 6 ± 13. 3	-	80	-	53. 5 ± 3. 2	6. 1 ± 1. 4	100	60
ET				**ET-C**			
132. 9 ± 14. 7	-	70	-	76. 0 ± 7. 1	1. 6 ± 0. 5	100	40

FIGURE 64.

Mortality of hyperthyroid (HT-C) and euthyroid (ET-C) male TO mice after intraperitoneal injection of leptazol 80 mg/kg at an ambient temperature of 22°C. Two groups (HT and ET respectively) were pretreated with chlordiazepoxide 5mg/kg subcutaneously 30 min before the administration of the convulsant. (see Table 64).

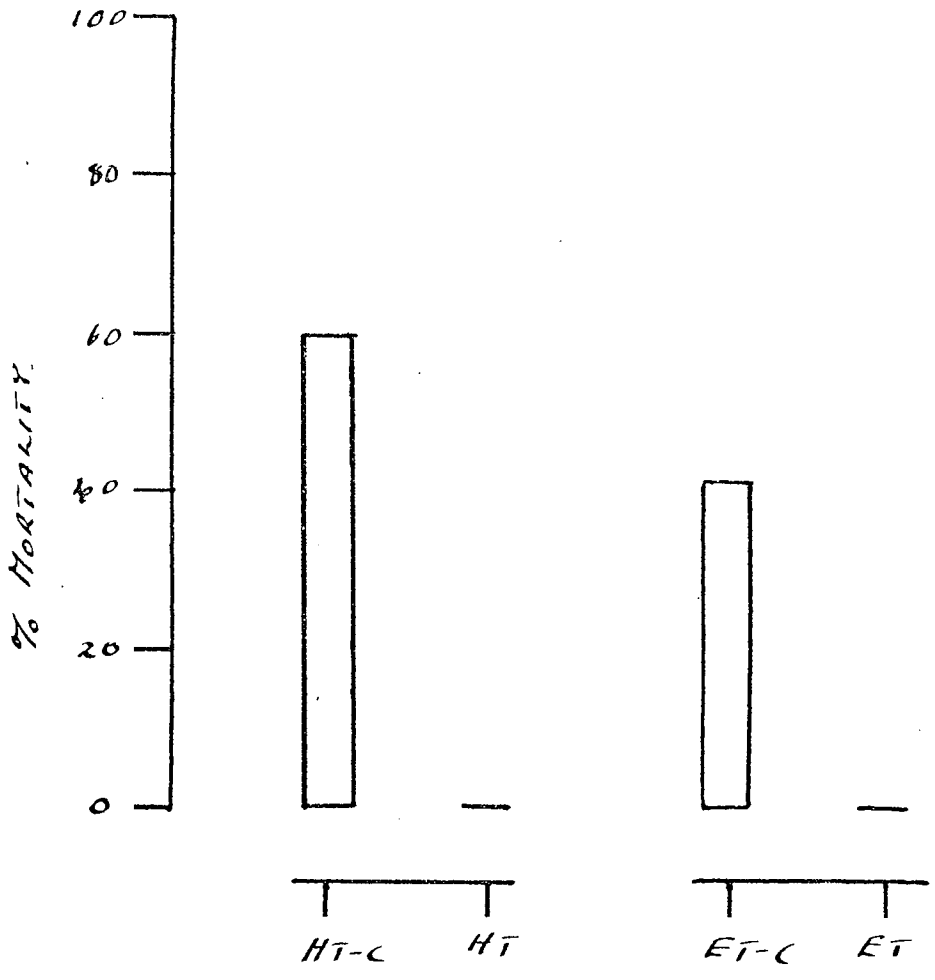


TABLE 65

Core temperatures of hyperthyroid (HT) and euthyroid (ET) male TO mice following intraperitoneal injection of leptazol (80 mg/kg) 30 min. after pretreatment with chlordiazepoxide (2.5 mg/kg) subcutaneously at an ambient temperature of 22°C. Control animals (HT-C and ET-C respectively) were pretreated with an equivalent volume of water by the same route. The table shows the mean core temperatures (°C ± S.E.) before (PRE) and 15 min. after the administration of the convulsant or at death (POST) and the mean changes in core temperature (DIFF) of groups of 10 mice.

	HT			HT-C			
PRE	POST	DIFF	P	PRE	POST	DIFF	P
39.1 ± 0.1	40.0 ± 0.3	+0.9	<0.02	38.3 ± 0.2	39.8 ± 0.2	+1.5	<0.001
	ET			ET-C			
38.3 ± 0.2	35.7 ± 0.4	-2.6	<0.001	37.4 ± 0.1	36.7 ± 0.3	-0.7	<0.05

TABLE 66

The effects of intraperitoneal injection of leptazol (80 mg/kg) in hyperthyroid (HT) and euthyroid (ET) male TO mice 30 min. after pretreatment with chlordiazepoxide 2.5 mg/kg subcutaneously at an ambient temperature of 22° C. Control animals (HT-C and ET-C respectively) were pretreated with an equivalent volume of water by the same route. The table shows the mean times of onset of convulsions (F sec S.E.), the mean times of death (D min ± S.E.), and the percentage mortality (%) produced by the convulsant in groups of 10 animals.

	HT		HT-C		
F	D	%	F	D	%
70.5 ± 13.3	2	10	63.3 ± 5.3	6.6 ± 1.1	80
	ET		ET-C		
92.8 ± 18.0	1.2	10	61.9 ± 3.2	3.6 ± 0.5	40

FIGURE 65.

Mortality of hyperthyroid (HT-C) and euthyroid (ET-C) male TO mice after intraperitoneal injection of leptazol 80 mg/kg at an ambient temperature of 22°C. Two groups (HT and ET respectively) were pretreated with chlordiazepoxide 2.5 mg/kg 30 min before administration of the convulsant (see Table 66).

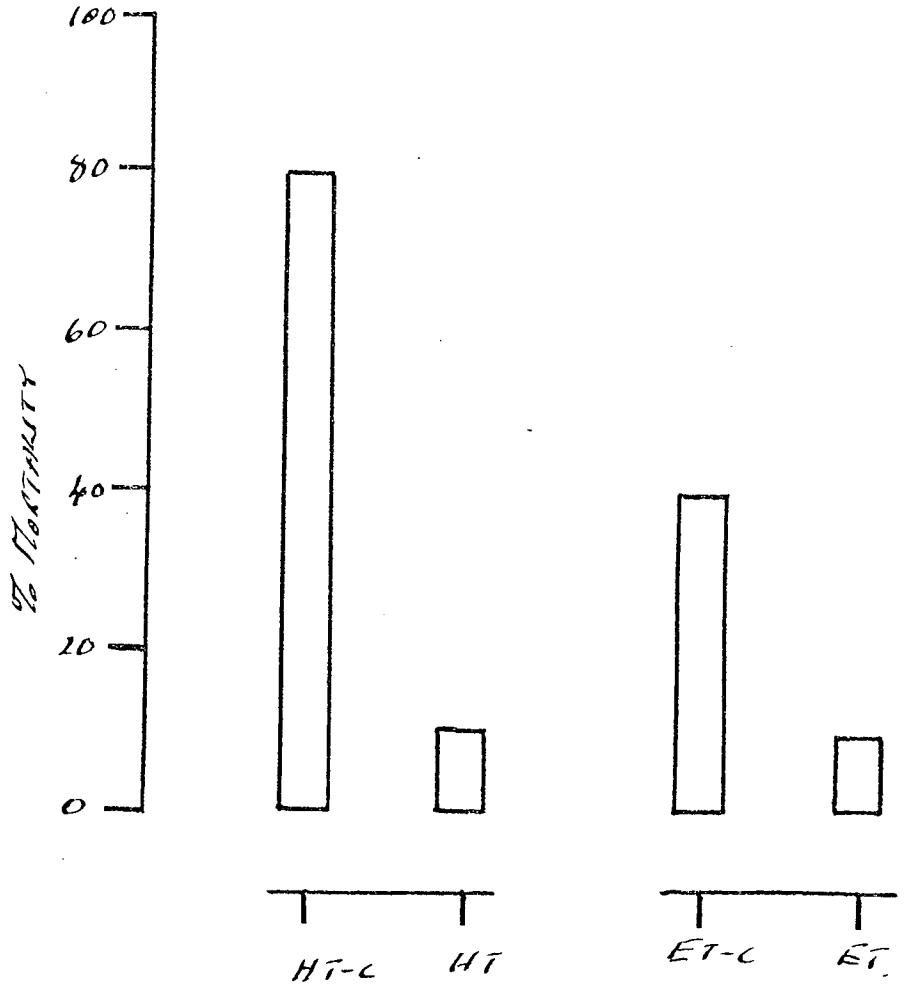


TABLE 67

The effects of intraperitoneal injection of leptazol (80 mg/kg) in hyperthyroid (HT) and euthyroid (ET) male TO mice 30 min. after pretreatment with chlordiazepoxide 5 mg/kg subcutaneously at an ambient temperature of 24°C. Control animals (HT-C and ET-C respectively) were pretreated with an equivalent volume of water by the same route. The table shows the mean times of onset of convulsions (F sec ± S.E.) the mean times of death (D min ± S.E.) and the percentage mortality produced by the drug (%) in groups of 10 animals.

HT			HT-C		
F	D	%	F	D	%
113.3 ± 10.6	2.5	10	62.0 ± 10.2	2.4 ± 0.8	90
ET			**ET-C**		
200.2 ± 18.6	3.1	10	80.5 ± 2.6	2.7 ± 0.4	50

FIGURE 66.

Mortality of hyperthyroid (HT-C) and euthyroid (ET-C) male TO mice after intraperitoneal injection of leptazol at an ambient temperature of 24°C. Two groups (HT and ET respectively) were pretreated with chlordiazepoxide 5 mg/kg subcutaneously 30 min before administration of the convulsant (see Table 67).

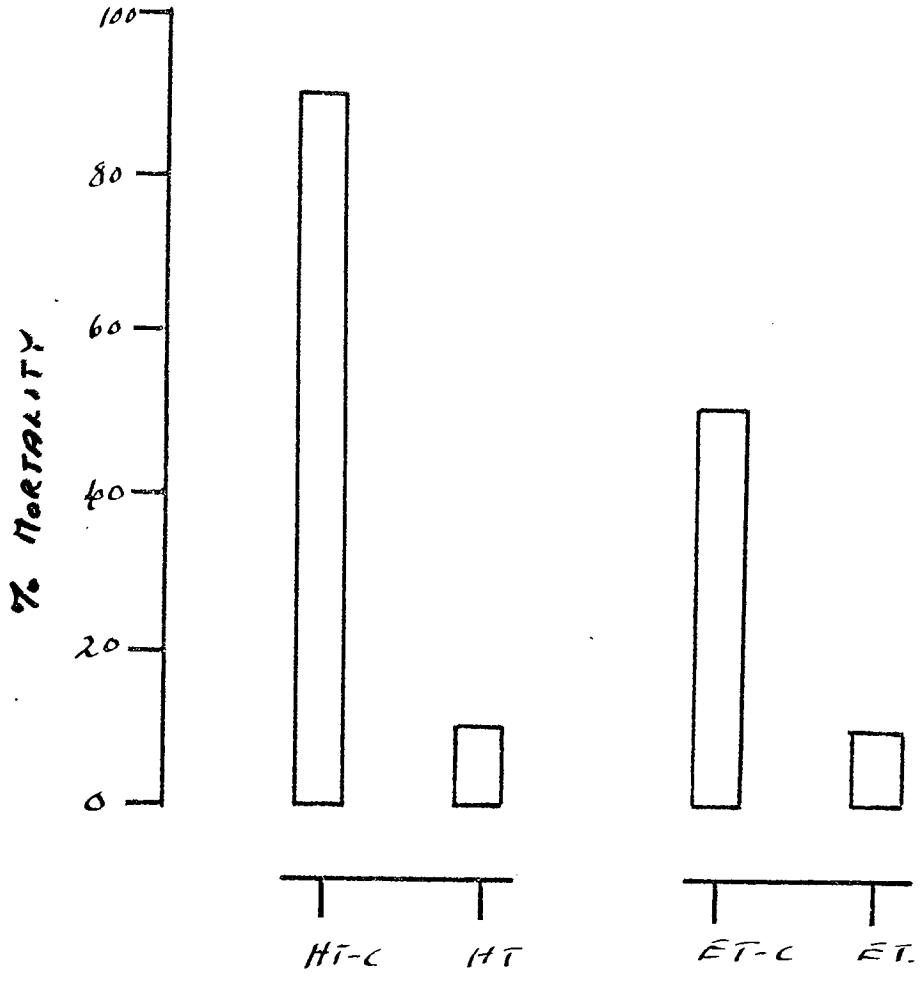


TABLE 68

The effects of leptazol (80 mg/kg) in euthyroid (ET) male TO mice 90 min. after pretreatment with 2,4-dinitrophenol 20 mg/kg subcutaneously at an ambient temperature of 22° C. Control animals (ET-C) were pretreated with an equivalent volume of normal saline by the same route. The table shows the mean times of onset of convulsions (F sec ± S. E.), the mean times of death (D min ± S. E.) and the percentage mortality (%) produced by the convulsant in groups of 10 animals.

ET			ET-C		
F	D	%	F	D	%
74.5 ± 13.6	5.3 ± 1.3	70	75.5 ± 6.7	5.4 ± 1.1	70

FIGURE 67.

Mortality of euthyroid (ET-C) male TO mice after intraperitoneal injection of leptazol 80 mg/kg at an ambient temperature of 22°C. One group (ET) had been pretreated with 2,4-dinitrophenol 20 mg/kg subcutaneously 90 min before treatment with the convulsant (see Table 68)

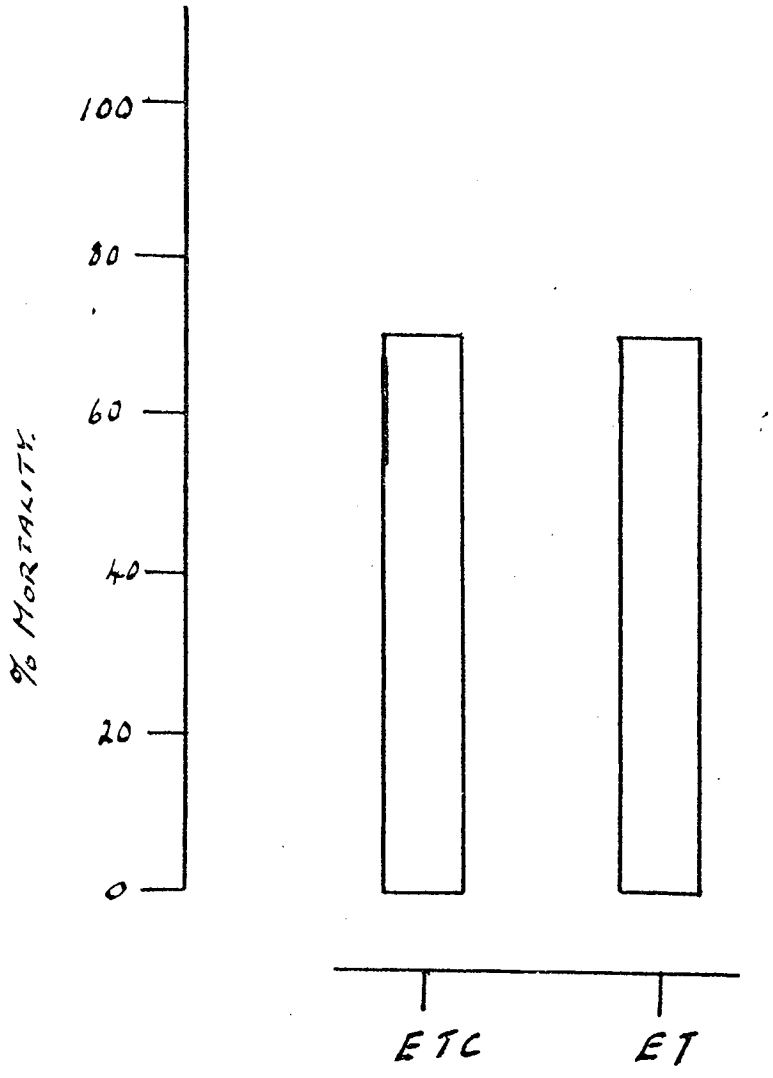


TABLE 69

Core temperatures of euthyroid (ET) male TO mice following intraperitoneal injection of leptazol (80 mg/kg) 90 min. after pretreatment with 2,4-dinitrophenol 20 mg/kg subcutaneously at an ambient temperature of 22°C. Control animals (ET-C) were pretreated with an equivalent volume of normal saline by the same route. The table shows the mean core temperatures (°C ± S.E.) before (PRE), 15 min. after treatment with the convulsant or at death (POST) and the mean changes in core temperature (DIFF) of groups of 10 animals.

ET				ET-C			
PRE	POST	DIFF	P	PRE	POST	DIFF	P
38.5 ± 0.2	35.0 ± 0.5	-3.5	<0.001	37.1 ± 0.2	35.6 ± 0.6	-1.3	<0.05

CHAPTER VIII.

THE EFFECTS OF MORPHINE IN HYPERTHYROID AND EUTHYROID MICE

CHAPTER VIII.

THE EFFECTS OF MORPHINE IN HYPERTHYROID AND EUTHYROID MICE

It has been reported (Bhagat, 1964) that the analgesic potency of morphine, as estimated by an electroshock method, is significantly potentiated in the hyperthyroid mouse.

It was decided to investigate the analgesic actions of morphine by three different tests in hyperthyroid and euthyroid mice. The tests used were the hot plate test, the tail clip test and the phenyl quinnone writhing test as detailed in the methods section.

1. The hot plate test.

An initial experiment revealed that with the plate at 54° C there was no difference in either the response times or in the proportion of the hyperthyroid and euthyroid mice responding to the pain stimulus (see Table 77). The responses of the animals of both groups were recorded 45 min. after intraperitoneal injection of morphine 10 mg/kg. The results show that the drug had a similar analgesic action in both the hyperthyroid and the euthyroid mice as shown by the increases in the animals response times and the reduction in the proportion of mice responding in the 30 sec. exposure period (see Table 77). There was, however, a slight difference in the number of animals in each group in which the response to the pain stimulus was completely abolished by the morphine, 50% of the hyperthyroid animals and 40% of the euthyroid mice failing to respond.

The results indicate that there is no major increase in the analgesic potency of morphine in the hyperthyroid animals as revealed by this test.

2. The tail clip test.

The results revealed that there was no difference in the responses of the hyperthyroid and euthyroid mice to the pain stimulus used in this test (see Table 72). Two doses of morphine (10 & 7.5 mg/kg intraperitoneally) were tested for analgesic potency in groups of hyperthyroid and euthyroid mice exposed to the tail clip test 45 min. after administration of morphine or normal saline (controls). The results (Table 72) revealed that although the drug did not significantly prolong the time interval before the animals of either group responded to the pain stimulus, there was a significant analgesic effect as shown by the proportion of mice responding. The high dose of morphine inhibited the response of 90% of the hyperthyroid and 70% of the euthyroid mice, whilst the lower dose the response of 60% of the hyperthyroid and 30% of the euthyroid animals was inhibited.

The results suggest that hyperthyroid mice are more susceptible to the analgesic actions of morphine when determined on a quantal basis.

3. The phenyl quinnone writhing test.

The results obtained in these experiments show, as before, that there was no difference in the responses of the hyperthyroid and euthyroid animals to the pain stimulus employed. As can be seen from the results (Table 73) 45 min. pretreatment of mice of both groups with morphine, 2.5 mg/kg intraperitoneally, produced a 70% reduction in the number of writhes of the hyperthyroid animals whilst reducing the score of the euthyroid group by only 40%.

This experiment showed that the analgesic effects of morphine are greater in hyperthyroid than in euthyroid mice.

DISCUSSION

Morphine is a potent analgesic agent which is believed to exert its action by a depressant action on the reticular formation (see Killam, 1962). The precise mechanisms involved in the production of the analgesic effect are not fully understood. Some workers suggest that the drug exerts its effects via a central cholinergic mechanism (Gordonoff 1963) whilst others suggest the involvement of adrenergic mechanisms (Nott. 1968).

The results presented here are in agreement with those of Bhagat (1964) who reported that the analgesic effects of morphine are potentiated by thyroxine pretreatment. In spite of variations between tests, the data shows that hyperthyroid mice are more sensitive to the effects of the drug. The increase in the analgesic potency be due thyroxine-induced changes in the metabolism of the drug, since Cochin and Sokoloff (1960) reported that thyroxine treatment inhibits the liver metabolism of morphine. However, other mechanisms may be involved; Kakunga, Kaneto and Hano (1966) reported that calcium ions inhibit the analgesic actions of morphine. These authors found that intracerebral injection of a chelating agent which reduced the concentration of free calcium in the brain potentiated the analgesic effects of the drug. Since thyroxine is known to be able to form chelation complexes (Lardy, 1955) it may be that this property of the hormone is involved in the thyroxine-induced potentation of morphine analgesia.

SUMMARY

1. In Thyroxine-induced hyperthyroidism in male TO mice renders these animals more sensitive to the analgesic effects of morphine than euthyroid mice.

TABLE 70

Responses of hyperthyroid and euthyroid male TO mice placed on a hot plate at 55°C before (HT-C and ET-C respectively) and 45 min. after (HT and ET respectively) subcutaneous injection of morphine 10 mg/kg. The table shows the mean time at which the first response to the pain stimulus occured (F sec S.E.), the total number of responses noted in groups of 20 animals and the percentage of animals not showing a response in the 30 sec. exposure period.

HT-C			HT		
F	No	%	F	No	%
19.9 ± 0.8	57	100	25.5 ± 1.3	12	50
ET-C			**ET**		
19.9 ± 1.1	53	100	24.8 ± 1.3	38	40

TABLE 71

Responses of hyperthyroid and euthyroid male TO mice exposed to the tail clip test before (HT-C and ET-C respectively) and 45 min after (HT and ET respectively) subcutaneous injection of morphine 10 mg/kg. The table shows the mean times at which the first response to the pain stimulus occured (F sec $\pm$ S. E.), and the percentage of animals not responding to the stimulus (%) in the 30 sec exposure period in groups of 30 animals.

HT-C		HT		ET-C		ET	
F	%	F	%	F	%	F	%
2.8 $\pm$ 0.5	0	3	90	2.1 $\pm$ 0.2	0	3.6 $\pm$ 1.0	50

TABLE 72

Responses of hyperthyroid and euthyroid male TO mice exposed to the tail clip test before (HT-C and ET-C) and after (HT and ET respectively) subcutaneous injection of morphine 7.5 mg/kg. The table shows the mean times at which the first response to the pain stimulus occured (F sec $\pm$ S.E.), and the percentage of animals not responding to the stimulus in the thirty second exposure period. In groups of 30 animals.

HT-C		HT		ET-C		ET	
F	%	F	%	F	%	F	%
2.1 $\pm$ 0.3	0	3.4 $\pm$ 0.2	60	2.0 $\pm$ 0.4	0	3.0 $\pm$ 1.6	30

TABLE 73

Writhing following intraperitoneal injection of phenyl quinnone in hyperthyroid and euthyroid male TO mice before (HT-C and ET-C respectively and after (HT and ET respectively) subcutaneous injection of morphine 2.5 mg/kg. The results are expressed as the mean number of writhes of groups of 10 animals.

HT-C	HT	ET-C	ET
51.5	15.7	59.0	36.7

SECTION 4

GENERAL DISCUSSION

GENERAL DISCUSSION

There is ample evidence to show that, in addition to the usual physical signs of hyperthyroidism, there are also definite changes in central nervous function. Therefore, the hyperthyroid patient as well as being treated specifically for the underlying condition, is likely to be given psychiatric drugs to alleviate the nervous symptoms of his disorder.

It is known that the toxicities of many centrally acting drugs are increased in hyperthyroidism, and some workers have suggested that the use of such drugs in clinical hyperthyroidism may be dangerous. It also seems likely that even in sub-clinical states of hyperthyroidism the use of these drugs may give rise to unexpected and undesirable side effects. This study was carried out to investigate to what extent hyperthyroidism was capable of changing the activities of a number of centrally acting drugs. It was hoped that any observed changes might provide the basis for an explanation of the mechanisms by which thyroid hormones affect behaviour, and also provide information about the basic actions of the particular drug used. A measure of success has been achieved in these aims and will be discussed further in this section.

In the case of the barbiturate groups of drugs (results section A), the results presented confirm the reports of previous authors that the hyperthyroid animal is more sensitive to the anaesthetic and toxic effects of both pentobarbitone and thiopentone. Existing information provides an adequate explanation of this on the basis of thyroid-induced changes in metabolic function: reduction in the rate of drug metabolism induced by thyroxine treatment (Prange et al, 1966; Conney & Garren, 1960), explains the observed increase in hypnotic potency of both pentobarbitone and

large doses of thiopentone in hyperthyroid mice. It also provides the basis for an explanation of the increased toxicities of both compounds. Conversely, the observed reduction in potency of small doses of thiopentone can be adequately explained on the basis of thyroid induced increased in cerebral blood flow (Sokoloff et al 1953; Sensenbach et al 1956). Both of these explanations however take no account of any thyroxine-induced changes in central nervous activity. The results presented in Chapter II provide strong support for the hypothesis that an uncoupling of oxidative phosphorylation is one of the mechanisms whereby the barbiturates exert their hypnotic activity. It is known that many uncoupling agents of widely different pharmacological properties will potentiate the hypnotic actions of the barbiturates. The results presented in this work show that 2, 4-dinitrophenol following intracerebral administration will produce a dose-dependent hypnosis accompanied by hypothermia and decreased oxygen consumption, which is similar to that produced by pentobarbitone given by the same route. DNP is the more potent of the two compounds by this route and is also known to be a more potent uncoupling agent. Recent experiments (the results of which are not presented here) show that although pentobarbitone has anti-leptazol activity following intracerebral administration, no anti-leptazol activity could be demonstrated with DNP. Though this may seem to undermine the idea that the two compounds pentobarbitone and DNP are acting in a similar manner, the result can be adequately explained by considering the physical properties of the two compounds. Pentobarbitone in its unionised form has an affinity for non-polar solvents; thus its physical properties favour its

retention in the brain and its distribution throughout the brain. The highly polar structure of DNP on the other hand renders it hydrophyllic and leads to its rapid removal from the site of injection in the continuous aqueous phase of CSF and then blood. Thus it is not surprising that DNP can give rise to hypnosis by a general depressant action on the reticular formation (which lies just beneath the intracerebral site of injection) yet produces no anticonvulsant effect, an effect which would perhaps necessitate diffusion of the compound across a relatively great distance in the brain into the cerebral motor cortex. In spite of this difference in the pharmacological properties of the two agents, it was found that the hypnosis produced by either compound can be reversed by intravenous administration of leptazol - indicating that there is a basic similarity in the effects of both agents.

These results considered together strongly implicate the uncoupling of oxidative phosphorylation as one of the basic mechanisms of barbiturate action. The failure of hyperthyroidism to potentiate significantly the hypnotic effect of intracerebrally-administered pentobarbitone or DNP can be adequately explained on the basis of an antagonistic increase in cerebral vascular flow in this condition. It is postulated that the uncoupling activity of thyroxine may be one of the underlying mechanisms of thyroxine-induced potentiation of peripherally-administered barbiturates. This remains, however, merely a hypothesis and there are reports which contradict it. For example, it was found by Prange et al (1966) that waking brain levels of pentobarbitone are the same in both hyperthyroid and euthyroid mice suggesting that there is no difference in brain sensitivity

of the hyperthyroid animals to the drug.

In the other results much emphasis has been put on the difference in the thermal response of hyperthyroid and euthyroid animals to the drugs tested. It is known that the thyroid gland plays a role in thermoregulation which is of particular importance when animals are exposed to extremes of cold. The thyroid hormone increases the obligatory heat production of the animal by causing a general increase in the BMR with a resulting increase in basal heat production (Morgans 1964). The thyroid also exerts an influence on the regulatory heat production of the animal by causing an increase in the calorigenic potency of catecholamines (Swanson 1956). Thus in the hyperthyroid animal at normal temperatures the thermoregulatory mechanisms are under constant strain to maintain a normal body temperature. This is reflected in the higher core and skin temperature of the hyperthyroid as opposed to the euthyroid mice. It follows that the effects of any drug which causes alterations in body temperature will be modified in hyperthyroidism due to the reduction in the critical temperature which thyroxine treatment causes. In this work it has been shown that the hypothermic effects of chlorpromazine, reserpine, tremorine and oxotremorine are antagonised or reversed in hyperthyroid animals, depending on the prevailing ambient temperature. The hyperthermic effects of amphetamine are markedly potentiated in hyperthyroidism. Further, the results obtained in the study of chemically-induced convulsions (Chapter VII) show that the proconvulsant action of thyroxine is accompanied by the production of significant hyperthermia in the hyperthyroid mice in response to convulsants at 22° C, whilst euthyroid animals under the same conditions respond with a significant hypothermia. In view of the observed

proconvulsant effects of an increase in ambient temperature, the hyperthermia may indicate one of the causes of an increased susceptibility of the hyperthyroid mice to convulsant drugs. However, the work of Timiras & Woodbury (1956) would suggest that changes in brain electrolyte distribution occurring in hyperthyroidism is an important factor in the increased brain excitability. Despite their evidence. however results presented in Chapter VII show that the anti-leptazol action of chlordiazepoxide is accompanied by a reduction of the hyperthermic response of hyperthyroid mice and a potentiation of the hypothermic response of euthyroid animals at 22° C. Similarly, the proconvulsant action of amphetamine is accompanied by a potentiation of the hyperthermia in hyperthyroid mice and an inhibition of hypothermia in euthyroid animals, in response to leptazol. These results add weight to the hypothesis that the thermal response of the animal to a convulsant drug is one of the factors governing their susceptibility to convulsion.

These results from temperature studies perhaps reveal one of the more significant factors involved in the changes in response to drugs observed in the hyperthyroid mouse. It has been noted repeatedly throughout this work that hyperthyroid mice are extremely susceptible to any environment or agent which produced hyperthermia. Exposure to a high environmental temperature alone is likely to cause 100% mortality in hyperthyroid mice without showing any lethal effect in euthyroid animals. Results of pilot studies not quoted in this thesis have shown that even at mildly elevated ambient temperatures (24 - 26° C), small doses of reserpine, chlorpromazine or amphetamine similar to those used in the experiments reported here, may produce very high or total mortality in hyperthyroid mice without causing

the deaths of any euthyroid animals. Death in the hyperthyroid mice always seems to be due to the severe hyperthermia produced by these agents under these conditions. This finding again points to the involvement of the thermal response of hyperthyroid mice in their increased mortal susceptibilities to many of the drugs investigated here and by other workers. The results also demonstrate the need for careful control of environmental temperature during any toxicity test. In fact, with any of the above mentioned drugs it is quite meaningless to quote any acute toxicity (LD 50) values unless the exact ambient temperature at which the experiment was conducted is also stated.

The hypothesis that there may be a potentiation of central adrenergic mechanisms in hyperthyroid animals was made in Chapter VI, a suggestion supported by the effects of tremorine and oxotremorine in hyperthyroid and euthyroid mice. It was found that the hyperthyroid animals, although showing signs of marked peripheral muscarinic activity after treatment with these drugs, were extremely insensitive to the tremor and hypothermia present in adjacent euthyroid mice. The absence of hypothermia is easily explained in the light of the previously discussed reduction in critical temperature in the hyperthyroid animal; this reduction in critical temperature is so great as to allow the hyperthyroid mice to maintain a core temperature only just below the control value even in the presence of a large increase in peripheral muscarinic activity (shown by salivation, peripheral vasodilation and diarrhoea which would lead to enhanced heat loss and usually to a marked hypothermia). The lack of tremor in hyperthyroid mice does not appear to be associated with the lack of concomitant hypothermia since tremor can be produced in euthyroid animals in the absence of hypothermia. Further, this insensitivity to both

tremorgenic agents is not due to any thyroid-induced changes in the permeability of the blood/brain barrier which might have prevented access of oxotremorine to the brain; intracerebral administration of oxotremorine produces marked tremor in euthyroid mice whilst causing only a very mild reaction (similar to that seen following peripheral administration) in the hyperthyroid animals. Thus the results suggest that there is a basic insensitivity of hyperthyroid mice due to thyroxine-induced changes in brain sensitivity. The tremorigenic effects of oxotremorine are known to be the result of central cholinergic stimulation. Thus it seems that to cause a reduction in the sensitivity of the brain to the effects of OTMN hyperthyroidism must be associated with some inhibition of central cholinergic function or potentiation of central adrenergic function. Of the two alternatives the potentiation of adrenergic mechanisms is perhaps the most attractive theory and several results may provide substantiating evidence. Firstly it is known that in hyperthyroidism there are changes in the peripheral adrenergic systems, (Parsons & Jewitt, 1967). Thus the metabolic and cardiovascular effects of the catecholamines are potentiated, though as mentioned before (Section I Chapter) this may be due to changes in tissue metabolism and not to their inherent sensitivity to the catecholamines (Van der Schoot & Moran, 1965). However, since thyroxine is reported to form chelation complexes with copper, an action which would be expected to result in potentiation of catecholamine activity (Chaberek & Martell, 1959), it may be that some potentiation does occur. Of the results presented in this thesis there are several which might be interpreted as being indicative of some increase in central adrenergic activity. The hyperthermic effects of amphetamine have been shown

to be increased in hyperthyroid mice, confirming the results of Askew, (1961) who reported that the locomotor stimulant activity of the drug is also increased in the hyperthyroid animal. Since amphetamine is known to exert its central stimulant actions by a sympathomimetic mechanism these results could indicate that hyperthyroidism is associated with some increase in central adrenergic activity. Turner and Spencer (1968) have shown that monoamine oxidase inhibitors with inherent sympathomimetic action have a proconvulsant effect. It has been shown in this work that hyperthyroidism is also associated with an increase in the potency of chemical convulsant agents. Since thyroxine is known to have monoamine oxidase inhibiting activity (Zile and Lardy, 1959) it may be that this effect in association with other forms of potentiation of central adrenergic activity is the underlying mechanism of the increase in brain excitability. Finally, since the analgesic effects of morphine have been shown to be potentiated in the hyperthyroid mouse (Chapter VIII) and it has been suggested (Heller, Saavedra & Fischer, 1968) that morphine exerts its analgesic action via a central sympathetic system, it may be that increased central adrenergic activity in hyperthyroidism is the cause of potentiation of morphine analgesia.

The potentiation of central adrenergic mechanisms in hyperthyroidism remains, however, a hypothesis, and no direct experimental work is quoted here to substantiate the theory. Preliminary work in this department (Ross personal communication) has indicated that there are increases in the levels of dopamine in the brains of hyperthyroid rats, and further work in this field may provide substantive evidence to support this theory.

Many possible mechanisms of thyroxine-induced changes in drug activity have not been investigated in this work. For example, the effects of the hormone on general metabolism may be the cause of many of the effects observed. Also the relationship between the thyroid gland and the other endocrines, particularly the adrenals, has not been investigated here. In view of the numerous reports that the size and output of the adrenal cortex is increased in hyperthyroidism it may be that many of the changes seen in hyperthyroidism are secondary effects mediated via the adrenal cortex.

In conclusion the results presented in this work have resulted in the formulation of three hypotheses:-

i. That the barbiturates exert their central depressant action, in part at least, by their ability to uncouple oxidative phosphorylation, and that the potentiating action of thyroxine may be related to the capacity of the hormone to uncouple.

ii. That the changes in thermoregulation seen in hyperthyroidism may be the cause of many of the changes in response to drugs seen in the condition including the increase in toxicity of a number of centrally acting drugs and the increased susceptibility of the hyperthyroid mice to convulsion.

iii. That there may be a increase in the activity of central adrenergic mechanisms in hyperthyroidism resulting in changes in response to several drugs including oxotremorine, tremorine, amphetamine, morphine and perhaps chemical convulsant agents.

Clinical implications:

Despite considerable evidence of changes in the actions and toxic effects of a number of drugs administered to hyperthyroid animals, there are very few similar clinical observations. This paucity of clinical observation may be due to two reasons: the occurrence of an unusual drug response in a hyperthyroid patient will occur relatively infrequently, and the endocrine disorder may be of a relatively low level (perhaps sub-clinical and unsuspected). Therefore, the occurrence of such unusual responses is more likely to be blamed directly on the drugs concerned. Such clinical reports as have been made of thyroid disease - drug interaction have been concerned with the effects of drugs to control blood pressure changes associated with hyperthyroidism (Prange, **et al** 1968).

The results presented in this work support the suggestion of Ashford and Ross (1968) that the use of certain centrally-acting drugs in uncontrolled hyperthyroidism may be dangerous. At a lower level not only is it possible that the hyperthyroid patient may show altered sensitivity to a drug, but there may be also qualitative changes in the normal response to that drug.

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SECTION 5

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