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CALORIGENIC EFFECT OF THYROID HORMONES AND NORADRENALINE IN WARM- AND COLD-ACCLIMATED MICE

by

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ABSTRACT

The effect of diacetyl-2,6-diiodohydroquinone (DDIH) on the calorigenic action of exogenous L-thyroxine and L-triiodothyronine was studied in normal and thyroidectomized male mice acclimated to 30° or 10°C. Two methods for determining the oxygen consumption rates (OCRs) were used: one was a modified Haclagen system in which the OCRs were determined on groups of eight mice; the other a system using single animals. The animals were pretreated with thyroxine or triiodothyronine in conjunction with DDIH, and their OCRs were determined using both methods. A comparison of the results showed that grouping had an effect on the measured response in both treated and untreated animals.

A series of [illegible] using ^{131}I-labelled hormones were administered did not reveal any correlation between the amount of ^{131}I excreted and the magnitude of increase in OCR in warm- and cold-acclimated mice.

The calorigenic response of untreated and pretreated mice to noradrenaline was measured under two sets of environmental conditions. In one, untreated and pretreated mice were tested with noradrenaline, their OCRs being determined at the temperature of acclimation. In the other, the noradrenaline test was conducted after the animals had been exposed to 10° or 30°C for one hour.

When noradrenaline was tested at the temperature of acclimation, the results showed that pretreatment had an effect on the sensitivity to noradrenaline; DDIH generally inhibited the sensitivity of thyroxine pretreated mice and enhanced the sensitivity of triiodothyronine pre-

treated ones. Thyroidectomy reduced the sensitivity to noradrenaline.

When cold-acclimated mice, sensitive to the higher dosage of noradrenaline were placed at 30°C for one hour, they lost this sensitivity. This lack of sensitivity of cold-acclimated mice was contrary to the sensitivity seen in cold-acclimated rats tested with a lower dosage of noradrenaline at 30°C and indicated a definite species difference. The difference in the response of mice to noradrenaline under the two environmental conditions indicated the importance of the temperature at which the test was conducted.

The rapidity with which cold-acclimated mice lost their sensitivity to noradrenaline indicates a larger role for the nervous system in thermogenesis than is seen in the rat, and perhaps different means of nonshivering thermogenesis are to be found in these two species.

RESUME

On a étudié l'effet de la diiodohydroquinone diacétylée (DDIH) sur l'action calorigénique de la thyroxine et de la triiodothyronine exogènes dans des souris males, normaux et thyroïdectomées, acclimatées à 30° ou 10°C. On a utilisé deux méthodes pour la détermination du taux de consommation d'oxygène (OCR): Dans un système de Maclagan modifié les OCRs ont été déterminés en groupes de huit souris; dans l'autre système on a fait la détermination avec une seule souris à la fois. On a traité les animaux avec la thyroxine ou la triiodothyronine conjointement avec la DDIH, et on a employé les deux méthodes pour la détermination du OCR. Une comparaison des résultats a montré que le groupement de souris avait un effet sur la réponse mesurée chez des animaux traités ou non-traités.

Une serie d'expériences pendant laquelle on a administré des hormones marquées de ^{131}I, n'a pas montré de corrélation entre la quantité de ^{131}I excrété et la grandeur de l'augmentation du OCR dans des souris acclimatées au chaud et au froid.

On a mesuré la réponse calorigénique à la noradrénaline dans des souris traitées et non-traitées en deux conditions de milieu: 1) On a examiné des souris traitées et non-traitées avec la noradrénaline, en déterminant les OCR à la température de l'acclimatization; 2) on a fait le test avec la noradrénaline apres avoir exposé les souris à 10° ou 30°C pendant une heure.

Quand on a examiné la noradrénaline à la température d'acclimatization, les résultats ont montrés que le traitement avait un effet sur la sensibilité à la noradrénaline; la DDIH a empêché généralement

la sensibilité des souris traitées à la thyroxine et a augmenté la sensibilité de ceux traitées à la triiodothyronine. La thyroïdectomie a reduit la sensibilité à la noradrénaline.

Des souris acclimatées au froid, sensible à une dose élevée de noradrénaline et exposé à 30°C pendant une heure, ont perdu cette sensibilité. Cette absence de sensibilité des souris acclimatées au froid était contraire à la sensibilité remarquée dans des rats acclimatés au froid et examinés avec une dose plus petite de noradrénaline à 30°C et indique une différence définie d'espèce. La différence de réponse des souris à la noradrénaline dans les deux conditions de milieu indique l'importance de la température pendant l'expérience.

La vitesse avec laquelle les souris acclimatées au froid ont perdu leur sensibilité à la noradrénaline indiquait le plus grand rôle du système nerveux dans la thermogénèse que celui observé dans le rat et peut-être un moyen différent de thermogénèse sans frisson est employé dans les deux espèces.

PART ONE

INTRODUCTION

A. General Aspects of Thermoregulation

All homeotherms which have been tested respond to cold by increasing their oxygen consumption rates (OCR's) (Hemingway 1963). This increase appears to have three basic origins: increased voluntary activity, shivering, and nonshivering thermogenesis.

1. Voluntary activity

When animals are initially exposed to cold, increased somatic voluntary activity and autonomic sympathetic pilo-erection are observed.

2. Shivering

An immediate fall in skin temperature indicating sympathetically induced peripheral vasoconstriction has been observed, as well as signs of increasing electrical activity from the muscles within fifteen minutes (Sellers et al. 1954) indicating the onset of shivering (Review von Euler 1961).

In shivering, the peripheral cold receptors pick up the initial cold stress. From here the impulse is sent via the spinal nerves to the spinal cord and on into the thalamus, then to the cortex and hypothalamus for integration.

The response to this stimulus is sent from the primary centre of the hypothalamus over the pons and bulbo-reticular facilitatory area

(particularly the Red nucleus) into the lateral funicles of the cord (Jung et al. 1937). From here the impulse follows primarily the reticulo-spinal pathways (Hemingway and Stuart 1963) and partially the pyramidal pathways (Keller 1948) and is transmitted to the respective muscle groups by motor nerves leaving via ventral spinal roots.

Studies on the muscle activity of unanesthetized cold-acclimated rats showed that they could increase their metabolic rate by about eighty per cent without appreciable change in EMG (Hart et al. 1956). This, according to the authors, indicated that shivering was not a major factor in the metabolic response to cold in these animals. The neurohumoral mechanism alone was apparently sufficiently rapid to account for the immediate response.

3. Nonshivering thermogenesis

Initially the concept of nonshivering thermogenesis as the ultimate stage in cold-acclimation was doubted because of the difficulty in demonstrating an elevation of oxygen consumption in the cold without shivering (Hemingway and Hathaway 1941, Burton and Edholm 1955). However the following were convincing evidence for the phenomenon of nonshivering thermogenesis: (1) Davis and Mayer (1955) showed that forty per cent of the total heat production of normal rats exposed to cold was due to nonshivering thermogenesis; (2) Sellers and co-workers (1954) demonstrated that there was a reduction of shivering accompanied by increasing production of heat in rats during chronic exposure to cold; (3) Cottle and Carlson (1956 a), using curare to prevent shivering, showed that cold-acclimated curarized rats could still increase their oxygen consumption and maintain their body temperature for a period

of two hours when exposed to cold. Warm-acclimated curarized rats showed only a small increase in oxygen consumption and were unable to maintain body temperature; (4) Héroux et al. (1956) and Hart et al. (1956) showed that cold-acclimated rats could approximately double their metabolic rate without shivering, whereas warm-acclimated rats showed pronounced shivering in the cold.

The main characteristics of nonshivering thermogenesis have been stated to be a high rate of heat production with actually decreased muscular electrical activity and the ability of curarized rats to increase their heat production when injected with calorigenic hormones.

In nonshivering thermogenesis, it would appear that the regulatory centre is the posterior hypothalamus (Jansky 1965) and that the efferent fibres are different from those of shivering. The sympathetic nervous system seems to be involved as well as the endocrine system in nonshivering thermogenesis.

Two principal factors have been stressed in an attempt to explain nonshivering thermogenesis. Since animals chronically exposed to cold have an elevated basal metabolic rate (BMR) (Sellers and You 1950) a role has been assigned to the thyroid. In rats, increased thyroid activity has been shown (Starr and Roskelley 1940, Rand et al. 1952, Cottle and Carlson 1956, Woods and Carlson 1956). However Leblond and Gross (1943) and Sellers and You (1950) showed that thyroidectomized rats could be maintained with minimal amounts of thyroxine (T_4). Weiss (1957) showed that the elevated tissue respiratory quotient was less but still present. Also unsuccessful attempts at artificial cold-acclimation with thyroxine (Sellers et al. 1951) and the capability of thyroidectomized curarized rats to respond to cold (Hsieh and

Carlson 1957) indicate that in cold-acclimated animals the chemical regulation of metabolism is not controlled solely by the thyroid gland.

The second factor involved in nonshivering thermogenesis appears to be the sympathetic nervous system (SNS). Cannon and co-workers (1926, 1929) showed that adrenalectomized and sympathectomized animals were not able to maintain body temperature when exposed to cold. Each of these factors, thyroid and SNS, will be discussed in more detail with regard to their role in thermoregulation.

4. Site of heat production in nonshivering thermogenesis

A number of sites might be possible in heat production, but the experiments of Depocas (1958, 1960) in which he showed that functionally eviscerated, curarized, cold-acclimated rats could increase their heat production to the same extent as intact animals, indicated the role of the skeletal muscle mass and the insignificant role of the viscera. The role of skeletal muscle was later confirmed by Jansky and Hart (1963) using the hind limb of curarized cold-acclimated rats, and by Davis (1963) on the muscles of cold-acclimated dogs.

More recent experiments by Jansky (1966) indicate that possibly the muscles are not the only organ participating in thermogenesis and that earlier experiments may have been influenced by the methods used. Jansky (1966) measured cytochrome oxidase activity and maximal metabolism on the entire muscle mass and showed that this tissue cannot account for all the increase in oxygen consumption in very cold environments. There is some evidence that part of this thermogenesis originates in brown fat (Smith and Roberts 1964). The oxygen consumption of the isolated perfused liver of cold-acclimated rats is not affected

by noradrenaline (Jansky et al. 1964) and thyroxine (Zeisberger 1966). Thus the liver does not seem to acquire an increased sensitivity to these hormones, but their indirect effect may be mediated through increased organ blood flow (Hannon et al. 1963) and on levels of substrate in blood (Hannon and Larson 1962, Rimmer et al. 1962), both of which would lead to increased liver metabolism.

Jansky (1965) points out that there may be species differences in the type of nonshivering thermogenesis since in the white mouse the muscle capacity for thermogenesis is only thirty-five per cent of the metabolic capacity.

B. The Role of the Thyroid in Thermoregulation

1. Metabolism of thyroxine and its actions

In attempting to elucidate the mode of action of thyroid hormones, two approaches have been used, each having drawbacks. In vitro experiments in which the effect of the hormone on a particular enzyme is studied are highly sensitive in the experimental conditions, and frequently the results are somewhat meaningless. For example, Dunne and Tapley (1960) showed that D- and L-thyroxine were equally active in vitro but not so in vivo. When studying the action in vivo, large and unphysiological doses of hormones frequently have to be administered before any measureable effect is observed.

The premise that the thyroxine molecule itself does not exert an effect on the metabolism but that it is altered to some active form has been put forth. Various metabolites of thyroxine have been suggested and subsequently tested to see whether they exhibit greater or less

metabolic action than T_4.

The three main pathways suggested have been: (1) conjugation of the phenolic hydroxyl group; (2) oxidative deamination or transamination of the alanine side chain; (3) deiodination of one or both benzene rings (Pitt-Rivers and Tata 1959).

Conjugation occurs primarily in the liver, and the formation of the β-glucuronides (Taurog et al. 1952, Roche et al. 1953) and their biliary excretion was originally thought to be an important mechanism for the elimination of excess hormone or the regulation of the hormone level in circulation. However further experimentation showing that only a small proportion of a large injected dose of T_4 is excreted in the conjugated form (Taurog 1955), casts doubt on the importance of this pathway. It seems also that rat livers do not handle an exogenous dose of hormone in the same manner as an endogenous one (Heninger et al. 1963), raising doubt as to the validity of extrapolating observations concerning the metabolism of an exogenous dose to the metabolism of endogenous hormone.

Roche et al. (1954) showed that β-glucuronide conjugation occured more readily with T_4 than with T_3, sulphate conjugation occuring predominantly with T_3.

No evidence has been presented to show that conjugation of thyroid hormones yields a compound of higher biological activity or is an important step for their physiological action.

The possible physiological importance of the conversion of iodothyronine to iodothyroacetic acid was suggested since these acids appeared to exert an immediate in vivo and in vitro action and did not exhibit the latent period which preceded the action of iodothyronines

(Thibault and Pitt-Rivers 1955, Thibault 1957). Objections to the importance of these compounds are presented by Pitt-Rivers and Tata (1959), one being that oxidative deamination of thyroid hormones is quantitatively a minor metabolic process in the body compared to deiodination and conjugation. It appears that there is no conclusive evidence to suggest that the deaminated derivatives of thyroid hormones are their "active" forms, although the derivatives themselves may be biologically active.

The third and major pathway is deiodination. The discovery that T_3 was more potent than T_4 (Gross and Pitt-Rivers 1953) as well as some reports of partial deiodination of T_4 (Larson et al. 1955, Albright and Larson 1959) suggested that this transformation was part of a mechanism for the activation of T_4. However, since partially deiodinated derivatives are rarely encountered in studies of deiodination, this view is generally discounted. Barker (1964) has postulated a protein complex containing 3, 5, 3'-T_3 derived by 5' deiodination of T_4 and this has led to renewed interest in the possibility of partial deiodination.

The problem still exists unresolved as to whether deiodination is the resultant or cause of hormonal action.

In recent studies using ^{14}C-labeled T_4 (Kot, P.A. and H.M. Klitgaard 1959, West et al. 1963, Shimizu and Pittman 1965), the pathways appear to be the same as those elucidated by the use of ^{131}I.

Another possible metabolic pathway of thyroid hormones is the rupture of the diphenyl-ether link (Nunez et al. 1964), 2,6-diiodohydroquinone (DIH) being one of the possible derivatives (Allegretti 1954). Serif and Seymour (1961) found that this compound, DIH, was very toxic when tested in animals, but that the diacetyl derivative,

DDIH was relatively non-toxic and had antithyroid effects.

From their observations that DDIH inhibited the OCR in mice injected with L-T_4 but not in those injected with L-T_3, they suggested that DDIH prevented deiodination of T_4 to T_3. Since they considered T_3 to be the active form of the hormone they felt that this would explain the inhibitory action of DDIH.

In rats, the major portion of injected DDIH was deacetylated to DIH which was conjugated with glucuronic or sulphuric acid and excreted via the urine (Serif and Seymour 1963). Deiodination did occur as a catabolic step since they detected free iodide ion. However deiodination did not yield monoiodohydroquinone or its excretion forms, suggesting simultaneous removal of two atoms of iodine. The low water solubility of DDIH could prevent rapid transport to enzyme sites. Also from their experiments, the low toxicity of large doses of DDIH would indicate little conversion to the very toxic quinone.

Serif and Seymour (1961) suggested that DDIH blocked the deiodination of T_4 to T_3 thus accounting for its inhibitory effect. However a large number of investigators fail to detect any significant conversion of T_4 to T_3 (Tata et al. 1957, Etling and Barker 1959, Lissitzky et al. 1959, Tata 1960, Lissitzky et al. 1961, Plaskett 1961). This would indicate that DDIH must act somewhere else since blockage of deiodination would not account for the observed inhibition of the calorigenic response.

2. Protein binding of thyroid hormones and their action

Another factor which is important in the action of thyroid hormone is the availability of the hormone to the cell and site of

action. It is known that T_4 is carried in the blood bound to proteins and that there is a very small amount of free T_4 in equilibrium with this bound form. T_3 is bound more loosely to thyroxine-binding globulin (TBG) and is not bound at all to thyroxine-binding pre-albumin (TBPA). Because of this difference in binding affinities, the percentage of free T_3 is much greater than that of free T_4. Since only the unbound T_4 or T_3 can diffuse into the tissues and thus exert an effect (Freinkel et al. 1957, Robbins and Rall 1957, Ingbar and Freinkel 1960), the free hormone levels are important. Thus the binding of the hormone plays a major role in its availability to the cells. In chickens, T_4 and T_3 exert equal biological activity (Shellabarger 1955) and the subsequent discovery that there was no TBG in chick serum led to the conclusion that this accounted for their similar biological effect.

When a single dose of T_3 is administered in man, there is a more rapid onset and higher degree of initial change produced than after an equimolecular dose of T_4, but the action of T_3 wears off more rapidly (Tata 1961 a). Thus if the time factor is considered, these two hormones have identical total calorigenic action. Ingbar and Freinkel (1960) point out that although binding may affect availability of the hormone to the cell and thus control to some extent the amount of hormone required for a given metabolic response, it seems unlikely that it would affect the intrinsic biological potency. Tata (1964 b) disagrees with this and feels that potency is linked with availability.

3. Dosage and its effect on the latent period of hormone action

Tata (1964 a) discusses the use of large doses of hormone and their effect on the latent period of action, which is defined as the

time that lapses between the administration of the hormone and the initial response of the subject. This period decreases with the size of the animal and varies according to the hormone or its derivatives used. These variations may be partially explained on the basis of rates of distribution of the active substance from the blood to tissue (Tata 1962). Administration of large doses of thyroid hormones may result in a shorter latent period, or its absence. The dose effect may have two explanations: (1) if the acceptor sites on the serum proteins were saturated, there would be more free hormone available to the cell and sites of action (Robbins and Rall 1957, 1960, Tata 1962, 1964 b); (2) this higher dosage might act differently than a normal dose, masking the latent period of action.

4. Intermediary metabolism in nonshivering thermogenesis

The striking similarities in the effect of increased thyroid function and in cold-acclimation have resulted in investigators assigning a role to thyroid hormones in chemical thermogenesis. It was thought that a change in a metabolic pathway caused by an excess of thyroid hormone would be similar to that incurred by cold-acclimation. However comparisons have been made and there definitely are differences in the ways that these two factors affect metabolism as well as similarities.

Héroux (1966) has pointed out that the increased production of thyroid hormones in cold-acclimation does not necessarily lead to the increased calorigenesis observed, since under the more natural outdoor conditions there is no evidence for increased thyroid activity in small mammals; resting metabolism in these animals appeared normal

and there was no sign of increased glandular activity. These animals did, however, show increased nonshivering thermogenesis (Héroux 1963). It seems that a distinction must be drawn between normal adaptation and laboratory acclimation, two processes which may result in entirely different end states in the animals.

Héroux also observed that warm- and cold-acclimated white rats, after an equilibration period, excreted ^{131}I in the urine (indicative of T_4 utilization) at the same rate. The fecal excretion was much greater in the cold-acclimated rats. The author states that the apparently normal utilization of thyroxine in the laboratory cold-acclimated rat, in spite of its increased hormone production, indicates that the thyroid is not involved in nonshivering thermogenesis to a much greater extent in cold-acclimated rats than in warm-acclimated ones.

There is also evidence that thyroid activity in rats reaches a maximum after about three weeks of exposure to cold and returns to near normal conditions after six to ten weeks (Cottle 1960).

It is difficult to assess the exact role of thyroid hormones in cold-acclimation, the situation being complicated by the multiplicity of effects that these hormones have and by a lack of knowledge as to their locus of action. Various areas have been studied; in particular are those which show an alteration in activity in the cold-acclimated animal such as p/o ratio, coenzyme A, NADPH generation, NAD-linked enzymes, and cholesterol synthesis. Most of these studies have been done in vitro, and it is assumed that the activity so determined reflects in vivo activity (Berry 1966); the possibility exists that enzyme levels may not be an index at all of the controlling mechanisms of cellular metabolism in temperature acclimation, but rather a result.

It is a fact that the animal in the cold needs to produce more heat, but it is not known whether this is done by intensifying existing processes controlled by ADP level or whether non-phosphorylating processes are involved.

It was thought that possibly the lowering of efficiency of oxidative phosphorylation (p/o ratio) seen in isolated mitochondria from cold-acclimated rats might be the explanation for increased heat production in nonshivering thermogenesis. According to Beyer (1963) the uncoupling of oxidative phosphorylation does not supply the additional heat in the steady state of the acclimated animal, but serves as a pathway allowing more than the normal number of electrons to flow through the electron transport chain than would be able to during tight respiratory control. Because of the difficulty in showing any uncoupling under physiological conditions, workers have tried to demonstrate augmentation of other enzymic routes of electron transport not under tight respiratory control.

Aerobic dehydrogenases and flavoprotein oxidases which are able to transfer electrons directly to oxygen are involved in the metabolism of noradrenaline, amino acids, and ascorbic acid, substrates which have been shown to increase in the cold-acclimated animal; thus this is a nonphosphorylating mechanism which could be involved in nonshivering thermogenesis (Smith 1960).

Other systems are affected by thyroxine: the hexose monophosphate shunt (HMPS) (Phillips and Langdon 1956) and 5α-steroid reductase are increased and NADPH-NAD transhydrogenase activity is decreased.

The thyroxine stimulation of HMPS results in a reduction of ATP yield, since the glycolytic synthesis of ATP at the substrate level does

not occur, and in the production of NADPH-NAD transhydrogenase. The glycerophosphate cycle in which thyroxine increases the activity of the intramitochondrial α-glycerophosphate dehydrogenase (Smith 1964) is probably involved in the transfer of cytoplasmic NADH to the mitochondria. Dihydroxyacetonephosphate (DHAP) is reduced to α-glycerophosphate by the NADH requiring enzyme outside the mitochondria and α-glycerophosphate oxidation is coupled to cytochrome c in the mitochondria. NAD is regenerated and only one phosphorylation occurs in this process. However the energetic importance of this pathway has not been established, and again, in vitro activity does not necessarily reflect in vivo activity. Masoro (1962) discredited the idea of the importance of the formation of NADPH in driving fat synthesis. The reverse may be true - fatty acid synthesis and other NADPH utilizing systems driving the HMPS by making NADP available.

Another system apparently not under tight respiratory control is that of DT diaphorase. In 1951, Lehninger and co-workers showed that NADH could be oxidized by an internal and an external pathway by mitochondria. Potter (1958) postulated that such nonphosphorylating pathways might be augmented in cold-acclimation and Beyer (1963) showed this to be the case in liver slices and diaphragms of cold-acclimated rats. Beyer (1963) gave evidence showing that mitochondrial DT diaphorase activity is elevated in cold-acclimated rats and the oxidation of NADH by diaphorase to coenzyme Q or cytochrome b lowers the yield of ATP by one to two thirds since no ATP is formed between NADH and the flavins and possibly not between cytochrome b and cytochrome c. This pathway may be important because it has an effect on the formation of ATP coming from all substrates (Jansky 1965).

Most of the experiments done to show various pathways have involved a rather indirect approach, and it has been assumed that if thyroxine augmented the activity of an enzyme, then cold-acclimation probably did the same. More advanced knowledge of protein synthesis has led to experiments which more directly try to elucidate the action of thyroid hormones.

Tata (1963) placed the locus of action of T_4 at DNA transcription. Experiments by Widnell and Tata (1963) and Tata (1964 c) in which T_3 induced an increase in DNA-dependent RNA polymerase activity and increased labeling of nuclear RNA substantiate this. The explanation presented by Rall (1965) of these facts is that T_4 displaces a hypothetical inhibitor of DNA transcription. This either results in unused DNA being "read" or accelerates the reading of previously available DNA. Nonspecific stimulation for the synthesis of most proteins could result from an increase in DNA-dependent RNA polymerase. It appears that the oxidation and reduction of cytochrome b is critical for thyroxine to be active (Rall 1965) and possibly T_4 effects the breadkown of X-Y or Y-P type of high energy intermediate.

In a series of experiments done by Pavlovic and Ardjus (1963), thyroid tissue was grafted into the anterior chamber of the eye of thyroidectomized rats. After a transitory increase, the metabolic rate at 30°C fell to the same level as that of thyroidectomized animals. However on exposure to 10°C, the grafted rats survived many months with a new basal metabolic rate lower than normal controls. The thyroidectomized rats died. The authors feel that even an undetectable increase in MR^{30° may be of importance for cold-acclimation as far as survival and metabolic responses are concerned.

Unsuccessful attempts at artificial cold-acclimation by using thyroid hormone (Sellers et al. 1951) and the adaptation to cold of thyroidectomized rats maintained on minimal amounts of thyroxine (Sellers and You 1951) indicated that the larger amounts of thyroxine secreted were not an absolute necessity for acclimation.

C. The Role of Catecholamines in Thermoregulation

The involvement of SNS in the resonse to cold exposure was first suggested by Cannon and his co-workers (1929). Since then considerable evidence has been accumulated emphasizing the role of the SNS in the response to cold stress (Leduc 1961).

1. Secretion and excretion

In a study done by DesMarais and Dugal (1951), it was shown that there was an initial depletion of adrenaline and possibly noradrenaline content of the adrenal glands in rats exposed to $0^{\circ}C$. The amine content of the gland then rose over a three week period to values higher than normal for noradrenaline and normal for adrenaline after which there was no change.

Studies concerning the excretion of catecholamines showed that there was an increased secretion of adrenaline (LeBlanc and Nadeau 1961) and of noradrenaline (Cottle 1960). Under moderate cold stress the adrenal contribution to the increased secretion was small (LeBlanc and Nadeau 1961, Leduc 1961) but was more important in severe cold stress.

In the experiments of Leduc (1961), the excretion of urinary catecholamines were followed during a one month period of acclimation of rats to $3^{\circ}C$. He observed a maximal output of noradrenaline (about

five times normal) within twenty-four hours which remained high for the monthly period although declining slowly with time. The adrenaline excretion reached a maximum after about one week and then decreased more rapidly than noradrenaline to a value still higher than the control after one month. In longer experiments, the noradrenaline output decreased further during the second month to a value maintained at approximately the same level for up to six months.

As to the origin of the noradrenaline excreted during cold exposure, Leduc (1961) using adrenalectomized rats and also using the ganglion blocking agent, mecamylamine, provided further evidence to that of LeBlanc and Nadeau (1961) that the increased amounts of noradrenaline came from adrenergic nerve endings.

2. The role of the sympathetic nervous system

The extent to which the SNS entered into nonshivering thermogenesis has been studied by the use of pharmacological agents.

The evidence for noradrenaline as the mediator in nonshivering thermogenesis has been summarized by Depocas (1961), the main points being: (1) Cottle and Carlson (1956 a) showed that adrenal demedullation lowered the increase in OCR of the curarized cold-acclimated rat at 6°C; (2) Hsieh and co-workers (1957) showed that previous injection of sympatholytic and ganglion blocking agents abolished the increase in oxygen consumption of curarized cold-acclimated rats at 6°C; (3) Hsieh et al. (1957) demonstrated that the fall in oxygen consumption caused by administration of hexamethonium, a ganglion blocking agent could be prevented by noradrena line but not by adrenaline; (4) Hsieh and Carlson (1957 a) showed that adrenaline caused a marked rise in

blood glucose whereas neither cold-acclimation nor noradrenaline did; (5) Hsieh and Carlson (1957 a) demonstrated that cold-acclimated rats (when oxygen consumption was measured at 30°C) showed greater sensitivity to noradrenaline than to adrenaline. Warm-acclimated rats did not show this sensitivity.

Depocas (1960) using infusions of noradrenaline showed that rats gradually developed this sensitivity to noradrenaline and that this increase was paralled by a decrease in muscular activity (Hart et al. 1956).

3. Stimulation of cyclic 3', 5'-AMP formation by catecholamines

Catecholamines have been shown to stimulate the formation or accumulation of cyclic 3',5'-AMP in a number of tissues such as skeletal muscle (Klainer et al. 1962, Posner et al. 1962), liver (Sutherland and Rall 1960) and adipose tissue (Butcher 1966, Sutherland et al. 1965, Klainer et al. 1962).

The enzyme responsible has been called adenylcyclase and sediments at low speeds appearing to be in the cell membrane (Sutherland et al. 1962); it generates cyclic 3',5'-AMP from ATP, requiring Mg^{++} or Mn^{++} for activity, with pyrophosphate as product (Rall and Sutherland 1962).

4. Adrenergic receptors and the adenylcyclase system

Belleau (1966) has speculated on the subject of how catecholamines stimulate the cyclase activity, and the previous concept of direct complex formation between catecholamines and adenylcyclase-bound ATP has been revised (Belleau 1967). He now states that instead of a single receptor describing the catalytic effect of catecholamines

at the α- and β-receptor levels, it would be more correct to designate it as a single catalytic mechanism with differences in its stereochemical application.

To date there is insufficient evidence to be able to say whether the β-receptor can always be equated with the adenylcyclase system (Sutherland and Robison 1966), and it seems possible that catecholamines might produce another messenger by α- as well as by some β-receptors (Furchgott 1959). However the classification of receptors does not seem applicable to the metabolic activities of catecholamines (Hagen and Hagen 1964). Receptors are classified on a pharmacological basis, and Furchgott (1967) states that adrenergic receptors mediating, for example free fatty acid (FFA) mobilization, cannot be clearly classified as α or β.

5. Effects of catecholamines on carbohydrate metabolism

Injections of adrenaline cause an immediate rise in blood glucose, and noradrenaline appears to be less effective in producing hyperglycemia (Bloom and Russel 1955). The reason for the lack of hyperglycemia caused by noradrenaline is that only liver glycogen utilization and not peripheral glucose utilization is affected by noradrenaline (Review Hagen and Hagen 1964).

The glycogenolytic effect of catecholamines may be mediated through the phosphorylase system (Sutherland and Cori 1951). The activation and inactivation of liver phosphorylase (Sutherland and Wosilait 1955) involved a mechanism comparable to that seen in cell free extracts involving phosphorylation and dephosphorylation of the enzyme (Krebs and Fischer 1956). Activated phosphorylase b kinase converts the in-

active phosphorylase b kinase to phosphorylase a kinase (Krebs et al. 1959). Cyclic 3',5'-AMP accelerates the ATP activation of the phosphorylase b kinase and maybe this is how adrenaline acts on the phosphorylase system (Murad et al. 1962, Rall and Sutherland 1962, Sutherland et al. 1962, Krebs et al. 1966).

6. Effects of catecholamines on lipid metabolism

Although noradrenaline has some effect on carbohydrate metabolism it is not nearly as great as that of adrenaline. However, noradrenaline exerts more of a lipolytic effect than adrenaline.

There are two aspects to lipid metabolism: one is to determine the relationship between the catecholamines and increased lipolysis, and the other is to determine what effect this increase has on the metabolism.

(a) Adipose tissue

(i) Lipolytic effect

Rizack (1961) and Vaughan et al. (1964) have shown that in rat adipose tissue, stimulation by catecholamines and other hormones results in a rapid rise in lipolytic activity. Steinberg (1966) studied the lipolytic response in adipose tissue; up to this time catecholamines had been shown to activate the phosphorylase system but it did not seem likely that this was the system responsible for the increased FFA release following noradrenaline injection. Butcher (1966) reported that cyclic 3',5'-AMP had an effect on fad pads, but the nature and number of steps between cyclic AMP and increased lipolysis are still unknown (Butcher and Sutherland 1967).

(ii) Activation of lipase

A higher cellular level of cyclic 3',5'-AMP appears to activate lipase and results in increased lipolysis (Butcher 1966). The activation of this lipase is too rapid to represent an enzyme induction caused by catecholamines (Steinberg 1966). It thus appears that the catecholamines, through stimulation of adenylcyclase, increase the conversion of inactive to active lipase, a situation similar to that of the phosphorylase system. The increased active lipase could then stimulate the release of FFA from adipose tissue (Vaughan et al. 1964). The hydrolysis seems to be complete and the glycerol so produced is not reused by adipose tissue being subsequently released into the circulation. The FFA are, to a great extent, rebuilt into triglycerides with only a few released to the circulation.

(iii) The effect of FFA on glucose utilization

The increased level of intracellular FFA concentration has an extensive effect on the glucose metabolism of adipose tissue (Hagen and Hagen 1964), one of these effects being an increase in glucose uptake.

Feigelson et al. (1961 a) and Havel and Goldfien (1959) have attributed the inability of adrenaline to maintain high FFA levels in vivo during infusion and the ability of noradrenaline to do so, to the higher glucose levels accompanying adrenaline administration.

(iv) Triglyceride synthesis

The rate of triglyceride synthesis in the intact adipose tissue might presumably be regulated by changes of ATP or one of the enzymes involved, by the availability of ATP or one of the other required co-factors, or by the availability of substrate. The availability of α-glycerophosphate may be an important factor in determining the rate

of glyceride synthesis (Vaughan 1961, Wertheimer and Shafrir 1960). in adipose tissue, no glycerokinase has been demonstrated (Wieland and Suyter 1957) and only very small amounts of ^{14}C-labeled glycerol are incorporated into adipose tissue triglyceride (Shapiro et al. 1957) so that glycerol released from the hydrolysis of exogenous or endogenous triglyceride probably cannot be reused. The α-glycerophosphate required for triglyceride synthesis is probably derived from the metabolism of glucose. Margolius and Vaughan (1962) have shown that each of the glycolytic intermediates between glucose-6-phosphate and α-glycerophosphate can form α-glycerophosphate in suitable homogenates of adipose tissue.

(b) Muscle

The effect of adrenaline and noradrenaline on lipid metabolism in muscle appears to be mediated solely by changes in the level of FFA in the blood supplying the muscle (Hagen and Hagen 1964).

(c) Liver

The uptake of FFA by the liver is proportional to their plasma concentration with prolonged noradrenaline infusion causing deposition of triglycerides in the liver (evidence cited by Hagen and Hagen 1964).

7. Effects of catecholamines on protein metabolism

The knowledge and understanding of catecholamine effect on protein metabolism is scanty.

The effect of adrenaline is thought to be mediated through its hyperglycemic activity. Noradrenaline has a much lower hyperglycemic effect than adrenaline and also has less effect on blood amino acid concentration (Ellis 1956).

8. The calorigenic action of catecholamines

The metabolic effects of catecholamines are diverse and affect a number of systems. The problem now is to associate these metabolic effects with calorigenic ones and to try to learn the mechanism whereby cold exposure increases heat production.

In normal adult animals, the calorigenic effect of noradrenaline is weaker than that of adrenaline (Lundholm 1950). However after continuous infusion, noradrenaline has more effect on the FFA content of plasma than adrenaline (Havel 1964). There is also an increase in the turnover of FFA resulting from increased oxidation and reesterification of the mobilized FFA accounting for the calorigenic effect of noradrenaline.

FFA serve as substrate for oxidation in most tissues. In liver the FFA are reesterified and then go back to the adipose tissue where they are reincorporated into adipose tissue triglyceride (Steinberg 1966). In muscle tissue, the FFA are esterified and oxidized proportionally to their plasma concentration (Eaton and Steinberg 1961). According to these same authors this mobilization and increased muscle metabolism could explain the calorigenic effects of catecholamines. Other factors which may contribute to the calorigenic effect are gluconeogenesis in liver and glycogen replacement in liver and muscle (Hagen and Hagen 1964).

It would be expected that if the prime function of NA were the mobilization of FFA followed by an increase in metabolism that the level of plasma FFA would be higher in cold-acclimated animals. Some workers (Mallov 1963, Wertheimer and Shafrir 1960, Hannon and Larson 1962) have observed higher FFA levels in cold-acclimated animals;

Masironi and Depocas (1961) observed lower FFA levels in cold-acclimated rats, and Rimmer et al. (1962) observed similar levels in both cases.

Hagen (1965) reported preliminary results indicating that the turnover of triglycerides was higher in cold-acclimated than in warm-acclimated animals after either exposure or injection of noradrenaline.

Hsieh et al. (1966) noted a higher level of FFA in cold-acclimated rats. He showed that the oxygen consumption increased with increasing doses of infused NA in both warm- and cold-acclimated rats. However the level of plasma FFA did not increase with dose in the cold-acclimated rats but did so in warm-acclimated ones. This indicates that the calorigenic effect of noradrenaline is not secondary to the increase in plasma FFA concentration and may be explained, according to the authors, by assuming that noradrenaline stimulates oxidation of fatty acids as well as lipolysis. Acclimation to cold would involve an increase in the ability of tissues to oxidize FFA. Hagen (1965) has shown that noradrenaline stimulated oxygen consumption in isolated adipose tissue of cold-acclimated rats.

Jansky (1965) draws attention to the fact that a number of systems activated by noradrenaline ar not so activated by cold-acclimation. He cites as examples the following: activation of phospnorylase was not affected by cold-acclimation (Hannon and Vaughan 1961); although noradrenaline activated glycogenolysis in the isolated perfused liver of cold-acclimated rats, it did not lead to increased liver metabolism; when warm- and cold-acclimated animals were killed at their temperature of acclimation, the release of FFA from the adipose tissue followed the same pattern in both groups (contrary to the findings of Mitchell and Longwell 1964, 1964 a).

There appears to be fairly divergent opinions as to the action of noradrenaline in cold-acclimation and more evidence must be gathered to indicate which if any of the possibilities presented is utilized.

D. Interaction of Thyroid and Sympathetic Nervous System

In nonshivering thermogenesis, the SNS and the thyroid each play a role, the SNS being the more important of the two. However the calorigenic action of noradrenaline is influenced by the activity of the thyroid gland (Harrison 1964).

Ring (1942) showed that the calorigenic response to adrenaline was potentiated by T_4 and that the log of the response was proportional to the BMR. This potentiation has been discussed by others (Schaeffer and Thibault 1945, 1945 a, Ellis 1956, Harrison 1964, Lundholm et al. 1966). Swanson (1956) showed that thyroidectomy inhibited and T_4 pre-treatment (3 to 48 µg/rat/day) increased the metabilic response to a standard injection of adrenaline (40 µg/100 g). She suggested as a possible explanation, that adrenaline increased the availability of substrate and T_4 accelerated its utilization. In addition to this she added that T_4 might also retard the breakdown of adrenaline so that it would have more time to exert its effects. Spinks (1952) and Spinks and Burn (1952) had previously observed lowered amine oxidase levels in blood vessels and in liver. Zile and Lardy (1959) correlated increased circulating levels of noradrenaline and adrenaline in hyperthyroid animals with a lowered monoamine oxidase activity in liver. Axelrod (1960) gave evidence that O-methylation was important in the rapid inactivation of circulating catecholamines and reported no effect

of T_4 pretreatment on this process. D'Iorio and Leduc (1960) obtained lowered hepatic O-methyltransferase and a smaller urinary excretion of metanephrine in T_4-injected animals.

However the importance of these enzymes remains difficult to assess, but thought not to be of prime importance for the termination of noradrenaline action after release (Iverson 1967). Inhibitors of these enzymes generally fail to produce any marked potentiation of the effects of noradrenaline released by sympathetic stimulation; nor do such inhibitors retard the rapid disappearance of noradrenaline from circulation. Nevertheless these enzymes may play a part, monoamine oxidase primarily, in regulating the storage concentration of noradrenaline in adrenergic nerves and may metabolize any excess of storage capacity.

Svedmyr (1966) working with fasted rabbits found that T_4 pretreatment potentiated the action of adrenaline on lactic acid production. He did not observe any effect of noradrenaline on this nor any effect of adrenaline or noradrenaline on the FFA levels of T_4 pretreated animals. Thyroidectomy decreased the calorigenic and lactate-increasing effects of adrenaline. Svedmyr (1966) also cites experiments which showed that T_4 treatment had no influence on the uptake of infused ^3H-labeled adrenaline or noradrenaline in different organs of the rat. Neither was the inactivation of catecholamines via catechol-O-methyltransferase affected by T_4 medication. It appeared unlikely that thyroid hormones increased the effect of adrenaline by inhibiting its inactivation.

Hyperthyroid animals have been used to study the interrelationship between thyroid hormones and catecholamines. According to Deykin

and Vaughan (1963) and Debons and Schwartz (1961), adipose tissue from hyperthyroid animals is hyperresponsive to the stimulating effects of catecholamines. It is not known definitely whether or not the high rate of FFA mobilization in the hyperthyroid state is due only to a hypersensitivity of adipose tissue to stimulation. The results of Eaton et al. (1965) showing that intravenously administered nicotinic acid, an inhibitor of catecholamine stimulation of adipose tissue, restored FFA to normal, are compatible with such a possibility.

Butcher (1966) quotes data from Bray (1966) which lends support to the concept that the changed responsiveness to noradrenaline is due to the level of cyclic 3',5'-AMP which is affected by thyroid hormone and is substantiated by the fact that the increase in cyclic 3',5'-AMP level in fat cells was one third lower in thyroidectomized than in normal rats.

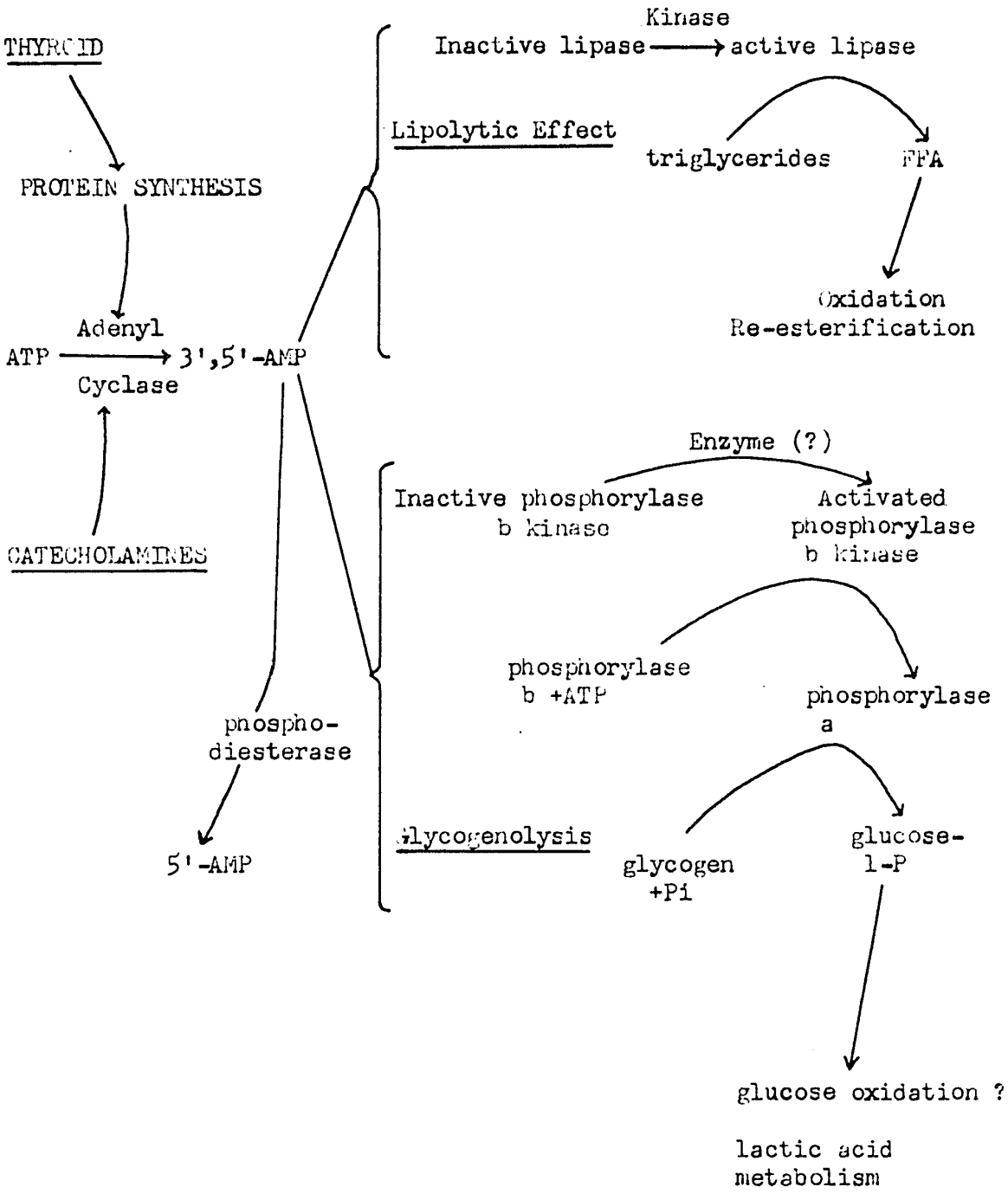
Myant and Whitney (1967) demonstrated a significant rise in basal oxygen consumption of rats given thyroid hormones before the FFA level changed. Eaton and Steinberg (1961) using in vitro studies, suggested that the effect of thyroid hormones on oxygen consumption might be due to a rise in FFA concentration. Myant and Whitney (1967) state that although there appears to be an initial dissociation between FFA and oxygen consumption, the further increase in oxygen consumption after chronic hormone treatment may be dependent upon a rise in FFA.

In attempting to elucidate the mechanism of nonshivering thermogenesis, numerous workers have drawn attention to the similarities between cold-acclimated and hyperthyroid animals (Smith and Hoijer 1962). The value of the similarities depends on the confirmation of some of the basic facts since one of the similarities observed was

the ability of injected adrenaline or noradrenaline to increase plasma FFA in vivo (Rimmer et al. 1962, Debons and Schwartz 1961, Schwartz and Debons 1959). Hsieh et al. (1966) stated that cold-acclimated rats were not hyperthyroid, and Héroux (1963 a) has pointed out the possible error in attempting to explain thermogenesis by increased thyroid activity.

The work of Hsieh and Carlson (1967) indicating that cold-induced nonshivering thermogenesis decreased with time following thyroidectomy and that of Johnson et al. (1967) using methimazole (an anti-thyroid compound) showed that there is a requirement for adequate thyroid secretion for maintenance of thermogenesis in the cold, and that a high secretion rate of noradrenaline is not sufficient to maintain heat production in the absence of T_4.

The current thoughts in the relationship and effects of thyroid hormones and catecholamines are presented on the next page.



Some Effects of Thyroid Hormones and Catecholamines on Metabolism

E. Statement of Problem

Diacetyldiiodohydroquinone (DDH) was shown to inhibit the calorigenic action of exogenous T_4 in warm-acclimated mice (Serif and Seymour 1961), was competitive in nature (DesMarais and LeMay 1964), and had no effect on the slight increase in metabolic rate of T_4-pretreated, cold-acclimated mice. DDH also was reported to have no effect on the calorigenic action of T_3.

There is evidence that DDH is deacetylated to [illegible] (Serif and Seymour 1963). Structurally this DDH molecule mimics the phenolic-moiety of T_4, and there is evidence also, that DDH analogs could act as competitive inhibitors of T_4 at the acceptor sites for deiodination (Barker 1962).

LeMay (1964) showed that cold-acclimated mice were much less sensitive to exogenous T_4 than warm-acclimated mice and theorized that partial saturation of T_4 acceptor sites in cold-acclimated animals might impair the efficiency of exogenous hormone. His was based on the fact that liver mitochondria of refrigerated animals had been shown to bind less T_4 in vitro (Tomomo and Matsumoto 1961). The possibility existed that DDH might block T_4 acceptor sites in the warm-acclimated animal in the same way that endogenous T_4 might saturate acceptor sites preventing exogenous T_4 from exerting its effect in cold-acclimated animals. Because of this it was suggested that DDH might be a useful tool in elucidating the role of thyroid hormones in cold-acclimation.

An attempt was made to study this disappearance of both the

calorigenic action of T_4 and the inhibitory effect of DDT by measuring the O_2 at intervals during the period of acclimation.

However, the methods used (grouped animal O_2 measurements) appeared to be too crude to reveal anything.

The inhibitory effect of DDT had only been studied using relatively high doses of T_4 and not at all using T_3 in cold-acclimated mice.

Therefore the present experiments were designed to study these effects of DDT on the calorigenic action of T_4 and T_3.

O_2 was determined using two methods: one was a modified Scholander (1950) system using groups of eight mice, the other, using single mice, was a modification, suggested by Dr. [illegible] of Scholander's method (1950).

Various doses of the hormones in combination with DDT were administered, and the responses measured in each O_2 [illegible] to enable a comparison of the methods.

The [illegible] of mice injected with ^{131}I-labelled hormones were measured in the single animal apparatus in an attempt to correlate the O_2 and excretion of ^{131}I.

T_3 is known to potentiate the action of adrenaline (Swanson 1954); temperature and thyroidectomy affect this response (Swanson 1954). On the other hand, cold-acclimated animals are much more sensitive to noradrenaline than to adrenaline (Feist and Carlson 1957a). It was also decided to study the effect of hormone and DDT pretreatment on the sensitivity to noradrenaline in an attempt to localize [illegible] of the hormones and DDT on the meta-

bolic rate. The animals were pretreated and re-tested with noradrenaline at the temperature of acclimation.

Because a number of workers (Hsieh and Carlson 1957a, Depocas 1960, Héroux 1967) had tested the responsiveness to noradrenaline by removing the animals from their temperature of acclimation to another temperature for the test, the effect of this temperature change was studied.

P A R T T W O

EXPERIMENTAL

A. MATERIALS AND METHODS

1. MATERIALS

a. Chemicals

(1) Hormones: L-thyroxine sodium and 3,5,3'-triiodo-L-thyronine, were obtained from Nutritional Biochemicals Corporation. Radioactively labeled thyroxine, 3' or 5' fifty per cent ^{131}I, and 3' ^{131}I, "Triomet", Both in fifty per cent propylene glycol were obtained from Abbott Laboratories.

(2) Diiodohydroquinone (DIH) was obtained from K and K Rare Chemicals.

(3) Diacetyl-2,6-diiodohydroquinone (DDIH) was synthesized in our laboratory.

(4) Noradrenaline (NA, Levophed Bitartrate 0.2% solution = 0.1% base), Winthrop Laboratories.

(5) Radioactive Iodine ^{131}I (Atomic Energy of Canada).

b. Animals

Male swiss mice descended from H.A./ICR - Charles River Laboratories, weighing between twenty-five and thirty grams, were obtained from Romain Robidoux Company, St. Constant, P.Q.

c. Diets

(1) Purina Lab Chow, iodine content 1.1 ppm

(2) Purina House Breeder Chow, iodine content 0.02 ppm

2. METHODS

a. Preparation and Maintenance of the Animals

(1) Normal Conditions

The mice were housed individually in wire mesh cages (25 cm x 17 cm x 17 cm) and fed Purina Lab Chow and tap water ad libitum. After a few days at room temperature they were randomly divided into two groups, one group being placed in a constant temperature room at 30±1° C(humidity 25%), the other at 10±1° C (humidity 65%). Bedding material was given when the animals were first at 10°C;after one week they were not given bedding. The animals were considered acclimated after one month of exposure to either temperature. The animals were acclimated and maintained individually for all experimentation.

(2) Low Iodine Diet

Mice were given Purina Mouse Breeder (low iodine) Chow for one week before exposure to 10°C or 30°C and were maintained on this diet during the period of acclimation and experimentation.

(3) Thyroidectomy

Animals acclimated to 10°C or 30°C were given Mouse Breeder Chow and one per cent potassium perchlorate in the drinking water for eight days, in order to deplete the thyroidal iodine pool. On the ninth day, tap water was substituted for the perchlorate solution, and on the eleventh day, 0.3 ml of a saline solution containing 50 μc ^{131}I was injected subcutaneously. The next day

these mice received a second injection of 50 μc ^{131}I. The animals were considered thyroidectomized (Tx) and thyroxine deficient when body weight failed to increase (about four weeks).

b. Injections

(1) Preparation and Administration of the Hormone Solutions

Thyroxine (T_4) and triiodothyronine (T_3) were dissolved in 0.4% saline (slightly basic). The doses administered were 0.5, 1.0, 1.6[illegible] and 2.0 mg/kg, the volume of injection being 0.01 ml/g body weight. All hormone injections were subcutaneous. The carrier doses, containing approximately 10 μc of ^{131}I, were administered at the level of 2.0 mg/kg for T_4 and 1.6[illegible] mg/kg for T_3.

(2) Preparation and Administration of the DDIH Suspension

2,6-diiodohydroquinone (DIH) was recrystallized from hot twenty per cent ethanol and then dissolved in a minimal amount of pyridine (dried over magnesium sulphate). Twice the molar concentration of acetic anhydride (dried over magnesium sulphate) was added, and after heating, the solution was poured over finely crushed ice. The resultant precipitate, diacetyl-2,6-diiodohydroquinone (DDIH), was then recrystallized from hot isopropanol.

Due to the water insolubility of DDIH, finely ground crystals were suspended in a 0.5% gelatin in 0.9% saline solution. DDIH was injected intraperitoneally at a constant dose of

200 mg/kg, the volume of injection being 0.01 ml per g body weight.

(3) Preparation and Administration of the Noradrenaline Solution

Noradrenaline was diluted with 0.9% saline and the following doses, 0.2, 0.4 0.6 mg/kg were injected subcutaneously, the volume of injection being 0.01 ml per g body weight.

c. Metabolic Cages and Sampling of Urine and Feces

(1) Metabolic Cages

Slightly modified Hammersmith Perspex Metabolism cages were used (Figure 1). An opening was cut in the side of the cage to accommodate a drinking tube. The clamp holding the tube was positioned to hold the lid down. The internal diameter of the cage was 9.5 cm, the height 10.0 cm. A wire mesh screen was fitted over the Erlenmeyer flask to catch the feces. This facilitated their removal and prevented any mixing with urine that occasionally splashed over to the bottom of the beaker.

(2) Feces Sample

All of the feces, free of food particles, excreted over the forty-eight hour period were collected and placed in as many counting tubes as required to hold them. The dry pellets were added to a constant level previously marked on the tubes to insure standard geometry, and the counts per minute

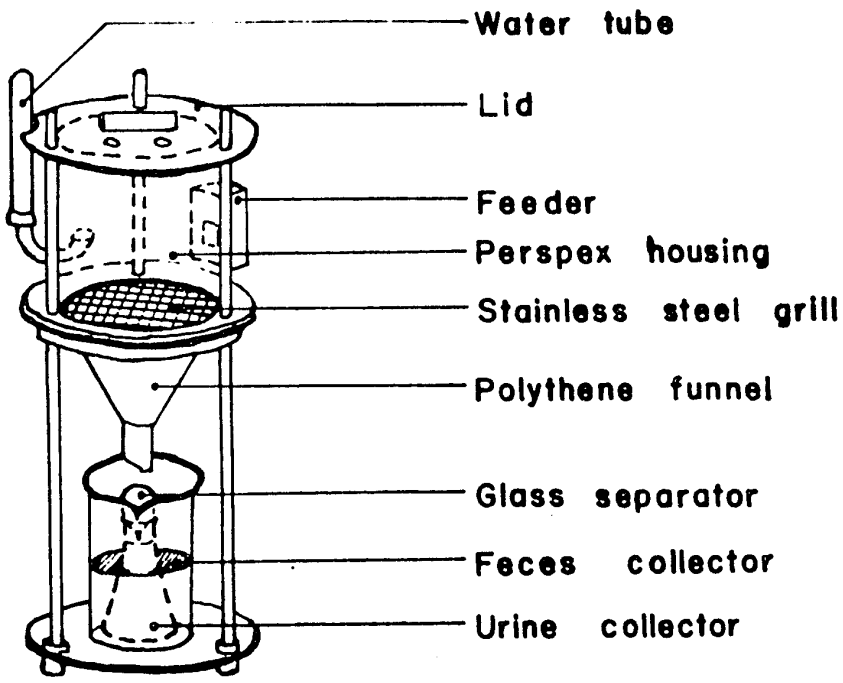


FIGURE 1

MODIFIED HAMMERSMITH METABOLIC CAGE

(cpm) from all the tubes were totalled. The uneven distribution of ^{131}I excreted in the feces necessitated counting all of the feces rather than a sample.

(3) Urine Sample

The flask, bulb, funnel, screen, and beaker of the metabolism cage were rinsed with distilled water, and these washings were added to the collected urine sample, the final dilution being one hundred ml. Four ml of this were then pipetted into a counting tube.

(4) Standards

The standard was prepared by removing 0.4 ml of the labeled hormone from the injection bottle and diluting this to one hundred ml with distilled water. Three four ml samples of this solution, containing approximately ten microcuries (equivalent to the amount injected into the animal), were pipetted into counting tubes. The standard was done in triplicate.

d. Determination of Radioactivity

A Nuclear Chicago Gamma Counting System was used (Timer and Scaler-Model 8166, Analyzer-Model 1810, Well Scintillation Detector Model DS 202 (V)).

All samples were counted for three one minute intervals and the average calculated. The standards were counted before

and after the samples from each animal. The before and after cpm were averaged to give the "standard cpm". By counting the excretion sample and a sample of the originally injected material at the same time it was possible to express the excretion rate in terms of a percentage of the originally injected material without the necessity of correcting for radioactive decay.

Since the animals were injected on a body weight basis, the "standard cpm" had to be corrected. The urine and "standard cpm" were multiplied by twenty-five because of their dilution.

The excretion of the label was expressed for both urine and feces, as a percentage of the administered dose.

e. Determination of Oxygen Consumption

In all cases, unless specified, the oxygen consumption was measured at the acclimation temperature.

(1) Grouped Animals

Oxygen consumption was measured in a closed system apparatus modified from that of Maclagan et al.(1950) in the following ways: a nine litre capacity desiccator was used; eight mice were placed in individual pie-shaped wire mesh compartments; a nitrogen to oxygen mixture (50:50) was flushed through the system; soda asbestos (Na_2O) replaced soda lime; magnesium perchlorate ($Mg(ClO_4)_2$) was added to absorb excess moisture; a thermometer was placed in the desiccator to determine the temperature rise during the experiment.

The mice were weighed, injected, and placed in the desiccator which was flushed through with the N_2:O_2 mixture at the rate of 8 l/min for three minutes to ensure an environment of 50% oxygen. After an equilibration period of thirty minutes, the zero reading was taken, and successive readings were taken at ten minute intervals for one hour. A correction factor was introduced to compensate for any change of temperature in the desiccator during the one hour reading period. The oxygen consumption rate (OCR) was expressed as ml of O_2 consumed per g of mouse per hr at S.T.P.

(2) Single Animals

A closed system, in which the oxygen consumed by the animal was replaced, was used.

The apparatus, Figure 2, modified from Ruhland and Heusner (1950) by Pace (1946), consisted of two main sections: the electrical part, Figure 3, entailed the use of an interlocking relay, built by W. Welsh, Riverdale Radio, Ottawa, a Durant Electric Counter (No. 4-y-943 B 110 V.), a platinum-mercury contact at the manometer, stainless steel contacts at the floater and an Ansco 3-way selenoid valve (IPS-8314-A43, 1/8 in. 110 V.); the non electrical part consisted of a container of approximately one litre capacity, and a wire mesh cage to contain the mouse.

A plexiglass floater (2 cm x 1 cm) with a stainless steel plate over one end was inserted in a tube in which an aperture had been bored at such a level that the floater displaced a known volume of water. When the apparatus was set up, the valve was in position b (Figure 2), forming a closed system containing

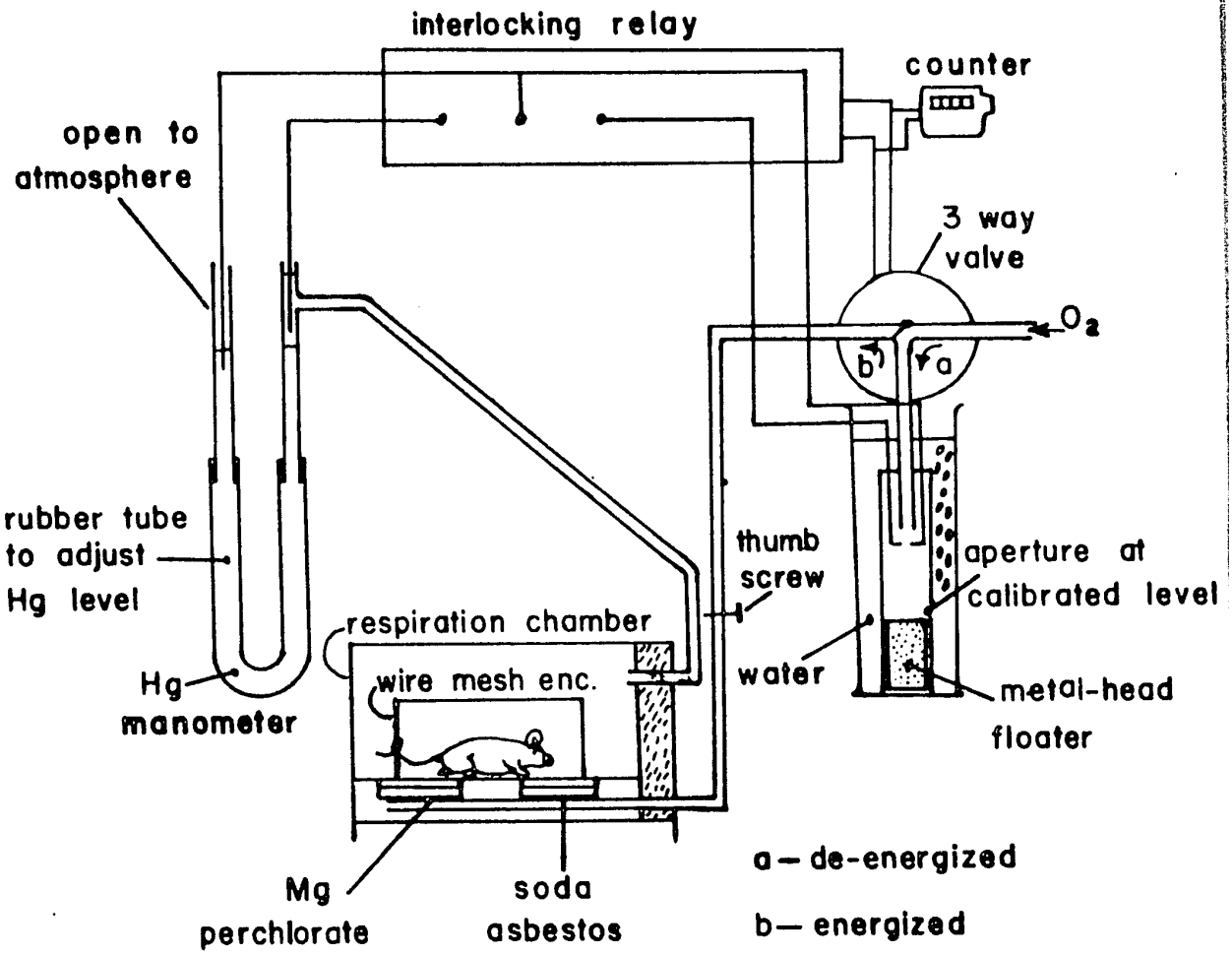


FIGURE 2

CLOSED SYSTEM FOR DETERMINATION OF OCRs IN SMALL ANIMALS

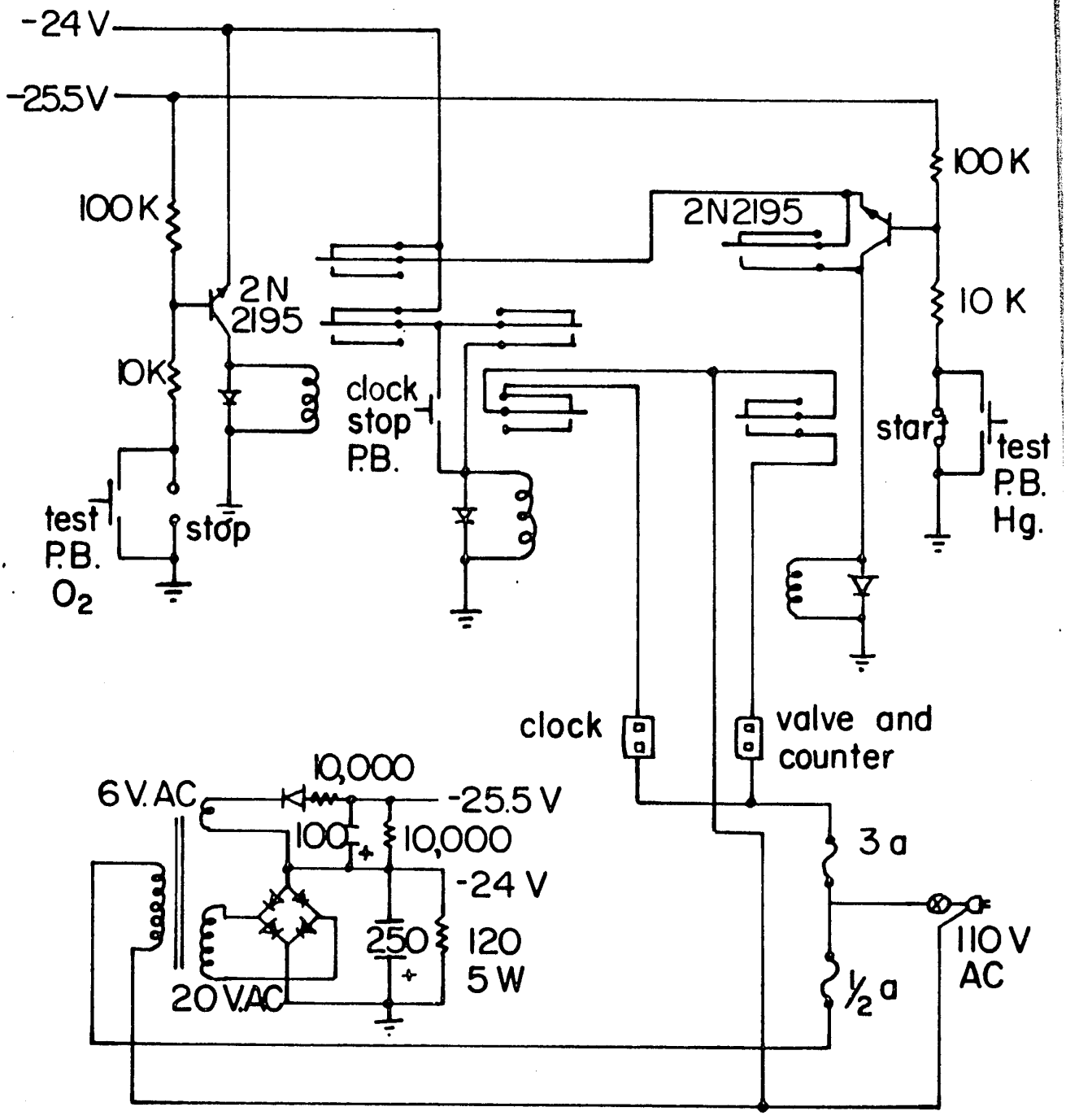


FIGURE 3

CIRCUIT DIAGRAM OF THE INTERLOCKING RELAY SYSTEM OF THE SINGLE ANIMAL OXYGEN CONSUMPTION APPARATUS

the mouse. The oxygen bubbled through the aperture displacing the floater. The carbon dioxide was absorbed by Soda asbestos, moisture by magnesium perchlorate, and as the pressure dropped in the cage, the mercury rose in the right side of the manometer and made contact with the platinum electrode. The mercury switch thus closed, de-energized the valve causing it to move to position a. The external source of oxygen was interrupted, allowing the water pressure and the negative internal pressure of the system to raise the floater and force the calibrated volume of oxygen into the container. A thumb screw was used to adjust the speed at which the floater rose. When the floater reached its upper limit, contact was made, the oxygen switch was closed,and the decreased level of mercury opened the mercury switch allowing the valve to be re-energized and return to position b. This completed one cycle. A clock, included in the circuit, started after initial contact of the floater, and a counter registered each contact made by the floater.

The mercury column was adjusted to maintain slight negative pressure in the container during the experiment.

Calculation of the oxygen consumption rate:

$$\text{OCR (ml/g/hr)} = \frac{\text{Counts} \times V \times P \times 273}{\text{wt} \times t \times 760 \times (273 + T)}$$

V = volume of oxygen replaced in each cycle

P = atmospheric pressure

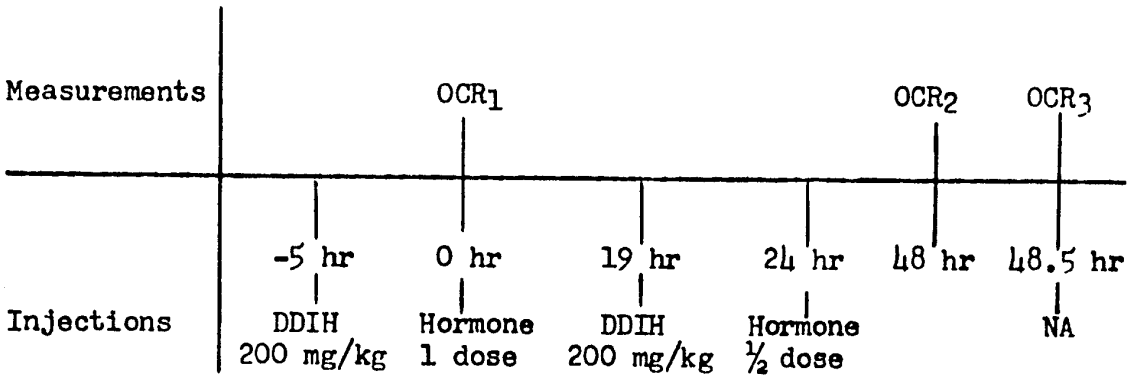
t = time (hr)

wt = weight of mouse (g)

T = temperature ^{o}C

No correction for temperature was necessary since it did not change in the respiration chamber during the experiment.

f. Treatment Protocol (Grouped and Single Animals)



g. Experimental Protocol

(1) Grouped Animals

(a) Variations in Oxygen Consumption Rate following increased Doses of Hormones measured in Warm- or Cold-acclimated Mice.

Parallel experiments were conducted on groups of eight mice acclimated to either 10°C or 30°C. The oxygen consumption of at least five groups of eight was read per treatment (Methods 2e(1)).

In one series, doses of 0.5, 1.0, 1.68 or 2.0 mg/kg T_4 or T_3 were injected subcutaneously as previously described. In a second series, all the hormone injections were given to mice previously injected with DDIH (2b(2)).

(b) The Effect of Grouping on Oxygen Consumption.

The OCR (ml/g/hr) was calculated by using groups of three,

six or eight mice. The effect of grouping was tested in warm- or cold-acclimated animals.

(2) Single Animals

(a) Variations in Oxygen Consumption Rate following increased Doses of Hormones, measured in Warm- or Cold-acclimated Mice.

This experiment was similar to that done with groups of animals (1(a)) except only two dosage levels of each hormone were used: 1.0 and 2.0 mg/kg T_4 or 1.0 and 1.68 mg/kg T_3. DDIH was administered as usual.

The oxygen consumption was measured in the single animal apparatus as described in Section 2e(2).

(b) Seasonal Variation and Duration of Acclimation.

Due to the period over which the experiments were conducted, it was possible to check whether the oxygen consumption varied with season or the length of time that the animals had been acclimated. Results from the grouped and single animal experiments were used.

(c) Effect of Diet on Oxygen Consumption Rate during Acclimation.

Mice were given one of two types of diet during acclimation, the regular lab chow having 1.18 ppm iodine, or Mouse Breeder Diet having only 0.02 ppm iodine. After one week at room temperature and on the diet, the mice were placed at 10° or at 30°C. Their OCRs were measured daily for a month.

(d) Concurrent Study of the Metabolic Effects of ^{131}I-labeled Hormones and the Rate of Excretion of the ^{131}I.

The animals were injected subcutaneously with ^{131}I-labeled hormones, 2.0 mg/kg T_4* ± DDIH or the equimolecular 1.68 mg/kg T_3* ± DDIH.

After the zero hour OCR, the mice were placed in metabolic cages for the forty-eight hour interval between the two OCRs. The urine and feces were removed and prepared as described in section 2, c and d.

(e) The Effect of Temperature Change on the Oxygen Consumption Rate.

The OCRs of untreated, cold-acclimated mice were measured. The animals were then moved immediately to the warm room and their OCRs remeasured at that temperature. There was a lag of approximately five minutes while the apparatus was being set up, following which the OCRs were measured at intervals up to twenty-four hours.

Animals pretreated with T_4 ± DDIH were tested as above. Other untreated and pretreated mice were moved from 30°C and measured at 10°C.

(f) Noradrenaline Test in Normal and Thyroidectomized Mice.

The animals used in this test were pretreated with hormone ± DDIH, and their OCRs measured at zero and forty-eight hours as described g(2)(c). At the conclusion of the forty-eight hour reading, the mouse was removed from the OCR apparatus, injected subcutaneously with 0.2 or 0.4 mg/kg NA and immediately returned to the

apparatus. The time of injection was noted; readings were then taken for the first ten minutes and at thirty minutes after the NA injection.

All the oxygen consumption measurements were taken at the temperature of acclimation and the results calculated as follows:

$$\% \text{ increase following NA} = \frac{OCR_3 - OCR_1}{OCR_1} \times 100$$

(g) Noradrenaline Test in Normal and Thyroidectomized Mice Exposed to 30° and 10°C.

To test this response, warm-acclimated mice were pretreated with 2.0 mg/kg T_4. The first oxygen consumption reading was taken at zero hour, the second forty-three hours later. The animals were then placed in the cold five, four, three, two or one hours or immediately after the forty-three hour OCR and were tested for their sensitivity to noradrenaline after the sixth hour. By using this schedule the animals had been in the cold from one to six hours but were all tested at the same time after the T_4 pretreatment. The reciprocal experiment, cold-acclimated mice moved to 30°C, was done also. The forty-three hour period was used to maintain this constant time after pretreatment. The results were calculated as in the noradrenaline test (f).

In subsequent experiments, a forty-eight hour OCR was measured and all NA testing done after one hour of exposure.

h. Statistical Analysis

A Bartlett's Test for homogeneity of variance was done on the results of each experiment. All except one were homogeneous. The homogeneous populations were analyzed by doing a nested analysis of variance on the temperature, concentration of NA, and time categories (Snedecor 1959, Davies 1960). Duncan's Multiple Range Test (Duncan 1955) was applied at the 5% level when there were significant F values.

To the non-homogeneous population (grouped animal experiment), the Dixon test for extreme values (Dixon 1951, 1953) was applied to remove possible outliers and the Bartlett's Test for homogeneity redone. The population was still not homogeneous, so a Kruskal-Wallis one way analysis of variance (Siegel 1956), a non parametric test, was applied (the outliers were included). This test showed there was significance. In order to find which values were significantly different, t tests were done on the ones of interest. Due to the error involved with repetitive use of the t test, it was applied only to values which seemed to overlap. Any treatments whose means $\pm$ S.E. did not overlap were considered statistically different as the number of animals adequately represented a population.

B. RESULTS

1. GROUPED ANIMAL EXPERIMENTS

a. Variations in Oxygen Consumption Rate following increased Doses of Hormones measured in Groups of Warm- or Cold-acclimated Mice.

The purpose of this experiment was to observe whether the administration of increasing amounts of either of the principal circulating thyroid hormones, T_4 or T_3, would cause a gradation of calorigenic response, and what the effect of DDIH would be on the response, in 30°C- or 10°C-acclimated mice. The results are shown in Tables 1 and 2 and their significance in Table 3.

As seen in Figure 4, the lowest dose of T_4 (0.5 mg/kg) had only half the calorigenic effect of the other three in warm-acclimated mice. Amongst the three higher doses, there was no significant difference of effect (Table 1, Figure 4). At 10°C there was no effect of dosage . At 30°C, the twenty-nine per cent increase in oxygen consumption after the administration of 2 mg/kg T_4, was eleven per cent lower than that reported by other workers (DesMarais and LeMay 1964). However, at 10°C, the response observed corresponded with their observations that exogenous T_4 had little or no calorigenic effect in further increasing the elevated oxygen consumption of cold-acclimated mice.

TABLE 1

THE EFFECT OF T_4 ± DDIH DOSAGE ON THE OCR (MEASURED IN GROUPS OF EIGHT) OF 30°- OR 10°-ACCLIMATED MICE

Treatment	30°C			10°C		
	OCR_1 (ml/g/hr)	change in OCR (OCR_2-OCR_1)	% change	OCR_1 (ml/g/hr)	change in OCR (OCR_2-OCR_1)	% change
T_4 (mg/kg B.W.)						
0.5	1.67 (7)" ±0.05'	0.21 ±0.03	12.8 ±1.9	2.59 (7) ±0.03	0.17 ±0.05	6.6 ±2.0
1.0	1.43 (5) ±0.06	0.38 ±0.04	26.3 ±2.9	2.47 (7) ±0.06	0.26 ±0.03	10.8 ±1.4
1.68	1.66 (7) ±0.07	0.40 ±0.06	25.0 ±4.8	2.43 (6) ±0.09	0.24 ±0.03	10.2 ±1.6
2.0	1.56 (19) ±0.04	0.44 ±0.04	28.5 ±2.9	2.53 (12) ±0.04	0.25 ±0.06	9.4 ±2.5
T_4 + DDIH						
0.5	1.51 (8) ±0.05	0.24 ±0.05	16.1 ±3.3	2.31 (9) ±0.07	0.22 ±0.12	9.3 ±1.9
1.0	1.44 (6) ±0.06	0.37 ±0.10	26.9 ±7.7	2.30 (5) ±0.03	0.42 ±0.11	18.3 ±4.8
1.68	1.32 (5) ±0.06	0.78 ±0.05	60.3 ±6.4	2.30 (5) ±0.08	0.35 ±0.11	15.5 ±4.9
2.0	1.50 (12) ±0.06	0.47 ±0.06	33.3 ±5.3	2.30 (8) ±0.11	0.26 ±0.07	11.9 ±3.4

' mean ± S.E.
" No. of replicates

TABLE 2

THE EFFECT OF T_3 ± DDIH DOSAGE ON THE OCR (MEASURED IN GROUPS OF EIGHT) OF 30°- OR 10°-ACCLIMATED MICE

Treatment	30°C			10°C		
	OCR_1 (ml/g/hr)	change in OCR (OCR_2-OCR_1)	% change	OCR_1 (ml/g/hr)	change in OCR (OCR_2-OCR_1)	% change
T_3 (mg/kg B.W.)						
0.5	1.74 (6)" ±0.05'	0.48 ±0.05	27.7 ±3.3	2.28 (6) ±0.14	0.43 ±0.11	19.8 ±5.7
1.0	1.72 (7) ±0.04	0.44 ±0.07	26.2 ±4.3	2.37 (8) ±0.12	0.33 ±0.11	15.4 ±5.7
1.68	1.67 (7) ±0.03	0.50 ±0.05	30.0 ±3.3	2.41 (12) ±0.09	0.36 ±0.06	15.5 ±3.1
2.0	1.61 (10) ±0.09	0.55 ±0.07	35.7 ±5.9	2.42 (5) ±0.12	0.40 ±0.08	17.3 ±4.7
T_3 + DDIH						
0.5	1.36 (5) ±0.07	0.70 ±0.08	52.8 ±9.4	2.10 (5) ±0.11	0.47 ±0.12	23.3 ±7.2
1.0	1.30 (5) ±0.07	0.69 ±0.11	54.5 ±10.4	2.12 (5) ±0.22	0.56 ±0.15	30.5 ±10.5
1.68	1.71 (6) ±0.16	0.46 ±0.21	30.1 ±7.6	2.37 (10) ±0.11	0.37 ±0.10	17.3 ±5.1
2.0	1.60 (5) ±0.06	0.57 ±0.05	35.6 ±3.1	2.36 (6) ±0.09	0.45 ±0.09	19.2 ±4.1

'mean ± S.E.

" No. of replicates

TABLE 3

A SUMMARY OF THE SIGNIFICANCE OF RESULTS OBTAINED USING GROUPED MICE.

T Tests were significant at the 5% level.

Treatment	Temp.°C	$Value_1$	is significantly different from $Value_2$	based on
T_4	30	0.5	1.0	no overlap
			1.68	t Test
			2.0	no overlap
T_4 + DDIH	30	1.68	0.5	no overlap
			1.0	no overlap
			2.0	no overlap
	30	T_4 (1.68)	T_4 (1.68) + DDIH	t Test
		T_3 (0.5)	T_3 (0.5) + DDIH	t Test
		T_3 (1.0)	T_3 (1.0) + DDIH	t Test
		T_4 (0.5)	T_3 (0.5)	t Test
	10	T_4 (0.5)	T_3 (0.5)	t Test
		30°C	10°C	
T_4		1.0	1.0	no overlap
		1.68	1.68	no overlap
		2.0	2.0	no overlap
T_3		1.68	1.68	no overlap

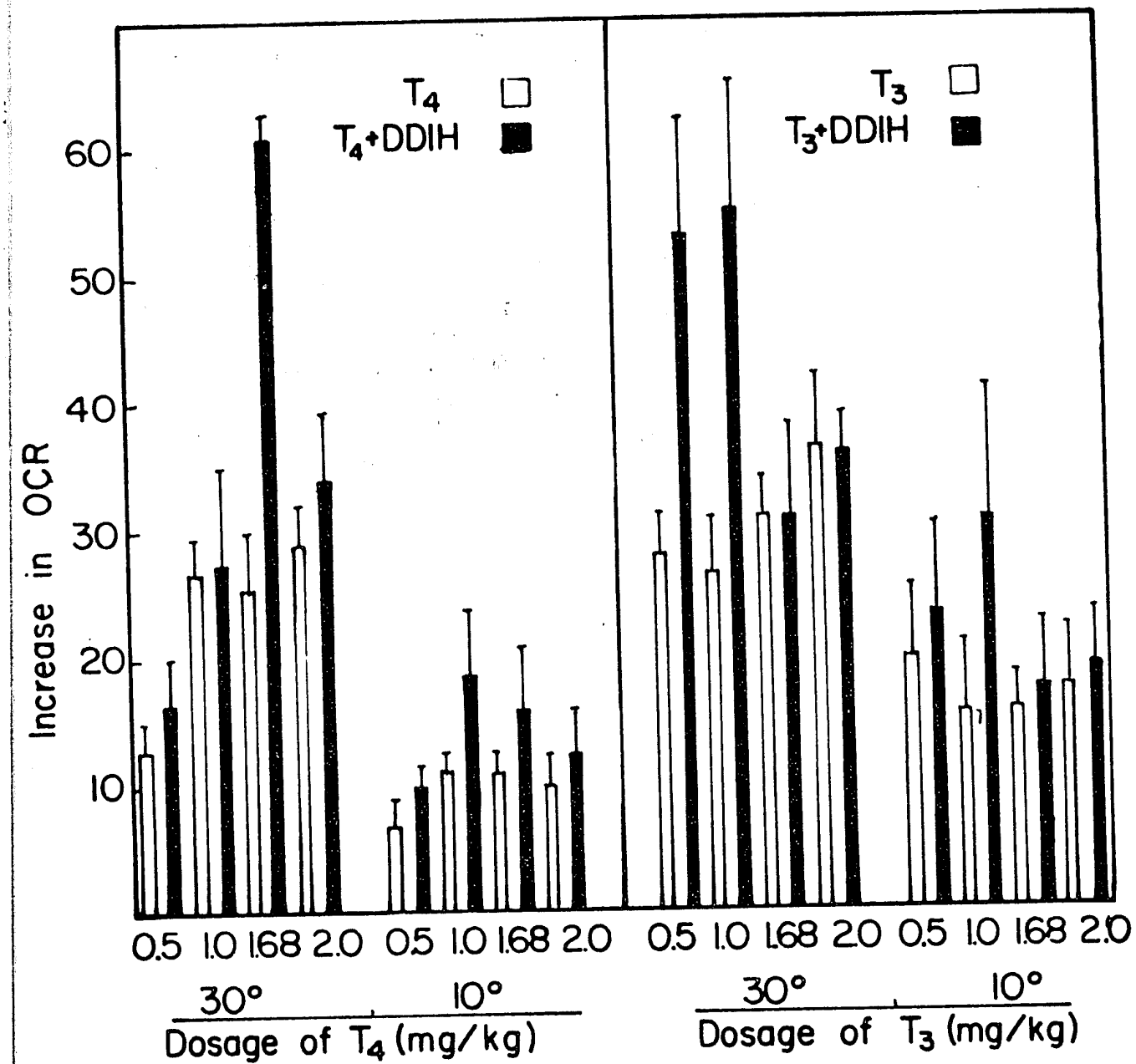


FIGURE 4

THE EFFECT OF T_4 ± OR T_3 ± DDIH DOSAGE ON THE OCR (MEASURED IN GROUPS OF EIGHT) OF 30°C- OR 10°C-ACCLIMATED MICE

DDIH administered five hours prior to the hormone, as previously described, had no significant effect on the calorigenic response of a warm-acclimated animal, injected with either 0.5 mg/kg or 1.0 mg/kg T_4 . At the 1.68 mg/kg level of T_4 , a greater than twofold increase in oxygen consumption was observed with DDIH. This enhancement of the calorigenic action of T_4 was not present at a higher dose of T_4 (2.0 mg/kg.)

These two observations: enhancement at 1.68 mg/kg of T_4 and lack of inhibitory effect at 2.0 mg/kg of T_4 , were contrary to earlier reports (Serif and Seymour, 1961, DesMarais and LeMay 1964) in which DDIH had decreased by fifty percent the calorigenic action of a 2 mg/kg dose of T_4.

In cold-acclimated mice, there was no enhancement of response with DDIH.

When T_3 was administered, there was no gradation of calorigenic response as the dosage was increased in either warm- or cold-acclimated animals (Table 2). The magnitude of response in warm-acclimated mice following an injection of T_3 (at all dosage levels) was similar to that elicited when T_4 was administered at the three highest doses. In cold-acclimated mice, T_3 at the four dosages increased the oxygen consumption approximately seventeen percent, whereas T_4 had had no significant effect at any dosage.

DDIH caused a twofold increase in oxygen consumption at the 0.5 and 1.0 mg/kg levels of T_3 in warm-acclimated mice, whereas a dose of 1.68 mg/kg T_4had been necessary before a similar enhancement was incurred. In cold-acclimated mice, DDIH did not

enhance the calorigenic effect of T_3.

Serif and Seymour (1961) using 1000 mg/kg DDIH with 1.68 mg/kg T_3 in mice at room temperature reported that DDIH had no effect on the calorigenic action of T_3 .This is consistent with the present observations using 400 mg/kg DDIH in mice acclimated to 30°C.

Summary:

30° C

T_4 Effect of Hormone:

- 0.5 mg/kg was only half as effective in increasing the OCR as the three higher doses.

Action of DDIH:

- not inhibitory to any dosage of T_4
- enhancement of response with 1.68 mg/kg T_4

T_3 Effect of Hormone:

- no effect of dosage
- response of the same magnitude as that elicited by T_4 at the three larger doses

Action of DDIH:

-not inhibitory to any dosage of T_3
-enhancement of response with 0.5 and 1.0 mg/kg T_3.

10° C

T_4

Effect of Hormone:

- no significant response to any dosage of T_4.

Action of DDIH:

- none

T_3

Effect of Hormone:

- no effect of dosage
- T_3 did increase OCR significantly

Action of DDIH:

- none

b. The Effect of Grouping on Oxygen Consumption Rate.

The oxygen consumption of warm- and cold-acclimated mice, measured in a group of eight, was lower than that obtained by Hart (1953) using single mice. To determine whether this was strictly a species difference or due to the method of OCR determination decreasing numbers of mice were grouped, and their OCRs calculated. The results obtained were expressed as the oxygen consumption per mouse and are shown in Table 4.

The oxygen consumption of warm-acclimated mice was not affected by grouping, but that of cold-acclimated ones was. By extrapolation of the curve, Figure 5, a value for a single mouse was obtained which was lower than that obtained by Hart (1953).

Thus it appeared that grouping had an effect on the OCR of cold-acclimated mice and that there was a species difference.

TABLE 4

THE EFFECT OF GROUPING ON THE OCR OF MICE ACCLIMATED TO 30° OR 10°C

Temp.	No. in Group	Av. O_2 Consumption (ml/g/hr)	Av. Wt. (g)	Av. O_2 Consumption per mouse
30°C	3	1.43	37.92	54.35
	6	1.51	37.92	56.97
	8	1.52	37.66	57.36
10°C	3	2.95	36.57	107.98
	6	2.55	36.50	92.97
	8	2.35	37.62	88.45

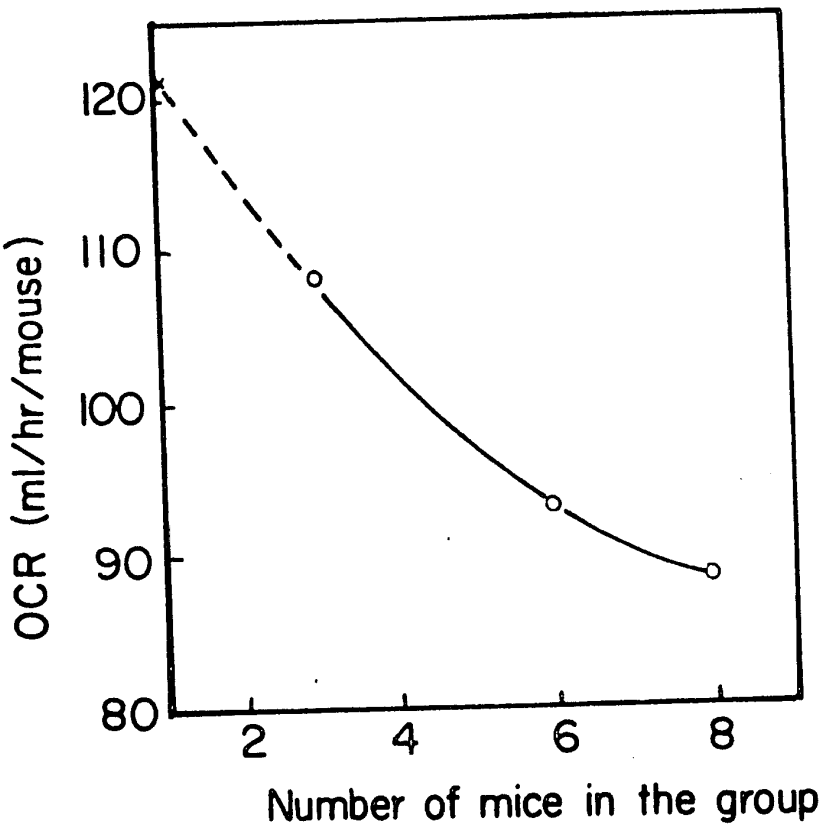


FIGURE 5

THE EFFECT OF GROUPING ON OCR OF MICE ACCLIMATED TO 10°C

2. SINGLE ANIMAL EXPERIMENTS

a. Variations in Oxygen Consumption Rate following increased Doses of Hormones, measured in single Warm- or Cold-acclimated Mice.

Since grouping had had an effect on the oxygen consumption of untreated mice in the cold, the likelihood arose that it might have an undefined effect on the values obtained from treated animals. This possibility was checked by repeating the 1.0 and 2.0 mg/kg doses of T_4 , and the 1.0 and 1.68 mg/kg doses of T_3, in conjunction with DDIH, in a series of experiments using the single animal apparatus. The results are shown in Tables 5 and 6 and Figure 6 and their significance in Figure 7. Since there was no significance of the dosage, the values were pooled for completion of the analysis of variance.

There was no temperature effect on the response to T_4 and T_4 + DDIH; T_3 and T_3 + DDIH were significantly more effective in increasing the OCR of warm-acclimated mice.

DDIH did not significantly alter the response to T_4 or T_3 at either temperature (Tables 5 and 6). The original inhibitory action of DDIH on a T_4 evoked increase, reported by Serif and Seymour (1961) and DesMarais and LeMay (1964), was not seen, nor was the enhancing effect that had been observed when groups of animals were treated with certain dosages of T_4 and T_3 observed in the single animal experiments.

TABLE 5

THE EFFECT OF T_4 ± DDIH DOSAGE ON THE OCR (MEASURED SINGLY) OF 30°- OR 10°-ACCLIMATED MICE

	30°C			10°C		
Treatment	OCR_1 (ml/g/hr)	change in OCR (OCR_2-OCR_1)	% change	OCR_1 (ml/g/hr)	change in OCR (OCR_2-OCR_1)	% change
T_4 (mg/kg)						
1.0	2.23 (20)" ±0.08'	0.51 ±0.05	24.9 ±3.7	4.71 (18) ±0.19	0.78 ±0.12	18.3 ±3.0
2.0	2.43 (34) ±0.05	0.44 ±0.03	18.5 ±1.5	4.89 (32) ±0.10	0.66 ±0.09	14.7 ±2.0
T_4 (mg/kg) + DDIH						
1.0	2.27 (15) ±0.08	0.42 ±0.08	19.2 ±4.2	4.62 (12) ±0.16	0.85 ±0.17	18.3 ±3.4
2.0	2.35 (27) ±0.05	0.39 ±0.05	17.4 ±2.4	4.62 (24) ±0.01	0.59 ±0.09	13.2 ±2.2

' mean ± S.E.

" No. of replicates

TABLE 6

THE EFFECT OF T_3 ± DDIH DOSAGE ON THE OCR (MEASURED SINGLY) OF 30°- OR 10°-ACCLIMATED MICE

	30°C			10°C		
Treatment	OCR_1 (ml/g/hr)	change in OCR (OCR_2-OCR_1)	% change	OCR_1 (ml/g/hr)	change in OCR (OCR_2-OCR_1)	% change
T_3 (mg/kg)						
1.0	2.61 (10)" ±0.07'	0.54 ±0.08	20.9 ±3.3	4.64 (9) ±0.14	0.62 ±0.16	13.8 ±3.7
1.68	2.55 (20) ±0.05	0.77 ±0.07	30.6 ±2.9	4.43 (20) ±0.11	0.83 ±0.10	19.5 ±2.8
T_3 (mg/kg) + DDIH						
1.0	2.26 (10) ±0.07	0.59 ±0.10	27.4 ±5.6	4.20 (10) ±0.16	0.45 ±0.17	12.0 ±4.4
1.68	2.48 (20) ±0.09	0.68 ±0.09	30.4 ±4.5	4.86 (20) ±0.13	0.64 ±0.07	13.5 ±1.7

' mean ± S.E.

" No. of replicates

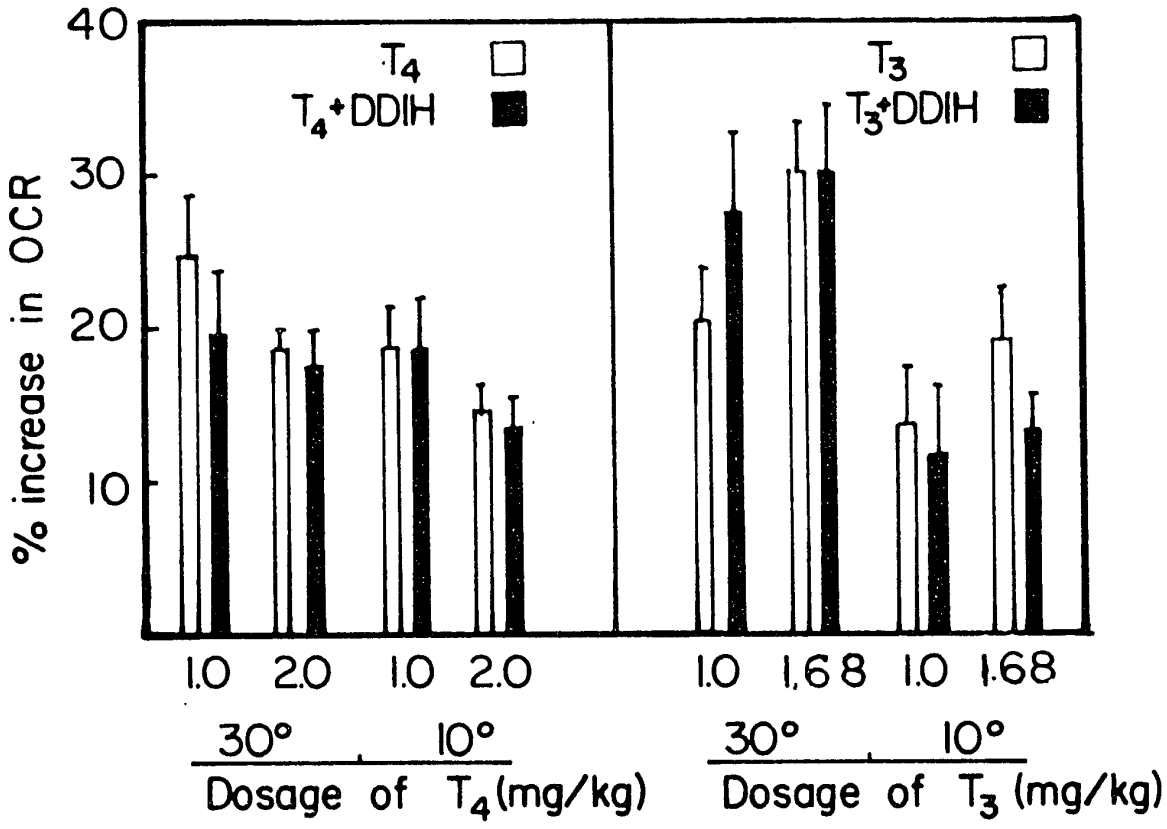


FIGURE 6

THE EFFECT OF T_4 ± OR T_3 ± DDIH DOSAGE ON THE OCR (MEASURED SINGLY) OF 30°C- OR 10°C-ACCLIMATED MICE

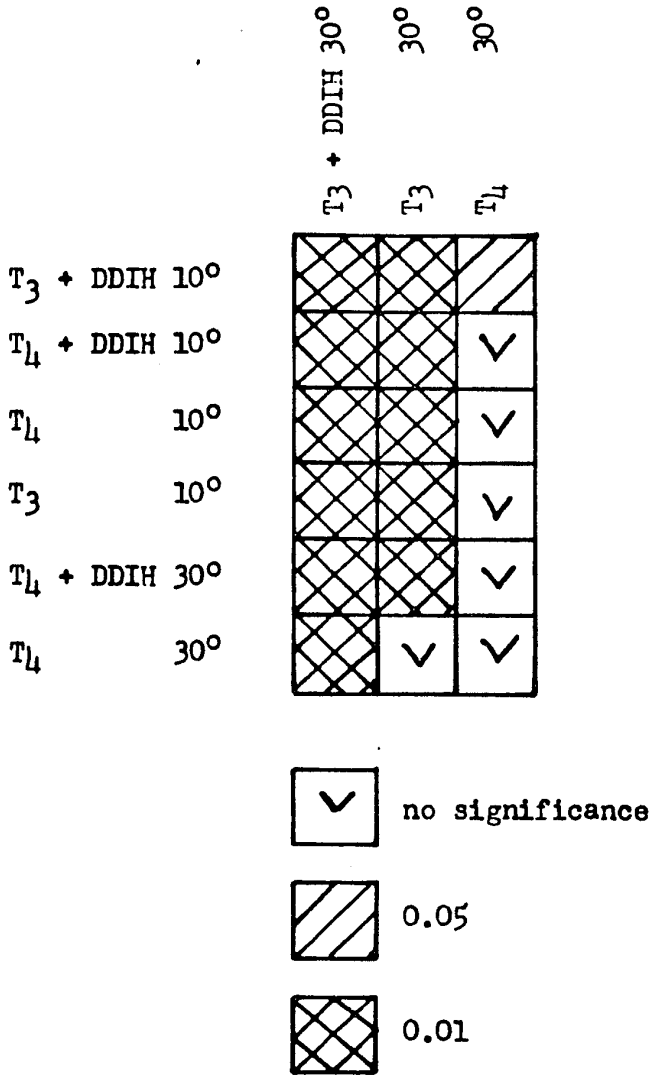


FIGURE 7

A SUMMARY OF THE RESULTS FROM THE DUNCAN'S MULTIPLE RANGE TEST FOR SIGNIFICANCE AMONGST CHANGES IN THE OCRs OF MICE PRETREATED WITH VARIOUS DOSES OF HORMONE ± DDIH

At 30° C, T_4 and T_3 were quantitatively similar in increasing the OCR but with DDIH the T_4 increase was significantly lower than that of T_3 + DDIH.

At 10° C, T_4 ± DDIH and T_3 ± DDIH showed no differences in effectiveness in increasing the OCR.

Summary:

30° C

T_4 Effect of Hormone:

- no dosage effect
- increased OCR by an average of 21.7%

Action of DDIH:

- none

T_3 Effect of Hormone:

- no dosage effect
- increased OCR by an average of 27.4%
- T_3 was significantly more effective in increasing OCR in 30° mice than in 10°C mice

Action of DDIH:

- no inhibitory action
- T_3 + DDIH was significantly more effective than T_4 + DDIH in increasing OCR
- T_3 + DDIH was more effective in 30° mice than in 10° C ones

10^{o} C

T_4 Effect of Hormone:

- no dosage effect
- increased OCR by an average of 16.5%

Action of DDIH:

- none

T_3 Effect of Hormone:

- no dosage effect
- increased OCR by an average of 13.0%

Action of DDIH:

- none

b. Variations in Oxygen Consumption over a yearly Period and the Effect of Duration of Acclimation (Groups and Single Animal Measurements).

Due to the period over which the experiments were conducted, it was possible to check if the oxygen consumption varied with season or with length of time that the animals had been acclimated. As seen in Figure 8 and Table 7, there was no apparent seasonal effect on the oxygen consumption of animals measured in groups or singly.

There were no significant differences in the OCRs of mice acclimated for varying periods, Figure 9 and Table 8. Most of the animals used in the group experiments had been acclimated for less than nine months and for less than twelve in the single animal experiments.

The differences in oxygen consumption values obtained by using the two methods were apparent Figure 9. The values obtained with the groups were lower at both temperatures of acclimation. The values obtained from cold-acclimated mice, measured in groups, approximated those from warm-acclimated mice measured singly. The OCRs of cold-acclimated mice measured in the single animal apparatus were the highest, approximating the extrapolated value obtained using decreasing numbers of mice (Figure 5) and were consistent with previous findings of Hart (1953).

TABLE 7

VARIATION IN OCR OVER A ONE YEAR PERIOD

Month	Oxygen Consumption Rate (ml/g/hr)			
	Groups		Single	
	30°C	10°C	30°C	10°C
Jan.	1.53 (4)" ±0.16'	2.30 (8) ±0.08	—	—
Feb.	1.58 (8) ±0.04	2.56 (6) ±0.02	—	—
Mar.	1.59 (11) ±0.07	2.44 (20) ±0.08	2.75 (4) ±0.24	4.05 (4) ±0.20
Apr.	1.69 (11) ±0.05	2.59 (1)	—	—
May	1.53 (4) ±0.07	2.30 (6) ±0.06	—	—
June	1.44 (21) ±0.04	2.29 (4) ±0.04	—	—
July	1.47 (5) ±0.07	2.34 (15) ±0.07	2.42 (39) ±0.05	4.68 (24) ±0.11
Aug.	1.59 (11) ±0.06	2.49 (7) ±0.07	2.27 (54) ±0.04	4.82 (51) ±0.09
Sept.	1.64 (15) ±0.07	2.31 (10) ±0.08	2.35 (13) ±0.09	4.88 (16) ±0.12
Oct.	—	2.27 (15) ±0.09	2.43 (11) ±0.10	4.13 (12) ±0.14
Nov.	1.59 (21) ±0.04	2.36 (15) ±0.06	2.62 (24) ±0.06	4.52 (31) ±0.07
Dec.	1.44 (9) ±0.05	2.43 (7) ±0.06	2.39 (10) ±0.07	4.86 (7) ±0.11

' mean ± S.E.
" No. of replicates

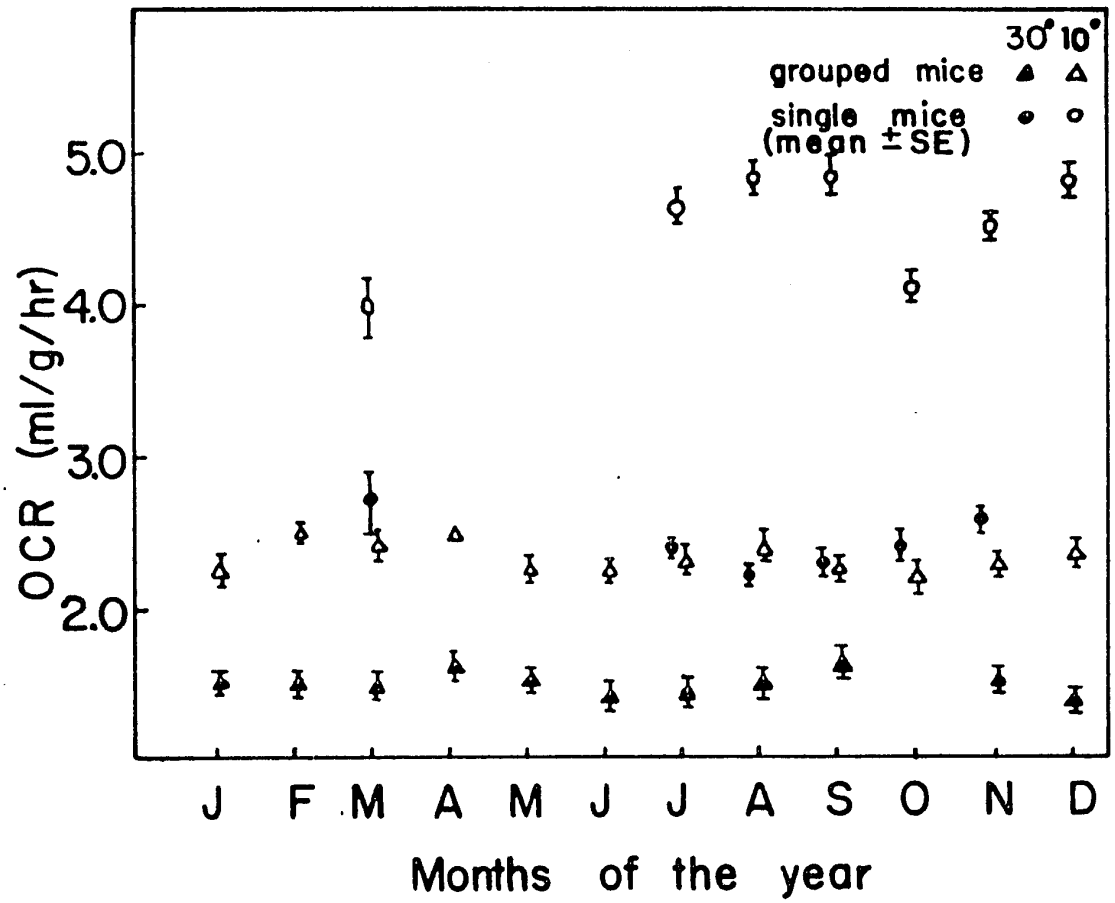


FIGURE 8

SEASONAL VARIATION IN OCR

TABLE 8

THE EFFECT OF THE DURATION OF ACCLIMATION ON OCR

Duration of Acclimation (months)	Oxygen Consumption Rate (ml/g/hr)			
	Groups		Single	
	30°C	10°C	30°C	10°C
1	1.52 (17)" ±0.05'	2.32 (27) ±0.06	2.72 (16) ±0.07	4.58 (23) ±0.08
2	1.61 (14) ±0.04	2.43 (26) ±0.05	2.39 (10) ±0.07	4.84 (6) ±0.13
3	1.59 (18) ±0.08	2.40 (13) ±0.08	1.99 (4) ±0.17	—
4	1.64 (19) ±0.04	2.40 (10) ±0.08	2.49 (27) ±0.05	—
5	1.58 (20) ±0.04	2.29 (18) ±0.06	2.29 (26) ±0.07	4.05 (4) ±0.20
6	1.46 (9) ±0.09	2.42 (12) ±0.06	2.35 (13) ±0.09	4.92 (13) ±0.13
7	1.49 (9) ±0.07	2.32 (3) ±0.14	2.34 (23) ±0.07	5.41 (9) ±0.25
8	1.47 (10) ±0.03	2.23 (5) ±0.16	2.29 (27) ±0.04	4.60 (34) ±0.11
9	—	—	—	4.95 (22) ±0.11
10	1.54 (4) ±0.05	—	—	4.57 (10) ±0.17
11	—	—	—	4.27 (18) ±0.11
12	—	—	2.75 (4) ±0.24	4.29 (6) ±0.21

' mean ± S.E.
" No. of replicates

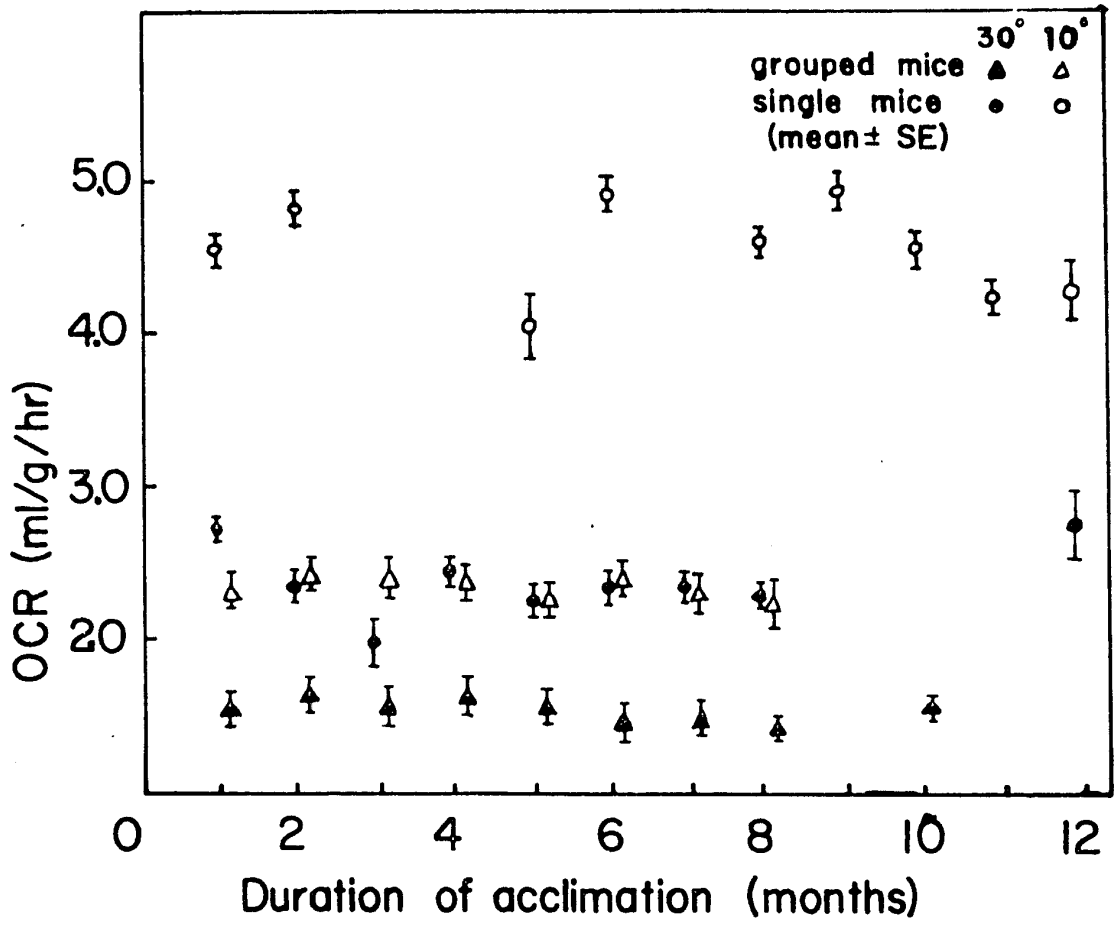


FIGURE 9

THE EFFECT OF THE DURATION OF ACCLIMATION ON OCR

c. The Effect on OCR of the Type of Diet given during Acclimation.

The effect of a low iodine diet (L.I.D.) given during acclimation on OCR is shown in Figure 10.

After one day in the cold room, the OCR of a mouse on L.I.D. was similar to that of one on regular diet. Both OCRs were quantitatively similar to that of a fully acclimated mouse. This was also true in the warm room.

The elevated OCR in the cold room was not simply due to an excess of iodine ingested with greater food consumption since the same OCR was attained on both diets.

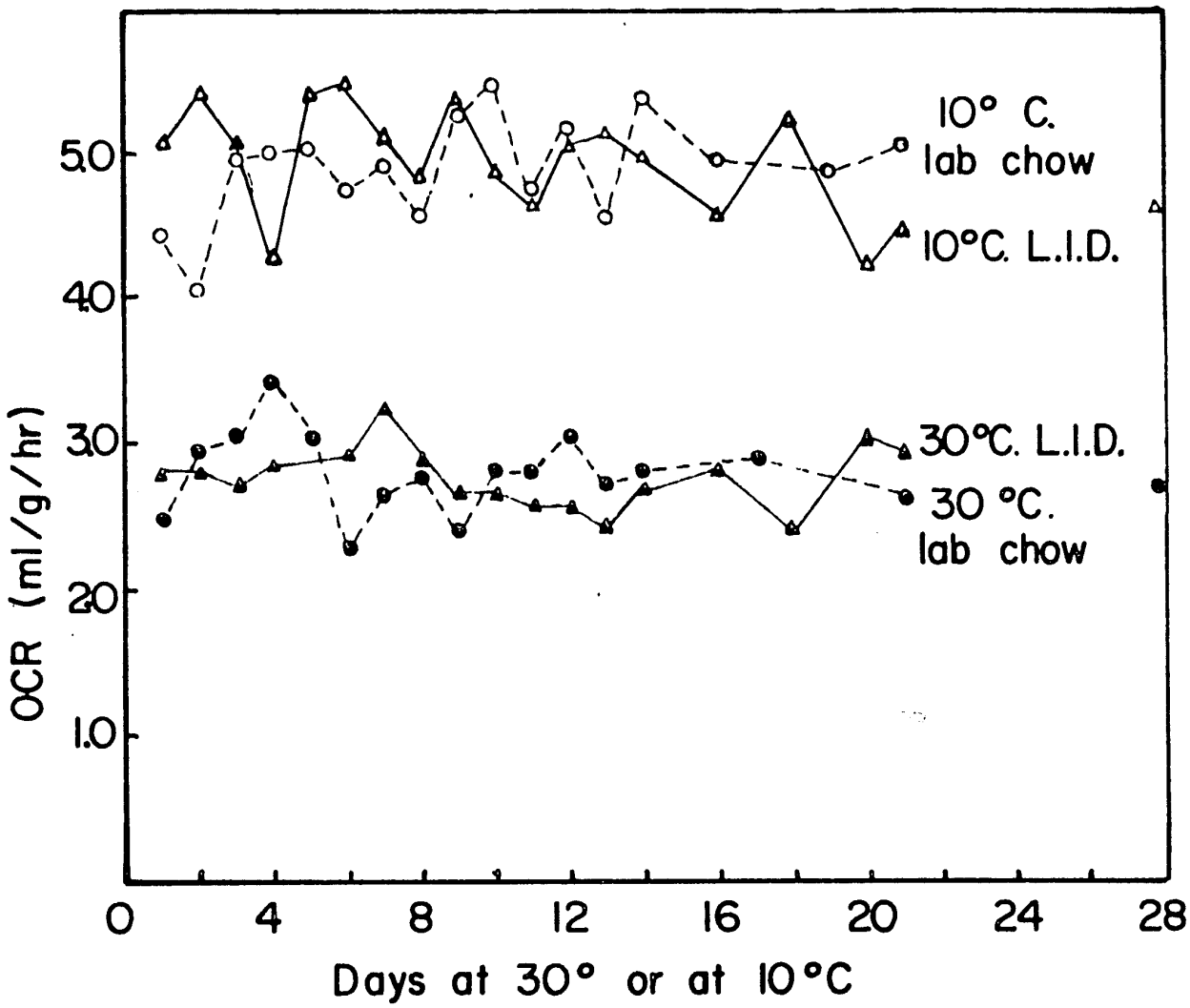


FIGURE 10

THE EFFECT OF A LOW IODINE DIET (L.I.D), GIVEN DURING ACCLIMATION TO 30°C OR 10°C, ON THE OCR

(each point represents the mean value for 2 different animals)

d. Concurrent Study of the Metabolic Effect of ^{131}I labeled Hormones and the Rate of Excretion of the Label

To further understand the correlation between oxygen consumption and the metabolism of either T_4 or T_3, mice were injected according to the protocol previously outlined and were kept in metabolism cages during this period. After forty-eight hours, and immediately prior to the second oxygen consumption measurement, the urine and feces samples were removed, prepared, and the radioactivity determined. The results for both excretion and oxygen consumption are shown in Tables 9 and 10.

Graphs in which the per cent change in OCR was plotted against the per cent ^{131}I excreted were drawn (Figure 11), to reveal any correlation between the amount of ^{131}I excreted and the metabolic response, ie. did a mouse excreting a higher percentage of the dose injected, have a higher OCR? There did not appear to be any relationship.

Further experiments were done in which the OCR was measured at twenty-four hour intervals from zero to ninety-six hours. The results are shown in Table 11 and Figures 12 and 13. The values for the percent excretion of ^{131}I after the administration of T_4 are from experiments done by McLennan*, and of T_3, from those by Champagne*. The significance of the results is summarized in Figure 14.

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* unpublished results from our laboratories

TABLE 9

THE CHANGE IN OCR FOLLOWING AN INJECTION OF T_4 ^{131}I ± DDIH AND THE % ^{131}I EXCRETED WITHIN 48 HOURS

	30°C		10°C	
	% ^{131}I excreted in urine + feces	% change in OCR	% ^{131}I excreted in urine + feces	% change in OCR
T_4* (2.0 mg/kg)	57.9	30.8	99.1	69.8
	52.2	24.0	68.9	22.1
	52.3	33.1	98.4	-5.9
	75.2	8.7	88.4	-12.1
	71.3	18.8	97.7	-17.4
	61.6	11.4	91.8	-17.2
	90.8	9.3	94.7	-15.5
	85.0	15.7	89.9	-14.7
	72.9	13.9		
	68.8	18.4	91.1	1.4
	±4.6[1]	±3.0	±3.5	±10.8
T_4* + DDIH	71.9	2.8	105.8	15.2
	83.3	50.3	98.2	17.6
	61.9	23.6	103.2	12.9
	66.2	21.3	94.5	28.8
	95.2	45.2	91.3	10.1
	90.5	32.7	84.7	-10.0
	73.8	37.3	96.8	15.3
	72.7	37.3	88.8	8.6
	76.9	31.3	95.3	12.3
	±4.1	±5.3	±2.5	±3.9

[1] mean ± S.E.

TABLE 10

THE CHANGE IN OCR FOLLOWING AN INJECTION OF T_3 ^{131}I ± DDIH AND THE % ^{131}I EXCRETED WITHIN 48 HOURS

	30°C		10°C	
	% ^{131}I excreted in urine + feces	% change in OCR	% ^{131}I excreted in urine + feces	% change in OCR
T_3* (1.68 mg/kg)	72.8	4.9	81.7	9.9
	79.9	2.6	76.4	-18.5
	84.2	7.8	83.7	19.1
	68.9	23.3	88.4	-18.4
	51.9	43.9	83.2	15.8
	75.5	14.3	92.1	-13.6
	95.2	30.3	70.4	29.4
			88.1	-7.9
			97.3	2.3
			90.6	13.9
	75.5	18.2	85.2	3.2
	±5.1'	±5.7	±2.5	±5.4
T_3* + DDIH	61.6	8.5	85.1	23.8
	66.6	23.3	75.7	-13.4
	63.9	14.9	84.6	19.8
	57.1	1.2	89.8	-3.3
	33.9	7.5	82.3	7.1
	62.9	33.9	87.6	5.6
	62.3	4.4	83.2	-3.5
	64.4	14.7	93.2	-17.4
	57.7	1.6	84.0	1.6
			82.2	-12.7
	58.6	12.2	84.8	0.3
	±3.3	±3.6	±1.5	±4.4

' mean ± S.E.

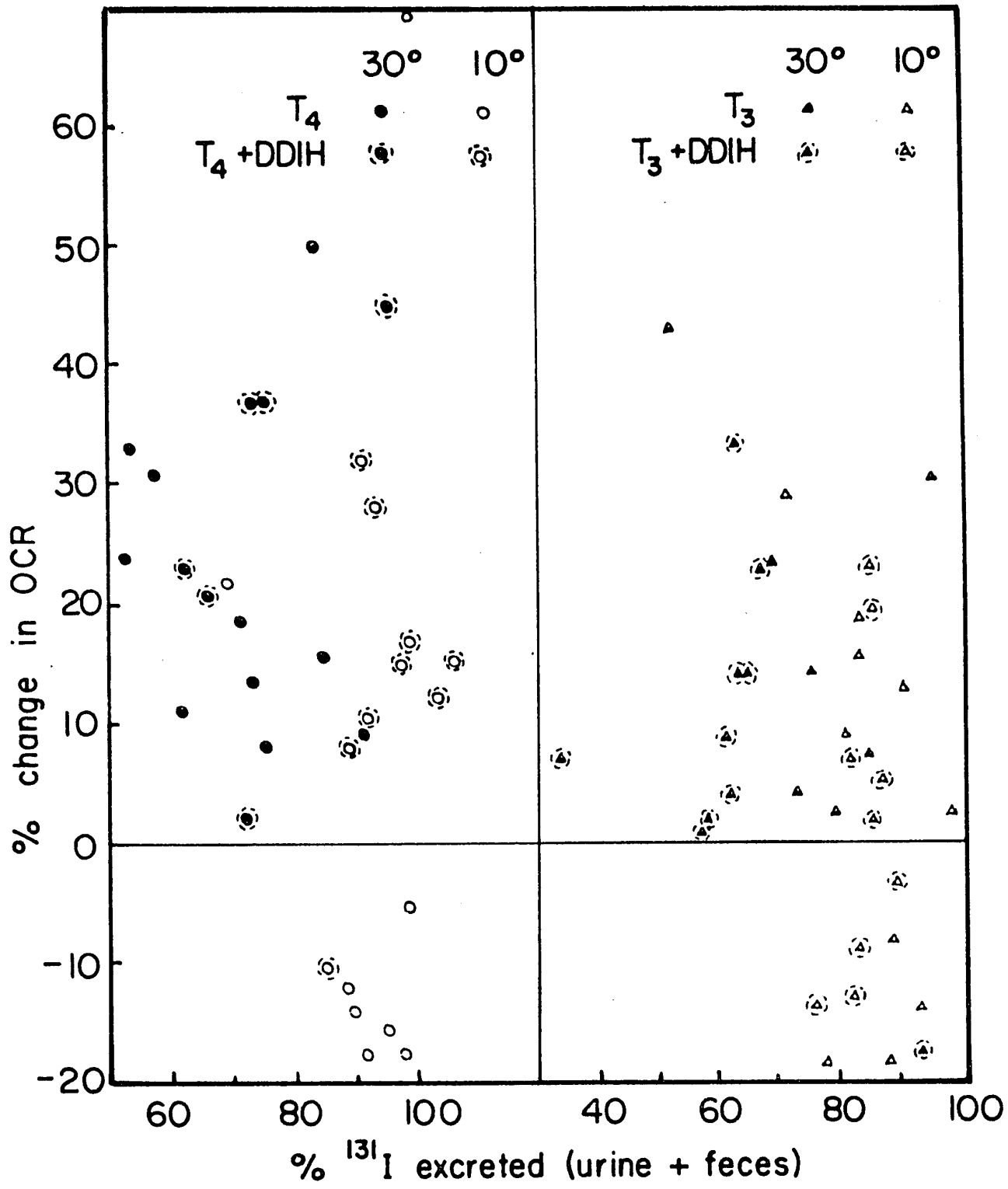


FIGURE 11

% CHANGE IN OCR VS. % ^{131}I EXCRETED IN THE URINE AND FECES OF 30°C- OR 10°C-ACCLIMATED MICE TREATED WITH ^{131}I HORMONE ± DDIH

Due to the following two facts from the data of the concurrent study using T_4: (a) The OCRs of mice injected with labeled hormones and kept in metabolic cages did not differ significantly (Student's t Test) from those of mice obtained using non-labeled hormones measured at forty-eight hours; and (b) their excretion of the dose administered did not differ significantly from the values obtained by McLennan and Champagne measured at forty-eight hours; it was thought feasible to correlate the OCR values obtained using mice injected with non-labeled hormone with the excretion values of different mice injected with labeled hormones. With the exception of the forty-eight hour values where OCR and the excretion values were obtained on the same animals (Tables 9 and 10) all the other OCR and the excretion values obtained after injection of T_4 ± or T_3 ± DDIH were done on different mice.

Cold-acclimated mice excreted approximately eighty-five per cent of the ^{131}I T_4 administered within forty-eight hours and only two per cent more within the next forty-eight hours. DDIH had no effect on the total excretion of ^{131}I.

T_4 ± DDIH had no metabolic effect in cold-acclimated mice at any time during the ninety-six hour experimental period.

When labeled T_3 ± DDIH was injected into cold-acclimated mice, more than ninety per cent of the ^{131}I was excreted within twenty-four hours and, effectively, one hundred per cent within forty-eight hours.

TABLE 11

% CHANGE IN OCR OVER A 96 HOUR PERIOD FOLLOWING INJECTION OF T_4 ± OR T_3 ± DDIH

Pretreatment	% change in OCR			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
30°C				
T_4	15.0 (9)" ±3.6'	18.9 (23) ±2.6	23.1 (15) ±4.0	26.0 (8) ±6.7
T_4 + DDIH	5.8 (3) ±3.2	27.3 (13) ±5.0	28.5 (4) ±9.1	28.7 (5) ±11.6
T_3	9.6 (5) ±3.7	16.6 (12) ±3.6	28.3 (5) ±3.5	24.6 (5) ±3.9
T_3 + DDIH	9.9 (5) ±4.2	15.2 (14) ±3.1	36.5 (5) ±7.9	31.2 (5) ±7.0
10°C				
T_4	6.8 (6) ±1.3	6.9 (7) ±2.5	10.9 (11) ±1.7	9.7 (11) ±1.6
T_4 + DDIH	4.6 (1)	8.7 (4) ±3.0	6.0 (7) ±1.8	5.1 (7) ±1.4
T_3	0.66 (5) ±4.0	0.11 (5) ±2.8	5.2 (5) ±1.4	-0.8 (5) ±1.5
T_3 + DDIH	6.5 (4) ±2.6	6.3 (4) ±2.3	8.0 (4) ±5.7	-1.1 (4) ±5.8

' mean ± S.E.

" No. of replicates

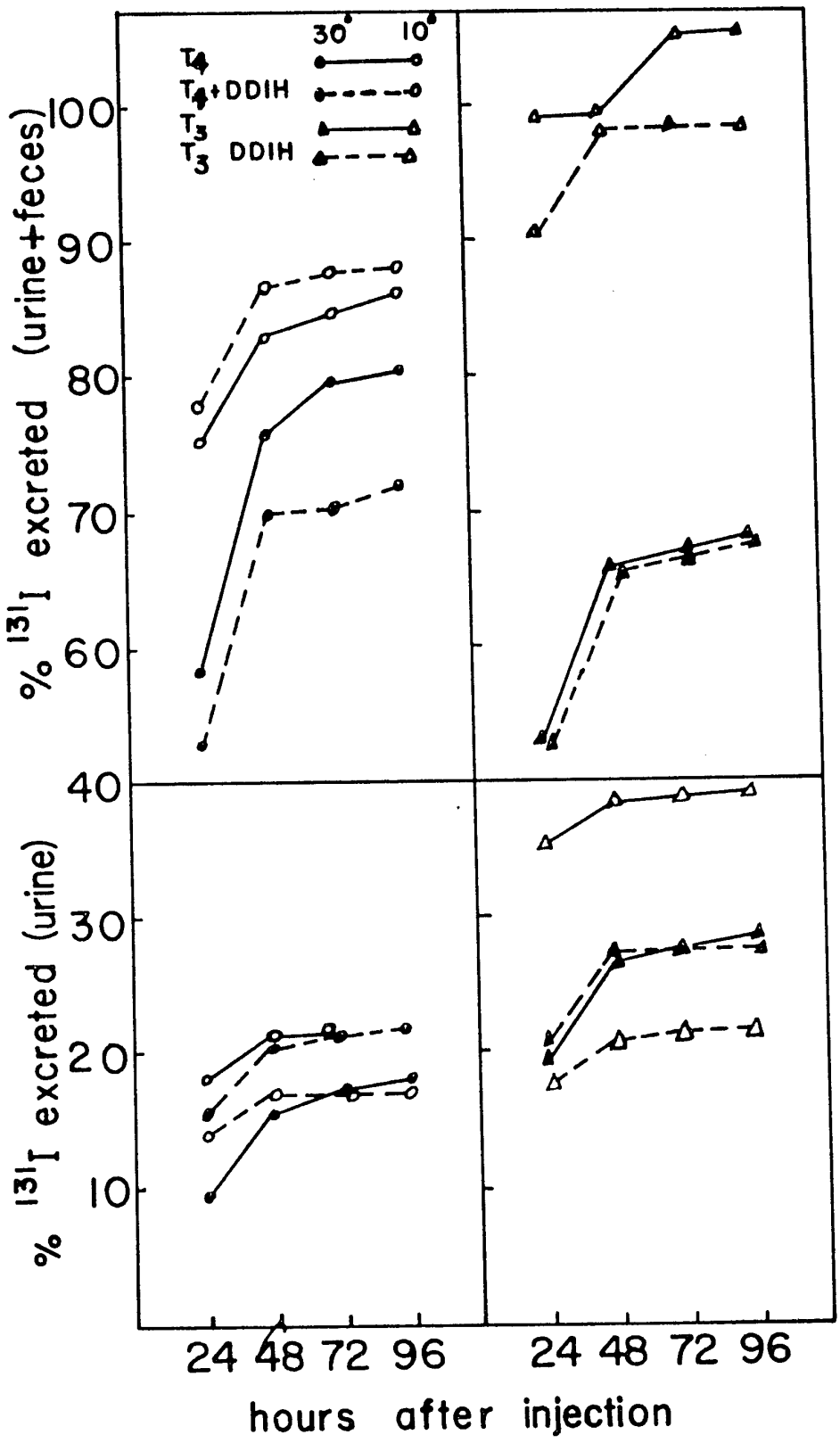


FIGURE 12

% ^{131}I EXCRETED AFTER TREATMENT WITH HORMONE ± DDIH

(From McLennan and Champagne)

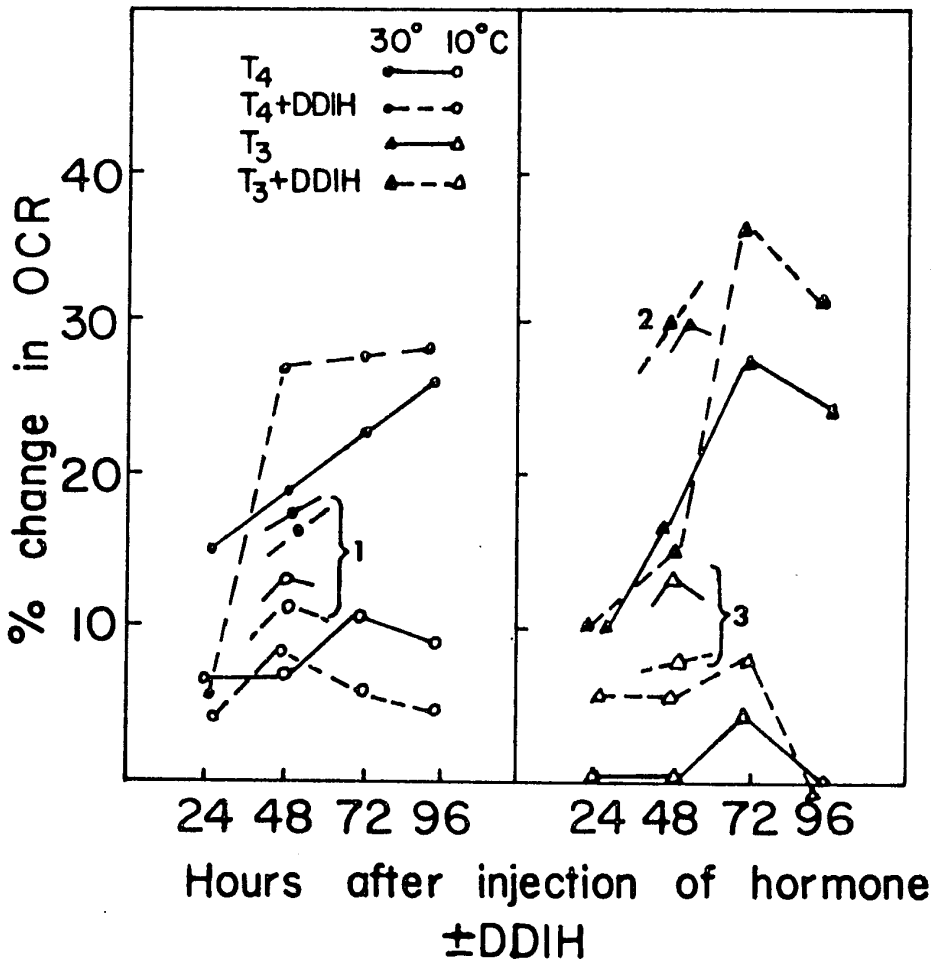


FIGURE 13

% CHANGE IN OCR OVER A 96 HOUR PERIOD AFTER TREATMENT WITH HORMONE ± DDIH. THE SEPARATE POINTS 1, 2 AND 3, REPRESENT THE OCR VALUES MEASURED IN ANIMALS KEPT IN NORMAL CAGE CONDITIONS AND INJECTED WITH NON-LABELED HORMONE

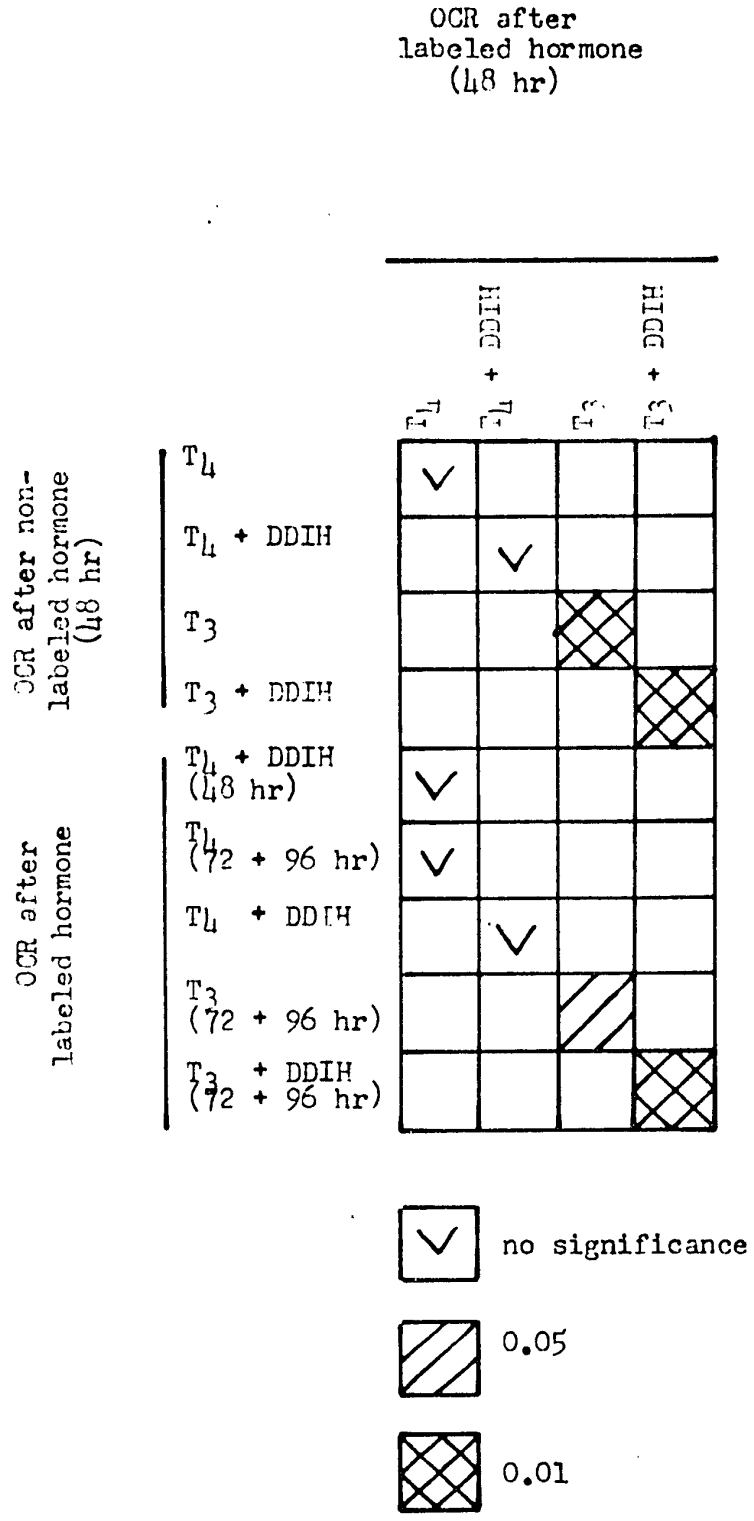


FIGURE 14

A SUMMARY OF THE RESULTS OF THE t TEST FOR SIGNIFICANCE BETWEEN OCR VALUES OF WARM-ACCLIMATED MICE INJECTED WITH LABELED OR NON-LABELED HORMONE

T_3 ± DDIH had no significant metabolic effect in cold-acclimated mice.

When warm-acclimated animals were tested, the excretion of ^{131}I was significantly lower than in cold-acclimated mice following injection of either of the labeled hormones ± DDIH. The excretion of ^{131}I was most rapid within the first twenty-four hours (approximately sixty per cent) and increased only slightly over the rest of the experimental period. DDIH again had no effect on the rate of excretion of ^{131}I.

Both T_4 and T_3 had an effect on the OCRs of warm-acclimated mice.

Although the OCR continued to increase up to ninety-six hours after injection of T_4, a Student's t Test showed that the pooled seventy-two and ninety-six hour OCRs were not significantly different from the forty-eight hour ones. DDIH had no effect at any time on the OCR.

When warm-acclimated mice were injected with ^{131}I-labeled T_3 ± DDIH and kept in metabolism cages, the per cent change in OCR after forty-eight hours was significantly lower (t Test) than that of mice injected with non-labeled hormone and kept in regular cages (Table 6). The seventy-two and ninety-six hour OCR values were obtained from mice kept in normal cage conditions. Thus the pooled seventy-two and ninety-six hour values were tested against the forty-eight hour values obtained from mice injected with non-labeled hormone

(30.6% after T_3 and 30.4% after T_3 + DDIH, Table 6). There were no significant differences. The discrepancy between the forty-eight hour OCR values obtained in warm-acclimated mice receiving labeled and non-labeled T_3 indicated that the caging did have an effect on the OCR. The values for cold-acclimated mice at forty-eight hours were lower than had been previously obtained. The OCR values obtained under normal conditions were also shown for comparison sake. When T_4 was used there seemed to be better agreement between results particularly in warm-acclimated mice.

The excretion values from these mice were compared to the ones obtained in experiments done by McLennan (using T_4) and Champagne (using T_3). There was good agreement with T_4 results and slightly different values after T_3 in cold-acclimated mice (the total excretion was lower and DDIH did not lower the amount of ^{131}I in the urine).

Caution should be exercised in interpreting particularly the T_3 results, and it is felt that chronic administration of ^{125}I in the drinking water and collection of urine and feces prior to administration of ^{131}I would have given more reliable results. This double isotope method has been discussed by Anbar et al. (1965 a).

e. The Effect of a Temperature Change on the Oxygen Consumption Rate.

The results are shown in Figure 15.

When cold-acclimated mice were put at 30°C their OCR dropped immediately. After a period of four hours the OCR was lower than the baseline representing the average OCR value of warm-acclimated mice. However after twenty-four hours at 30°C, the OCR of the cold-acclimated mice was similar to that of warm-acclimated ones.

When warm-acclimated mice were exposed to 10°C their OCRs rose immediately and within ninety minutes were similar to those of cold-acclimated mice.

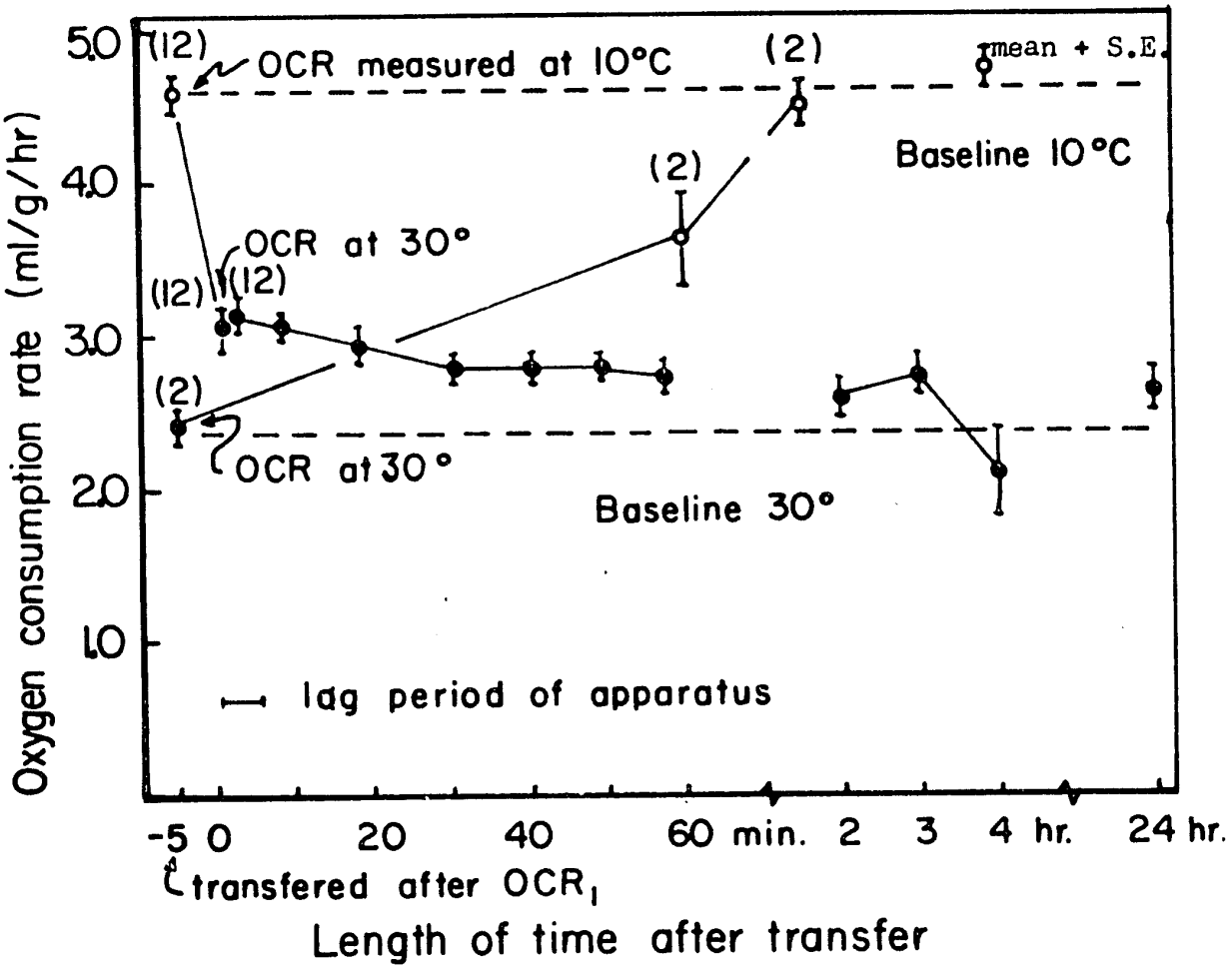


FIGURE 15

OCR OF COLD- AND WARM-ACCLIMATED MICE BEFORE AND AFTER TRANSFERRAL TO 30° AND 10°C

(No. = 5 unless specified otherwise)

f. Noradrenaline Test in Normal and Thyroidectomized Mice

This experiment was divided into two main sections. In thefirst section, warm- and cold-acclimated mice were tested with various dosages of NA to determine their sensitivity if any. In the second section, warm- and cold-acclimated mice were given a variety of pretreatments; T_4±DDIH, T_3±DDIH, or DDIH alone, and then tested with 0.2 or 0.4 mg/kg NA to reveal any alteration in the sensitivity due to pretreatment. This experiment was repeated in thyroidectomized mice.

Composite summaries are presented, Figure 18 pertaining to normal mice, Figure 19 to thyroidectomized ones.

(1) Normal Mice

(a) The Sensitivity of Untreated Warm- and Cold-acclimated Mice to Noradrenaline.

(i) Sensitivity to o.2 mg/kg NA

When o.2 NA was injected into warm-and cold-acclimated mice, small(less than 10%) increases in OCR were observed (Figure 17). To ascertain whether these small increases were significant it was necessary to compare the actual oxygen consumption value expressed in ml/g/hr, the untreated mice having no per cent change, rather than comparing the per cent change as was done in other instances.

In Table 12 the actual OCR values are represented. The value given for untreated mice is after OCR_1, the value given for the NA treated animals is after OCR_2. Although the actual OCR value from

NA treated warm-acclimated animals is lower than that of untreated animals, the 2.22 ml/g/hr reading was 7.3% higher than the OCR obtained from these treated mice.

When student's t tests were done on the actual OCR values before and after NA treatment there were no significant differences indicating that neither warm- nor cold-acclimated mice were sensitive to 0.2 NA.

After the injection of the other dosages of NA, any value significantly greater than that observed after 0.2 NA indicated sensitivity.

Statistical Analysis

The increases in OCR's of untreated mice tested with NA and discussed in the following sections (ii) and (iii) were analyzed by means of a nested analysis of variance, and a Duncan's Multiple Range Test was applied. The results are given in Table 13 and Figure 16.

(ii) The Effect of Increasing Dosages of NA

Three dosages of NA, 0.2, 0.4, 0.6 mg/kg, were tested in normal mice. the results are shown in Figure 17.

Warm-acclimated mice were insensitive to both 0.2 NA (section(i) above) and to 0.4 NA(Figure 17, andl, Figure 18).

These animals were sensitive to 0.6 NA and were visibly stressed as evidenced by increased respiratory rate, excessive salivation and exophthalmia.

Cold-acclimated mice were insensitive to 0.2 NA(section (i)above). They were sensitive to 0.4 NA(Figure 17 and 2, Figure 18).

TABLE 12

THE AVERAGE OCR (ml/g/hr) OF NORMAL MICE BEFORE AND AFTER TREATMENT WITH 0.2 mg/kg NA

	average OCR (ml/g/hr)	
	30°C	10°C
Untreated	2.36 (40)" ±0.06'	4.62 (40) ±0.10
NA (0.2 mg/kg)	2.22 (10) ±0.10	4.75 (10) ±0.20

' mean ± S.E.

" No. of replicates

There were no significant differences between OCRs of untreated and treated mice at the same temperature (Students t Test).

TABLE 13

A SUMMARY OF THE NESTED ANALYSIS OF VARIANCE APPLIED TO THE INCREASES IN OCRs OF UNTREATED MICE ACCLIMATED TO 10° AND 30°C AND TESTED WITH 0.2, 0.4, AND 0.6 NA.

Sources of Variation	df	Sum of Squares	Mean Square	F
Temp.	1	479.02	479.02	0.258 NS
Conc. Temp.	4	7,414.89	1853.72	14.48**
Error	33	4,225.84	128.05	
Total	38	12,119.75		

** is significant at 0.01

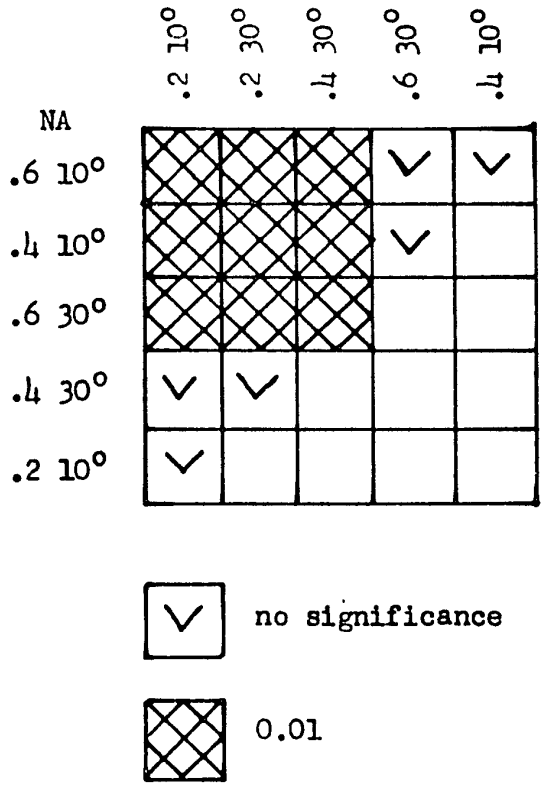


FIGURE 16

A SUMMARY OF THE RESULTS FROM THE DUNCAN'S MULTIPLE RANGE TEST FOR SIGNIFICANCE BETWEEN INCREASES IN OCRs FOLLOWING INJECTION OF INCREASING DOSES OF NA

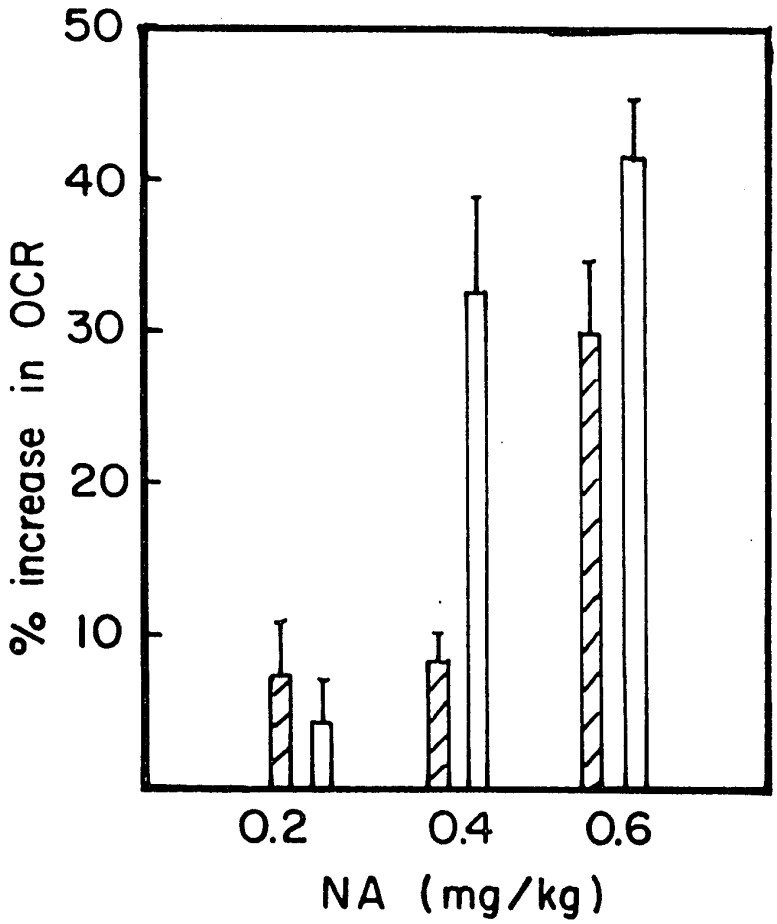


FIGURE 17

RESPONSE TO VARYING DOSES OF NORADRENALINE BY WARM-ACCLIMATED MICE (HATCHED BAR) AND COLD-ACCLIMATED MICE (OPEN BAR). THE MEAN ± S.E. IS SHOWN

Cold-acclimated mice were also extermely sensitive to 0.6NA and showed similar signs of stress as had the warm-acclimated mice.

(iii) The Effect of Temperature

The temperature of acclimation affected the sensitivity to NA. The response of warm-acclimated mice to 0.2 NA was not significantly different from that of cold-acclimated, similarly tested, ones (3, Figure 18). The increase in OCR of cold-acclimated mice 0.4 NA was significantly greater than that in warm-acclimated mice(4,Figure 18). At 0.6 NA the response in warm-acclimated mice was not significantly different from that of cold ones.

In summary, warm- and cold-acclimated mice showed no sensitivity to 0.2 NA, marked sensitivity to 0.6 NA, and only cold-acclimated mice were sensitive to 0.4 NA.

(b) The Effect of Pretreatment on the Sensitivity of Normal Warm- and Cold-acclimated Mice to Noradrenaline.

Warm- and cold-acclimated mice were pretreated with T_4, T_3,DDIH, and a combination of hormone and DDIH; they were then tested with 0.2 and 0.4 NA to determine whether pretreatment had altered their sensitivity to NA. The results are given in Tables 16, 17,18,and 19.

Readings had been taken at ten and thirty minutes after the injection of NA. However, the nested analysis of variance revealed no significance between the values at these two times. Since the ten minute readings were extremely variable, they were eliminated, reducing the levels of nesting. The analysis was redone on just the thirty minute values(Table 14). A Duncan's Multiple Range Test was

TABLE 14

A SUMMARY OF THE NESTED ANALYSIS OF VARIANCE APPLIED TO THE INCREASES IN OCRs OF NORMAL MICE ACCLIMATED TO 10° AND 30°C AND TESTED WITH 0.2 AND 0.4 NA.

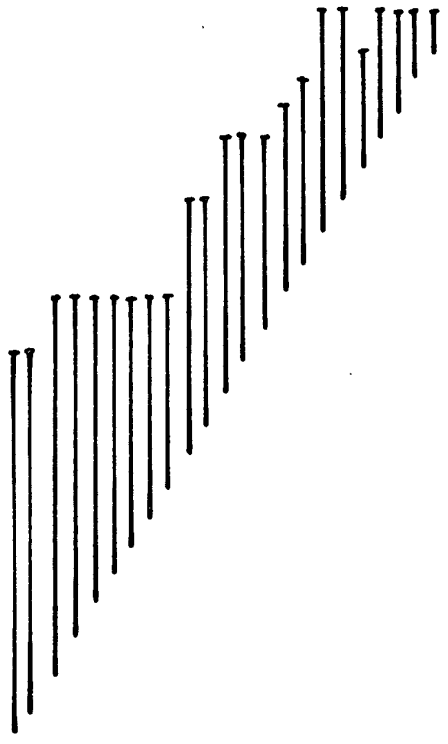
Sources of Variation	df	Sum of Squares	Mean Squares	F
Conc.	1	5,284.938	5284.938	16.304 NS
Temp. Conc.	2	648.312	324.156	0.124 NS
Trt. Temp. Conc.	20	52,393.625	2619.681	7.893 **
Error	138	45,803.125	331.906	
Total	161	104,130.000		

** is significant at 0.01

TABLE 15

A SUMMARY OF RESULTS FROM DUNCAN'S MULTIPLE RANGE TEST FOR SIGNIFICANCE BETWEEN INCREASES IN OCRs OF UNTREATED AND PRE-TREATED MICE FOLLOWING NORADRENALINE TEST.

Pre-treatment	NA mg/kg	Temp. °C	Repl.	Mean Value
DDIH	0.2	10°	3	-7.900
none	0.2	10°	10	4.111
none	0.2	30°	10	7.248
none	0.4	30°	4	8.545
DDIH	0.4	30°	5	16.218
T_4 + DDIH	0.2	10°	10	19.500
DDIH	0.2	30°	3	19.887
DDIH	0.4	10°	4	20.820
T_4 + DDIH	0.2	30°	9	24.379
none	0.4	10°	5	32.524
T_4	0.2	30°	10	34.673
T_4 + DDIH	0.4	10°	5	39.988
T_3	0.4	10°	5	41.194
T_3	0.2	10°	10	42.374
T_4	0.2	10°	10	43.489
T_3	0.2	30°	10	47.927
T_3 + DDIH	0.2	10°	10	50.543
T_3 + DDIH	0.4	10°	5	52.788
T_4	0.4	10°	5	52.832
T_3 + DDIH	0.2	30°	9	55.997
T_4 + DDIH	0.4	30°	5	56.476
T_4	0.4	30°	5	59.582
T_3	0.4	30°	5	62.396
T_3 + DDIH	0.4	30°	5	64.082



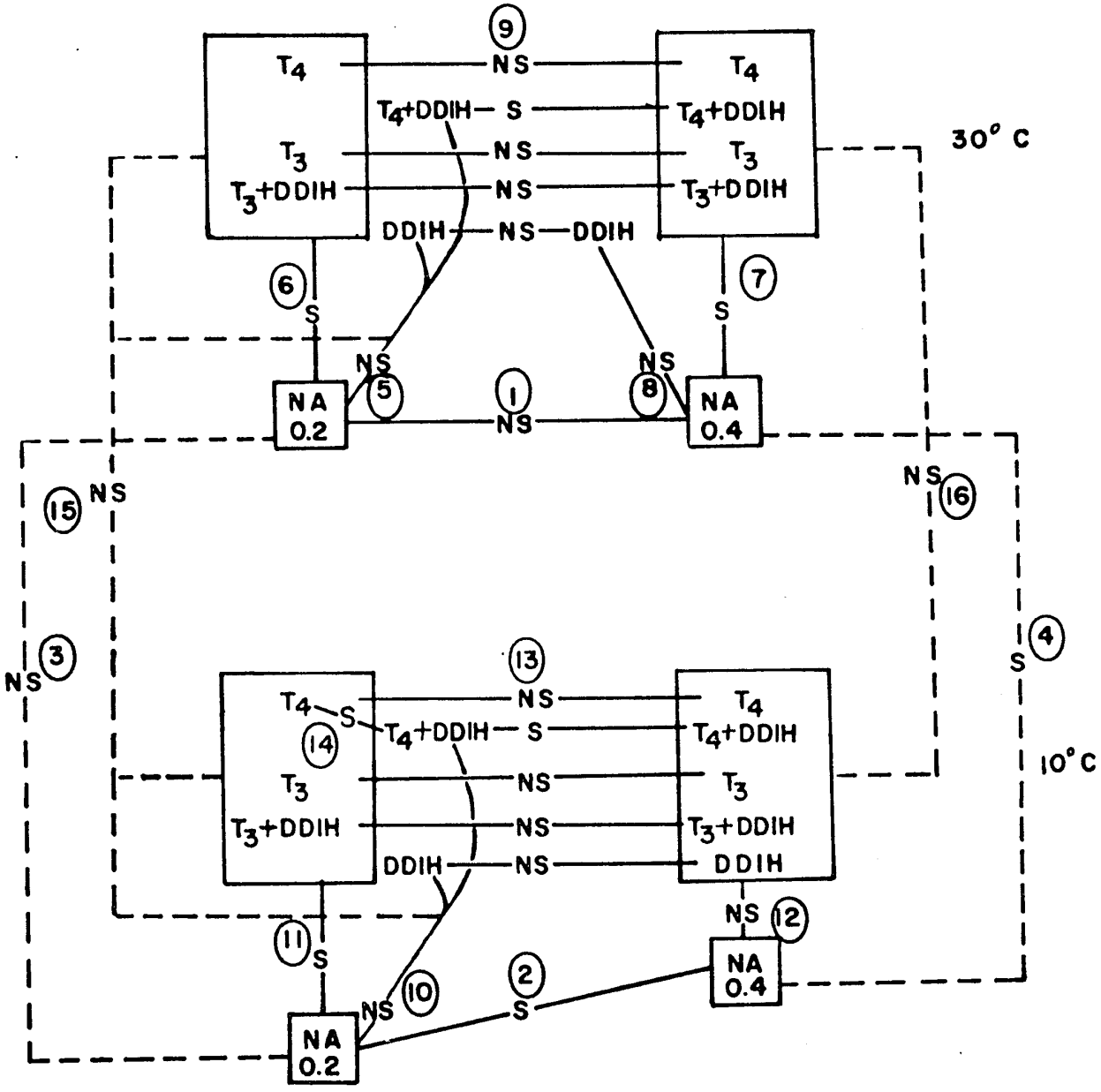


FIGURE 18

SUMMARY OF THE NORADRENALINE TEST IN NORMAL MICE

(NS = not significant, S = significant)

TABLE 16

NORADRENALINE TEST IN MICE ACCLIMATED TO 30°C

(NA = 0.2 mg/kg)

Pretreatment	% change in OCR_2	% change in OCR_3 30 min after 0.2 mg/kg NA	OCR_3-OCR_2 (change due to NA)
none		7.3 (10)" ±3.7'	7.3
DDIH	15.7 (4) ±5.6	19.9 (3) ±4.2	4.2
T_4 (2 mg/kg)	16.3 (10) ±3.2	34.7 (10) ±4.5	18.4
T_4 + DDIH	14.0 (10) ±2.8	24.4 (9) ±4.3	10.4
T_3 (1.68 mg/kg)	30.8 (10) ±3.9	47.9 (10) ±5.2	17.1
T_3 + DDIH	25.1 (10) ±5.8	56.0 (9) ±6.0	30.9

' mean ± S.E.

" No. of replicates

TABLE 17

NORADRENALINE TEST IN MICE ACCLIMATED TO 30°C

(NA = 0.4 mg/kg)

Pretreatment	% change in OCR_2	% change in OCR_3 30 min after 0.4 mg/kg NA	OCR_3-OCR_2 (change due to NA)
none		8.5 (4)" ±2.0'	8.5
DDIH	-1.5 (5) ±4.1	16.2 (5) ±6.9	17.7
T_4 (2 mg/kg)	20.4 (5) ±12.7	59.6 (5) ±10.0	39.2
T_4 + DDIH	10.4 (5) ±4.6	56.5 (5) ±8.3	46.1
T_3 (1.68 mg/kg)	25.2 (5) ±4.7	62.4 (5) ±6.7	37.2
T_3 + DDIH	26.9 (5) ±6.8	64.1 (5) ±5.4	37.2

' mean ± S.E.

" No. of replicates

applied to the means and the significance of results is shown in Table 15.

The main questions raised relative to this section were (i) the effect of pretreatment on the sensitivity to NA; (ii) the effect of DDIH on the sensitivity of hormonally pretreated animals; and (iii) the effect of the temperature of acclimation on the sensitivity to NA in these pretreated mice.

(i) The Effects of Pretreatment

Warm-acclimated mice: The results are given in Tables 16 and 17, and their significances summarized in Figure 18.

The increases in OCRs of warm-acclimated mice, pretreated with T_4- DDIH and DDIH tested with 0.2 NA (Table 16) were not significantly different from the increases observed in untreated mice given 0.2 NA (5, Figure 18), but pretreatment with T_4, T_3, and T_3-DDIH did increase their sensitivity to 0.2 NA (6, Figure 18).

All pretreatments (7, Figure 18), with the exception of DDIH (8, Figure 18), increased the sensitivity of warm-acclimated mice to 0.4 NA; therefore thyroid hormones increased the sensitivity to NA without any effect of DDIH.

Generally the increase in OCR after pretreatment plus 0.2 NA did not differ significantly from the increase after the same pretreatment plus 0.4 NA (9, Figure 18). T_4-DDIH was the only pretreatment that showed an effect relative to NA dosage. The response was significantly lower when warm-acclimated mice were tested with 0.2 NA.

Cold-acclimated mice: The results are given in Tables 18 and 19 and their significances summarized in Figure 18.

Cold-acclimated mice, pretreated with T_4-DDIH or DDIH did not

TABLE 18

NORADRENALINE TEST IN MICE ACCLIMATED TO 10°C

(NA = 0.2 mg/kg)

Pretreatment	% change in OCR_2	% change in OCR_3 30 min after 0.2 mg/kg NA	OCR_3-OCR_2 (change due to NA)
None		4.1" ±3.8'	4.1
DDIH	6.6* ±2.1	-7.9* ±2.1	-14.5
T_4 (2 mg/kg)	22.7 ±7.0	43.5 ±11.9	20.2
T_4 + DDIH	10.3 ±4.0	19.5 ±5.3	9.2
T_3 (1.68 mg/kg)	19.0 ±5.0	42.4 ±7.4	23.4
T_3 + DDIH	17.6 ±2.3	50.5 ±5.7	32.9

' mean ± S.E.

" No. of replicates = 10, except * where No. = 3

TABLE 19

NORADRENALINE TEST IN MICE ACCLIMATED TO 10°C

(NA = 0.4 mg/kg)

Pretreatment	% change in OCR_2	% change in OCR_3 30 min after 0.4 mg/kg NA	OCR_3-OCR_2 (change due to NA)
None		32.5" ±6.4'	32.5
DDIH	1.3 ±4.6	20.8* ±1.2	19.5
T_4 (2 mg/kg)	10.8 ±5.1	52.8 ±8.8	42.0
T_4 + DDIH	-1.6 ±2.7	40.0 ±7.1	41.6
T_3 (1.68 mg/kg)	0.5 ±4.2	41.2 ±7.6	40.7
T_3 + DDIH	2.2 ±4.5	52.8 ±8.9	50.6

' mean ± S.E.

" No. of replicates = 5, except * where No. = 4.

show any sensitivity to 0.2 NA(Table 18 and 10,Figure 18).Pretreatment with T_4, T_3, or T_3-DDIH caused them to become sensitive(11, Figure 18).

None of the pretreatments modified the greater sensitivity of cold-acclimated mice to 0.4 NA (Table 19, and 12, Figure 18).

The OCRs of pretreated cold-acclimated mice were not significantly different after 0.2 or 0.4 NA(13,Figure 18), with one exception: T_4-DDIH pretreatment decreased the sensitivity to 0.2 NA.

In cold-acclimated mice as in warm-acclimated ones, differences in pretreatment could only be observed at the lower NA dosage.

(ii) The Effect of DDIH:

The only significant effect of DDIH on the sensitivity of hormonally pretreated mice was to inhibit the response of cold-acclimated mice pretreated with T_4 and tested with 0.2 NA(14,Figure 18). However,DDIH lowered the calorigenic effect of T_4 in warm-acclimated mice when tested with 0.2 NA. It also slightly enhanced T_3 effect.

(iii) The Effect of Temperature:

When 0.2 NA was injected into pretreated warm-acclimated mice, the increases in OCR were similar to the increases in OCR of cold-acclimated pretreated mice(15,Figure 18). The same was true when 0.4 NA was injected (16,Figure18).

(c) Comment on the Statistical Analysis.

The significance of the temperature effect at the 0.4 NA level in untreated mice was taken from a nested analysis of

variance and Duncan's Test applied to the values obtained specifically in section (a) Figure 16 and summarized in Figure 18. However, when a nested analysis of variance and Duncan's Test were applied to the results of section (b), Tables 16,17,18 and 19) which included the results of section (a), this effect of temperature at 0.4 NA was not significant. The variance in the second experiment may have been sufficiently large to mask the significance apparent in the analysis of the first experiment.

It seemed reasonable to accept the significance of the analysis of the first experiment, i.e. temperature did have an effect when 0.4 NA was injected into untreated animals (4, Figure 18), since this experiment was specifically designed to test the effect of dosage and of temperature in untreated mice.

(d) Summary

Sensitivity of Untreated Mice to NA

Warm- and cold-acclimated mice

-showed no sensitivity to 0.2 NA,
marked sensitivity to 0.6 NA, and
only cold-acclimated mice were
sensitive to 0.4 NA.

Sensitivity of Pretreated Mice to NA

Warm-acclimated mice

-hormonal pretreatment increased the sensitivity to 0.2 and 0.4 NA.

-DDI[II] significantly lowered the effect of T_4 when 0.2 mg/kg was used in the NA test and enhanced, although not significantly, the effect of T_3 on the NA test at the same dosage level.

Cold-acclimated mice

-hormonal pretreatment increased the sensitivity to 0.2 NA and had no effect on the greater sensitivity to 0.4 NA.

-DDI[II] significantly lowered the response of T_4 when 0.2 was used and slightly enhanced, although not significantly, the effect of T_3 on the NA test at the same dosage level.

-in cold- and warm-acclimated mice differences in pretreatment could only be observed at the lower NA dosage.

(2) Thyroidectomized Mice

(a) The Effect of Thyroidectomy on OCR

The OCRs of normal and Tx mice acclimated to 30°C and 10°C are given in Table 20. Student's t tests revealed no significance, thus thyroidectomy had no effect on the OCR of these untreated mice.

(b) The Sensitivity of Untreated Warm- and Cold-acclimated Thyroidectomized Mice to Noradrenaline.

(i) Sensitivity to 0.2 mg/kg NA.

When 0.2 NA was injected into warm- and cold-acclimated Tx mice, small increases in OCRs were observed(Figure 20). To ascertain whether these small increases were significant, it was necessary to compare the actual oxygen consumption value expressed in ml/g/hr, the untreated Tx mice having no per cent change in OCR, rather than comparing the per cent change as was done in other instances.

In Table 20 the actual OCR values are represented. The value given for the untreated Tx mice is that obtained after OCR_1; the value given for NA treated Tx mice is after OCR_2. Although the actual values obtained from NA treated mice are lower than those of the untreated controls, they represent an increase in OCR above their base.

Student's t tests done on the OCR values expressed in ml/g/hr before and after NA treatment revealed no significant differences, indicating that neither warm- nor cold-acclimated Tx mice were sensitive to 0.2 NA.

Statistical Analysis

The increases in OCRs of untreated and pretreated mice discussed in the following sections (ii), (iii), and (c) were analyzed by

TABLE 20

THE AVERAGE OXYGEN CONSUMPTION RATE OF UNTREATED, NORMAL AND THYROIDECTOMIZED MICE ACCLIMATED TO 30° AND 10°C

	average OCR (ml/g/hr)	
	30°C	10°C
Normal Mice	2.36 (40)" ±0.06'	4.62 (40) ±0.10
Thyroidectomized Mice	2.56 (24) ±0.14	4.30 (37) ±0.10

' mean ± S. E.

" No. of replicates

TABLE 21

THE AVERAGE OCR (ml/g/hr) OF THYROIDECTOMIZED MICE BEFORE AND AFTER THE ADMINISTRATION OF 0.2 mg/kg NA

	average OCR (ml/g/hr)	
	20°C	10°C
Untreated	2.56 (24)"	4.80 (37)
	±0.14'	±0.10
NA (0.2 mg/kg)	2.55 (8)	4.74 (8)
	±0.09	±0.18

' mean ± S.E.

" No. of replicates

TABLE 22

A SUMMARY OF THE NESTED ANALYSIS OF VARIANCE APPLIED TO THE INCREASES IN OCRs OF THYROIDECTOMIZED MICE ACCLIMATED TO 10° AND 30°C AND TESTED WITH 0.2 AND 0.4 NA.

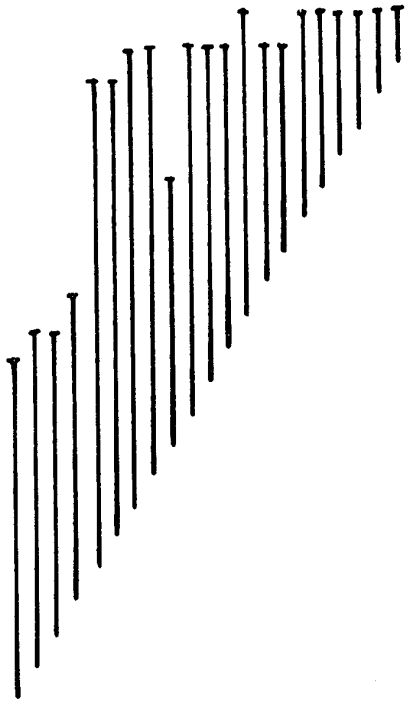
Sources of Variation	df	Sum of Squares	Mean Squares	F
Conc.	1	4,799.805	4799.805	2.917
Temp. Conc.	2	3,291.316	1645.658	2.022
Trt. Temp. Conc.	18	14,648.430	813.802	3.030 **
Error	49	13,158.566	268.542	
Total	70	35,898.117		

** significant at 0.01

TABLE 23

A SUMMARY OF RESULTS FROM DUNCAN'S MULTIPLE RANGE TEST FOR SIGNIFICANCE BETWEEN INCREASES IN OCRs OF UNTREATED AND PRE-TREATED THYROIDECTOMIZED MICE.

Pre-treatment	NA mg/kg	Temp. °C	Repl.	Mean Value
T_4 + DDIH	0.2	10°	4	-11.282
none	0.4	30°	2	-3.895
T_3	0.2	30°	2	2.995
T_4	0.2	10°	4	3.457
none	0.2	10°	9	5.474
none	0.4	10°	2	6.785
DDIH	0.4	30°	2	7.305
T_4 + DDIH	0.2	30°	5	15.000
none	0.2	30°	7	15.571
T_3 + DDIH	0.2	10°	3	17.180
T_3	0.2	10°	3	19.103
DDIH	0.4	10°	2	23.360
T_3 + DDIH	0.4	10°	3	29.427
T_4	0.2	30°	7	31.401
T_3	0.4	30°	2	32.670
T_3	0.4	10°	2	34.960
T_4 + DDIH	0.4	30°	2	37.310
T_4 + DDIH	0.4	10°	2	41.960
T_3 + DDIH	0.4	30°	2	51.870
T_3 + DDIH	0.2	30°	2	52.975
T_4	0.4	10°	2	53.620
T_4	0.4	30°	2	59.705



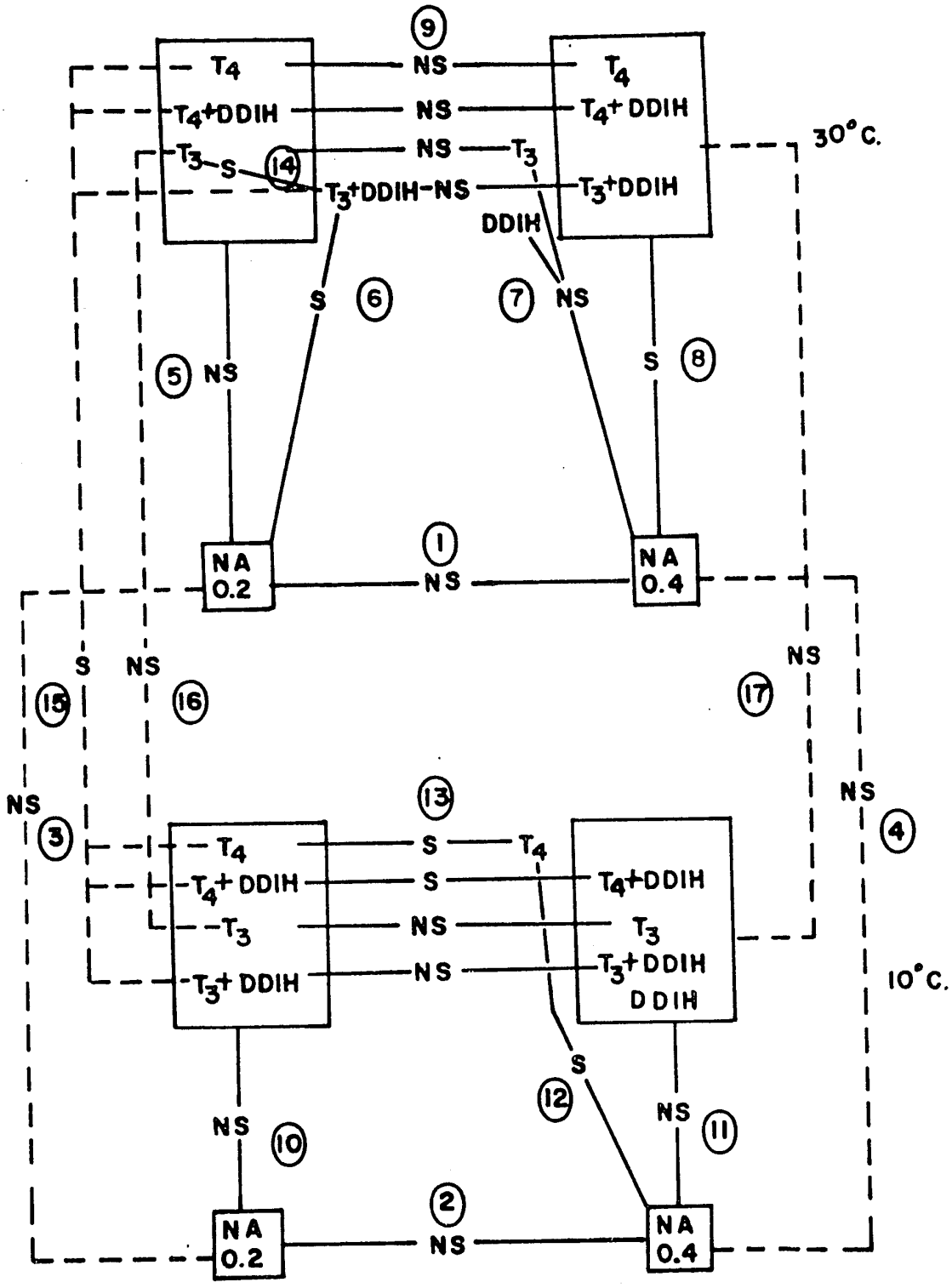


FIGURE 19

SUMMARY OF THE NORADRENALINE TEST IN THYROIDECTOMIZED MICE

(NS = not significant, S = significant)

means of a reduced nested analysis of variance(see section f(b)), and a Duncan's Multiple Range Test was applied. The results are presented in Tables 22 and 23, and are summarized in Figure 19.

(ii) The Effect of the Dosage of NA

Two dosages of NA, 0.2 and 0.4 mg/kg were tested in Tx mice. The results are shown in Figure 20.

Warm-acclimated mice were insensitive to both 0.2(section (i) above) and 0.4 NA(1 Figures 19 and 20). This was similar to normal.

Cold-acclimated mice were insensitive to both 0.2(section (i) above and 0.4 NA(2 Figures 19 and 20). Normal mice had been sensitive to 0.4NA;cold acclimation reduced sensitivity of Tx mice to NA.

(iii) The Effect of Temperature

The temperature of acclimation did not affect the sensitivity to NA. The response of warm-acclimated mice to 0.2 NA was not significantly different from that of cold-acclimated mice similarly tested (3, Figure 19).

The responses of warm-and cold-acclimated mice tested with 0.4 NA were not significantly different (4, Figure 19).

(c) The Effect of Pretreatment on the Sensitivity of Thyroidectomized Warm- and Cold-acclimated Mice to Noradrenaline.

(i) The Effects of Pretreatment

Warm-acclimated mice:The results are given in Tables 24 and 25 and their significances summarized in Figure 19.

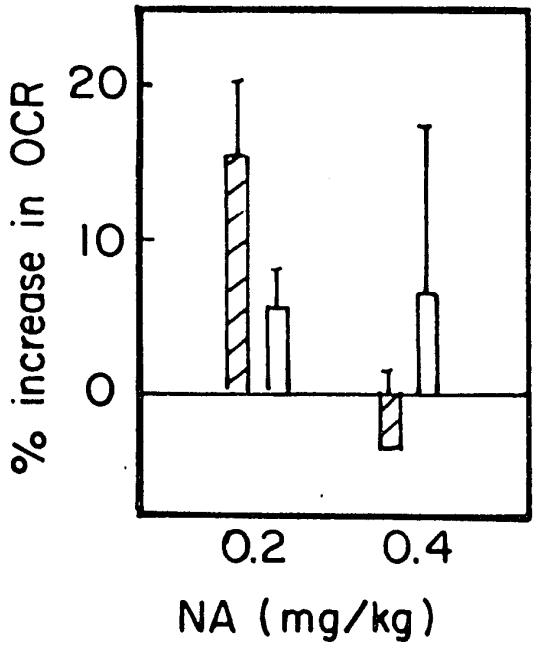


FIGURE 20

RESPONSE TO TWO DOSES OF NORADRENALINE BY WARM-ACCLIMATED (HATCHED BAR) AND COLD-ACCLIMATED (OPEN BAR) THYROIDECTOMIZED MICE

(The S.E. of mean is shown)

TABLE 24

NORADRENALINE TEST IN THYROIDECTOMIZED MICE ACCLIMATED TO 30°C

(NA = 0.2 mg/kg)

Pretreatment	% change in OCR_2	% change in OCR_3 30 min after 0.2 mg/kg NA	OCR_3-OCR_2 (change due to NA)
none		15.6 (7)" ±6.2'	15.6
T_4 (2 mg/kg)	23.4 (5) ±7.9	31.4 (7) ±8.8	8.0
T_4 + DDIH	7.6 (5) ±9.7	15.0 (5) ±6.2	7.4
T_3 (1.68 mg/kg)	10.1 (2) ±8.5	3.0 (2) ±0.1	-7.1
T_3 + DDIH	39.0 (2) ±11.5	53.0 (2) ±3.4	14.0

' mean ± S.E.

" No. of replicates

TABLE 25

NORADRENALINE TEST IN THYROIDECTOMIZED MICE ACCLIMATED TO 30°C

(NA = 0.4 mg/kg)

Pretreatment	% change in OCR_2	% change in OCR_3 30 min after 0.4 mg/kg NA	OCR_3-OCR_2 (change due to NA)
none		-3.9[n] ±5.3[1]	-3.9
DDIH	17.5 ±17.8	7.3 ±20.7	-10.2
T_4 (2 mg/kg)	19.6 ±12.0	59.7 ±23.4	40.1
T_4 + DDIH	22.2 ±5.8	37.3 ±16.1	15.1
T_3 (1.68 mg/kg)	7.3 ±4.2	32.7 ±16.5	25.4
T_3 + DDIH	25.1 ±0.4	51.9 ±18.0	26.8

[1] mean ± S.E.

[n] No. of replicates = 2

Warm-acclimated Tx mice when pretreated with T_4±DDIH, T_3, and DDIH, showed no sensitivity to 0.2 NA(5,Figure 19). When the mice were pretreated with T_3-DDIH, DDIH enhanced the response causing an increase in OCR_2 of 39.0%(Table 24). When 0.2 NA was administered the OCR further increased to 53.0%. This increase in OCR_3 was significantly higher than that observed after the other pretreatments but may have been due not to an increased sensitivity to NA, but simply due to the pretreatment.

When 0.4NA was tested, warm-acclimated Tx mice pretreated with T_3 and with DDIH were not sensitive(7,Figure 19). However, pretreatment with T_4, T_4-DDIH,and T3-DDIH increased sensitivity to 0.4NA(8,Figure19).

The increases in the OCRs of warm-acclimated, pretreated, Tx mice tested with 0.2NA did not differ significantly from the response of correspondingly pretreated mice tested with 0.4NA (9, Figure 19).

Cold-acclimated Mice: The results are given in Tables 26 and 27 and their significances summarized in Figure 19.

Pretreatment had no effect on the sensitivity of these mice when tested with 0.2 NA (10, Figure 19).

When 0.4 NA was tested, T_4-DDIH, T_3±DDIH and DDIH did not sensitize these mice(11,Figure 19). T_4 pretreatment sensitized them to 0.4 NA (12, Figure 19).

The increase in the OCR of cold-acclimated, Tx, mice, pretreated with T_4 and T_4-DDIH, was significantly greater when the mice were tested with 0.4 NA than when tested with 0.2NA(13, Figure19). The OCRs of mice pretreated with T_3 and T_3-DDIH were the same when tested with 0.2 and 0.4 NA (13, Figure 19).

TABLE 26

NORADRENALINE TEST IN THYROIDECTOMIZED MICE ACCLIMATED TO 10°C

(NA = 0.2 mg/kg)

Pretreatment	% change in OCR_2	% change in OCR_3 30 min after 0.2 mg/kg NA	OCR_3-OCR_2 (change due to NA)
none		5.5 (9)" ±2.7'	5.5
T_4 (2 mg/kg)	10.9 (5) ±1.7	3.5 (4) ±3.6	-7.4
T_4 + DDIH	-7.9 (4) ±2.2	-11.3 (4) ±3.3	-3.4
T_3 (1.68 mg/kg)	1.1 (3) ±6.3	19.1 (3) ±8.7	18.0
T_3 + DDIH	9.5 (3) ±5.2	17.2 (3) ±3.7	7.7

' mean ± S.E.

" No. of replicates

TABLE 27

NORADRENALINE TEST IN THYROIDECTOMIZED MICE ACCLIMATED TO 10°C

(NA = 0.4 mg/kg)

Pretreatment	% change in OCR_2	% change in OCR_3 30 min after 0.4 mg/kg NA	OCR_3-OCR_2 (change due to NA)
none		6.8" ±11.0'	6.8
DDIH	4.5 ±1.9	23.4 ±6.3	18.9
T_4 (2 mg/kg)	21.3 ±2.2	53.6 ±10.5	32.3
T_4 + DDIH	14.6 ±9.2	42.0 ±8.1	27.4
T_3 (1.68 mg/kg)	16.1 ±14.7	35.0 ±11.3	18.9
T_3 + DDIH	11.6* ±11.3	29.4* ±15.9	17.8

' mean ± S.E.

" No. of replicates = 2, except * where No. = 3.

(ii)The Effect of DDIH:

The only significant effect of DDIH on the sensitivity of hormonally pretreated mice, was to enhance the response of warm-acclimated mice pretreated with T_3 and tested with 0.2 NA (14, Figure 19). However it did appear to enhance the response in mice pretreated with T_3 when they were tested with 0.4 NA and to inhibit the response in T_4 pretreated mice when they were tested with 0.2 and 0.4 NA.

(iii) The Effect of Temperature:

When 0.2 NA was injected, the increase in the OCRs of warm-acclimated Tx mice, pretreated with T_4, T_4 + DDIH, and T_3 + DDIH was significantly greater than the increase in the OCRs of cold-acclimated Tx mice similarly pretreated (15, Figure 19). The increase in the OCR of warm-acclimated Tx mice pretreated with T_3 was not significantly different from the increase of cold-acclimated Tx mice pretreated with T_3 (16, Figure 19).

The changes in the OCRs of pretreated warm-acclimated Tx mice tested with 0.4 NA (17, Figure 19) were not significantly different from the changes in OCRs of cold-acclimated Tx ones.

Attention should be drawn to the extremely large variance in the section of the experiment dealing with 0.4 NA. The number of animals used was small, two in most cases, and no doubt was the major contributor to the large variance.

It is felt that more experimentation with Tx mice might reveal significant effects not seen in this experiment.

(d) Summary

Sensitivity of Untreated Tx Mice to NA

Warm- and cold-acclimated mice

-showed no sensitivity to 0.2 or 0.4 NA.

-this meant a loss of the sensitivity which had been observed in normal cold-acclimated mice given 0.4 NA.

Sensitivity of Pretreated Tx Mice to NA

Warm-acclimated Tx mice

-Tx mice pretreated with T_3 + DDIH were sensitive to 0.2 NA. None of the other pretreatments had any significant effect in increasing sensitivity but DDIH did lower the effect of T_4 and enhance significantly the effect of T_3 when NA was administered.

-Tx mice pretreated with T_4, T_4 + DDIH, and T_3 + DDIH were sensitive to 0.4 NA; T_3 and DDIH were not effective in increasing sensitivity to NA. Again DDIH lowered the effect of T_4 and enhanced the effect of T_3 when NA was administered.

-these responses differed from those of normal mice where hormonal pretreatment increased the sensitivity to 0.2 and 0.4 NA.

Cold-acclimated Tx Mice

-hormonal pretreatment did not increase the sensitivity of these mice to 0.2 NA. They differed from normal mice which had been sensitized by hormone pretreatment.

-only T_4 pretreatment sensitized these mice to 0.4 NA. T_4 pretreatment restored the sensitivity of Tx mice to the level observed in normal mice.

-in normal mice, the sensitivity to 0.4 NA was not further affected by hormonal pretreatment.

-in Tx mice DDTH had a slight inhibitory effect on the action of T_4 but did not potentiate the action of T_3 as previously indicated in normal mice.

c. Noradrenaline Test in Normal and Thyroidectomized Mice Exposed to 30° or 10°C.

(1) Normal Mice

(a) The Effect of Exposure to 10° or to 30°C on the Oxygen Consumption Rates of Warm- and Cold-Acclimated Mice (Untreated Controls).

Warm-acclimated mice were exposed to 10°C and cold-acclimated mice to 30°C to study the effect on their OCRs. First, their OCRs were measured at the temperature of acclimation; then, the mice were exposed to either 10° or 30°C for one hour and their OCRs remeasured. At the completion of the second oxygen consumption reading, the mice had been exposed for ninety minutes. The results are shown in Figure 21.

Warm-acclimated mice increased their OCR by 82.3% from 2.47 to 4.53 ml/g/hr when exposed to 10°C. The OCR of cold-acclimated mice dropped 46.8% from 4.89 to 2.62 ml/g/hr when exposed to 30°C. The actual change in both cases was approximately the same (2.05 ml/g/hr increase for warm-acclimated mice, and 2.27 ml/g/hr decrease for cold-acclimated ones). The percentage formulation of the change in OCR accounted for the apparently greater increase in OCR by warm-acclimated mice than decrease by cold-acclimated ones.

(b) The Effect of Exposure to 10°C or to 30°C on the Sensitivity to Noradrenaline.

Preliminary experiments had indicated no significant

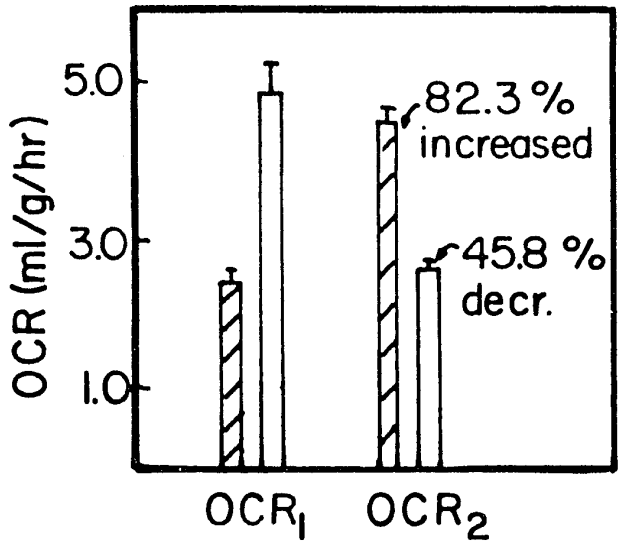


FIGURE 21

THE CHANGE IN OCR AFTER EXPOSURE OF WARM-ACCLIMATED MICE TO 10°C FOR NINETY MINUTES (HATCHED BAR) AND OF COLD-ACCLIMATED MICE TO 30°C FOR NINETY MINUTES (OPEN BAR). OCR_1 WAS MEASURED AT THE TEMPERATURE OF ACCLIMATION, OCR_2 AT THE TEMPERATURE OF EXPOSURE

differences in the response of mice exposed for a period of up to six hours. Therefore all further experimentation was done after one hour of exposure. At the completion of each experiment the mice had been exposed for ninety minutes.

In untreated acclimated animals, the OCR was first determined at the acclimation temperature; they were then exposed to either 30° or 10° C for one hour and a second OCR determination was made at the end of which the animals had been at the temperature of exposure for ninety minutes.

In Thpretreated, acclimated animals, the hormone was injected and an OCR measurement performed immediately to determine the baseline level; forty-eight hours later a second OCR determination was made to evaluate the effect of the hormone. These animals were then transferred to the proper temperature of exposure for one hour and a third OCR determination was made, at the end of which the animals had been at the temperature of exposure for ninety minutes.

For the NATest, NA at either 0.2 or 0.4 mg/kg was injected immediately prior to the last OCR determination, that is the second OCR in untreated and the third OCR in pretreated animals.

The results obtained are given in Tables 28 and 29; the analysis of variance was completed by a Duncan's Multiple Range Test and the significance of results summarized in Figures 22, 23, and 24. When the NA administration proved lethal during or immediately after the last OCR determination, the values were not included in the analysis.

(i) Warm-acclimated Mice exposed to 10°C.

Control experiments: Pretreatment with T_4 resulted in a 28.1% higher OCR than that of the untreated mice (Table 28). However, after exposure to 10°C for ninety minutes, the OCR of the pretreated mice was not significantly different from that of the untreated ones.

NA Test: In this test, an increase in OCR greater than that of the control indicated sensitivity. The increase after 0.2 and 0.4 NA and exposure was compared to the untreated exposed control. The increase in the OCR of T_4 pretreated mice after 0.2 and 0.4 NA and exposure was compared to the pretreated, exposed control.

Untreated Mice: Warm-acclimated mice exposed to 10°C, were insensitive to 0.2 and 0.4 NA, the increases in OCR being lower than that of the control (1 and 2, Figure 22). Similarly it had been shown that warm-acclimated mice tested at 30°C were insensitive to both doses of NA (1, Figure 18); thus exposure to cold for a short period of time did not alter the response by making warm-acclimated mice sensitive to NA. The response was similar when either 0.2 or 0.4 NA was tested (3, Figure 22).

Pretreated mice: Pretreatment with T_4 before exposure did not elicit any sensitivity to either 0.2 or 0.4 NA (4 and 5, Figure 22). This was contrary to the response observed when warm-acclimated mice were tested at 30°C.

TABLE 28

NORADRENALINE TEST IN WARM-ACCLIMATED MICE AFTER ONE HOUR OF EXPOSURE TO 10°C

Pretreatment	% change in OCR_2	% increase in OCR_3 30 min after exposure[1] to 10°C
Control		
none	—	82.3 (2)" ±2.7'
T_4	28.1 (5) ±2.5	70.6 (6) ±8.0
NA Test		% change in OCR_3 30 min after exposure[1] and NA Test
none (NA 0.2)	—	65.2 (3) ±7.1
none (NA 0.4)	—	58.5 (18) ±6.2
T_4 + NA 0.2	15.5 (12) ±3.2	71.4 (12) ±3.7
T_4 + NA 0.4	27.3 (4) ±6.4	70.6 (4) ±9.1

1 animals had been exposed to 10°C for 90 min at completion of OCR_3

' mean ± S.E.

" No. of replicates

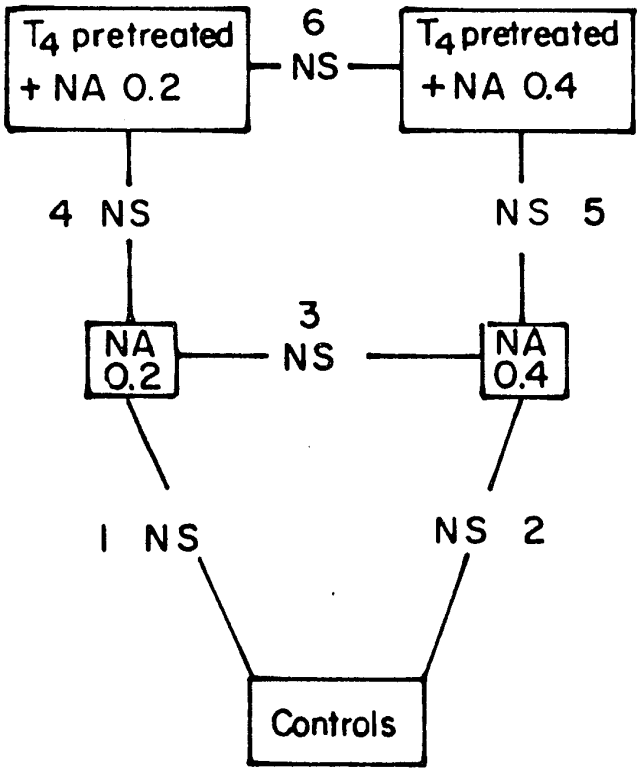


FIGURE 22

A SUMMARY OF THE NORADRENALINE TEST IN WARM-ACCLIMATED MICE EXPOSED TO 10°C FOR NINETY MINUTES

(NS = not significant, S = significant)

In that case, pretreatment with T_4 had sensitized the mice to 0.2 and 0.4 NA (6 and 7, Figure 18). T_4 pretreated exposed mice responded similarly to 0.2 or 0.4 NA (6, Figure 22).

(ii) Cold-acclimated Mice exposed to 30°C.

Control experiments: Pretreatment with T_4 did not increase the OCR measured at 10°C nor did it prevent the drop in OCR after exposure to 30°C (Table 29).

NA Test: In this Test, sensitivity to NA was indicated by an increase in OCR above the appropriate control.

Untreated Mice: The increases in OCRs of cold-acclimated mice exposed to 30°C and then tested with 0.2 and 0.4 NA, did not differ significantly from their controls (Figure 23 and 1 and 2, Figure 24). This represented a loss of sensitivity to 0.4 NA since cold-acclimated mice tested at 10°C had been sensitive to 0.4 NA (2, Figure 18).

The response to 0.2 or 0.4 NA was similar (3, Figure 24).

Pretreated Mice: Pretreatment with T_4 before exposure, significantly increased the sensitivity of these mice to 0.2 NA (4, Figure 24). When 0.4 NA was tested, pretreated exposed mice exhibited hypersensitivity (5, Figure 24). Two of the four mice tested died before the completion of OCR3. The remaining two were in shock and showed signs of pulmonary edema when removed from the apparatus. They died within three hours. The sensitivity to 0.4 NA was significantly greater than to 0.2 in T_4 pretreated mice (6, Figure 24).

TABLE 29

NORADRENALINE TEST IN COLD-ACCLIMATED MICE AFTER ONE HOUR OF EXPOSURE TO 30°C

Pretreatment	% change in OCR_2	% drop in OCR_3 30 min after exposure[1] to 30°C	Sensitivity[2]
Control			
none	—	-45.8 (2)" ±8.1'	
T_4	-0.2 (6) ±2.3	-40.8 (6) ±2.0	
		% change in OCR_3 30 min after exposure[1] and NA Test	
NA Test			
none (NA 0.2)	—	-45.5 (3) ±4.6	0.3
none (NA 0.4)	—	-23.8 (18) ±2.8	22.0
T_4 + NA 0.2	3.7 (18) ±2.1	-16.4 (18) ±5.3	24.4
T_4 + NA 0.4	-1.0 (2)	-11.6 (2)[3]	lethal

1 animals had been at 30°C for 90 min at completion of OCR_3
2 Sensitivity is expressed as the increase in OCR above the appropriate control
3 animals died immediately after OCR_3 from NA hypersensitivity

' mean ± S.E.
" No. of replicates

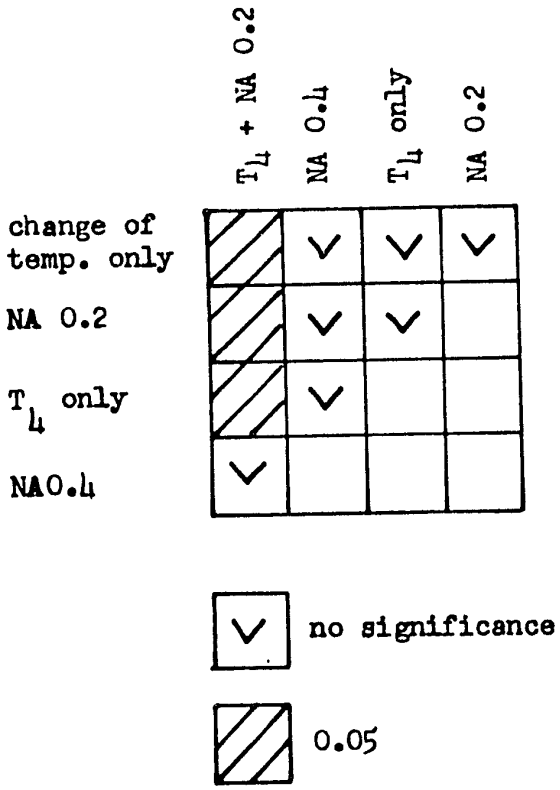


FIGURE 23

A SUMMARY OF THE RESULTS OF THE DUNCAN'S MULTIPLE RANGE TEST FOR SIGNIFICANCE AMONGST CHANGES IN THE OCRS OF COLD-ACCLIMATED MICE (PRETREATED OR NOT) AFTER EXPOSURE TO 30°C AND NA TEST

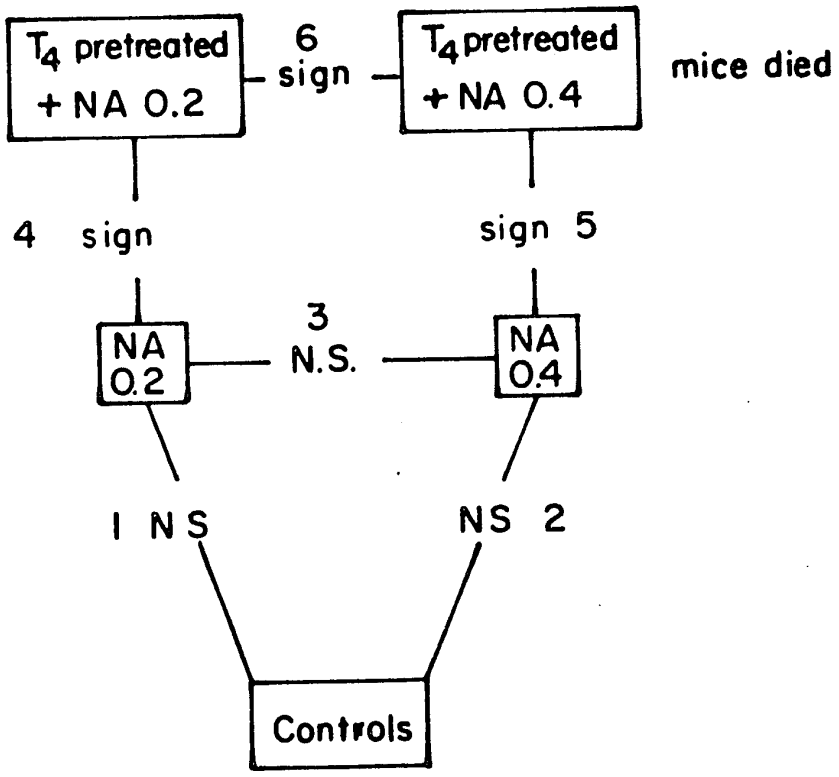


FIGURE 24

A SUMMARY OF THE NORADRENALINE TEST IN NORMAL COLD-ACCLIMATED MICE EXPOSED TO 30°C FOR NINETY MINUTES

(NS = not significant, S = significant)

(c) Summary

Effect of Exposure to 10° or to 30°C

Warm-acclimated mice exposed to 10°C

-increased their OCR to a value similar to that of cold-acclimated mice within ninety minutes of exposure.

-although mice pretreated with T_4 had a higher initial OCR, the OCR after exposure was the same as that of the untreated mice.

Cold-acclimated mice exposed to 30°C

-decreased their OCR to a value similar to that of warm-acclimated mice within ninety minutes of exposure.

Effect of Pretreatment and Exposure on Sensitivity to NA

Warm-acclimated mice exposed to 10°C

Untreated mice

-were insensitive to 0.2 and 0.4 NA.

-this was similar to the response observed in warm-acclimated mice measured at 30°C.

Pretreated mice

-T_4 pretreatment had no effect on their sensitivity to NA.

-this was different from the response observed in warm-acclimated mice measured

at 30°C. These pretreated mice had been sensitive to 0.2 and 0.4 NA.

Cold-acclimated mice exposed to 30°C

Untreated mice

-were insensitive to 0.2 and 0.4 NA

-this represented a loss of sensitivity to 0.4 NA since cold-acclimated mice tested at the temperature of acclimation had been sensitive.

Pretreated mice

-T_4 pretreatment increased the sensitivity to 0.2 and was fatal when 0.4 NA was used.

-from the results, warm-acclimated mice did not appear to gain sensitivity to NA within a six hour period of exposure to 10°C, and cold-acclimated mice lost their sensitivity to NA within one hour after transfer to 30°C.

(2) Thyroidectomized Mice

(a) The Effect of Exposure to 10° or to 30°C on the Oxygen Consumption Rates of Warm- and Cold-Acclimated Tx Mice (Untreated Controls).

Warm-acclimated Tx mice were exposed to 10°C and cold-acclimated ones to 30°C. The same procedure was followed as with normal mice. The results are shown in Figure 25.

Warm-acclimated Tx mice increased their OCR by 73.7% from 2.16 to 3.76 ml/g/hr when exposed to 10°C. The OCR of cold-acclimated Tx mice dropped 39.4% from 4.79 to 2.90 ml/g/hr when exposed to 30°C. Although the actual decrease in the OCR of cold-acclimated mice was slightly larger than the increase, the percentage formulation made the drop appear smaller.

The OCR of warm-acclimated Tx mice, after exposure to 10°C, was significantly lower than that of cold-acclimated Tx mice and the OCR of cold-acclimated Tx mice after exposure to 30°C was significantly higher than that of warm-acclimated Tx mice.

(b) The Effect of Exposure to 10° or to 30°C on the Sensitivity to Noradrenaline.

Experimental procedures were the same as in 5(1)(a).

The results are given in Tables 30 and 31. A one way analysis of variance was done on the results excluding any treatments which were lethal (Table 31). The treatment T_4 + NA 0.4, in warm-acclimated mice, was also excluded because of its unduly large standard error (Table 30). Duncan's Multiple Range Tests were applied to the means and the significance of results summarized in Figures 26, 27, 28 and 29.

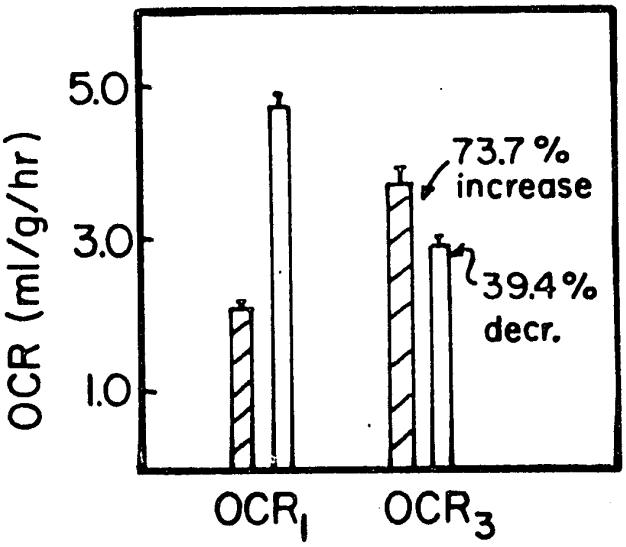


FIGURE 25

THE CHANGE IN OCR AFTER EXPOSURE OF WARM-ACCLIMATED Tx MICE TO 10°C FOR NINETY MINUTES (HATCHED BAR) AND OF COLD-ACCLIMATED Tx MICE TO 30°C FOR NINETY MINUTES (OPEN BAR). OCR_1 WAS MEASURED AT THE TEMPERATURE OF ACCLIMATION OCR_3 AT THE TEMPERATURE OF EXPOSURE

(i) Warm-acclimated Tx Mice exposed to 10°C.

Control experiments: The warm-acclimated control, pretreated with T_4, had a slightly higher (10.1%) OCR than the untreated control (Table 30). However after exposure to 10°C for ninety minutes the OCR of the pretreated control was significantly lower.

NA Test: In the NA Test, sensitivity to NA was again indicated by an increase in OCR significantly larger than that of the appropriate control.

Untreated Mice: The increase in OCR of warm-acclimated Tx mice, exposed to 10°C was significantly lower than that of their controls when tested with 0.2 and 0.4 NA (1 and 2, Figure 27) indicating no sensitivity. The increase in OCR was significantly lower with 0.4 NA than with 0.2 NA (3, Figure 27) suggesting an inhibitory action. The increase in OCR of normal mice, similarly tested had been lower, but not significantly, than that of their controls (Table 28).

Pretreated Mice: Pretreatment with T_4 before exposure, had no significant effect on the OCR when tested with 0.2 NA (4, Figure 27). When 0.4 NA was used, pretreatment with T_4 did not enhance the the action of NA but did prevent the decrease in OCR observed when 0.4 NA was administered alone. Because of the unduly large standard error of the result when 0.4 NA was administered to T_4 pretreated mice, it was excluded from the analysis.

TABLE 30

NORADRENALINE TEST IN WARM-ACCLIMATED THYROIDECTOMIZED MICE AFTER ONE HOUR OF EXPOSURE TO 10°C

Pretreatment	% change in OCR_2	% increase in OCR_3 30 min after exposure[1] to 10°C	Sensitivity[2]
Controls			
none	—	73.7 (2)" ±3.6'	
T_4	10.1 ±3.7	53.6 ±5.6	
		% change in OCR_3 30 min after exposure[1] and NA Test	
NA Test			
none (NA 0.2)	—	52.6 ±2.4	none
none (NA 0.4)	—	29.6 ±5.9	none
T_4 + NA 0.2	15.4 ±9.7	64.9 ±5.8	11.3
T_4 + NA 0.4	19.3 ±8.8	66.4 ±22.1	12.8

1 mice had been at 10°C for 90 min at completion of OCR_3
2 Sensitivity is the increase in OCR_3 above appropriate control

' mean ± S.E.
" No. of replicates = 2 in all cases

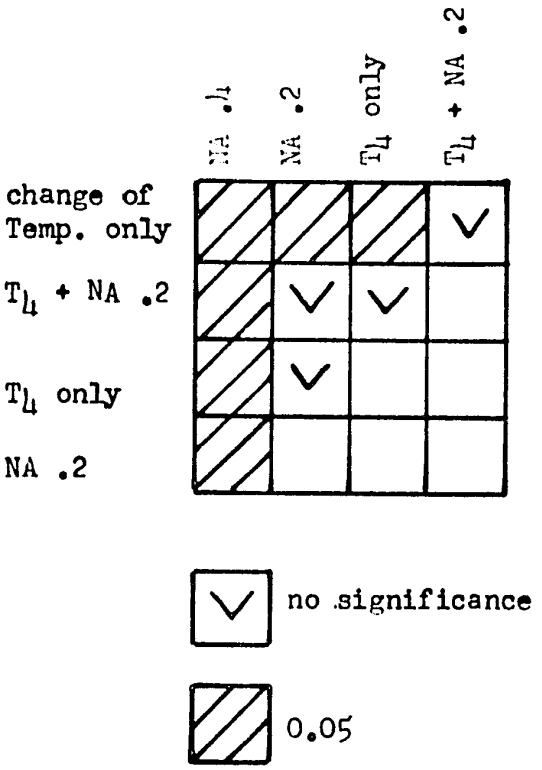


FIGURE 26

A SUMMARY OF THE RESULTS OF THE DUNCAN'S MULTIPLE RANGE TEST FOR SIGNIFICANCE AMONGST CHANGES IN THE OCRs OF WARM-ACCLIMATED TX MICE (PRETREATED OR NOT) AFTER EXPOSURE TO 10° C AND NA TEST

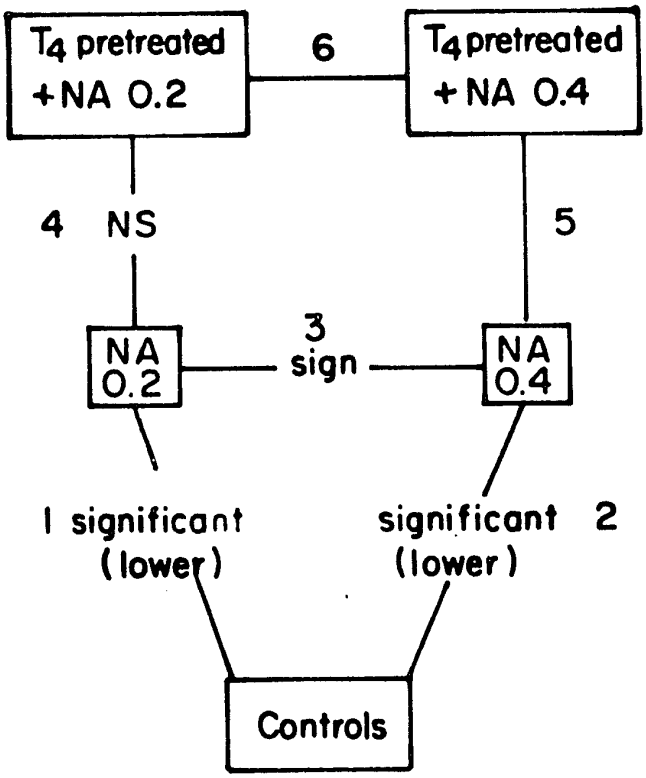


FIGURE 27

A SUMMARY OF THE NORADRENALINE TEST IN THYROIDECTOMIZED WARM-ACCLIMATED MICE EXPOSED TO 10°C FOR NINETY MINUTES

(NS = not significant, S = significant)

(ii) Cold-acclimated Tx Mice exposed to 30°C.

Control experiments: The cold-acclimated control mice, pretreated with T_4, had a slightly higher OCR than that of the untreated controls (Table 31). However after exposure to 30°C for ninety minutes their OCRs were not statistically different.

NA Test: In the NA Test, sensitivity was indicated by an increase in OCR above the appropriate baseline control.

Untreated Mice: 0.2 NA had no effect on the OCR after exposure (1, Figure 29), but these mice were extremely sensitive to 0.4 NA exhibiting signs of noradrenaline shock when removed from the apparatus (2, Figure 29). Because of the condition of these mice at the completion of the OCR_3, these results were considered significant although not included in the analysis. Neither Tx nor normal mice showed sensitivity to 0.2 NA, but Tx mice were more sensitive to 0.4 NA than were normal mice.

Tx mice exposed to 30°C were much more sensitive to 0.4 than to 0.2 NA (3, Figure 29).

Pretreated Mice: Pretreatment with T_4 appeared to sensitize these mice to 0.2 NA (4, Figure 29). However 0.4 NA in pretreated mice caused shock, pulmonary edema and death before completion of OCR_3 (5, Figure 29). The value given represented the change in OCR ten minutes after NA and was not included in the statistical analysis.

T_4 pretreated mice were much more sensitive to 0.4 than to 0.2 NA (6, Figure 29).

TABLE 31

NORADRENALINE TEST IN COLD-ACCLIMATED THYROIDECTOMIZED MICE AFTER ONE HOUR OF EXPOSURE TO 30°C

Pretreatment	% change in OCR_2	% drop in OCR_3 30 min after exposure[1] to 30°C	Sensitivity[2]
Control			
none	——	-39.4 (3)" ±1.6'	
T_4	10.4 ±8.2	-37.6 ±3.5	
NA Test		% change in OCR_3 30 min after exposure[1] and NA Test	
none (NA 0.2)	——	-43.8 ±5.1	none
none (NA 0.4)	——	-21.5[3] ±8.5	NA shock
T_4 + NA 0.2	14.3 ±7.9	-29.8 ±1.5	7.8
T_4 + NA 0.4	9.3 ±2.3	-27.1[4] ±5.7	lethal

1 mice had been at 30°C for 90 min at completion of OCR_3
2 sensitivity is expressed as the increase in OCR_3 above appropriate control
3 mice were in shock at completion of OCR_3
4 OCR 10 min after NA. Mice were dead before completion of OCR_3.
' mean ± S.E.
" No. of replicates = 3 in all cases

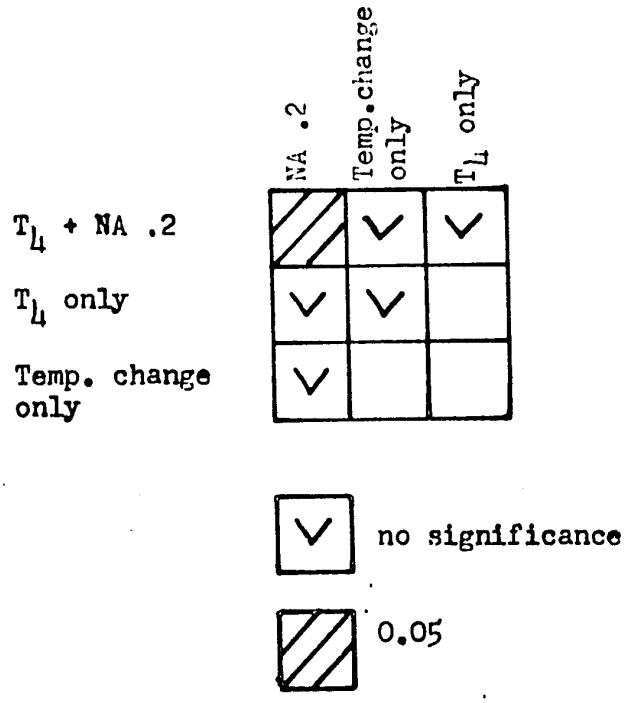


FIGURE 28

A SUMMARY OF THE RESULTS OF THE DUNCAN'S MULTIPLE RANGE TEST FOR SIGNIFICANCE AMONGST CHANGES IN THE OCRs OF COLD-ACCLIMATED TX MICE (PREATEATED OR NOT) AFTER EXPOSURE TO 30°C AND NA TEST

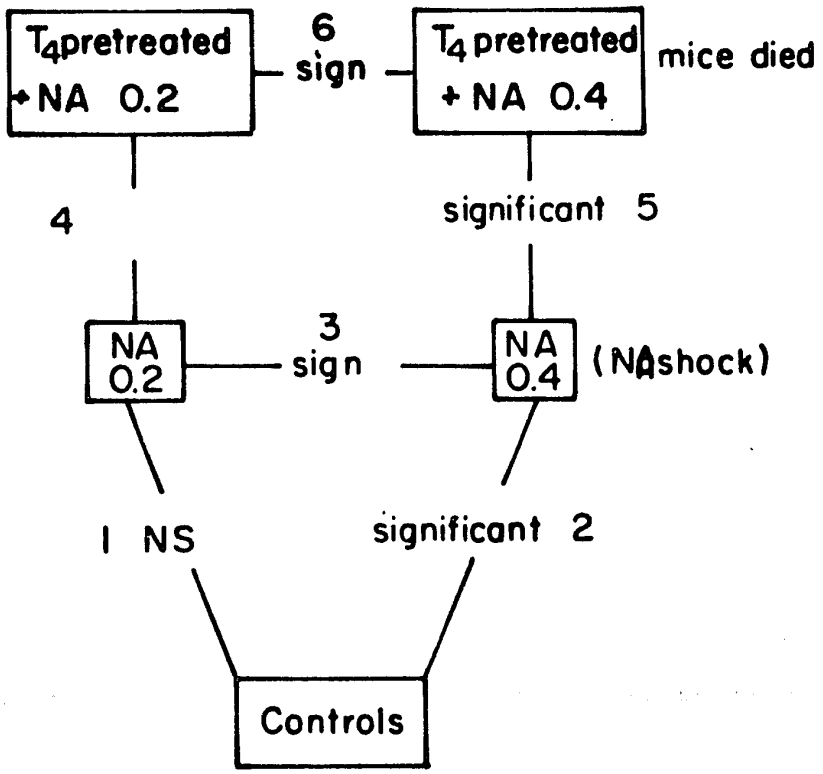


FIGURE 29

A SUMMARY OF THE NORADRENALINE TEST IN THYROIDECTOMIZED COLD-ACCLIMATED MICE EXPOSED TO 30°C FOR NINETY MINUTES

(NS = not significant, S = significant)

(c) Summary

Effect of Exposure to 10° or to 30° C

Warm-acclimated Tx mice exposed to 10°C

-increased their OCR but it was significantly lower than the OCR of cold-acclimated Tx mice.

-the increase in OCR of Tx mice, after exposure was significantly lower than the increase observed in normal mice.

-although Tx mice pretreated with T_4 had a higher initial OCR, the OCR after exposure was significantly lower than that of untreated Tx mice.

-thyroidectomy resulted in a loss of the rapid OCR readjustment observed in normal mice when moved from their temperature of acclimation.

Cold-acclimated Tx mice exposed to 30° C

-showed a decrease in OCR but the value was still significantly higher than that of warm-acclimated mice.

-although Tx mice pretreated with T_4 had a higher initial OCR, the OCR after exposure was not significantly different from the untreated mice, and T_4 administration did not restore the ability to respond rapidly to a temperature change as observed in normal animals.

Effect of Pretreatment and Exposure on sensitivity to NA

Warm-acclimated Tx mice exposed to 10° C

Untreated mice

-not sensitive to 0.2 or 0.4 NA. The OCRs were significantly lower after NA than in the controls. 0.4 NA had a greater inhibitory effect than 0.2 NA.

Pretreated mice

-pretreatment with T_4 did not increase the sensitivity but did bring the OCR back to the control value thus preventing the inhibitory effect observed with 0.4 NA.

Cold-acclimated Tx mice exposed to 30 °C

Untreated mice

-not sensitive to 0.2 NA but were extremely sensitive (shock) to 0.4 NA.

Pretreated

-T_4 pretreatment did sensitize these mice to 0.2 NA but was fatal when 0.4 NA was injected.

C. DISCUSSION

1. THYROID HORMONES AND COLD-ACCLIMATION

a. Grouped and Single Animal Determinations of OCR

The OCRs were determined on both grouped and single animals. The oxygen consumption values obtained using groups of eight mice were significantly lower in both warm- and cold-acclimated mice when compared to the values obtained by Hart (1953) who determined the OCRs on individual mice. Pearson (1960) explained this discrepancy in terms of heat transfer between grouped mice and of a different air-body surface ratio. Since decreasing the number of cold-acclimated mice in the group caused the OCR to approximate that of a single animal, LeMay (1964) assumed that the fraction of the OCR that he measured with the grouped animals might be a reliable index of the actual metabolic rate.

The inhibitory effect of DDIH on the calorigenic action of exogenous T_4 in warm-acclimated mice reported by Serif and Seymour (1961) and DesMarais and LeMay (1964) was not observed. In fact DDIH had a potentiating effect on various dosages of T_4. When T_3 was administered, DDIH not only failed to inhibit the calorigenic action, as noted by Serif and Seymour (1961), but using lower doses than they had, it had an enhancing effect on the action of T_3.

When the OCRs were determined on single animals, it became apparent that a number of the effects previously observed had been due to grouping. The response to T_4 was affected by grouping; under

these circumstances, cold-acclimated mice were only slightly affected by exogenous T_4 and warm-acclimated mice showed increased OCRs. However when the OCR determinations were made on single animals there were no significant differences in the response to T_4 by warm- and cold-acclimated mice. Grouping did not affect the response to T_3; however the enhancement of hormone effect by DDIH, apparent when groups were used disappeared when tested in single animals.

Grouping has been shown to affect hormone levels in rats (Chance 1956). The hormonal and DDIH pretreatment may have made the animals hyperexcitable and grouping could have compounded this effect. The situation was further complicated in the cold room since grouping lowered the OCRs of untreated cold-acclimated mice. However this lowering of OCR may have been sufficient to mask the effect of hyperexcitability on the OCR. When OCRs were measured in single animals there was no temperature change in the respiration chamber and no contact with other mice; under these circumstances the calorigenic action of T_4 was similar in both warm- and cold-acclimated mice.

Even in the single animal apparatus, T_3 was more effective in increasing the OCR of warm- than cold-acclimated mice although the magnitude of response was lower than it had been in the grouped animal experiment. This differs from the experiment of Hsieh (1963) where he observed no effect of 5 or 15 μg of T_3 administered daily during acclimation period on the OCR of cold-acclimated rats measured at 5°C.

In the grouped animal experiments the only effect of the size of hormonal dosage was observed with T_4. At the lowest dosage level there was a significant decrease in calorigenic effect.

All the other T_4 and all the T_3 dosages were sufficiently large to maximally stimulate the OCR. T_3 did not exert a greater calorigenic effect than T_4 in either warm- or cold-acclimated mice when measured on single animals. However the doses used were sufficiently large and all capable of inducing calorigenesis that any difference in effect due to dosage size would no doubt have been masked. In the grouped animal experiment T_3 was significantly more effective than T_4 at the lowest dosage level. Tata (1964 a) draws attention to the unphysiological response after large doses of hormone. Thus the lack of differentiation between the effect of T_4 and T_3 in this experiment does not necessarily mean that there is no difference.

All further discussion refers to experiments done on single animals, unless otherwise specified.

b. Duration of Effect and Excretion of ^{131}I

When the OCRs were determined at twenty-four hour intervals for ninety-six hours a difference of effect between the two hormones was observed. The results indicated that forty-eight hours after injection in warm-acclimated mice T_3 did not cause a significantly greater increase in OCR than did T_4. Also, the effect of T_3 did not last as long as T_4. The increase in OCR began to decline seventy-two hours after injection of T_3 whereas the effect of T_4 was still increasing and did so up to ninety-six hours. Tata (1961 a) noted that the calorigenic action of T_3 was higher initially and wore off more quickly than the action of T_4 but that the total calorigenic response was the same.

In warm-acclimated mice the excretion of ^{131}I in the urine was similar after an injection of either T_4 or T_3. There was no indication that the increase in OCR preceded the deiodination of T_3 as suggested by Anbar et al. 1965 a. However the limitations of this experiment must be realized. The volume of urine excreted within twenty-four hours was extremely small and varied considerably and was thus particularly susceptible to error. The non-physiological doses of hormones probably disturbed the latent period of action as described by Tata (1964 a) masking any association or dissociation of deiodination and calorigenic action. The calorigenesis produced by toxic amounts of thyroid hormones is a catabolic process which can be best explained by an uncoupling of oxidative phosphorylation through a direct action on the mitochondrion (Tata 1964 a).

DDIH had no effect on the metabolic pattern of T_4 or T_3 at room temperature. Neither did it change the excretion of the ^{131}I after administration of labeled hormones.

In cold-acclimated mice, the magnitude of calorigenic response was similar after T_3 or T_4 injection, and was not affected by DDIH administration.

The route for excretion of ^{131}I after administration of labeled hormones was primarily via the feces; approximately twenty to thirty per cent was excreted in the urine. After injection of labeled T_4 the total ^{131}I was slightly but not significantly increased in cold-acclimated mice with the slight excess being excreted in the feces; the urinary iodide remained constant. If one assumes that the ^{131}I in the urine is a measure of the T_4 metabolized, it would appear that warm-

and cold-acclimated mice metabolize an exogenous dose of T_4 in a similar fashion, cold-acclimated mice exhibiting a greater turnover of T_4. This substantiates the view that although more endogenous hormone is produced by cold-acclimated animals this increased thyroid activity is not essential for the maintenance of nonshivering thermogenesis (Heroux 1963).

DDIH was without effect on the excretion of ^{131}I after administration of T_4 at both temperatures.

Although most of the dose of exogenous T_4 was excreted within forty-eight hours, the OCR continued to rise up to ninety-six hours. It would appear that the small amount of T_4 left in the animal was sufficient to increase the OCR and again indicates the difficulties involved in working with non-physiological doses.

The excretion of ^{131}I in warm-acclimated mice, after administration of labeled T_3, was similar to that observed after T_4.

In cold-acclimated mice the total amount of ^{131}I excreted was greater than in warm-acclimated mice. The amount of ^{131}I excreted in the urine was significantly greater in cold-acclimated mice in one experiment and DDIH reduced the urinary elimination of ^{131}I in the same experiment. In another experiment the ^{131}I excreted in the urine was similar in both cold- and warm-acclimated mice and DDIH showed no effect.

The results of this experiment clarified two main points - DDIH does not inhibit either the calorigenic action or the deiodination of an exogenous dose of T_4 in warm- or cold-acclimated mice. Also DDIH

had no effect on the calorigenic action of T_3 and may or may not alter its deiodination. A preferable way to study the action of DDIH on excretion would be to endogenously label the hormone pool by chronic administration of ^{125}I after which a single injection of ^{131}I could be given (Anbar et al. 1965). The excretion of both isotopes could then be followed and a more complete idea obtained on the effect of DDIH.

2. CATECHOLAMINES AND COLD-ACCLIMATION

a. General Considerations

Since Hsieh and Carlson (1957 a) demonstrated an increased sensitivity to noradrenaline in cold-acclimated animals a great deal of work has been done in an attempt to elucidate the role of noradrenaline as the mediator of nonshivering thermogenesis. Curarized cold-acclimated rats returned to the cold have a greater capacity for heat production than similarly treated warm-acclimated rats (Cottle and Carlson 1956 a). Exogenous noradrenaline administered to cold-acclimated rats left at room temperature for an undefined length of time caused an even greater increase in the OCR than did re-exposure to cold (Hsieh and Carlson 1957 a). Because noradrenaline simulated cold exposure, these authors have suggested that it is the mediator in chemical regulation of heat production. Various studies have shown that the metabolic changes induced by exogenous noradrenaline are not necessarily the same as those induced by cold. Much the same situation existed with respect to the role of the thyroid gland - hyperthyroidism and cold-acclimation were compared, and the two did not always have the

same effect. It does seem apparent that both the thyroid and the sympathetic nervous system are necessary for acclimation and evidence for this has been discussed.

One striking feature of much of the work is the variety of experimental conditions under which it is done making it extremely difficult to compare results. In an attempt to point out the necessity for standardization, the experiments were done under two sets of environmental conditions - one in which noradrenaline was administered to mice at the temperature of acclimation, the other to acclimated mice exposed to 10 or 30°C.

The first set of environmental conditions was felt to best approximate steady state conditions and the better situation in which to study the effect of hormonal and drug treatment.

Hsieh and Carlson (1957 a) used spinal or curarized rats to eliminate the excessive movement observed in rats after injection of adrenaline (Swanson 1956); Depocas (1960) used anesthetized rats. These are experimental conditions which alter the steady state of the animal to an undefined extent. 'Equithesin' a mild anesthetic (a mixture of chloral hydrate and sodium pentobarbital) had, in our experience, deleterious effects on cold-acclimated mice tested at 10°C. Sellers (1957) also noted a similar effect in clipped rats where anesthesia with sufficient sodium pentobarbital for measurement at 1.5°C resulted in death during the half hour experimental period. Since anesthetic could not be used successfully in the cold-acclimated mice at 10°C, it was also not used in the second series where the OCRs of cold-acclimated mice were determined at 30°C.

In contrast to the observed effect of adrenaline on rats, warm- and cold-acclimated mice were no more restless after noradrenaline than untreated mice. Thus at least in mice tested under the present circumstances there did not seem to be any necessity for anesthesia.

The variety of experimental conditions and the use of various species makes comparison with the work of others difficult. Because of the multiplicity of effects of noradrenaline it is difficult to know upon which grounds to base inter-species comparisons. Should a standard dose of noradrenaline be used in all species and its effect on the parameter in question be compared i.e. metabolic rate? This would not take into consideration the difference in initial metabolic rate of the species being compared, and what might be a stimulatory dose in one, might not be in another. Another approach would be to determine the amount of noradrenaline necessary to elicit a certain response. Both approaches have limitations but the situation might gain more clarity if the physical condition of the animal at the dosage of noradrenaline used to obtain a certain effect was described.

When the three dosages of noradrenaline were used three categories of metabolic effect were observed. At the lowest dosage, neither warm- nor cold-acclimated mice were sensitive to noradrenaline; at the highest dosage, both were, and at the intermediate dosage only cold-acclimated mice responded metabolically. At the two lower dosages, the mice appeared to respond similarly in a physical sense (respiratory, vascular) but were visibly stressed by the largest dosage showing increased respiration, salivation and exophthalmia.

Hsieh and Carlson (1957a) do not state what the physical condition of the rats was following the dosage of noradrenaline capable of eliciting the extremely large metabolic response observed. This makes it difficult to decide which dose used in mice should be compared with the dose used in rats (Hsieh and Carlson 1957a) when comparing the metabolic effect.

Since the mice could not be successfully anesthetized single injections of noradrenaline rather than infusions were used.

b. The Effect of Pretreatment and Thyroidectomy on the Responses to Noradrenaline Administered at the Temperature of Acclimation.

Warm-acclimated mice were not sensitive to noradrenaline at either of the dosages used. A similar lack of response was observed in rats (Hsieh and Carlson 1957a). However, Heroux (1967) using anesthetized animals observed a twenty-one per cent increase in the OCR of warm ($28^{o}C$) -acclimated white rabbits and a twenty-five per cent increase in the OCR of white rats infused with noradrenaline (1.18 µg/kg/min in rabbits and 1.34 µg/kg/min in rats). Depocas (1960) observed that an infusion of 0.1 to 3.0 µg/min/374 g into anesthetized rats acclimated to 30^{o} C did not increase the OCR measured at 20^{o} C. He stated that the temperature of measurement had a negligible effect on the OCRs of both warm- and cold-acclimated rats. However when some animals were infused at 30^{o} C he did note a slight increase which he explained as a temperature effect - the small calorigenic effect of noradrenaline at 20^{o} C being balanced by the decrease in oxygen

consumption due to body cooling. There thus does not seem to be general agreement as to whether warm-acclimated rats are sensitive to noradrenaline or not. Nevertheless they do not appear to be nearly as sensitive as cold-acclimated animals.

The only sensitivity to noradrenaline apparent in untreated mice tested at the temperature of acclimation was that observed after administration of 0.4 mg/kg noradrenaline to cold-acclimated mice. Thyroidectomy caused this sensitivity to disappear indicating the necessity of at least a minimal amount of thyroid hormone for the calorigenic response to noradrenaline. Swanson (1956, 1957) has shown a similar relationship between adrenaline and thyroxine.

Mice made hypermetabolic by pretreatment with T_4 or T_3 exhibited a variety of responses to noradrenaline. In general hormonal pretreatment increased the sensitivity to noradrenaline except in the case of cold-acclimated mice given 0.4 noradrenaline. These mice, sensitive to 0.4 noradrenaline, were incapable of further increasing their metabolic response after T_4 or T_3 administration.

Hormonal pretreatment of thyroidectomized mice did not increase the sensitivity to 0.2 noradrenaline, and only T_4 increased their sensitivity to 0.4. The lack of a calorigenic effect of T_3 in these animals may be related to its faster elimination (Tata 1964 b), most of the dose being excreted before noradrenaline was administered.

DDIH, when given in conjunction with T_4 and T_3, modified the responsiveness to noradrenaline in some way. In general, DDIH lowered the sensitivity of T_4 pretreated mice and enhanced the sensitivity of T_3 pretreated mice to noradrenaline. However the effect was statistically

significant in only a few instances. The effect of DDIH on the hormonal response was more apparent at the lower dosage of noradrenaline, thus again the importance of hormonal dosage level is seen. Also T_4 did not sensitize the mice to as great a degree as did T_3 when the lower dose of noradrenaline was used. This difference of effect between the two thyroid hormones disappeared at the higher noradrenaline level.

The differential effect of DDIH on the calorigenic action of T_4 and T_3 is difficult to explain. It may indicate different metabolic paths for the two hormones, or it may simply have a kinetic basis - T_3 reaching the sites of action more quickly than T_4 and acting with DDIH at a time when the distribution of DDIH is different than it is when T_4 acts. This is strictly speculative and would need further study before anything could be said with certainty. However if a differential effect of DDIH on the in vitro response to T_4 and T_3 were observed, it would indicate a metabolic rather than kinetic difference.

It is also difficult to state where DDIH is exerting its effect. It does not inhibit or enhance either T_4 or T_3 alone, nor does it have any effect when just noradrenaline is administered. However, when mice are pretreated with T_4 or T_3, DDIH has an effect on their response to noradrenaline. From OCR measurements it is impossible to say whether DDIH modifies the action of T_4 or T_3 which then alters the response to noradrenaline, or whether DDIH acts directly on the action of noradrenaline, the response in this case being different depending on whether T_4 or T_3 was used.

DDIH is deacetylated in vivo and is excreted as a hydroquinone conjugate (Serif and Seymour 1963). This does not mean that DDIH did

not appear at some stage as the quinone, a relatively toxic compound.

DDIH could exert an effect as a quinone and in some way alter the calorigenic effect of T_4. Tata (1964 a) has stated that large doses of thyroid hormones exert their effect through uncoupling oxidative phosphorylation. The region of electron transport and the phosphorylation linked to cytochrome b has been studied by the blockage of other acceptors before and after this site. By this method it has been shown that electron transport through at least some portion of the NAD-flavoprotein or cytochrome b part of the electron transport system is required for thyroxine activity (Rall 1965). Speculation has it that thyroxine possibly effects the breakdown of one of the hypothetical electron carriers at the coupling sites of the respiratory chain (Ernster 1965). Quinones may stimulate or depress the rate of electron flow in the total sequence or differentially alter the rates in different segments of the sequence. They may affect oxidative phosphorylation by interferring with the generation of ATP directly or by an uncoupling action (Webb 1966). Quinones are also potent inhibitors of the glycolytic cycle affecting particularly hexokinase and 3-phosphoglyceraldehyde dehydrogenase (Webb 1966). Thus, DDIH could exert an effect at any of these loci.

There is also a possibility that DDIH could affect the metabolism of catecholamines exerting an effect at this locus. Substituted iodophenols have been shown to inhibit catechol-O-methyltransferase (D'Iorio and Mavrides 1963) an enzyme which is responsible in part for the degradation of catecholamines. DDIH might affect this enzyme. However the uncertainty as to where thyroxine and noradrenaline act

and the manner in which they interact make it practically impossible to predict where DDTH might exert its effect.

c. The Effect of Exposure to 30° or to 10°C on the OCR of Untreated Acclimated Mice.

In a number of experiments noradrenaline was administered to cold-acclimated animals and the OCR determinations were done at a higher ambient temperature.

Hsieh (1963) discusses the variety of responses reported concerning removal of cold-acclimated animals from their temperature of acclimation. Some workers report a higher resting OCR in cold- than warm-acclimated rats at 30°C. In various studies the OCRs have been based on body weight, body surface or fractions of body weight. Hsieh (1963) observed that cold-acclimated rats left at 28°C for six hours had an eighteen per cent higher metabolic rate than that of 28°C-acclimated rats, all measured at 30°C.

When acclimated mice were exposed to 30° or 10°C a number of differences were observed when the values were compared with those obtained using rats. Normal cold-acclimated mice, after ninety minutes of exposure to 30°C, had an OCR similar to that of warm-acclimated mice. In cold-acclimated rats it was only after twenty-four hours that the OCR dropped to a value similar to warm acclimated animals (Hsieh 1963). Normal warm-acclimated mice exposed to 10°C increased their OCR immediately to a value similar to that of cold-acclimated mice.

Thyroidectomized mice exposed to 30° or 10°C were unable to

make the rapid metabolic readjustment observed in normal mice. Warm-acclimated Tx mice exposed to 10°C increased their OCRs but the value was significantly lower than that of cold-acclimated Tx mice. Similarly cold-acclimated Tx mice after ninety minutes at 30°C decreased their OCR but it was still significantly higher than the OCR of warm-acclimated Tx mice. This fact that thyroidectomized mice were unable to adjust their OCR as rapidly as normal mice indicates the necessity of at least a minimal amount of thyroid hormones.

Since cold-acclimated mice adjusted to a change of temperature more rapidly than did rats, the possibility existed that mice might respond differently than rats when noradrenaline was administered and that various environmental conditions might affect this response. The next experiment was designed to show the effect of this temperature change on the response to noradrenaline and to enable comparison with the response of rats tested at 30°C.

d. The Effect of Exposure to 30° or to 10°C on the OCRs of Untreated and Pretreated Mice Tested with Noradrenaline.

Mice were pretreated with T_4 and then tested with noradrenaline after one hour of exposure. Warm-acclimated mice exposed to 10°C showed no sensitivity to noradrenaline. Depocas (1960) also observed that rats were insensitive to noradrenaline when first exposed and gradually became more sensitive after fifteen days in the cold. Pretreated (T_4) mice had a higher OCR than the untreated ones in the warm room, but their OCRs were similar after exposure to 10°C; they were also insensitive to noradrenaline indicating that a hypermetabolic

state does not "pre-acclimate" (Sellers and You 1950).

Noradrenaline had an inhibitory effect in thyroidectomized warm-acclimated mice exposed to 10°C. Noradrenaline is accepted as causing vasoconstriction at arterioles (Carlson 1966). In acute exposure tests such as these, the effect of noradrenaline is most probably vascular rather than metabolic. Animals rely heavily upon shivering when first placed in the cold; the vasoconstrictive effect of noradrenaline could decrease the calorigenic efficiency of shivering by lowering the blood supply to the skeletal muscles with a concomitant loss of heat production. T_4 administered prior to exposure prevented this noradrenaline inhibition. Pretreatment with thyroid hormones would in effect return these animals at least to the level of intact mice. These pretreated Tx mice did not show signs of increased sensitivity to noradrenaline apparent in normal mice.

Cold-acclimated mice tested at 10°C had shown sensitivity to the higher dosage of noradrenaline. However when they were transferred to 30°C for one hour before noradrenaline was administered, they lost this sensitivity. This is definitely a different response than that observed in rats. Hsieh and Carlson (1957) noted a ninety-three per cent increase in the OCR of rats after a single injection of noradrenaline, Heroux (1967) reported that cold-acclimated rats infused with 5.36 µg/kg/min noradrenaline for sixty minutes increased their OCR by 113 per cent. However when rabbits were similarly tested they showed only a forty-one per cent increase in OCR.

Cold-acclimated thyroidectomized mice tested with noradrenaline after one hour of exposure to 30°C were insensitive to 0.2 NA and

were in a state of collapse after 0.4 noradrenaline. After pretreatment with T_4 and the same higher dosage of noradrenaline these mice died. Although it was difficult to take accurate OCRs under these conditions, these mice appeared to increase their OCR before dying. Possibly circulatory failure and inadequate dissipation of the extra heat produced was the cause of death. These mice did not become hyperexcitable but seemed to lose energy, and collapsed. More experimental data is necessary to determine whether it was circulatory or metabolic effects or both that were responsible for death.

e. Hyperthyroidism and Cold-Acclimation.

There are a number of similarities between hyperthyroid and cold-acclimated animals. Thyroxine turnover is increased in the cold-acclimated rat, the thyroxine requirement to maintain growth and metabolism is doubled although animals can survive with minimal thyroxine levels; and TSH levels are high. The rise of plasma free fatty acids in response to noradrenaline is increased.

Heroux (1966 a) has warned against placing too great an emphasis on the role of the thyroid; although thyroid activity is increased in cold-acclimation, the actual role of this increase in thermogenesis has not yet been elucidated.

In the present experiment, warm-acclimated mice made hypermetabolic by injection of T_4 or T_3 were compared with untreated cold-acclimated mice to see if both responded to noradrenaline in a similar fashion. They did not.

Warm-acclimated mice made hypermetabolic by injection of T_4 or T_3 were more sensitive to 0.2 noradrenaline than untreated cold-acclimated mice. After T_3, warm-acclimated mice were still more sensitive to 0.4 noradrenaline than were cold-acclimated mice (even though these cold-acclimated mice were sensitive to 0.4 noradrenaline). When T_4 and 0.4 noradrenaline were used, both hypermetabolic warm- and untreated cold-acclimated mice responded similarly.

DDIH had no effect on this response.

These observations were opposite to those obtained by Hsieh et al. (1966). They observed that warm-acclimated rats rendered hypermetabolic with daily doses of T_3 reacted quite differently to noradrenaline infusion than did cold-acclimated rats. After thirty minutes of an infusion of 8 µg/kg, the OCR of cold-acclimated rats was much higher than that of hypermetabolic warm-acclimated rats similarly tested.

Warm-acclimated thyroidectomized mice pretreated with T_4 were more sensitive to 0.2 and 0.4 noradrenaline than were cold-acclimated animals. DDIH significantly lowered the sensitivity after which the pretreated warm- and untreated cold-acclimated mice responded similarly to noradrenaline. T_3 pretreated warm-acclimated mice were not more sensitive to either 0.2 and 0.4 noradrenaline than were untreated cold-acclimated mice.

DDIH enhanced the sensitivity to noradrenaline of these hypermetabolic warm-acclimated mice and had no such effect on the cold-acclimated ones.

These results indicate that a hypermetabolic state induced by cold differs from that induced by exogenous hormones.

D. SUMMARY AND CONCLUSIONS

1. Thyroid Hormones

a. Grouped animals

Comparison of results using the two methods of determining oxygen consumption revealed that grouping animals had an effect not only on their normal OCR but also on their response to treatment.

The only effect of the dosage of hormones was seen when T_4 was administered. The response was significantly lower when the smallest dosage was used. There was no such effect when T_3 was administered.

DDIH did not inhibit the calorigenic action of T_4 as previously reported and potentiated the action of certain doses of T_4 and T_3.

b. Single animals

Single animals responded similarly to either doses of hormone used and DDIH had no effect.

Warm-and cold-acclimated mice were equally sensitive to T_4.

Warm acclimated mice were more sensitive to T_3than cold-acclimated animals.

There did not appear to be any correlation between the rate of excretion of ^{131}I following injection of labeled

T_4 or T_3 and the increase in OCR.

DDIH did not affect the deiodination of T_4 as predicted by earlier workers. It is uncertain whether it had any effect on the metabolism of T_3 since the results from the experiments were not the same. In one DDIH lowered the deiodination of ^{131}I - T_3 and had no effect in the other.

When the OCR was followed for ninety-six hours, the effect of T_3 was slightly higher and wore off more rapidly than that of T_4.

c. Exposure of acclimated mice to 10° or 30°C.

Warm-acclimated mice exposed to 10°C increased their OCR to a value similar to that seen in cold-acclimated mice. Thyroidectomized mice increased their OCR but it was significantly lower than that of cold-acclimated mice. Normal and thyroidectomized cold-acclimated mice decreased their OCR within ninety minutes to the value observed in warm-acclimated mice.

2. Catecholamines

a. Noradrenaline test at the temperature of acclimation

Warm- and cold-acclimated mice were insensitive to 0.2 mg/kg noradrenaline, extremely sensitive to 0.6 mg/kg and only cold-acclimated mice were sensitive to 0.4 mg/kg

noradrenaline. Thyroidectomized cold-acclimated mice were not sensitive to 0.4 noradrenaline. Hormonal pretreatment in general enhanced the sensitivity of warm- and cold-acclimated mice.

DDIH seemed to inhibit the action of T_4 and enhance the action of T_3 on noradrenaline calorigenesis.

b. Noradrenaline test after exposure to 10° or 30°C.

Cold-acclimated mice sensitive to 0.4 noradrenaline when tested at the temperature of acclimation were insensitive after one hour in the warm room. This indicated a definite species difference between mice and rats. The fact that mice lost their sensitivity to noradrenaline so quickly seems to imply that the sympathetic nervous system, under continuous stimulation by the cold stress, plays a significant role in the nonshivering thermogenesis of mice. Also the immediate response by normal mice to a temperature change indicates the importance of doing these experiments under carefully controlled temperature conditions. This eliminates the possibility of attributing responses to noradrenaline which in effect are due to temperature changes.

A number of the responses observed when mice were tested with noradrenaline after exposure to 10° or 30°C may be due to actions other than the metabolic ones and further experimentation is necessary to clarify this.

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