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AGE-RELATED CHANGES IN THE PHYSIOLOGY OF TEMPERATURE REGULATION

by

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"Those who do not know the torment of the unknown cannot have the joy of discovery...Even in science itself, the known loses its attraction, while the unknown is always full of charm."

- Claude Bernard (1847)

ABSTRACT

Experiments were undertaken to investigate whether the thermal environment which an animal experiences in early postnatal life influences the development of temperature regulation. New Zealand White rabbits and Sprague Dawley rats were born and raised at 33.0°C. The colonic temperatures of animals reared in this way fell significantly during cold exposures which had no effects on the body temperature of control and warm-acclimated animals. Also, the febrile response of warm-reared rabbits was found to be significantly reduced in comparison to both control and warm-acclimated groups.

Investigation of the effector mechanisms of temperature regulation showed no deficits which could explain these modified thermoregulatory responses. Shivering was observed in warm-reared animals during cold exposure, and oxygen consumption was increased during fever. A deficit in non-shivering thermogenesis was observed in both warm-reared and warm-acclimated animals. This change was, therefore, a result of the normal acclimation process, rather than a specific effect of early environmental manipulation.

Microinjection studies in which putative thermoregulatory neurotransmitters were administered into the AH/POA of the brain showed changes in the effects on body temperature of noradrenaline, serotonin, and PGE_2 in warm-reared as compared to control and warm-acclimated rats. The thermoregulatory effects of dopamine were modified in both warm-acclimated and warm-reared animals. It was concluded that the hypothalamic mechanisms of body temperature control were altered by raising rats from birth at 33.0°C.

Evidence suggesting that the "critical period" for this thermoregulatory plasticity is during the first 30 days of life in the rat was obtained by examining the thermoregulatory responses of animals placed at 33.0^{o}C at a variety of different ages.

The effects of increasing age on the febrile response of the New Zealand White rabbit was examined. Reduced fevers were found to occur in rabbits greater than 3 yrs. old, when compared to animals less than 1 yr. of age. Plasma adrenaline concentrations during fever were significantly greater in old as compared to young animals, although the calorigenic actions of infused adrenaline and noradrenaline were not observed in the older group. However, this deficit was not the primary cause of the reduction in fever.

The results presented in this thesis demonstrate that the early postnatal thermal environment influences the development of temperature regulation. Also, fever and the thermoregulatory mechanisms associated with it are shown to be altered with increasing age.

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I. INTRODUCTION

The body temperature of the majority of mammals is maintained at a relatively constant level despite the wide variety of environmental temperatures experienced. The hibernators, during hibernation, are a notable exception to this general rule. The importance of the maintenance of body temperature was emphasized in 1805 by Currie who wrote, "that a few degrees of increase or diminution of the heat of the system, produces diseases and death." He concluded that, "A knowledge therefore of the laws which regulate the vital heat, seems to be the most important branch of physiology." Later in the nineteenth century the concept that body temperature was maintained as the result of a fine balance between the heat produced and the heat dissipated developed (Wünderlich, 1871).

In this introduction, the mechanisms by which the thermoregulatory systems of mammals regulate body temperature, despite a constantly changing environment, will be considered. Fever, the pathological process by which body temperature is elevated during disease, will then be examined, and this section will be followed by a review of our current understanding of the ontogeny of temperature regulation. Finally, recent experimental evidence will be presented which demonstrates clearly that the development of many sensory systems is significantly affected by the afferent input in early post-natal life, and this concept will be related to the temperature regulating system.

A. The Thermoregulatory System

The maintenance of body temperature depends on the ability of cold and warm receptors to monitor the absolute temperature, as well as changes in temperature, of both the internal and external environment. This thermal information is then integrated within the central nervous system, and ultimately elicits the relevant thermoregulatory responses by either the activation or inhibition of a variety of effector mechanisms. The system will be reviewed here in terms of these three major divisions.

1) The Afferent System

There are two functionally distinct types of cutaneous thermoreceptors, warm and cold receptors, which are classified according to the way in which their primary afferent fibres respond to changes in temperature. It was originally proposed that Krauses's endbulbs were the cold receptors, while Ruffini cylinders were the warm receptors (Bazett & McGlone, 1932; Kadanoff, 1966), although there is now evidence to suggest that this may not be the case (Hensel et al., 1974), at least in the case of the cold receptors.

The neurophysiological characteristics of these two types of thermal receptors are more clearly defined. In 1935 the first demonstration of cold receptors by electrophysiological means, suggested the primary afferent fibres to be myelinated A δ axons (Zotterman, 1935), while in the case of the warm receptors these fibres have been shown to be unmyelinated C fibres (Hensel, 1973a). The primary afferents of both receptors show static responses to temperature which may be described by the classical bell shaped curve (Hensel, 1966). The

primary afferents subserving cold receptors show maximal firing rates between 26 → 30°C, while those subserving warm receptors show optimum firing rates between 40 → 45°C (Hensel, 1973a). Thus, in terms of static firing properties, the only difference between the two groups of receptors is seen in the temperature to which they respond. In contrast, there are major differences between the dynamic response characteristics of warm and cold receptor afferents. The dynamic firing properties of the primary afferents are related to the rate of change of temperature at the receptor. Warm receptor fibres respond to increases in temperature with an increased firing rate, and to decreases in temperature with a reduction in firing rate. In contrast, cold receptor afferents decrease firing rate during increases in temperature and are excited during temperature falls (Hensel & Zotterman, 1951; Dodt & Zotterman, 1952; Iggo, 1969).

Thus, in terms of temperature sensation, there are two functionally distinct types of thermoreceptors which are able to signal four, rather than two, specific thermal sensations, although all are related to the same physical factor - the absolute temperature at the receptor. The warm receptors, when activated, will signal "heat" and, when silent, will signal "unheat" while in contrast the cold receptors will signal "cold" when silent and "uncold" when activated.

The primary afferent fibres from the warm and cold receptors enter the spinal cord through the dorsal roots and synapse in the dorsal horn. Second order neurons arise in this area and send axons across the midline in the anterior commissure to the anterior lateral portion of the lateral funiculus, forming the lateral spinothalamic tract. This tract then runs through the pons in the lateral margin of the medial

lemniscus to the second relay station in the thalamus. At this level of the nervous system, neurons subserving temperature reception show a more finely tuned response to the static properties of temperature at the receptor, that is each has a far more specific range of temperatures to which it responds (Jahns, 1975). The second major change in the functional characteristics of thalamic thermosensitive neurons is that, at this level, no dynamic response to peripheral temperature changes is observed (Hellon & Misra, 1973b; Martin & Manning, 1971). Thus, it would appear that integration of thermal inputs occurs, at least to some extent, in the spinal cord and brain stem. Also, there is evidence that the afferent thermal inputs which are responsible for the reflex vasodilatation in the hand during warming of the legs with radiant heat are anatomically located in the sympathetic chain (Cooper & Kerslake, 1953). Support for such a pathway is provided by work in which electrical stimulation of the lumbar sympathetic chain is shown to result in hand vasoconstriction (Cooper & Kerslake, 1955). One must presume that such integration will combine the input from both peripheral and visceral thermoreceptors throughout the body. Obviously, the comparison of temperature between these two groups of receptors will be the most important single factor in the normal control of body temperature. Neurons involved in thermal sensation then project from the thalamus to the sensory cortex. A second destination of thermal afferents from the thalamus, is believed to be the hypothalamus, although there is no definitive physiological evidence to support the existence of such a thermoregulatory pathway. Anatomical studies have demonstrated the existence of such pathways, but their thermoregulatory function tends to be assumed, more owing to the significance of the

hypothalamus in the control of body temperature, than due to direct physiological evidence.

2) The Role of the Hypothalamus in the Control of Body Temperature

The importance of the central nervous system in the control of normal body temperature became accepted during the latter part of the nineteenth century when the effects of various brain stem lesions on body temperature were examined (Richet, 1885; Ott, 1887). The acceptance of the hypothalamus as the major centre involved in the control of body temperature followed a number of studies which showed thermoregulation to be impaired after damage to this specific region of the brain (Bazett & Penfield, 1922; Keller & Hare, 1932; Bazett et al., 1933). Initially it was demonstrated that discrete lesions in the anterior hypothalamus specifically impaired thermoregulatory responses during heat stress (Teague & Ranson, 1936; Ranson et al., 1937) while lesions in the caudal part of the lateral hypothalamus caused prolonged hypothermia (Ranson et al., 1937; Clark et al., 1939). Thus, the concept of two brainstem centres for the regulation of body temperature, one responsible for heat loss, and one for heat production; originally proposed by Meyer (1913) was supported. However, it has been shown since that loss of the anterior hypothalamic preoptic area impairs regulation against the cold also (Andersson et al., 1965; Squires & Jacobson, 1968; Lipton et al., 1974; Veale and Cooper, 1975).

Lesions of the posterior hypothalamus have been shown to cause animals to become poikilothermic (Clark et al., 1939) but it has been suggested that this is due to a disruption of the motor pathways involved in temperature regulation (Satinoff, 1974). Also, a specific

impairment of behavioural thermoregulation has been demonstrated in rats in which the lateral hypothalamus has been destroyed (Satinoff & Shan, 1971).

The above evidence demonstrates clearly that it is no longer realistic to consider two anatomically and functionally distinct thermoregulatory centres in the hypothalamus, independently controlling heat production and heat loss.

i) Effects of Stimulation

Electrical stimulation of specific hypothalamic areas has been shown to elicit thermoregulatory responses. In the goat, stimulation of the preoptic area results in an inhibition of shivering, panting, and cutaneous vasodilatation (Andersson et al., 1956), while stimulation of the septum results in shivering, vasoconstriction, and piloerection (Andersson, 1957). In cats, Hemingway and co-workers (1954) demonstrated that stimulation of several other areas of the CNS inhibited shivering, whereas stimulation of the septum or the dorsal medial posterior hypothalamus resulted in intense electromyographic activity (Stuart et al., 1961).

ii) Thermoreceptors in the Central Nervous System

Experiments in which heating or cooling of the carotid blood supply by means of a cuff around the artery elicited thermoregulatory responses provided circumstantial evidence that thermosensitive structures are found within the brain (Kahn, 1904; Moorhouse, 1911). Newman and Wolstencroft (1960) showed changes in blood pressure and respiration which occurred when brain temperature reached 41.0^{o}C. They postulated these changes to be mediated by temperature sensitive structures in the medulla, as these effects were observed in the decerebrate, but not in

the spinal preparation. Downey et al. (1964) first approached this problem in the conscious animal and measured changes in oxygen consumption during warming or cooling of the blood in various vessels. They were able to elicit the largest changes by altering the temperature of the blood in the internal carotid artery, and thus, concluded "that an important collection of temperature sensitive receptors must lie in the territory supplied by the internal carotid artery."

Further support for specific thermosensitive areas in the central nervous system came from experiments in which thermodes were used to change the temperature of small areas of brain tissue. There is now clear evidence demonstrating that heating the anterior hypothalamus causes a fall in body temperature which is associated with sweating, panting, and vasodilatation (Beaton et al., 1941, Ingram et al., 1963; Hammel et al., 1963; Phillips & Jennings, 1972). In contrast, cooling of the same area results in shivering, vasoconstriction, reduction in respiratory rate, and a rise in body temperature (Freeman & Davis, 1959; Hammel et al., 1963; Calvert & Findlay, 1975). Also, behavioural thermoregulation has been shown to be influenced by anterior hypothalamic temperature manipulation (Satinoff, 1964; Carlisle, 1966). Other areas of the central nervous system which have been shown to be thermosensitive include: the medulla (Lipton, 1971; Lin & Chai, 1974); the midbrain (Hardy, 1969); the spinal cord (Thauer & Simon, 1972; Hensel et al., 1973); and the posterior hypothalamus (Edinger et al., 1969).

iii) Thermosensitive Neurons in the Hypothalamus

In 1950, von Euler first reported changes in the electrical potentials of the hypothalamus which were related to brain temperature. However, the first recordings of changes in the electrical activity of single nerve cells of the hypothalamus in response to local temperature changes were made by Nakayama et al. (1961). This group reported only warm sensitive neurons in the hypothalamus, i.e. neurons that increased their firing rate during local heating. Cold sensitive neurons, which respond to decreases in temperature with an increase in firing rate, were reported later by Stuart et al. (1963). Recent work has suggested that the cold sensitive units of the hypothalamus may be dependent on synaptic input from warm sensitive units for their thermosensitivity (Kelso et al., 1980), although there is also evidence against such a possibility (Hori et al., 1980). Intracellular recordings from neurons in the preoptic area of the green sunfish support the former hypothesis (Nelson & Ladd Prosser, 1981). These workers found a small percentage of warm-sensitive neurons which showed exponential firing rate responses to temperature changes. Such cells, discharged rhythmically, lacked visible synaptic input and showed slowly depolarizing potentials resulting in action potentials. These neurons may be true thermodetectors.

Neurons which change their firing rate in response to local temperature changes in the spinal cord (Simon, 1972; Hellon & Misra, 1973a; Iggo, 1974; Necker, 1975); the medulla (Lee & Chai, 1976) and in the sensorimotor cortex (Barker & Carpenter, 1970) have all been shown to exist. The exact significance of these thermosensitive neurons in the normal control of body temperature is not known, although it has

been suggested that they represent a hierarchical organization of the thermoregulatory system (Satinoff, 1978).

The above evidence quite clearly demonstrates the importance of the anterior hypothalamic/preoptic region of the brain in the control of body temperature. However, there are also data showing other regions of the brain to be involved in temperature regulation. Such findings provide a timely warning against consideration of the hypothalamus as the only "thermoregulatory centre" in the nervous system.

iv) Neurotransmitters and Hypothalamic Control of Body Temperature

There is an abundance of evidence suggesting a variety of neurotransmitters to be involved in the hypothalamic control of body temperature. In 1963, Feldberg & Myers microinjected small quantities of serotonin and noradrenaline into the cerebral ventricles of the unanaesthetized febrile cat. They observed that noradrenaline lowered body temperature, possibly owing to an inhibition of shivering, while serotonin caused an increase in body temperature which was accompanied by shivering. This was the first definitive evidence that these neurotransmitters were involved in thermoregulation. Since this time, much further evidence has emerged in the literature indicating a role for these, and other, putative neurotransmitters in the control of body temperature (reviewed by Feldberg, 1970; Bligh et al., 1971; Veale & Cooper, 1973; Myers , 1974b; Hellon, 1975). Noradrenaline, acetylcholine, serotonin, and dopamine have now gained general acceptance as hypothalamic thermoregulatory neurotransmitters. Unfortunately, the literature is cluttered with contradictions as to the effects of these substances when administered into the brain (for reviews see Clark, 1979; Clark & Clark, 1980a; Clark & Clark, 1980b).

Such inconsistencies may be explained by many factors including: species differences, use of anaesthetized as opposed to unanaesthetized preparations, dose response relationships and precise anatomical region of administration. However, if a single species (the rat) and a single route of administration (directly into the AH/POA) are considered, the available evidence repeatedly demonstrates the following effects: Noradrenaline microinjected in a dose of 5-20 µg causes a short latency hypothermia (Myers & Ruwe, 1978; Poole & Stephenson, 1979; Day et al., 1979; Metcalf & Myers, 1978; Ferguson et al., 1981). Similarly, both acetylcholine (10-50 µg) and carbachol (0.1-5 µg) when administered into the AH/POA cause short latency hypothermia (Beckman & Carlisle, 1969; Netherton et al., 1977; Poole & Stephenson, 1979). Dopamine (5-600 µg), when given into the hypothalamus, has similar effects (Cox & Lee, 1977,1979) Small, intrahypothalamically administered doses of serotonin (0.05-5.0 µg) cause rises in body temperature (Crawshaw, 1972). Although such studies provide some evidence as to the role of these neurotransmitters in the control of body temperature, a true understanding of the neurophysiological functions of these substances is not yet a reality.

The possible complexity of CNS control of body temperature has been highlighted recently by numerous studies in which; bombesin (Brown et al., 1977; Tache et al., 1979), β-endorphin (Martin & Bacino, 1978), thyrotropin releasing hormone (Brown & Vale, 1980), vasopressin (Kasting et al., 1979), as well as a number of other peptides and amino acids, have been implicated in the central nervous system control of body temperature (for review see, Clark, 1979; Metcalf et al., 1980).

v) The Set-Point for Body Temperature

All of the substances mentioned above, with the exception of vasopressin, share a common feature in their thermoregulatory actions. These materials all act to change body temperature in a way which does not alter the set-point of body temperature, i.e. the animal will always attempt to return its temperature to pre-injection levels. In 1970, Feldberg et al. demonstrated that hyperthermia could be induced by perfusing the lateral cerebral ventricle with salt solutions containing no calcium. The normal physiological concentration of calcium produced no changes in body temperature while high concentrations of calcium resulted in hypothermia. Myers & Veale (1970) push-pull perfused various regions of the brain with perfusates containing differing concentrations of sodium and calcium. They demonstrated that high calcium-low sodium, lowered the body temperature set-point when perfused in the posterior hypothalamus; while high sodium-low calcium raised the set-point when perfused in the same region. These workers proposed that the set-point was controlled by the ratio of Na/Ca ions in the posterior hypothalamus.

3) Effector Mechanisms of Temperature Regulation

Ultimately, the control of body temperature depends on the effector mechanisms by which it can be altered, that is the fine balance which is maintained between heat production, heat conservation, and heat loss, whether by behavioural or reflex thermoregulatory mechanisms.

i) Behavioural Thermoregulation

One of the most significant effector mechanisms involved in the normal maintainence of body temperature is the behaviour of the animal. In the ectotherms, for example, this is the only way in which body temperature can be controlled. In contrast, endothermic animals, which have the ability both to conserve and to produce heat, do not share this total dependence on behavioural means of thermoregulation of the ectothermic animals. Amongst the mammals, the human population is possibly now the most extensive utilizer of this particular thermoregulatory mechanism.

ii) Heat Production

The significance of heat production mechanisms to survival is quite clearly demonstrated by the fact that the majority of homeotherms maintain their body at a temperature significantly higher than that of the surrounding environment (Jansky, 1979). Thermogenesis is normally considered under two major classifications; shivering and non-shivering thermogenesis and will be discussed here under these two subdivisions.

a) Shivering Thermogenesis

Shivering has been described as "rhythmic involuntary movements of muscles, consisting of an oscillation about the mid point of one or several limbs, the movement not causing any change in body position" (Jung, 1941). Shivering is not a highly economical means of heat production as the increase in peripheral blood flow which accompanies the muscular activity means that only 48% of the heat produced is retained in the body (Hardy, 1961). Shivering may be induced by a reduction in either peripheral or core temperature and the difference between the two temperatures appears to exert some influence on the

intensity of the response (Benzinger, 1969). However, it has been demonstrated that changes in hypothalamic temperature are the most effective in terms of the stimulation of shivering (von Euler, 1961). Downey et al. (1964) showed the sensitivity of the hypothalamus to a reduction in temperature by cooling the blood in the internal carotid artery of the rabbit. They found increases in oxygen consumption and shivering in response to changes in blood temperature of as little as $0.2^{o}C$. It has been suggested that the true function of shivering thermogenesis may be to provide a coarse method of adjusting body temperature, while fine control is maintained by changes in both vasomotor tone, and non-shivering thermogenesis (Cooper, 1972).

b) Non-Shivering Thermogenesis

Claude Bernard (1876) first proposed that shivering was not the only source of heat production involved in the maintenance of thermal balance during cold exposure. He demonstrated cold induced heat production which persisted after spinal transection, and after blockade of the neuromuscular junction with curare. The importance of the sympathetic nervous system in non-shivering thermogenesis was established by Hsieh & Carlson (1957), who demonstrated also that this non-muscular heat production could be stimulated by both noradrenaline and adrenaline. This calorigenic response to intravenous infusion of the catecholamines is inhibited by the β-blocking agent propranolol (Bertin et al. 1968), suggesting a direct involvement of the β-adrenergic receptor.

A variety of tissues have been implicated as contributors to catecholamine induced non-shivering thermogenesis, including the liver (Grayson & Mendel, 1956), the kidney (Burlington, 1966), muscles (Jansky

& Hart, 1963), and brown adipose tissue (Smith, 1962). It is now clear that all of these tissues are involved in heat production by non-shivering mechanisms (Jansky, 1971). It is of interest that the particular significance of each of these tissues in non-shivering thermogenesis may change with age; for example, in newborn rabbits, about 66% of non-shivering heat production has its origin in the brown adipose tissue (Heim & Hull, 1966), while the thermogenic significance of this tissue in the adult may be substantially less (Jansky, 1971). However, non-shivering thermogenic capacity has been shown to be significantly potentiated in adult rats by cold exposure, and this effect may be, at least in part, due to an increased amount of brown adipose tissue (Foster & Frydman, 1979). Non-shivering thermogenic mechanisms have now been shown to be activated by electrical stimulation of the ventromedial hypothalamus (Perkins et al., 1981).

The sympathetic nervous system is only one of several hormonal systems activated during non-shivering thermogenesis. Thyroxine is known to increase during long term cold exposure, although non-shivering thermogenesis is still observed in thyroidectomized cold-adapted rats (Jansky, 1979). It has been suggested that one important function of this hormone during cold exposure may be to potentiate the calorigenic effects of the catecholamines (Swanson, 1956; Arner et al., 1971).

Other hormones which have been implicated in non-shivering thermogenesis include ACTH, growth hormone, corticosterone, glucagon and insulin (Jansky, 1973).

iii) Heat Conservation and Heat Loss

Alterations in peripheral vasomotor tone (PVMT) result in changes in blood flow in the distal extremities of the skin. Such changes are

mediated by the sympathetic nervous system (Lewis, 1927), and may result in increases in either heat conservation or heat loss. It has been suggested that only the blood flow to the extremities of the body, such as fingers and ears, is under the direct control of the nervous system (Grayson & Kuehn, 1979). It has been demonstrated also that vasoconstriction in the extremities is not directly controlled by the core temperature, rather the core temperature sets the sensitivity of the periphery to changes in temperature; i.e., a subject with reduced core temperature will produce vasoconstriction to a cold stimulus which would have no effect if body temperature was normal (Grayson, 1949).

Other thermoregulatory mechanisms of importance in the control of body temperature by heat loss include sweating and panting (Bligh, 1973). The former mechanism is activated during increases in core temperature and results in the discharge of fluid onto the skin from where it evaporates, this process being mediated by acetylcholine (Grayson & Kuehn, 1979). Panting may be simply defined as "the use of forced movement of air over the moist surfaces of the upper respiratory tract to facilitate evaporative heat loss" (Bligh, 1973). The use of the respiratory surfaces as a method of "injecting" heat into hypothermic victims (Lloyd, 1971) provides an interesting clinical application of this particular thermoregulatory mechanism.

4) Acclimatization & Acclimation

In 1970, Bruck et al. stated "There is no no doubt that warm blooded animals and man are able to adapt to stressful climatic conditions. Our knowledge is quite incomplete, however, as for the principles and basic mechanisms of the numerous modifications occurring in the course of long term adaptation." This adaptation may be referred

to by one of two terms, depending on its exact nature. Acclimatization is the process by which an animal adapts to a new natural environment, and all of the factors involved in that environment. In contrast, acclimation refers to the laboratory situation where a single environmental factor may be changed, while others remain constant. For example, the processes involved in the acclimatization of an animal which moves from a temperate to a tropical environment may not be identical to the changes which take place during warm acclimation of the same species of animal under laboratory conditions. A number of thermoregulatory changes will now be considered which are thought to occur during both acclimatization and acclimation. Chronic heat exposure results in many changes in the thermoregulatory system. In man, there is no significant increase in the capacity to lose heat from the skin (Ladell, 1964). However, sweating is increased (Bligh, 1973) and it has been suggested that the increased water loss which occurs as a result of this sweating may be responsible for elevations in plasma vasopressin during the acclimation process (Sief & Robinson, 1979; Harrison et al., 1981). Basal metabolic rate has been shown to change only slightly during heat exposure (Collins & Wiener, 1968), however, these workers point out that a 10% reduction in the basal metabolic rate could raise the air temperature at which survival is possible by some 2.0^{o}C. It is not known whether changes in thyroid hormone levels underlie these changes in the metabolic rate.

Cold acclimatization has been demonstrated to result in a decrease in the critical temperature (the temperature at which increased heat production is necessary to balance heat production and heat loss) (Gelineo, 1934). Presumably, such an effect is due to either a

reduction in thermal conductivity or an increase in the basal metabolic rate. There is little evidence to support the former possibility ; and no consensus of opinion regarding the latter . Some studies show no change in basal metabolic rate (Scholander et al., 1950; Heroux et al., 1959), while others report an increase (Hart, 1957; Heroux, 1967). However, possibly the best understood, and most significant factor in cold acclimatization and acclimation is the increase in non-shivering thermogenic capacity which occurs. This possibility was first proposed by Bernard (1876) and more recent work has demonstrated that cold acclimation increases the β-adrenergic receptor mediated non-shivering thermogenic capacity (Depocas, 1960; Bertin et al, 1968).

B. Fever

Fever is the pathological process by which regulated body temperature is elevated during infection. Hippocrates, despite his recognition of fever, considered the febrile response to be a disease in its own right (in Atkins & Bodel, 1979). In 1871, Wünderlich first commented on the possible diagnostic value of fever, "Because one can conclude, without hesitation, from an aberration of temperature on the presence of a disorder." Some eighteen years later, Welch (1888) hypothesized that the central nervous system controlled body temperature in health as well as during fever. He suggested that bacterial pyrogens acted indirectly through the release of a leucocytic "ferment" in causing fever, and concluded that fever may be beneficial to survival during infection (in Atkins & Bodel, 1979). Over the past century, a vast body of research has advanced the understanding of fever to the state of one of the more recent models proposed illustrated in Fig. 1 (Kasting, 1980).

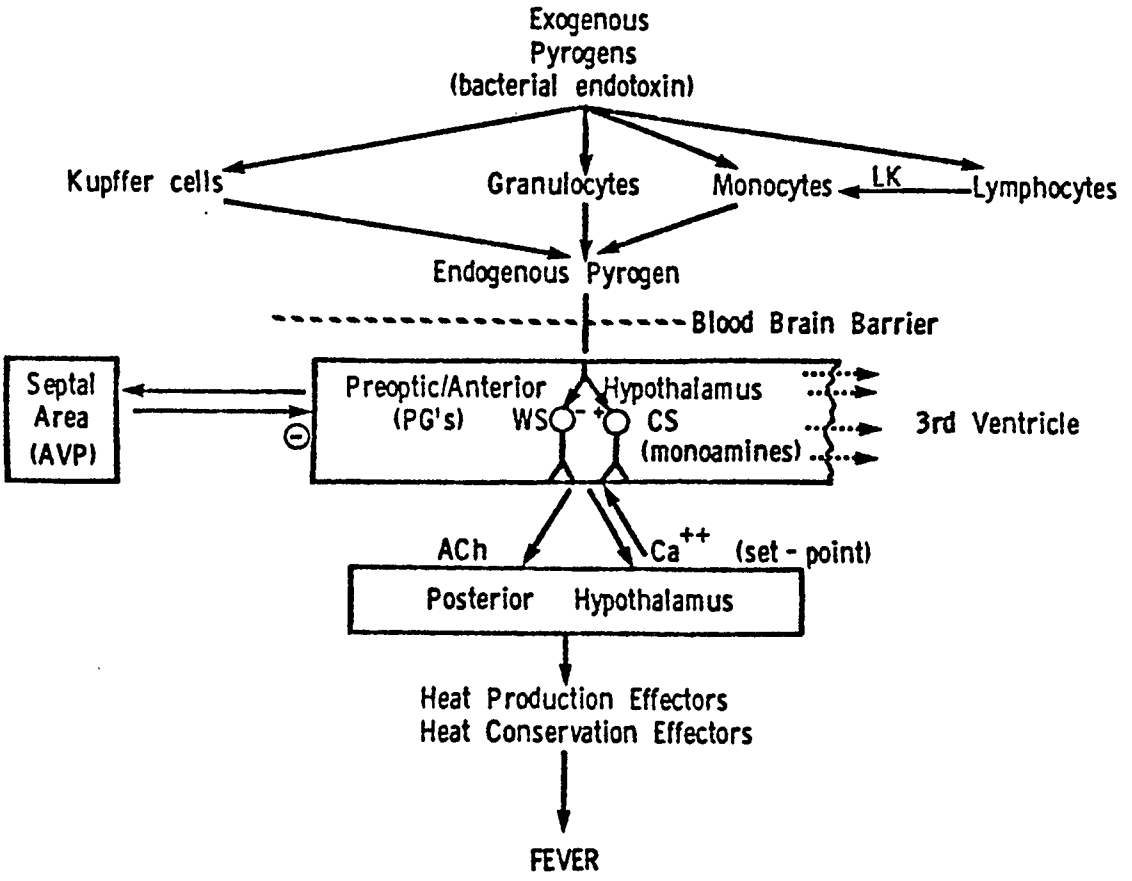


Figure 1: A schematic diagram showing the sequence of steps involved in the genesis of fever. LK, lymphokine: PG's, prostaglandins; WS, warm sensitive neurons; CS, cold sensitive neurons; ACh, acetylcholine; AVP, arginine vasopressin (from Kasting 1980).

1) Pyrogens

The general term used to describe fever causing materials is pyrogen. Any substance which results in fever following administration to experimental animals or humans is said to be pyrogenic. Exogenous pyrogens originate outside the host and show great diversity (Dinarello, 1979), although the most frequently utilized is the bacterial endotoxin derived from gram negative bacteria. This is the most potent of the exogenous pyrogens. A dose of 5 ng/kg results in fever in both rabbits and humans (Dinarello & Wolff, 1976; Dinarello et al, 1978). Exogenous pyrogens result in the production of endogenous pyrogen (E.P.), a heat labile protein with a molecular weight of approximately 15,000 daltons (Dinarello & Wolff, 1977) which may be present in the plasma as a trimer (Dinarello, 1980). EP has been shown to be produced by many different reticuloendothelial system cell types, including: blood granulocytes (Beeson, 1948), monocytes (Bodel & Atkins, 1967), eosinophils (Mickenberg et al., 1972), peritoneal exudate macrophages (Hahn et al., 1967), and hepatic Kupffer cells (Gander & Goodale, 1975). It is thought that EP is carried in the circulation from its various sites of production to the brain, where it acts on neurons in the anterior hypothalamic/preoptic area (AH/POA) to produce fever.

2) The Role of the Central Nervous System in Fever

The AH/POA has been shown to be the only site in the brain sensitive to pyrogenic effects of both exogenous (Bennett et al., 1957) and endogenous (Cooper et al., 1967) pyrogens. The spontaneous activity of both warm and cold sensitive neurons in the AH/POA has been demonstrated to be affected by the administration of exogenous pyrogens (Wit & Wang, 1968; Nakayama & Hori, 1973). The former show a decrease

in their firing rate while the latter show an increase. Such changes in activity are similar to those observed during cold exposure. In fact, it has been suggested that pyrogens might modulate the central pathways leading from the skin thermoreceptors (Hensel, 1973b) and that the chill sensation which follows intravenous administration of endotoxin in humans may be essential to the development of the normal febrile response (Guieu & Hellon, 1980). Fever is known to raise the set-point around which body temperature is regulated (Cooper et al., 1964) and may be functionally distinguished from hyperthermia (an undefended rise in body temperature) in that the ratio of Na^{+}:Ca^{++} in the posterior hypothalamus is elevated during fever as assessed by measurement of $^{45}Ca^{++}$ and $^{22}Na^{+}$ efflux into the third ventricle (Myers, 1974a).

A number of different neurotransmitters have been implicated in the hypothalamic pathways involved in the development of fever. Evidence demonstrating noradrenaline, serotonin, and acetylcholine involvement in thermoregulatory pathways has already been discussed and these transmitters' functions appear to be similar during fever. The role of prostaglandins in the development of the febrile response has received much attention in recent years. In 1971, prostaglandins of the E series (PGE) were demonstrated to have potent pyrogenic effects when microinjected into the cerebral ventricles of many different species (Feldberg & Saxena, 1971a; Milton & Wendlandt, 1971). The prostaglandins have since been shown to act directly in the AH/POA (Feldberg & Saxena, 1971b; Stitt, 1973; Veale & Cooper, 1975), and PGE levels have been found to increase in the CSF during pyrogen fevers (Feldberg & Gupta, 1973).

Evidence against PGE involvement in fever includes studies in which, following lesion of the AH/POA, PGE no longer caused fevers while a febrile response was observed following intravenous administration of endogenous pyrogen (Veale & Cooper, 1975). Cranston et al. (1976) have demonstrated that specific PGE antagonists antagonize only PGE fevers while arachadonic acid and endogenous pyrogen fevers were unaffected. These studies suggest that a close derivative of PGE, rather than the substance itself, may be involved in the febrile response.

Another putative neurotransmitter specifically involved in the control of fever is vasopressin. This peptide has been shown to act as an endogenous antipyretic in the brain of both the sheep (Kasting et al., 1978) and the rat (Eagan and Veale, 1982) and, as such, forms a negative feedback loop in the control of fever.

3) The Survival Value of Fever

Over the past decade the concept that fever may be beneficial to survival during infection has received support from several experimental studies. However, the idea of an adaptive value for fever is by no means new. As early as the seventeenth century the English physician Thomas Sydenham suggested that "Fever is a mighty engine which Nature brings into the world for the conquest of her enemies" (in De Kruif and Simpson, 1940). Similarly, in 1946, Dubois stated that "Fever is merely a symptom and we are not sure that it is an enemy. It may be a friend." More recently, Bennett & Nicastri (1960) carried out an extensive review of the literature but reached no positive conclusions as to whether or not fever may serve as a mechanism of resistance.

Experiments utilizing lower vertebrates provided some of the first evidence that fever may play a role in survival during infection.

Although all ectotherms do not appear to be capable of developing fever (Laburn et al., 1981), the lizard, Dipsosaurus dorsalis, which regulates its body temperature by behavioural means, will develop a fever in response to endotoxin by such means if they are available (Vaughn et al., 1974). Thus, fever may be prevented in these animals by altering the external environment available to them. When this was done after administration of live bacteria, the majority of febrile animals survived, while most afebrile animals died (Kluger et al., 1975).

More recent studies have extended such observations to a mammalian species, the New Zealand White rabbit. The survival of animals infected with live Pasteurella multicida was correlated with the fever height and the results indicated that fever, up to a certain level, was beneficial to survival, whereas, above this level, it was detrimental (Kluger & Vaughn, 1978). Also, it was found that the reduction of fever by the administration of antipyretics directly into the anterior hypothalamic/preoptic area of the brain resulted in an increase in the mortality rate during infection (Vaughn et al., 1980).

The mechanisms underlying this fever related resistance to infection are not known, although evidence exists to support several possibilities. The phagocytic activity of leukocytes has been demonstrated to be potentiated in humans and guinea pigs at febrile as compared to afebrile temperatures (Ellingson & Clark, 1942). Circulating iron concentrations have been shown to be reduced during fever (Kampschmidt et al., 1973), which may be of significance in view of the requirement of many bacteria for iron during growth (Weinberg, 1978).

Thus, the above evidence indicates that an experimental basis now exists to support the concept that fever may act to enhance the resistance of the host organism during infection.

C. Age-Related Changes in Temperature Regulation

1) Temperature Regulation in the Newborn

There is much evidence demonstrating that the temperature of the foetus *in utero* is 0.3 - 0.8°C above maternal temperature (Abrams et al., 1969; Pittman et al., 1973). Also, it has been suggested that heat production in the foetus may be 1.6 - 2.0 times that of the adult sheep when calculated on a per kg. basis (Abrams et al., 1970). These data provide evidence that the foetus is constantly losing heat to the mother. However, it has been shown also that when maternal temperature is elevated, either during heat stress (Morishima et al., 1975), or during pyrogen fever (Pittman et al, 1975), the foetal maternal temperature gradient is maintained. Such maintenance of this gradient, specifically in the former case, clearly suggests that the foetus *in utero* is not actively regulating its temperature. However, newborn lambs do shiver and increase metabolic heat production during cold exposure, as does the newborn rat, rabbit, guinea pig, dog, cat and human (Alexander et al., 1972; Bruck, 1961). The role of brown adipose tissue in metabolic heat production is of great significance in the newborn (Hull, 1966). The large amounts of brown adipose tissue found in neonatal animals apparently regress rapidly unless animals are reared in a cold environment (Zeisberger et al., 1967). Similarly, the ability to lose heat appears to be functional in the newborn (Bruck, 1961; Foster et al., 1969; Hensel et al., 1973). Also, peripheral vasomotor

tone is increased in neonates during cold exposure (Bruck, 1961).

The newborn regulates its body temperature at approximately the same level as the adult (Veale et al., 1979), although neonatal temperature often fluctuates far more (Hensel et al., 1973).

Thus, the majority of evidence indicates that the newborn mammal possesses many functional thermoregulatory effectors. It may well be that the problems the newborn encounter in the regulation of body temperature are more related to physical factors, such as high surface to mass ratio (Bruck, 1961), or decreased insulation (Hill, 1961) rather than to a direct immaturity of thermoregulatory mechanisms.

2) Fever in Neonatal Animals

The febrile response of newborn humans (Epstein et al., 1971; Smith et al., 1956), guinea pigs (Blatteis, 1975), rabbits (Szekely & Szelenyi, 1979b), and lambs (Pittman et al., 1974) have all been found to be reduced in comparison to the adult of the species.

There is evidence which suggests that a sensitization process may be a part of the development of the normal febrile response mechanisms. Pittman et al. (1974, 1975) demonstrated that sensitized 60 hr. old lambs developed a significantly larger fever to endotoxin than animals which had not been sensitized (sensitization involved a prior endotoxin treatment at 4 hrs. of age). Similarly, Podoprigori (1978) reported that mini-pigs raised in a germ-free environment were unable to develop fevers when 3 weeks old.

Thus, the febrile response mechanism of the newborn is not fully functional despite the already discussed maturity of many of the thermoregulatory effector mechanisms at birth.

3) Thermoregulation in the Aged

The increased incidence of both hypothermia (Taylor, 1964) and hyperthermia (Butler & Shalowitz, 1978) in the aged human population indicates that changes in thermoregulatory function occur with increasing age. Such an idea is not totally new, in fact, the writings of Hippocrates tell us that, "In old persons the heat is feeble.... On this account, also, fevers in old persons are not equally acute, because their bodies are cold" (in Benzinger, 1977).

There is little experimental evidence to support this concept apart from a small number of clinical studies in which thermoregulatory ability has been assessed by a variety of different means. The variability in methodology, age groupings, and subject history makes general interpretation of these studies difficult, however, some relevant findings will be reviewed in this section.

There is conflicting evidence as to whether or not resting body temperature is changed in the elderly population. Finch & Hayflick (1977) found no evidence to support the concept that normal body temperature is altered with increasing age, while other groups have reported reduced temperatures in a section of the elderly population (Fox et al., 1973; Collacott, 1975).

There are some experimental data which suggest that old people have a reduced ability to feel the cold. Such data were obtained by simple thermal preference tests in which both young and old subjects were placed at low temperatures ($10^{o}C$), the former complained of discomfort in the cold, while the latter group did not (Horvath et al., 1955; Watts, 1972; Collins et al., 1977). Changes in thermoregulatory effector mechanisms have been reported also in old human subjects.

Collins et al. (1977) found that out of 43 elderly people placed in the cold for 15 mins, only four were observed to shiver. The short time period of exposure rules this test out, however, as a definitive test of the ability to shiver in these subjects. Other studies have suggested deficits in shivering ability in the elderly (McMillan et al., 1967; Horvath, 1955). Interestingly, MacMillan's group were able to show that aged subjects with a previous history of hypothermia were unable to shiver while, in old people with no such history, shivering was observed during cold exposure. Also, in the same study these workers demonstrated that during cold exposure the former group did not increase O_2 consumption while the latter did by an average of 67%.

The importance of peripheral vasomotor tone to the regulation of body temperature has already been discussed. Deficits in the control of this mechanism have been reported in the elderly. Old subjects who demonstrate no vasoconstriction in the cold and/or no vasodilatation in the warm are not uncommon (MacMillan et al., 1967; Collins et al., 1977). These changes could be due to alterations in the sensitivity of peripheral blood vessels to vasoconstrictor or vasodilator substances with increasing age, as has been demonstrated recently in animal studies (Fleisch et al., 1980).

Although the etiology of age-related changes in the thermoregulatory system is not well understood, the evidence which suggests such changes do occur is convincing. As Collins et al. (1977) stated recently, "Indeed accidental hypothermia is now recognized as one of the natural hazards of old age."

D. Developmental Plasticity

The concept that the early environment which an animal experiences may influence the development of the nervous system is not new. Charles Darwin (1874) speculated that, "The brains of domestic rabbits are considerably reduced in bulk in comparison with those of the wild rabbit or hare and this may be attributed to their having been closely confined during many generations so that they have exerted their intellect, instincts, senses, and voluntary movements but little." Similarly, Spurzheim (1815) and Cajal (1895) suggested that experience may play a role in the development of the nervous system. The first scientific evidence really to support this hypothesis was provided by the findings that rats raised in an isolated environment developed significantly smaller brains than littermate controls raised in an enriched environment (Bennett et al., 1964).

Since this initial report, a number of other studies have demonstrated the importance of sensory input in early life to the development of the nervous system. The functional characteristics of single neurons in the visual cortex have been shown to be modified by visual deprivation of the animal in early life (Hubel & Wiesel, 1965; Hirsch & Spinelli, 1970; Blakemore & Cooper, 1970; Shlaer, 1971; Pettigrew & Freeman, 1973; Olson & Pettigrew, 1974). Visual deprivation only results in such effects if it occurs during the early "critical period" of development (Hubel & Wiesel, 1970; Blakemore & Van Sluyters, 1975), or so it was originally believed. There is evidence, however, which suggests that this "critical period" may be simply the period in the animals' life when it is most sensitive to environmental deprivation, and that some degree of recovery does occur if the animal

receives normal visual experience after visual deprivation throughout the "critical period" (Cynader & Mitchell, 1980; Cynader et al., 1980; Juraska et al., 1980; Olson & Freeman, 1980; Spear et al, 1980; Timney et al., 1980).

Although modified synaptic populations have been reported in the striate cortex following visual deprivation (Rothblat & Schwartz, 1979), a much clearer example of anatomical plasticity is found in the work of Van Der Loos and Woolsey (1973). These workers demonstrated that selective injury to individual mouse vibrissae at birth influences the development of the specific region of somatosensory cortex to which that vibrissae would normally project, in that the differentiation of the cortical barrel does not occur. The "critical period" for this effect of vibrissal damage has been shown to be during the first five days of postnatal life (Weller & Johnson, 1975; Woolsey & Wann, 1976).

Although the above are probably two of the best understood examples of developmental plasticity in the mammalian central nervous system, there is considerable additional evidence to support the hypothesis that the early environment influences the development of the nervous system. Knudsen et al. (1982) have demonstrated that the auditory experience of the barn owl in early life modifies the ability of this animal to localize sound in later life. Graziadei et al. (1979) show a recovery of olfactory function following unilateral bulbectomy in neonatal mice which does not occur in the adult. Also, exercise during postnatal development has been demonstrated to induce an increase in the size of the dendritic trees of Purkinje cells of the cerebellum (Pysh & Weiss, 1979; Floeter & Greenough, 1979).

The cellular mechanisms controlling developmental plasticity are poorly understood. However, there is now some experimental evidence to suggest that the duration of the critical period during visual cortical development may be under the control of the neurotransmitters noradrenaline and serotonin (Kasamatsu & Pettigrew, 1976). These workers demonstrated that 6-hydroxydopamine perfusion over the kitten's visual cortex blocked the normal visual cortical changes observed following deprivation; serotonin had the same effect. Similarly, cortical noradrenaline perfusion in the adult cat was found to induce a phase of plasticity during which deprivation induced changes similar to those observed in the neonate were shown to occur.

Further evidence supporting the possible role of chemical mediators in the development of the central nervous system comes from an understanding of the processes involved in the sexual differentiation of the brain. It is now well established that estradiol in specific areas of the brain is intimately involved in the normal development of a phenotypic male during an early critical period in development (Korenbrot et al., 1975; Clemens et al., 1978). In the rat, regardless of genetic sex, a phenotypic female will develop if this steroid hormone does not reach these areas of the brain during the first 10 days of life. The long-term anatomical effects of such a brief exposure to this hormone are so profound as to induce the development of the sexually dimorphic nucleus of the medial preoptic area (Gorski et al., 1978; Gorski et al., 1980; Jacobson et al., 1980).

Thus, it is now well established that a variety of both internal and external factors during early life play a significant role in the development of the nervous system.

E. Rationale for this Research

This work was designed to examine the effect of restricting thermal experience on the normal development of the thermoregulatory system and thus to establish whether a period of "thermoregulatory plasticity" occurs during early life. The problem has been approached by raising both New Zealand White rabbits and Sprague Dawley rats at an environmental temperature of 33.0°C from birth. Such a thermal environment will result in minimal cold stimulation. The functional characteristics of the thermoregulatory system of animals reared in this manner may then be examined in later life.

In order to clearly demonstrate developmental plasticity in the thermoregulatory system, it is essential to show that animals which have been acclimated to this elevated environmental temperature as adults (warm acclimated) do not display similar thermoregulatory deficits to warm reared animals which were born at high temperature. Changes observed in both these groups may be considered to be an effect of the normal acclimation process, while changes found in the latter group only reflect the effects of the differential environmental experience in early life.

A second part of this thesis has examined the changes in the thermoregulatory system which occur with increasing age and many parallels will be shown to exist between the effects of age and those of cold deprivation on the ontogeny of temperature regulation.

II. General Materials & Methods

Male and female New Zealand White rabbits or Sprague Dawley rats were used in these experiments. Three groups of animals were utilized, two control and one experimental. The latter group were raised from birth in an environmentally controlled chamber maintained at 33.0 ± 1.0°C with a relative humidity of 50% and on a 12 hour light/dark cycle. Food and water at a temperature of 33.0°C were provided ad libitum. In order that these animals would be born under these environmental conditions, pregnant females were placed in the environmental chamber at least 7 days before term. They gave birth to their litters at an air temperature of 33.0°C and tended their young until weaning, which took place at 28 days postpartum in the case of the Sprague Dawley rats and at 40 days of age for New Zealand White rabbits. Animals raised in this way will be referred to as the "warm reared" group.

The "control" group of animals were raised in the same manner as the warm reared group except that the environmental temperature was 20.0 ± 1.0°C. All experiments performed on these two groups were carried out when the animals were 90 - 240 days of age.

A third, "warm acclimated", group was employed in these studies. These animals were raised in the same way as the control group and, when adult, were placed in the environmental chamber at an air temperature of 33.0°C for a minimum time period of 3 months. Thus, by necessity, the age of this group of animals when experiments were performed, was approximately 6 months greater than that of the other two groups. The postnatal experience of these three groups of animals is summarized in Table 1.

TABLE 1: SUMMARY OF THE ENVIRONMENTAL HISTORY OF EXPERIMENTAL GROUPS OF ANIMALS.

GROUP	HISTORY
Warm Reared	33° C Only
Warm Acclimated	20° C→Adult Then minimum 3 months at 33° C
Control	20° C Only (Same age as warm reared)

Throughout all experimental procedures, the movement of New Zealand White rabbits was restricted by the use of conventional neck restrainers (Hampton et al., 1973). The Sprague Dawley rats were allowed complete freedom of movement. Colonic temperature was monitored continuously throughout all experiments by use of a Yellow Springs thermistor probe (YS 401) inserted 7.0 cm beyond the anus, and gently taped to the tail. Permanent records of these temperature recordings were made by use of a Beckman Dynograph (R 612). In experiments where ear skin temperature was monitored, another thermistor was taped to the ear (YS 709) and a digital readout of temperature was obtained (United Systems Digitec) at 5 minute intervals. These recording systems were accurate to ± 0.1^{o}C. The dynograph and thermistor probes were calibrated in a stirred water bath, the temperature of which was measured with a mercury thermometer calibrated at 0.1^{o}C intervals. Prior to all experimental procedures, a baseline body temperature recording was obtained for a minimum time period of 60 minutes at room temperature (21.0 ± 2.0^{o}C). All experiments were carried out between 9:00 a.m. and 6:00 p.m..

Statistics

All temperature data are presented as the mean ± the standard error of the mean for grouped data. In some cases these measurements are normalized, while in others they are given in the units as measured. The body temperature responses of different groups of animals were statistically compared by use of the unpaired Student's "t" test. In the majority of cases, the areas below the temperature response curves were calculated for each animal and then used for this comparison. In

some experiments changes in temperature at specific times were compared by this statistical test. Unless otherwise stated, the former of these two methods was used. The paired Student's "t" test was used to test for significance in experiments where data from the same group of animals, during different experimental procedures, was evaluated.

Throughout this dissertation, a p value of < 0.05 will be taken to show statistically significant differences between groups of data.

III. Temperature Regulation in Warm Reared Animals

In order to assess whether the reduced cold stimulation in early life affected the development of thermoregulation in warm reared animals, this group was exposed initially to a number of simple tests of their ability to control body temperature. These included exposure to low and high environmental temperatures and an evaluation of their febrile responses to exogenous pyrogens.

Methods

New Zealand White rabbits and Sprague Dawley rats from all three experimental groups were exposed to an environmental temperature of 2.0 ± 1.0°C for time periods of 240 mins and 270 mins respectively. After cold exposure, both warm reared and warm acclimated animals were returned to the environmental chamber, while the control group was returned to their normal 20.0°C environment. Two days later, all three groups of rats were again exposed to the cold.

As a heat stress, control and warm reared Sprague Dawley rats were exposed to an ambient temperature of 38.0 ± 1.0°C for a time period of 300 mins.

In all three groups of New Zealand White rabbits, fever was induced at room temperature by intravenous injection of endotoxin derived from Salmonella abortus equi (Difco) through the lateral ear vein. A dose of 0.2 µg was administered to young animals, while the older warm acclimated group and their age matched controls received a dose of 0.25 µg. In the Sprague Dawley rat, endotoxin derived from Escherichia coli (Difco) was administered intraperitoneally in a dose of 100 µg and the changes in colonic temperature were recorded.

Results

The changes in colonic temperature recorded in New Zealand White rabbits during a 4 hr cold exposure are presented in Fig 2. The data show that in the warm-reared animals there was an average fall in colonic temperature of 2.7 ± 0.5^{o}C which was significantly different to that observed in warm acclimated and control groups, both of which maintained colonic temperature throughout cold exposure ($p < 0.01$).

Similar falls in body temperature were observed in warm-reared Sprague Dawley rats during cold exposure as illustrated in Fig 3. The data presented here show the change in colonic temperature during first and second exposure to the cold 270 mins after the animals were placed in the cold as compared to baseline recordings. Significant falls in colonic temperature occurred in both warm-reared (-6.9 ± 0.9^{o}C) and warm acclimated (-3.0 ± 0.5^{o}C) groups, but not in control rats (+0.4 ± 0.1^{o}C) during first cold exposure. In contrast, body temperatures fell significantly only in the warm-reared group of animals during the second cold exposure 2 days later (-6.5 ± 1.05^{o}C).

In Fig 4, the mean colonic temperatures of warm reared and control rats during heat stress are presented graphically. No significant differences between the rises in colonic temperatures of these two groups of animals were observed.

The normal febrile response of the rabbit to intravenous administration of endotoxin is a biphasic rise in body temperature. Such febrile responses were observed in the control group of animals as shown in Fig 5. The first peak of fever occurred 60 - 90 mins, and the second peak approximately 180 mins after the administration of endotoxin. The febrile responses of warm-reared animals are presented

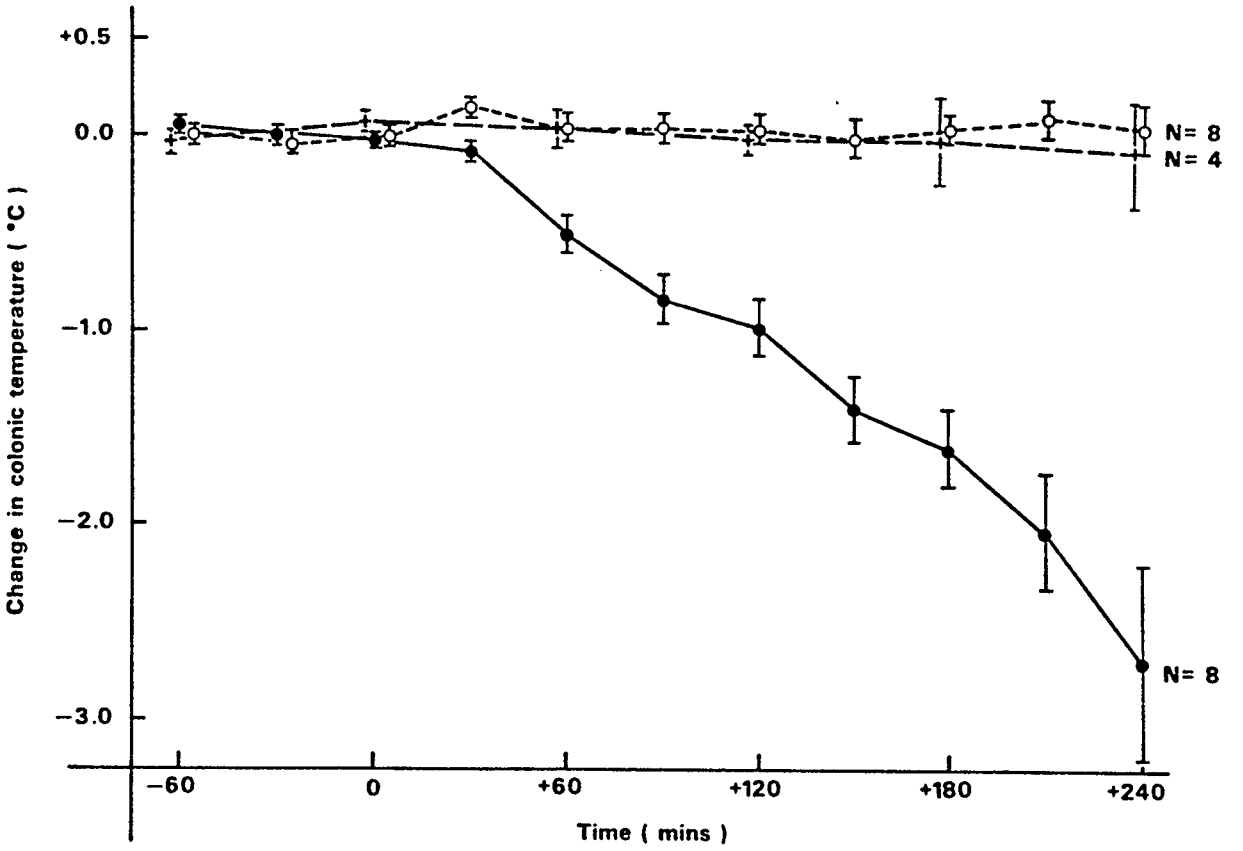


Figure 2: Records of changes in colonic temperature of warm-reared (●—●), warm-acclimated (+- -+), and control (○- -○) rabbits during a 4.0 hr cold exposure. Each point represents the mean and the vertical bars show the standard error of the mean.

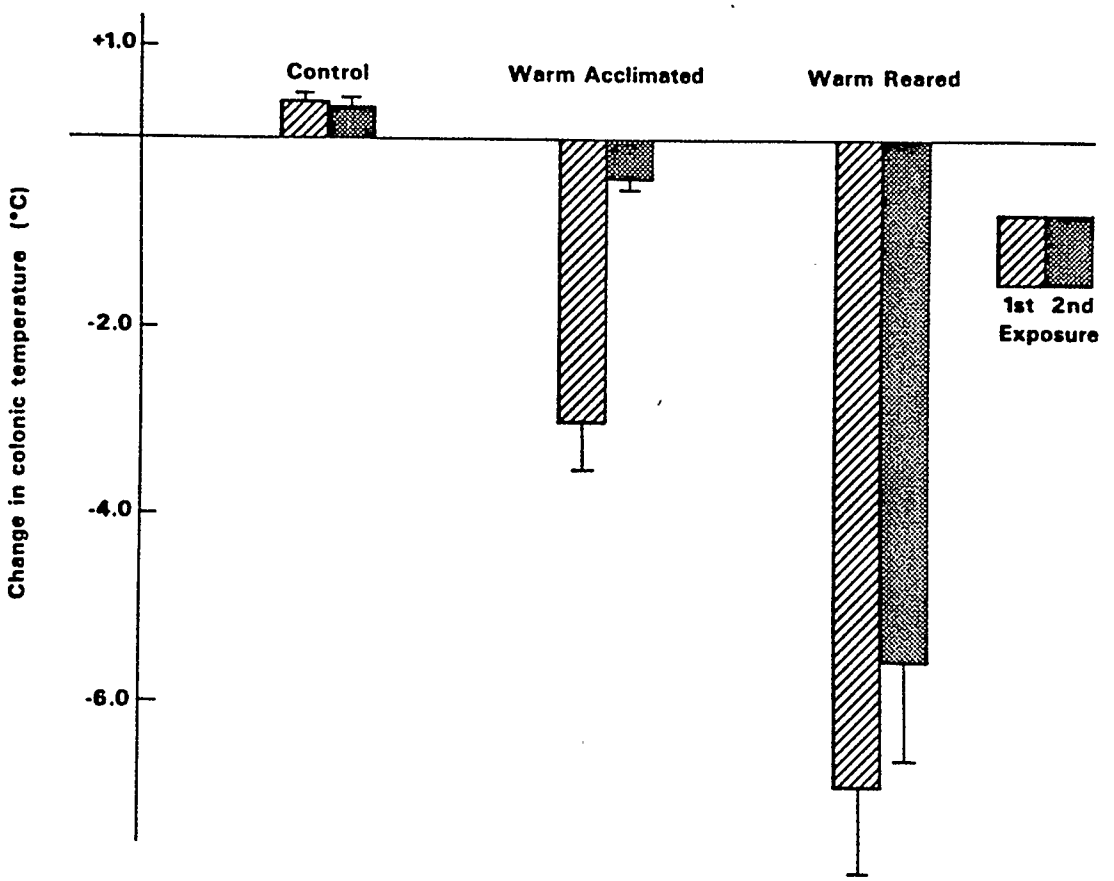


Figure 3: Absolute changes in colonic temperature of control (N = 11), warm -acclimated (N = 5), and warm-reared (N = 10) rats during 1st and 2nd cold exposures. Each bar represents the mean and the line vertical bars show the standard error of the mean.

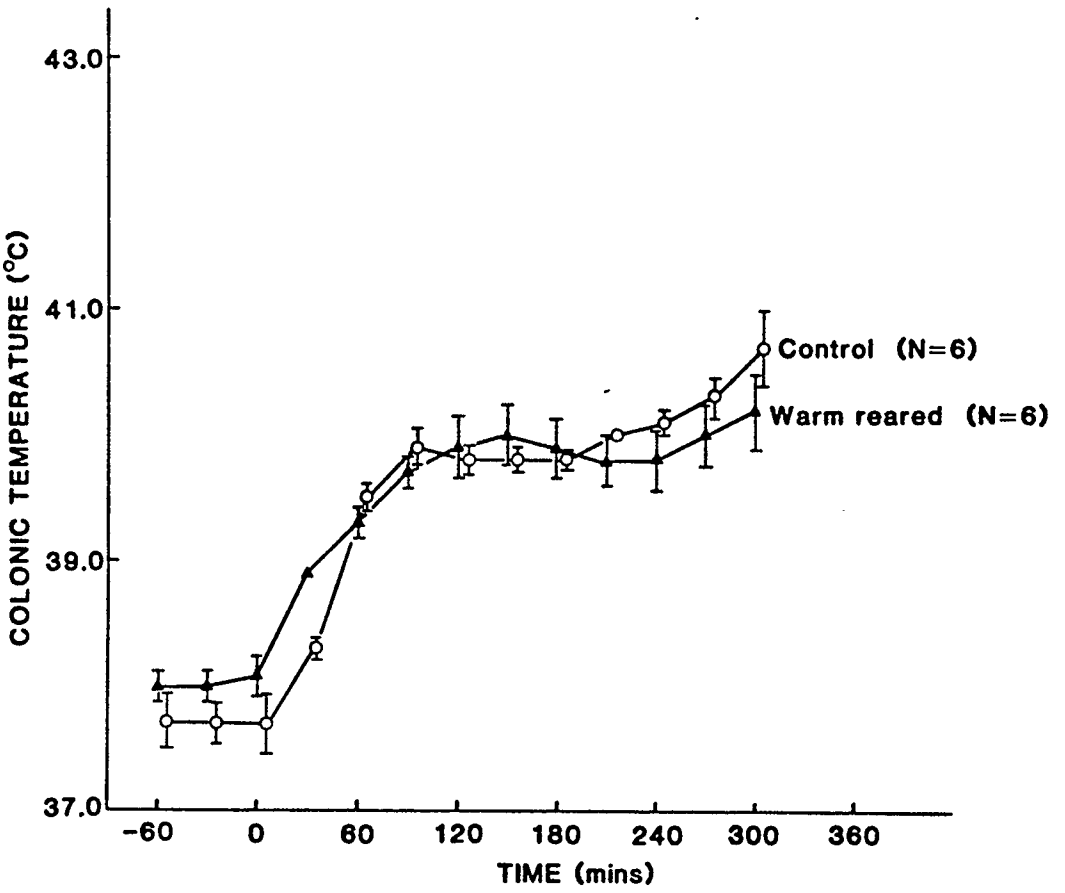


Figure 4: Graphs showing the mean (± SEM) colonic temperature of rats exposed to 38.0°C for 5 hrs.

in Fig 5, also. This group of rabbits showed a reduced first peak of fever and an absent second peak. Comparison of the areas below the temperature response curves showed that the febrile response of warm-reared animals were significantly reduced in comparison to the control group ($p < 0.001$). The temperature response of warm-acclimated rabbits to endotoxin are illustrated in Fig 6. As these animals were adult, they were compared to fully grown controls. No statistically significant differences between the two groups were observed ($p > 0.1$).

The changes in temperature which occur in Sprague Dawley rats after intraperitoneal injection of endotoxin were unusual in that no fever was observed. This lack of fever was common to both control and warm-acclimated rats (Fig 7). In both of these groups, body temperature did not change significantly following endotoxin administration. However, warm-reared animals showed significant falls in colonic temperature in response to endotoxin ($-0.75 \pm 0.16^{o}C$).

There were no statistically significant differences between the baseline temperatures of the three experimental groups of animals.

Discussion

The specific aim of this series of experiments was to establish whether reduced cold stimulation in early life, as a result of rearing animals at $33.0^{o}C$ from birth, affected the functional characteristics of the thermoregulatory system in later life. Initially, it was essential to demonstrate that the ability to regulate body temperature in animals reared at $33.0^{o}C$ was different to that of animals raised at $20.0^{o}C$. In order to propose that a "critical period" during early life may exist for such environmentally induced changes, it was necessary also to

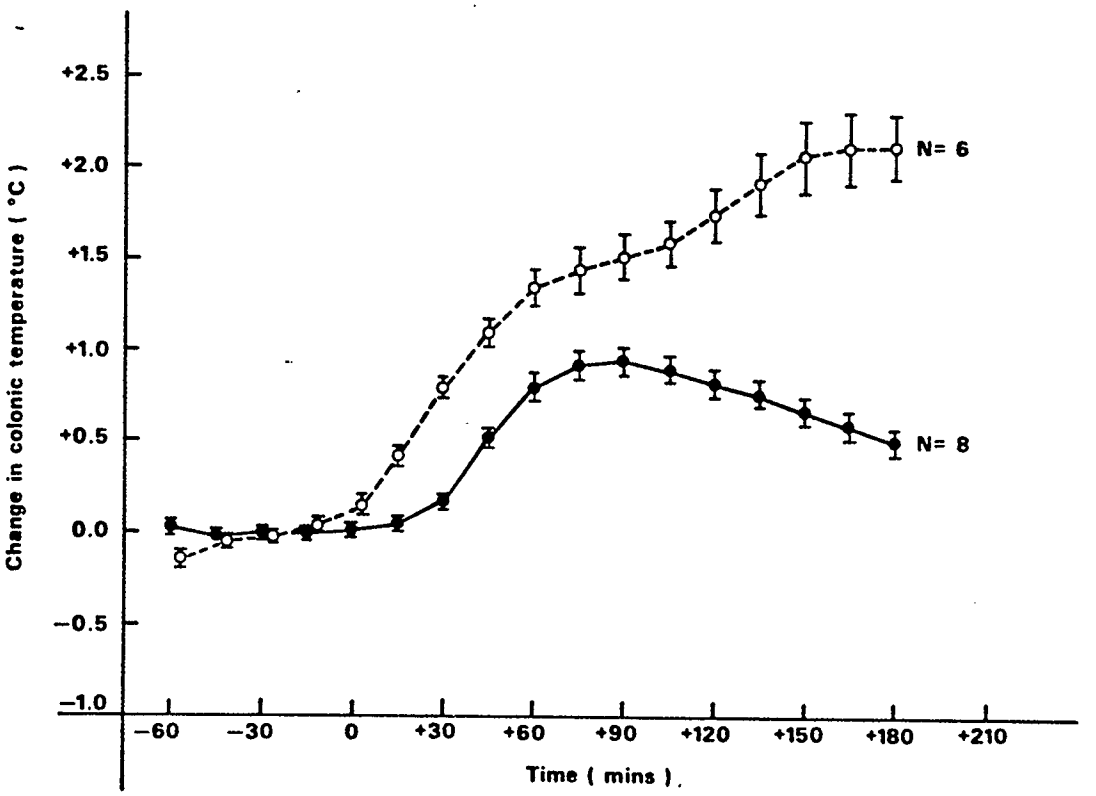


Figure 5: Records of colonic temperature of warm-reared (●——●) and control (o- -o) rabbits in response to 0.2 µg endotoxin. Each point shows the mean (± SEM).

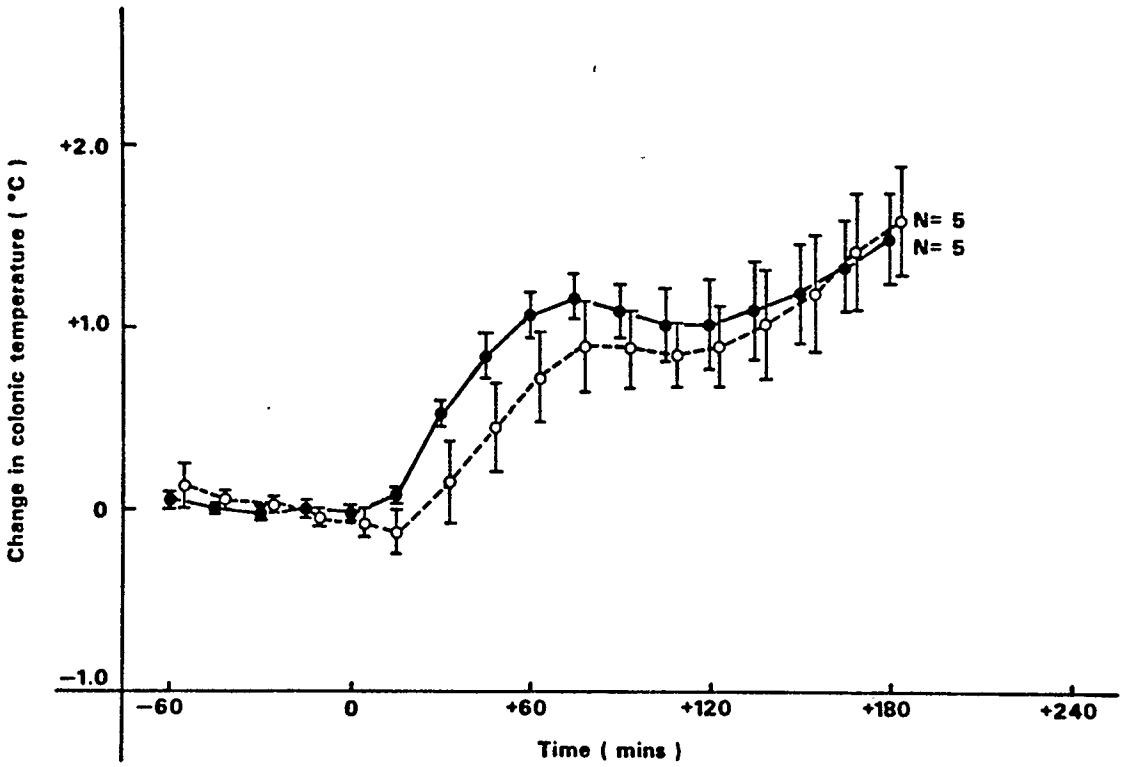


Figure 6: Records of colonic temperature of warm-acclimated (●—●) and control adult (○- -○) rabbits in response to 0.25 µg endotoxin. Each point represents the mean (± SEM).

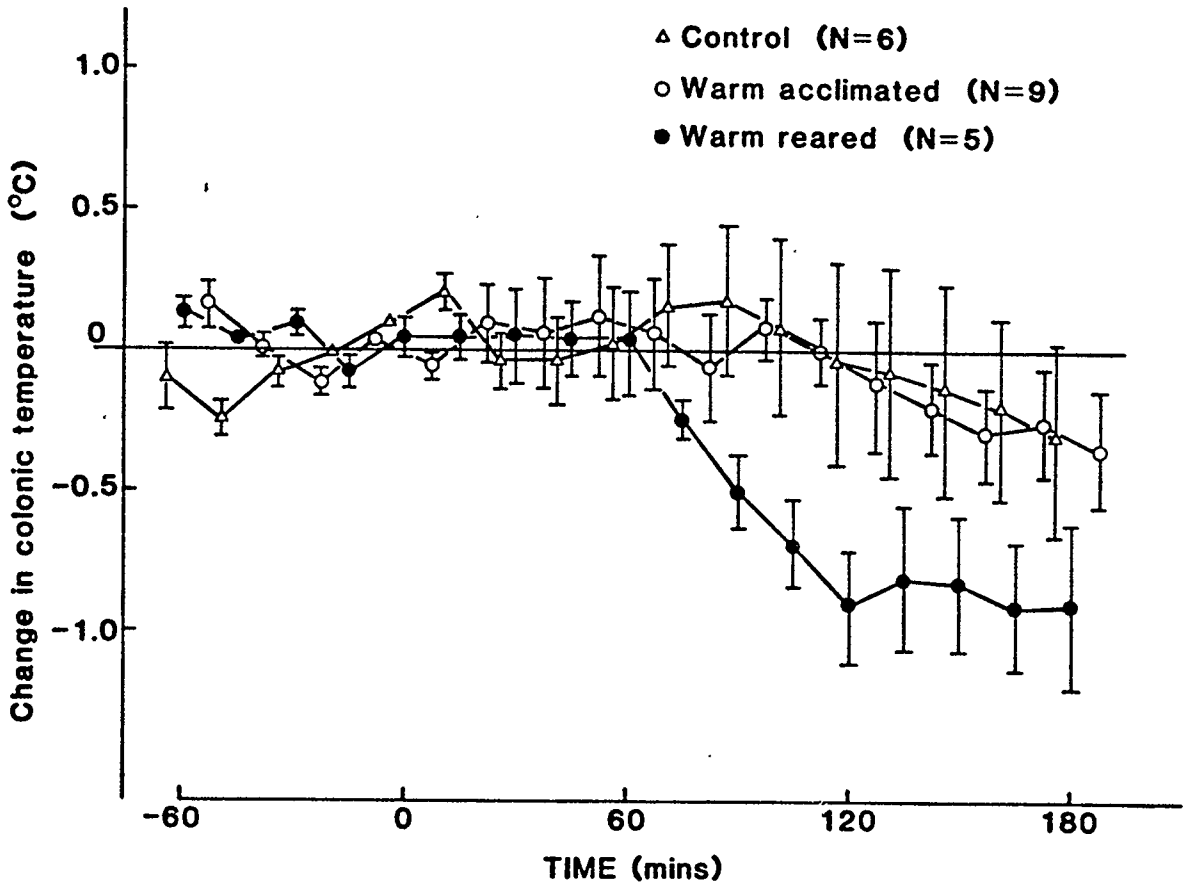


Figure 7: Changes in colonic temperature of Sprague Dawley rats in response to intraperitoneal administration of endotoxin. Each point represents the mean (± SEM).

demonstrate that similar environmental manipulation of the adult animal did not result in similar changes in thermoregulatory responses. It is for these reasons that the most significant observations are those which show differences between warm- acclimated and warm-reared groups of animals, that is changes in thermoregulatory responses which do not occur during the normal acclimation process.

Animals reared at elevated environmental temperature were first challenged by a cold exposure, as this is one of the simplest tests of these animals' ability to maintain body temperature. In the case of the New Zealand White rabbits, both control and warm-acclimated animals maintained body temperature throughout the duration of cold exposure. In contrast, the colonic temperature of warm-reared animals fell significantly during the same exposure to cold. The results were similar, but not identical, in the case of the Sprague Dawley rats. In this species, colonic temperature was maintained during first and second cold exposure in control animals, but only during second exposure in the warm-acclimated group. The fall in temperature observed in the latter group during first exposure to cold shows the effect of simple acclimation to 33.0^{o}C and, when compared to second exposure, indicates that this effect may be reversed quite readily. Such a recovery from the effects of acclimation is suggested also by the observation that the colonic temperature of warm-reared animals fell less during the second cold exposure than during the initial exposure to cold. However, it should be stressed that the colonic temperature of the warm-reared group fell to significantly lower levels than the warm-acclimated animals during both cold exposures. Thus, these data show clear differences between warm-reared animals on the one hand and control and warm-

acclimated animals on the other, and their ability to maintain colonic temperature during a 4 hr cold exposure.

More recent work in support of the above findings has demonstrated similar falls in colonic temperature of Sprague Dawley rats raised at 30.0^{o}C (Dawson et al., 1981). However, this group did not compare warm-reared animals to a warm-acclimated group. Doi & Kuroshima (1979) have manipulated the thermal environment in early life by exposing newborn rats to four 1 hr cold exposures at 5.0^{o}C each day for the first 14 days of life. They reported that, when anaesthetized, animals raised in this way were better able to maintain colonic temperature during cold exposure than either untreated animals, or adult animals which had undergone the same repetitive cold exposure treatment.

As a further test of thermoregulatory ability, a febrile response was induced by administration of endotoxin to all three groups of animals in both species. In the New Zealand White rabbit, the observed rise in temperature occurs as a result of the activation of heat conservation and/or heat production mechanisms (Palmes & Park, 1965; Atkins & Bodel, 1972). Warm reared rabbits developed a significantly reduced fever following intravenous administration of endotoxin as compared to control animals. This evidence supports the hypothesis that the early environment may have modified the development of the thermoregulatory mechanisms involved in the development of fever in response to intravenous injection of endotoxin, as no such deficit was observed in the warm-acclimated group. It should be noted also that the warm-reared rabbits, despite the observed deficits, are able to increase body temperature significantly, as demonstrated by the existence of the first peak of the febrile response. The fact that the first peak of fever is rapidly followed by a return toward baseline temperature

suggests either, that the effector mechanisms can only maintain this rise in temperature for a short period of time, or, that the usual febrile drive to increase body temperature does not exist after the initial rise in body temperature.

In the Sprague Dawley rat, the effects of intraperitoneal administration of E. coli are difficult to interpret, as administration of this pyrogen did not result in fever.

Some controversy exists as to whether or not rats are able to develop fever in response to intraperitoneal administration of pyrogens. Avery & Penn (1974) report that endotoxin, given by this route, results in the development of fever, while other workers find no significant changes in colonic temperature (Feldberg & Saxena, 1975; Szekely & Szelenyi, 1979a). Despite these contradictions, the results reported here show that the changes in colonic temperature, which occurred as a result of endotoxin administration in warm-reared rats, were significantly different from those observed in both warm-acclimated and control groups.

The finding that during heat stress the changes in colonic temperature of warm-reared and control animals were not significantly different was unexpected. However, on closer examination, these data may in fact provide some indication as to the nature of the thermoregulatory deficits of warm-reared animals. When exposed to an environmental temperature of 38.0^{o}C, information regarding thermal sensation normally will be derived almost totally from the warm-receptors as the cold receptors, which in most cases respond only between 10^{o}C and 35^{o}C (Zotterman, 1953), will be silent. Therefore, these particular experiments show that the control of body temperature

in warm-reared rats is normal during a thermoregulatory stress which is responded to as a consequence of warm-receptor afferent input. In contrast, during cold exposure, when the input from cold receptors is most significant, a thermoregulatory deficit was observed. Thus, it would seem possible that the restriction of cold stimulation in early life may specifically affect the thermoregulatory mechanisms which are controlled by the cold receptor input.

The data presented in this section demonstrate that raising both New Zealand White rabbits and Sprague Dawley rats at 33.0^{o}C from birth results in the modification of the functional characteristics of the thermoregulatory systems of these animals. The changes observed are different from those which occur during the acclimation of adult animals to 33.0^{o}C. The following chapters of this thesis describe a series of experiments designed to investigate the changes in the thermoregulatory system underlying the deficits observed in warm-reared animals.

IV. Investigation of Thermoregulatory Effector Mechanisms of Warm Reared Animals

The evidence presented in the previous chapter demonstrates that the thermal environment in early life may influence the development of the thermoregulatory system in a way different from the normal acclimation process. The experiments reported in this section were designed to investigate whether the thermoregulatory deficits specific to warm-reared animals may be explained by changes in the thermogenic mechanisms of the temperature regulating system in this group of animals.

MATERIALS & METHODS

Electromyogram recordings (E.M.G.) - Electrophysiological recordings of muscle activity were made by placing bipolar needle electrodes in the gluteus muscle of the hind leg of the rabbit. A third ground electrode was inserted into the back of the animal and all three electrodes were gently taped in place and connected to a Beckman E.M.G. coupler, such that muscle activity could be recorded directly on a Beckman Dynograph (R 612).

Noradrenaline infusion - Sterile 23 gauge stainless steel needles were implanted acutely in the lateral ear vein of New Zealand White rabbits. Polyethylene tubing (Intramedic PE 50) was attached to the distal end of this needle and noradrenaline (Noradrenaline HCl, Sigma), dissolved in sterile saline, was infused at a rate of 2 μg/kg/min (Hull & Segall, 1965) for 60 mins by use of a Harvard infusion pump.

Propranolol treatment - In one group of experiments, the β-adrenergic receptor was blocked by intravenous administration of propranolol (Sigma HCl) in a priming dose of 1.0 mg/kg followed by a further 0.25 mg/kg every 30 mins (Heim & Hull, 1966). This drug was given through cannulae implanted acutely in the lateral ear vein of the rabbit, as for the infusion of noradrenaline.

Blood sampling - At least one week prior to experimentation, animals were implanted with chronically indwelling jugular cannulae. Surgery was performed under halothane anaesthesia. An incision was made ventral to the right external jugular vein, a section of which was then isolated from surrounding tissue, and tied off at the rostral end. Silastic tubing (Dow-Corning PP100) was inserted through a small incision in the vein and advanced approximately 6.0 cm towards the heart. It was then tied in place and run dorsally through the subcutaneous tissue and exteriorized through the inside of the ear. All cannulae were filled with heparinized saline (50 units/ml) and sealed with a stainless steel stylet. Approximately 66% of cannulae implanted in this way remained patent for 3 - 6 weeks, such that blood samples could be drawn readily from the conscious animal.

Blood samples for catecholamine assay were drawn at 60 min intervals in a volume of 0.5 mls. These samples were placed on ice immediately after collection, were then centrifuged, the plasma retained and stored at -20^{o}C until later determination of both adrenaline and noradrenaline concentrations by use of a radioenzymatic assay utilizing α-methyldopa as an internal standard (Bauce et al., 1980). A minimum of two baseline samples were drawn from all animals prior to any experimental manipulations.

Samples for assay of thyroid hormones were taken at 120 min intervals and 4.0 mls of blood was withdrawn for each sample, was given time to clot, and was then centrifuged, the serum being retained and stored at -20°C until later assay for tri-iodothyronine by use of radio-immunoassay. Concentrations of plasma catecholamines and of serum thyroid hormones were compared by use of the Student's "t" test applied to the raw data. Within group comparisons were made with the paired "t" test, while between group evaluation of changes in these hormone levels were made with the unpaired "t" test.

Histological Examination of Adipose Tissue - After the animals were killed, small samples of adipose tissue were excised from the interscapular region of both New Zealand White rabbits and Sprague Dawley rats. These samples were fixed in a 10% formalin solution for 14 days, were then embedded in paraffin and 50 μm sections were cut, mounted, and stained for later histological examination.

Measurement of Oxygen Consumption During Fever - Rabbits were placed and restrained in an airtight perspex cage, to which was fitted both gas inlet and outlet pipes. One further hole was drilled in this cage and a rubber stopper was fitted. Both an acutely implanted intravenous injection cannulae and the thermistor probe were exteriorized through this stopper. Air was passed through this cage throughout experiments, the rate of flow being monitored by use of a simple gas flow meter (Singer DTM-115). The percentage of oxygen in the air leaving the perspex chamber was monitored continuously with a Beckman Oxygen Analyzer (OM-11) which was connected to a Beckman Pulse/Pressure coupling unit and recorded on a Beckman Dynograph (R 612). The calibration of this unit was checked daily by passing gas mixtures of

varying known oxygen concentrations through the system. Each animal was weighed after experimentation. Oxygen consumption could then be calculated at any point during experimentation according to the formula:

$$O_2 \text{ cons. cc/kg/min} = \frac{([O_2]^A - [O_2]^C)\ F \times 10}{W}$$

Where $[O_2]^A$ = % oxygen in air

$[O_2]^C$ = % oxygen in air leaving cage

F = Flow rate in litres/min

W = Weight of animal in Kg.

In all experiments, basal oxygen consumption was monitored for at least 60 mins prior to, and for 240 mins after, the induction of fever by intravenous injection of endotoxin.

RESULTS

The ability of all three groups of New Zealand White rabbits to shiver during cold exposure was examined qualitatively in two separate ways. Observation of animals while at 2.0°C demonstrated that, within 60 mins of being placed in the cold room, all animals shivered, in some

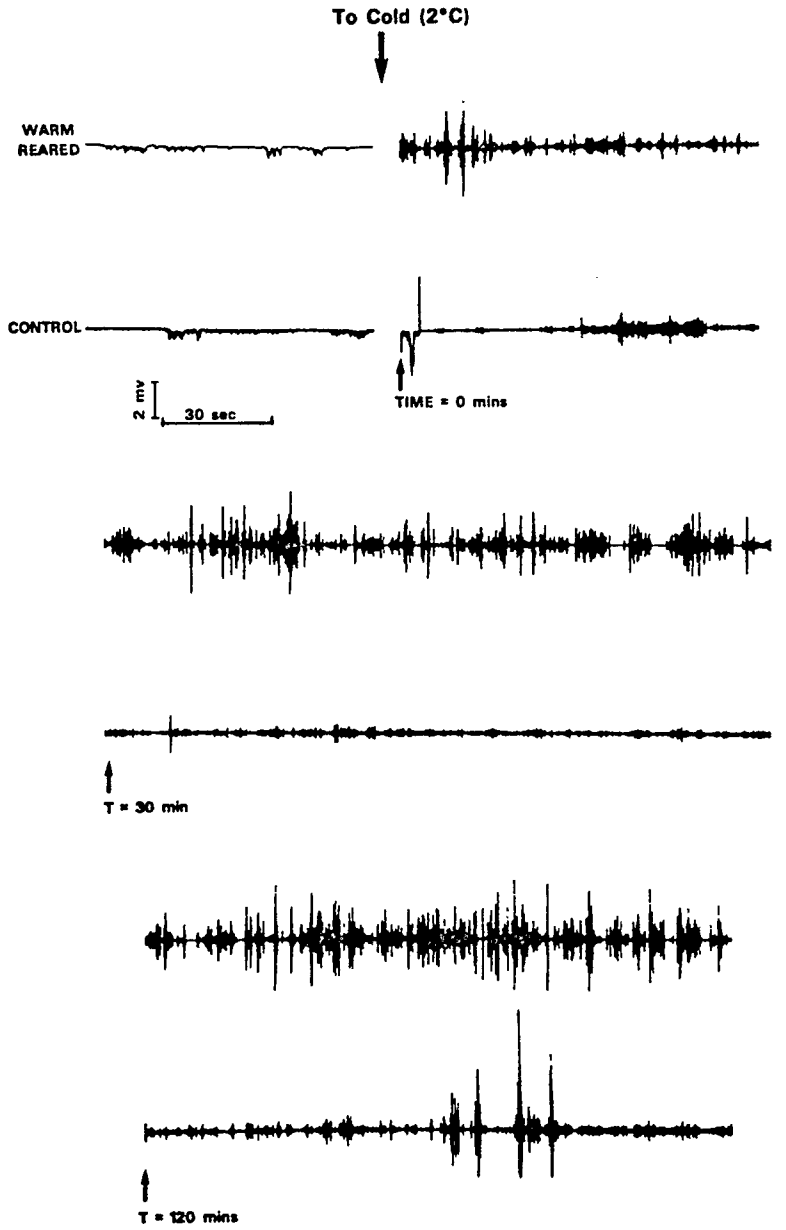


Figure 8: Typical recordings of E.M.G. activity during cold exposure, taken from warm-reared and control rabbits.

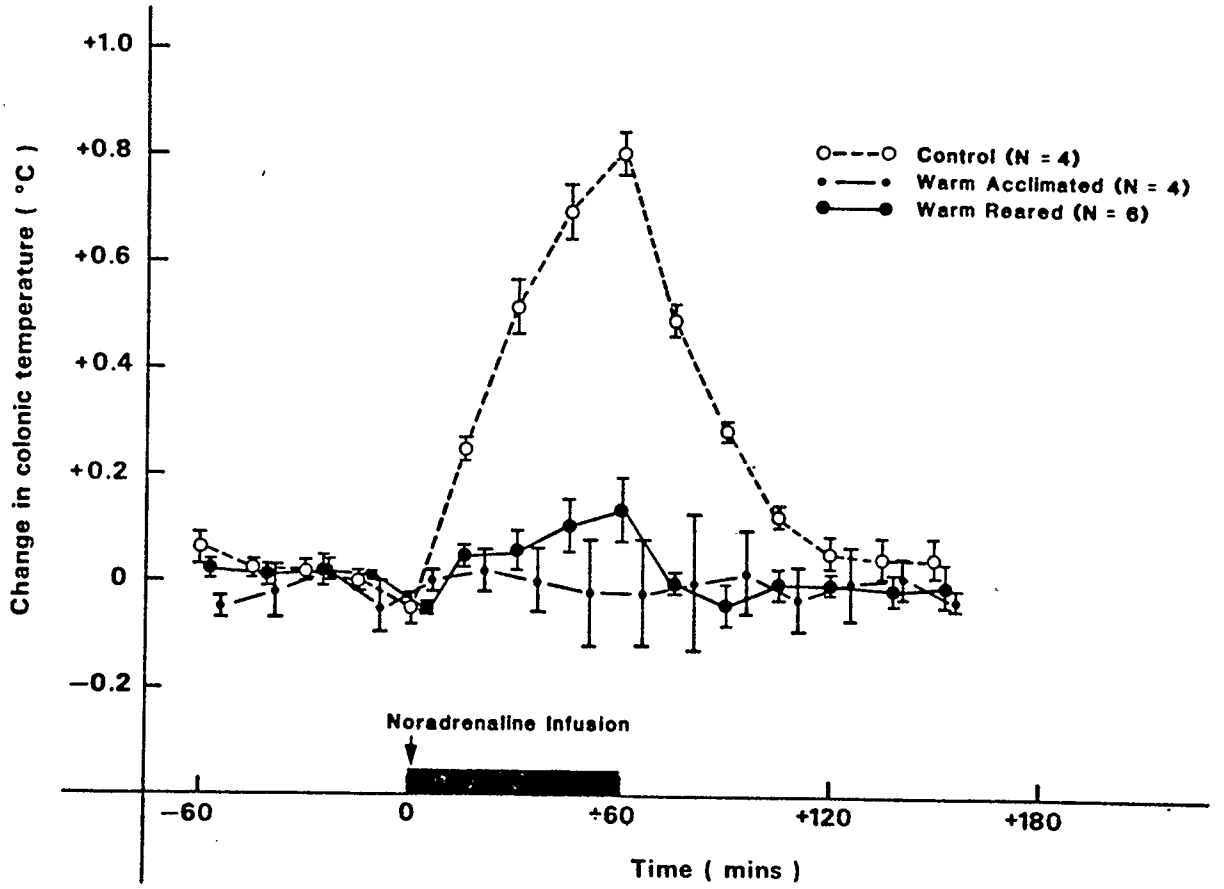


Figure 9: Graph showing the changes in colonic temperature of warm-reared, warm-acclimated, and control rabbits during a 60 minute infusion of noradrenaline. Each point shows the mean (± SEM).

cases continuously, and in others only sporadically. Secondly, E.M.G. recordings of muscle activity in warm-reared and control animals during cold exposure demonstrated that the former group began shivering as soon as they were placed at 2.0^{o}C, while, in control animals, the shivering began later, and did not appear to be as intense. Typical E.M.G. recordings from warm-reared and control animals are presented in Fig 8.

The calorigenic action of intravenous infusion of noradrenaline was examined in the rabbit. Following infusion for 60 mins at a rate of 2 µg/kg/min, plasma noradrenaline concentrations were found to be elevated above 5000 picograms/ml. In control animals, administration of this monoamine resulted in a increase in body temperature throughout the time period of infusion, followed by a rapid return to baseline temperature. In contrast, no significant changes in body temperature were observed in either warm-reared or warm-acclimated animals (Fig 9). Comparison of the areas below the temperature response curves showed the hyperthermia which occurred in control animals to be significantly greater than the two other groups (Student's "t" test $p < 0.001$).

Plasma adrenaline and noradrenaline concentrations were measured during cold exposure in all three groups of rabbits. The baseline levels of catecholamines measured in plasma samples taken immediately

Table 2: BASAL LEVELS OF PLASMA CATECHOLAMINES

	Adrenaline (pg/ml)	Noradrenaline (pg/ml)
Control (N=4)	28 ± 11	362 ± 14
Warm-Acclimated (N=3)	68 ± 42	185 ± 52
Warm-Reared (N=4)	339 ± 72 *	645 ± 165

* $p < 0.05$ compared to controls

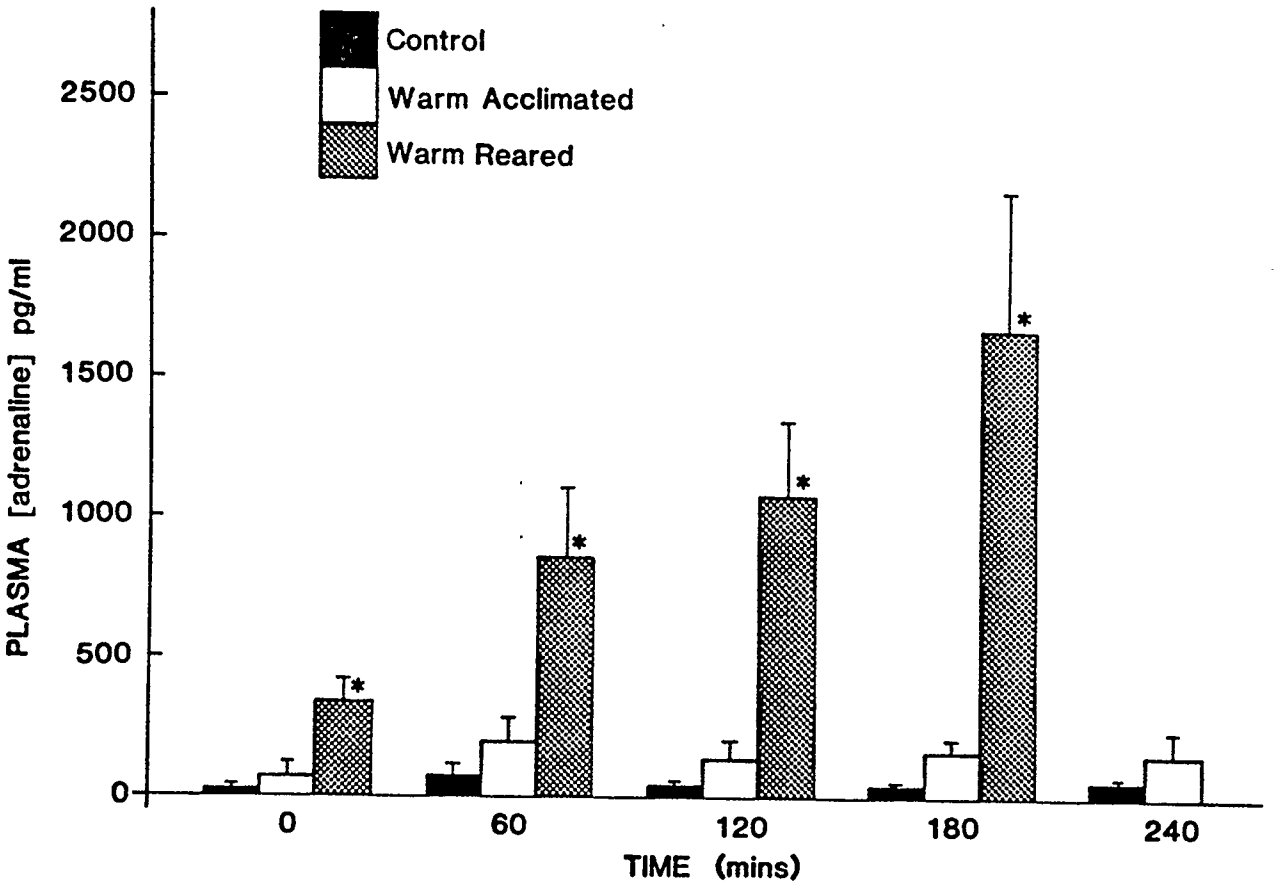


Figure 10: A histogram in which the bars represent the mean (± SEM) plasma concentrations of adrenaline in control (N = 4), warm-acclimated (N = 3) and warm-reared (N = 4) rabbits, immediately before and during cold exposure, beginning at time = 0. * $p < 0.05$ compared to controls.

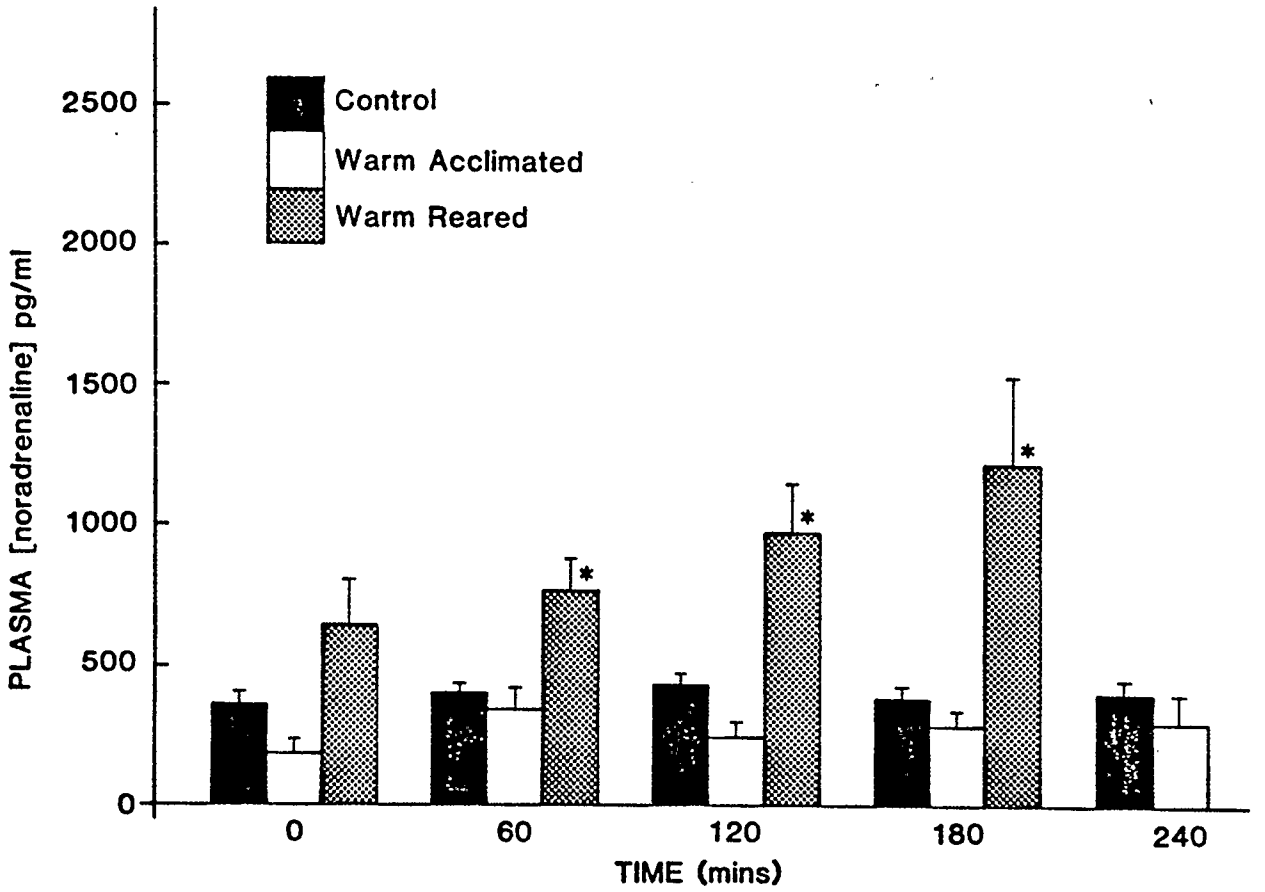


Figure 11: This histogram shows the mean (± SEM) plasma concentrations of noradrenaline in control (N = 4), warm-acclimated (N = 3), and warm-reared (N = 4) rabbits, immediately before and during cold exposure, beginning at time = 0.

* $p < 0.05$ compared to controls.

before the animals were placed in the cold are presented in Table 2. Adrenaline concentrations were found to be statistically significantly elevated in warm-reared animals (Student's "t" test $p < 0.05$). Histograms showing plasma adrenaline and noradrenaline concentrations at 60 min intervals during cold exposure are presented in Fig. 10 and 11, respectively. In all samples taken during cold exposure, both plasma adrenaline and noradrenaline concentrations in warm-reared rabbits were found to be significantly elevated above the values obtained from either warm-acclimated or control groups ($p < 0.05$). Also, it was found that, during cold exposure, plasma adrenaline and noradrenaline concentrations increased significantly above baseline levels in warm-reared animals ($p < 0.05$), while no statistically significant changes occurred in either control or warm-acclimated groups ($p > 0.05$). It should be noted that for warm-reared rabbits data are presented for 3 hrs of cold exposure only as, following large falls in colonic temperature, two animals from this group were removed from the cold after this time period. Serum concentrations of tri-iodothyronine (T_3) were measured in all three groups of rabbits during cold exposure also and the mean group values are shown in Fig 12. These data demonstrate that there were no statistically significant changes in T_3 concentrations in serum as a result of cold exposure ($p > 0.1$). All groups demonstrate stable levels of this hormone throughout cold exposure. However, both warm-reared and warm-acclimated groups of animals had significantly lower basal levels of T_3 than control animals (Table 3).

Table 3: BASAL SERUM CONCENTRATIONS OF TRI-IODOTHYRONINE

	[tri-iodothyronine] ng/dl
Control (N=4)	111 ± 12
Warm-Acclimated (N=3)	35 ± 2 *
Warm-Reared (N=4)	41 ± 7 *

* $p < 0.01$ as compared to control group

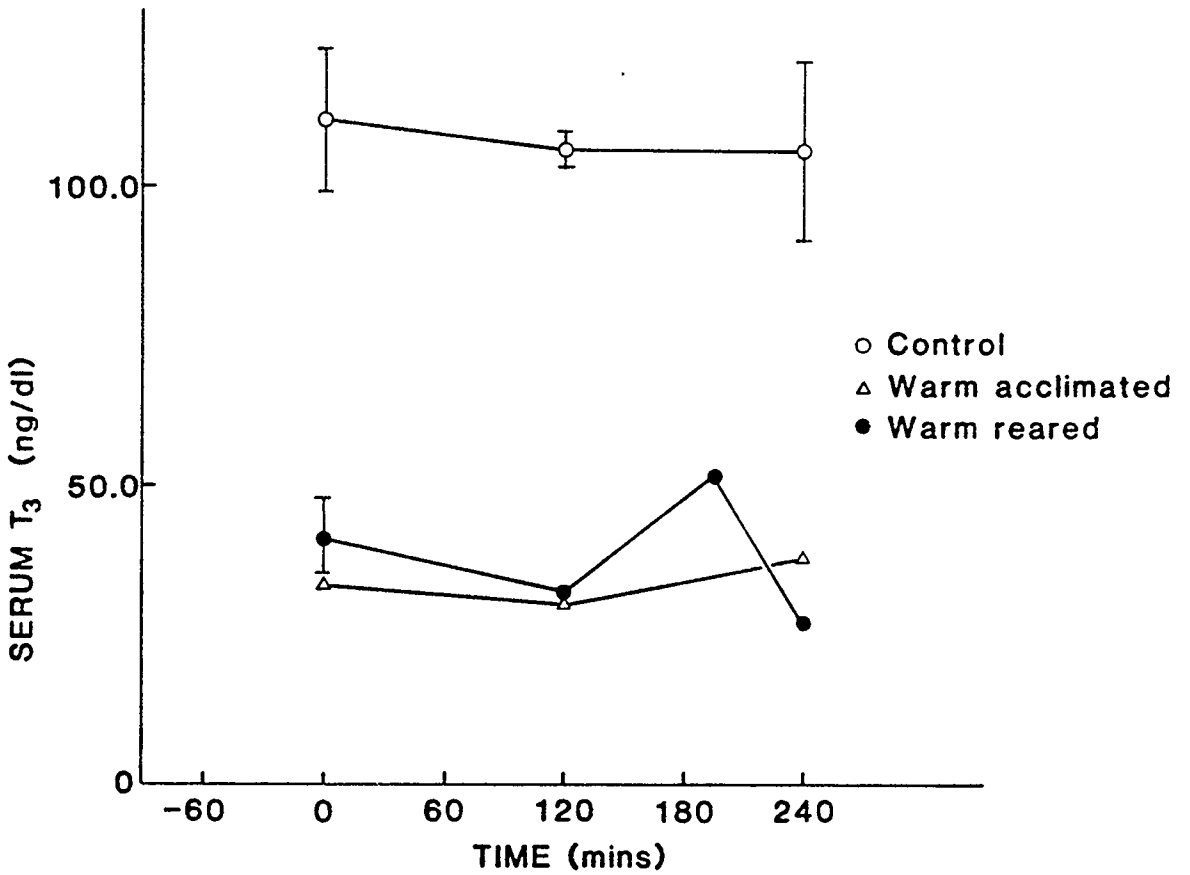


Figure 12: Graph showing serum concentrations of tri-iodothyronine before and during cold exposure, beginning at time = 0, in control (N = 3), warm-acclimated (N = 3), and warm-reared (N = 3) rabbits. Each point represents the mean value, and standard error bars are shown only when they are greater than the limits of the point.

Figure 13: Photomicrographs of 50 μm sections of adipose tissue excised from the interscapular region of the rat.

a - e magnification X40, f magnification X160

a) Brown and white fat cells from a control rat.

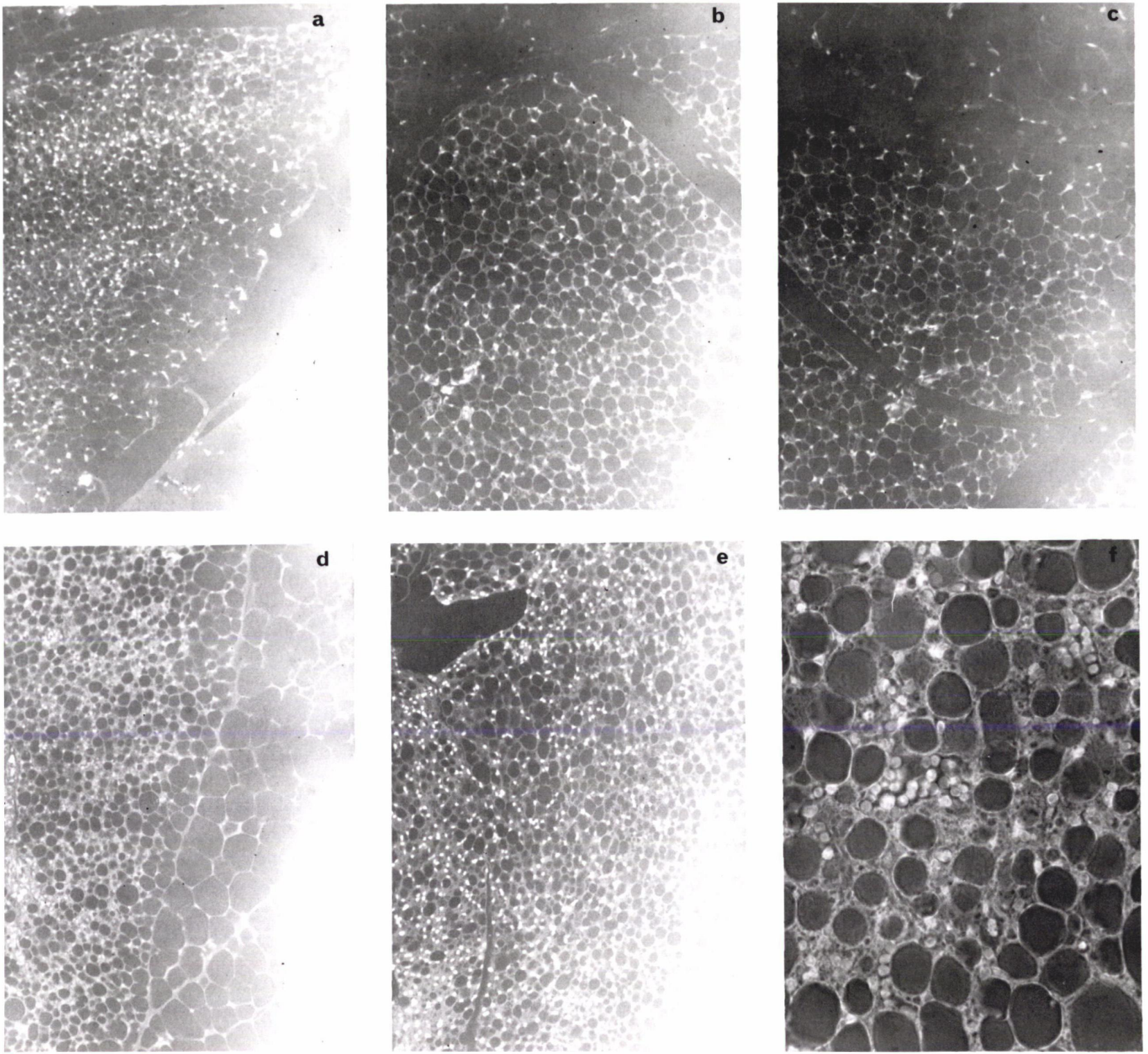
b) Brown adipose tissue taken from a control rat.

c) Brown and white fat cells from a warm-acclimated rat.

d) Brown and white fat cells from warm-reared rat.

e) Brown fat cells in a section removed from a warm-reared rat.

f) High magnification picture of brown adipose tissue taken from a warm-reared rat.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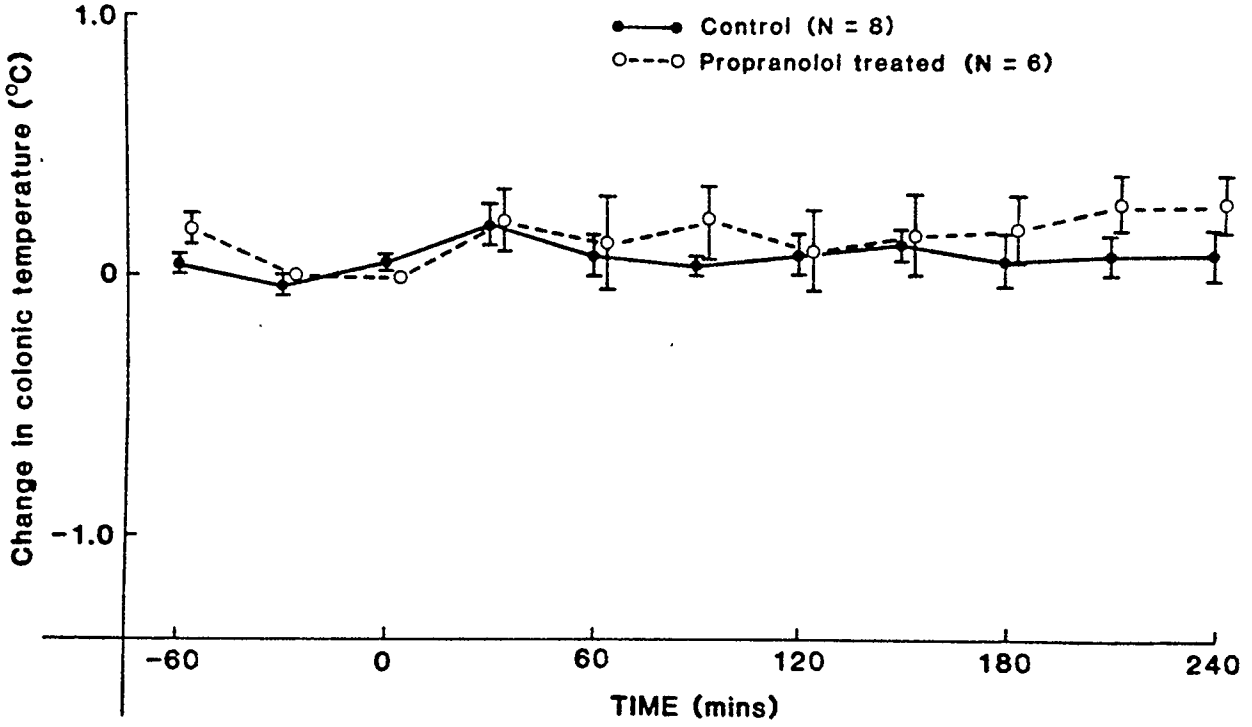


Figure 14: This graph shows the changes in colonic temperature which occurred in control rabbits during cold exposure when non-shivering thermogenesis was functional or when it was blocked by propranolol treatment. Each point represents the mean (± SEM).

Interscapular adipose tissue of animals from each different environmental background was examined microscopically after histological preparation. Photomicrographs were taken, examples of which are presented in Fig 13. These photographs of tissue excised from Sprague Dawley rats provide qualitative evidence that both brown adipose tissue and white adipose tissue cells were present in the interscapular tissue of all groups of animals. Similar results were obtained from rabbit tissues. No attempt was made to quantify the brown adipose tissue content of these groups of animals.

The role of non- shivering thermogenesis in the thermoregulatory responses of control animals during cold exposure was examined by using the β-adrenergic receptor blocking agent propranolol to block the activation of this system by catecholamines in control animals. The results of this procedure (Fig 14) showed that pharmacological treatment with propranolol did not affect the ability of control animals to maintain colonic temperature during cold exposure.

The oxygen consumption of warm-reared, warm-acclimated and control groups of New Zealand White rabbits during fever induced by intravenous injection of endotoxin was measured and the results of these experiments are shown in Table 4. Oxygen consumption was increased significantly in all groups following injection of endotoxin ($p < 0.05$), but no significant differences were observed among the three groups of animals ($p > 0.1$). Considerable variability was observed within each experimental group and thus the changes in oxygen consumption of individual warm-reared and control rabbits are shown in Fig 15.

Table 4: CHANGES IN OXYGEN CONSUMPTION DURING FEVER

	Mean fever height (^{o}C)	Increase in O_2 Consumption (% above baseline)
Control (N=4)	2.85 ± 0.25	32.5 ± 8 *
Warm-acclimated (N=3)	2.13 ± 0.10	52.6 ± 15 *
Warm-reared (N=4)	1.60 ± 0.27	41.0 ± 9 *

* $p < 0.05$ compared to basal O_2 consumption

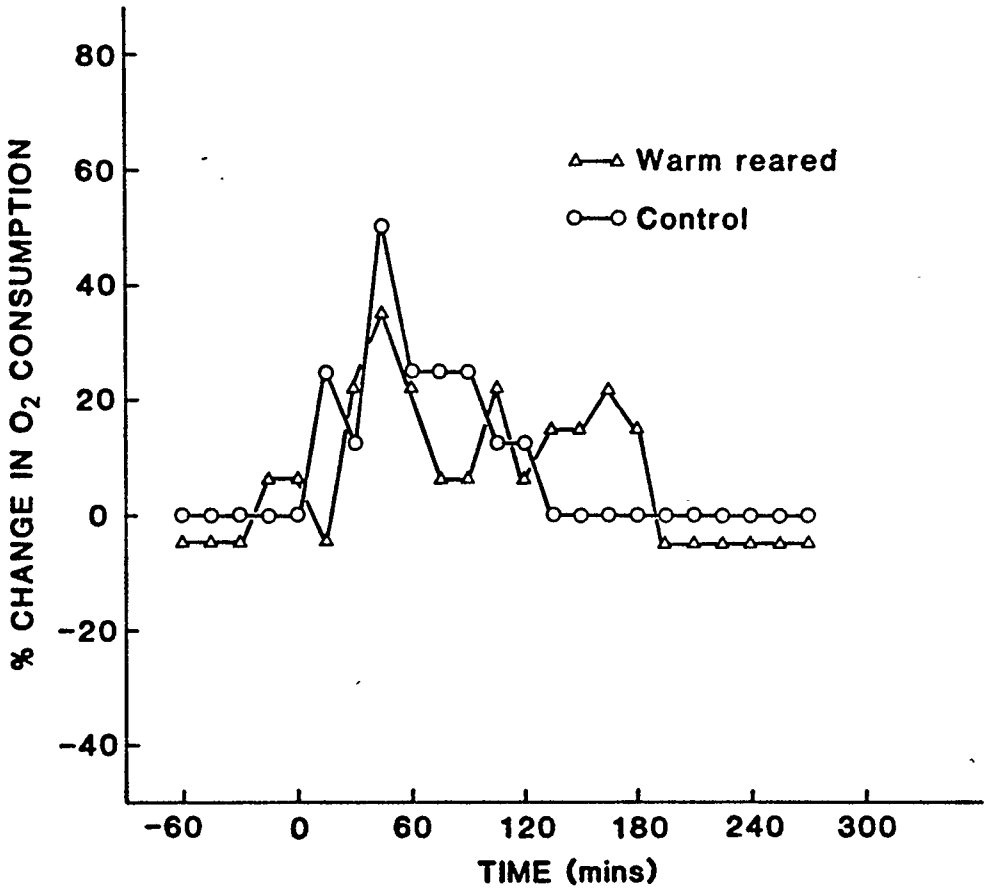


Figure 15: Graph showing typical changes in oxygen consumption which were measured in control and warm-reared rabbits following intravenous injection of endotoxin at time = 0.

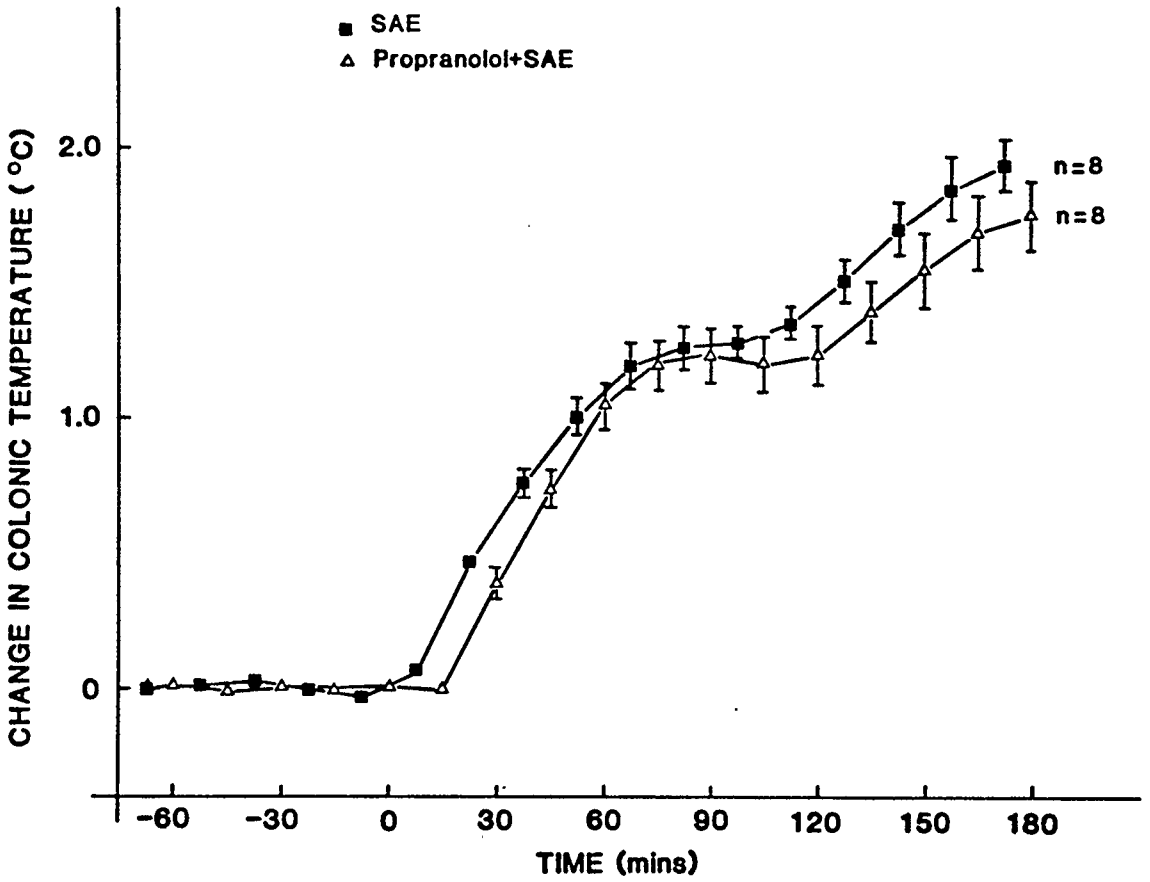


Figure 16: This graph shows the mean changes in colonic temperature (± SEM) which were observed in control rabbits following intravenous injection of endotoxin and the effect of propranolol treatment on this response.

The effect of blockade of non-shivering thermogenesis on the febrile response of control animals was examined. Propranolol administration was found to have no statistically significant effects ($p > 0.1$) on the rises in colonic temperature which occurred following intravenous injection of endotoxin (Fig 16).

DISCUSSION

The major effect of rearing at 33.0°C would be expected to be the fact that animals would be deprived of cold receptor input from the periphery during early postnatal development. Originally it was postulated that such sensory deprivation may influence the ability of animals to feel the cold and thus affect their thermoregulatory responses. The observation that warm-reared animals are able to shiver during cold exposure provides evidence against such a hypothesis. However, it should be pointed out that reduced temperature could be signalled also by warm receptors and that evidence has already been presented which suggests that this group of temperature sensors are functionally normal in warm-reared animals. The greater intensity of shivering, as well as the shorter latency of onset observed in warm-reared animals, may be related to the fact that colonic temperature is falling in this group of animals, while, in contrast, baseline body temperature is maintained during cold exposure in both warm-acclimated and control rabbits. Thus, the observed shivering of warm-reared animals shows conclusively only that this thermoregulatory effector mechanism is functional in animals reared at 33.0°C.

Non-shivering thermogenesis is stimulated by intravenous infusion of noradrenaline (Hsieh & Carlson, 1957) and thus the non-shivering

thermogenic capacity of an animal can be assessed by administration of this catecholamine (Depocas, 1960). The recorded increase in colonic temperature gives an indication of the calorigenic action of the hormone. A significant reduction in the non-shivering thermogenic capacity of both warm-acclimated and warm-reared animals was demonstrated by the results of noradrenaline infusion. The increases in plasma catecholamine concentrations during cold exposure in warm-reared rabbits suggests that the deficit in this system may be at the level of the peripheral effectors, although the histological identification of brown adipose tissue in these animals provides evidence against such a possibility. It should be realized, however, that many other tissues do contribute to non-shivering thermogenesis (Jansky, 1971) and also that the identification of brown fat cells in warm-reared and warm-acclimated animals reported here is a qualitative rather than a quantitative observation. A further possibility is that the biochemical machinery by which plasma catecholamines stimulate thermogenesis in target tissues may be suppressed, as has been demonstrated to occur in isolated brown fat cells from warm-acclimated animals (Kuroshima & Yahata, 1979). The increases in oxygen consumption observed in warm-reared rabbits during fever suggest that these animals are able to increase their metabolic rate in an attempt to increase body temperature. It is surprising that the changes in oxygen consumption during fever of the three groups of rabbits were similar, despite the differences in the febrile responses. No changes in oxygen consumption in the warm-reared group would have demonstrated a deficit which could explain the reduced fevers. However, the observed results suggest that the deficit in the febrile response is not due to a failure of heat production mechanisms. Presumably the heat

produced is derived from shivering, as the non-shivering thermogenic capacity of the warm-reared animals was shown to be minimal. Another possibility is that the plasma concentrations of noradrenaline during infusion may not produce high enough levels of this hormone at its site of action to stimulate thermogenesis (although the high plasma levels achieved during such experiments make such a possibility unlikely) and that, during fever, higher catecholamine concentrations at the site of action may stimulate non-shivering thermogenesis. The reduced T_3 concentrations found in warm-reared animals may be of significance also as the calorigenic action of the catecholamines has been shown to be potentiated by the thyroid hormones (Arner et al., 1981; Fain, 1981).

Further work is necessary to clarify the exact nature of the observed deficits in non-shivering thermogenesis. However, most relevant to the current discussion is the fact that all of the deficits in the effector mechanisms of warm-reared animals also have been demonstrated to occur in the warm-acclimated group. Thus, it would appear that, although a deficit in non-shivering thermogenesis does exist in warm-reared animals, this deficit is a part of the normal acclimation process rather than a specific effect of warm-rearing. This hypothesis is supported by experiments in which non-shivering thermogenesis of control rabbits is inhibited by pharmacological treatment with the β-adrenergic receptor blocker propranolol. Control animals treated in this way would be expected to display the thermoregulatory deficits of the warm-reared group if these deficits were specifically related to a failure of non-shivering thermogenesis. Such deficits do not occur in the febrile response, or during cold exposure, in such pharmacologically treated control animals.

The data presented in this section demonstrate that warm-reared animals are able to react to the cold by shivering and that they increase their metabolic rate in response to pyrogens. Also, they demonstrate a peripheral deficit in non-shivering thermogenesis which is a function of the normal acclimation process and, therefore, does not explain the thermoregulatory deficits of warm-reared animals. Thus, it would appear that warm-rearing does not specifically affect the effector mechanisms of heat production, and that the modified thermoregulatory responses of animals raised at 33.0^{o}C may be due to the modification of other areas of the temperature regulating system.

V. Hypothalamic Mechanisms in the Control of Body Temperature in Warm-Reared Animals.

This section describes experiments designed to investigate whether the thermoregulatory deficits of warm-reared animals may be associated with changes in the role of specific neurotransmitters in the control of body temperature by the AH/POA of the brain. These studies were carried out by monitoring changes in body temperature in awake, behaving animals following intrahypothalamic microinjection of substances which have been suggested to act in this region of the brain as neurotransmitters involved in the control of body temperature.

MATERIALS AND METHODS

Male and female Sprague Dawley rats (250 → 400 g) were used in these experiments. Chronic, bilateral stainless steel guide cannulae were implanted stereotaxically in these animals such that the tips of these cannulae rested approximately 3.0 mm above the AH/POA of the brain. Surgery was performed under sodium pentobarbitol anaesthesia (45 mg/kg Somnotol). A dorsal midline incision was made in the skin above the skull and holes were drilled through the bone 2.0 mm anterior to bregma and 1.5 mm lateral to the coronal suture. The guide cannulae were then lowered 5.0 mm below the dura by use of a stereotaxic manipulator. This assembly was anchored to the skull by two jewellers screws, inserted posterior to bregma, and dental cement. Guide cannulae were kept patent by the use of stainless steel stylets which were removed prior to each experiment. All animals were allowed a recovery period of at least 7 days prior to experimentation. Stereotaxic

coordinates were determined according to the atlas of Pellegrino et al. (1979).

Various thermogenically active agents were microinjected bilaterally into the AH/POA of rats prepared in this way. All drugs were dissolved in sterile non-pyrogenic saline and were delivered through a stainless steel microinjection cannula lowered into the brain through the guide cannulae following removal of the stylets. A constant volume of 1 µl was injected per side over a time period of 60 seconds, as controlled by a microinjection syringe (10 µl Unimetrics) and a Harvard infusion pump.

The following pyrogenic agents and proposed thermoregulatory neurotransmitters were microinjected into the AH/POA. The doses shown represent the quantity of the substance administered to each side of the brain: a) noradrenaline (Sigma, HCl, 10.0 µg), b) serotonin (Sigma, Creatinine Sulphate, 10.0 µg, 5.0 µg), c) dopamine (Sigma, HCl, 20.0 µg), d) carbachol (Sigma, 2.0 µg), e) endotoxin derived from Salmonella abortus equi (5.0 µg), f) prostaglandin E_2 (Upjohn, 100.0 ng) and g) control injections of non-pyrogenic saline. All these doses are commonly administered to the AH/POA and produce thermoregulatory effects (Clark, 1979; Clark & Clark, 1980a, Clark & Clark, 1980b).

After completion of experiments, animals were anaesthetized with sodium pentobarbitol. Microinjection sites were marked by infusion of 1 µl of 1.0% bromophenol blue over 60 seconds, and physiological saline, followed by a 10% formalin solution, was then perfused through the left ventricle of the heart. The brains of these animals were then removed and placed in 10% formalin. Thin, 50 µm sections were later cut on a

freezing microtone and the anatomical location of microinjection sites was verified. Data from animals in which either these sites were found to lie outside the AH/POA or microinjection of sterile saline caused short latency changes in body temperature of more than $0.5^{o}C$ are not included in the results.

RESULTS

The effects of intrahypothalamic microinjection of putative neurotransmitters thought to be involved in the normal control of body temperature were examined initially.

a) Noradrenaline

In control animals, administration of noradrenaline was observed to cause a short latency hypothermia ($-0.7 \pm 0.2^{o}C$). A similar hypothermia occurred in the warm-acclimated group of animals ($-0.8 \pm 0.18^{o}C$). In contrast, the thermoregulatory responses of warm- reared rats to intrahypothalamic infusion of this monoamine consisted of significant rises in colonic temperature ($+1.08 \pm 0.28^{o}C$). The thermoregulatory responses of these three groups of experimental animals are illustrated in Fig 17. Statistical evaluation of these results shows that the changes in colonic temperature observed in warm-reared animals were significantly different to both warm-acclimated ($p < 0.01$) and control ($p < 0.01$) groups, while no significant differences between the thermoregulatory responses of the latter two groups of animals were observed ($p > 0.1$).

b) Serotonin

Serotonin was microinjected in two different doses, 5.0 µg/side and 10.0 µg/side. The changes in colonic temperature elicited in

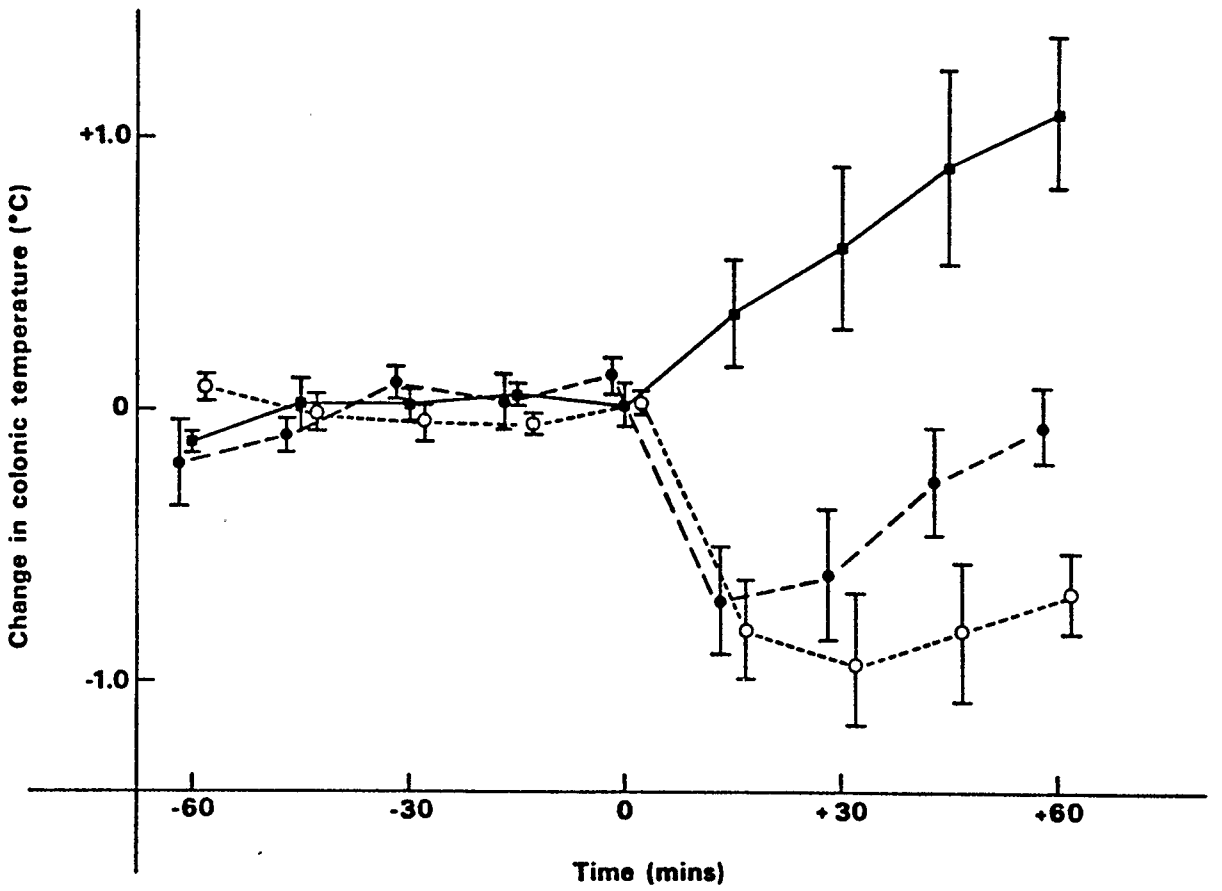


Figure 17: This figure shows the mean (± SEM) changes in colonic temperature, following intrahypothalamic infusion of noradrenaline (10.0 µg/side) observed in control, (●——●, N = 4), warm-acclimated (○---○, N = 5) and warm-reared (■——■, N = 5) rats.

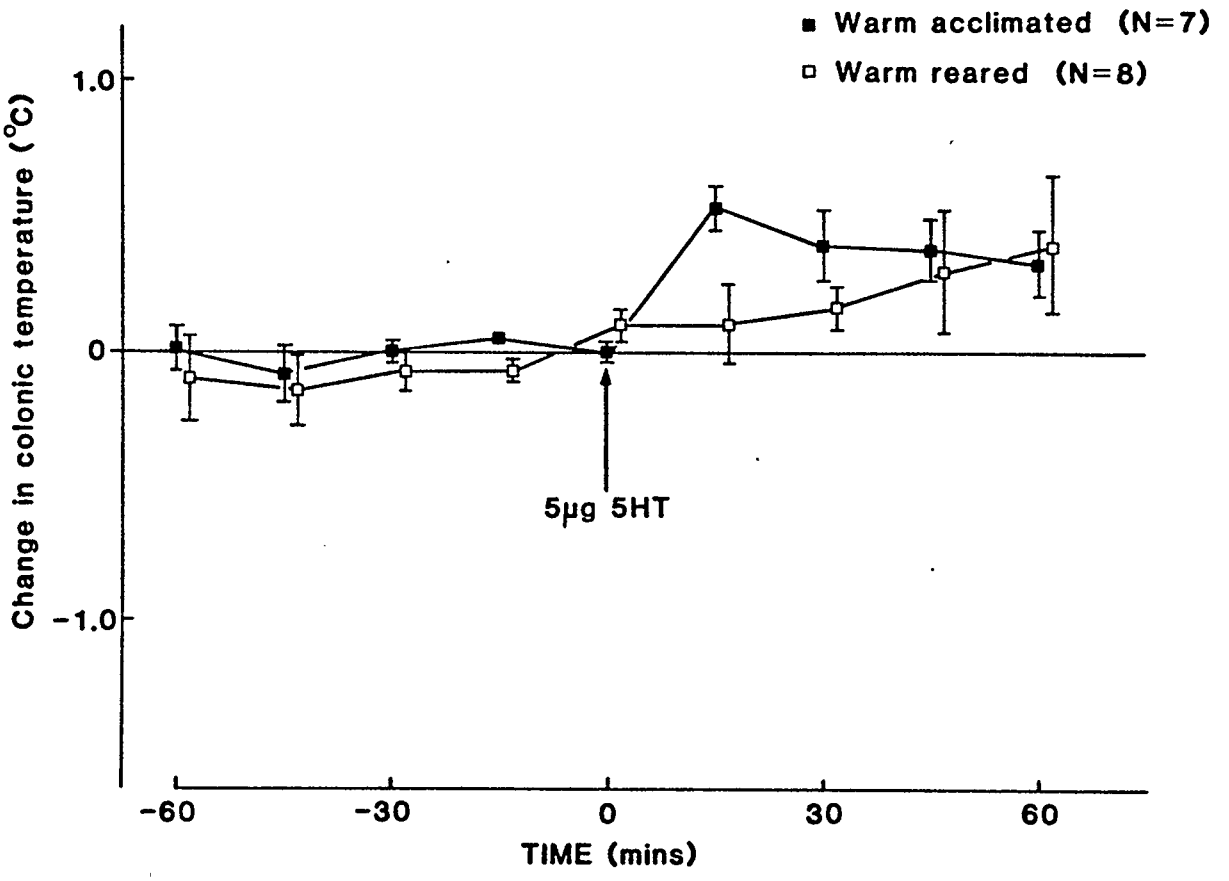


Figure 18: The changes in colonic temperature observed in warm-reared and warm-acclimated rats following intrahypothalamic administration of serotonin (5.0 µg/side). Each point represents the mean (± SEM).

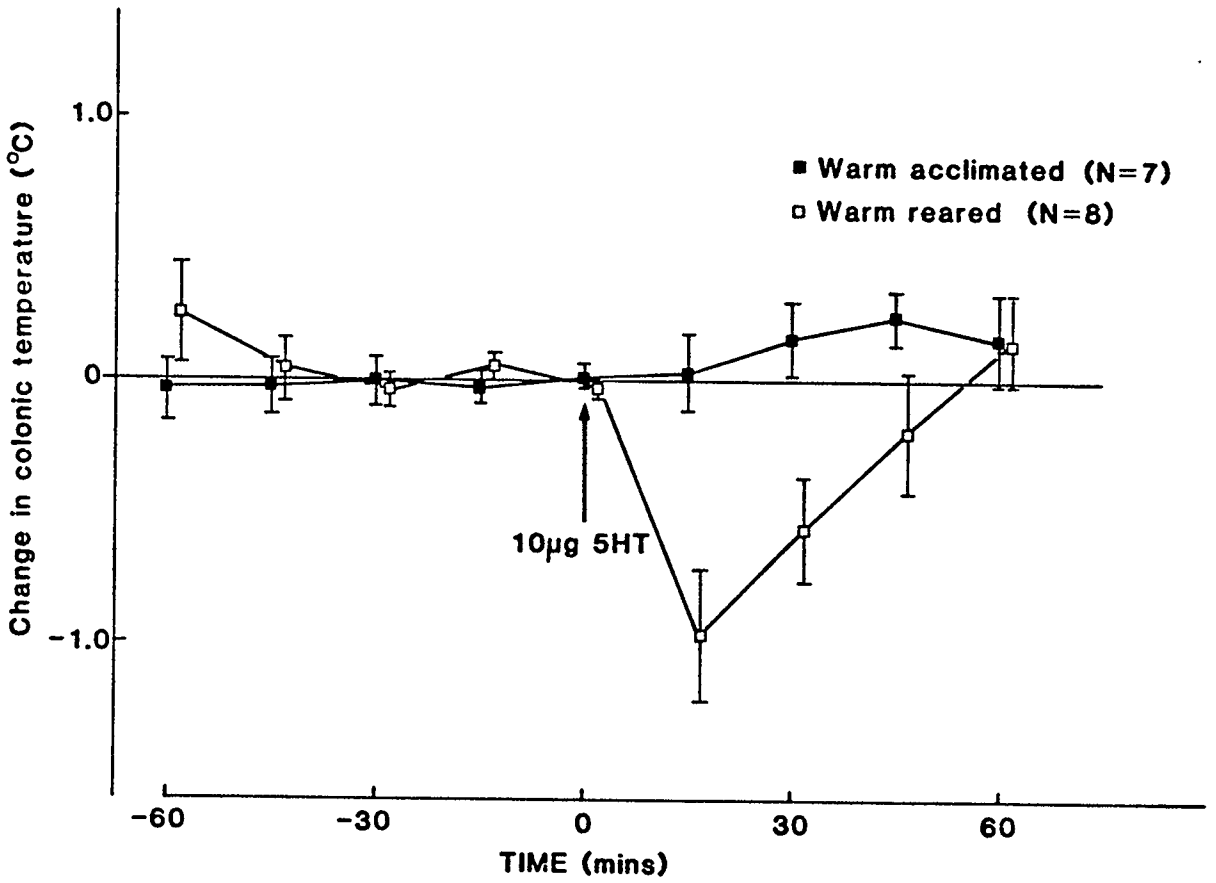


Figure 19: Each point represents the mean (± SEM) change in colonic temperature observed after intrahypothalamic microinjection of serotonin (10.0 µg/side) in warm-reared and warm-acclimated rats.

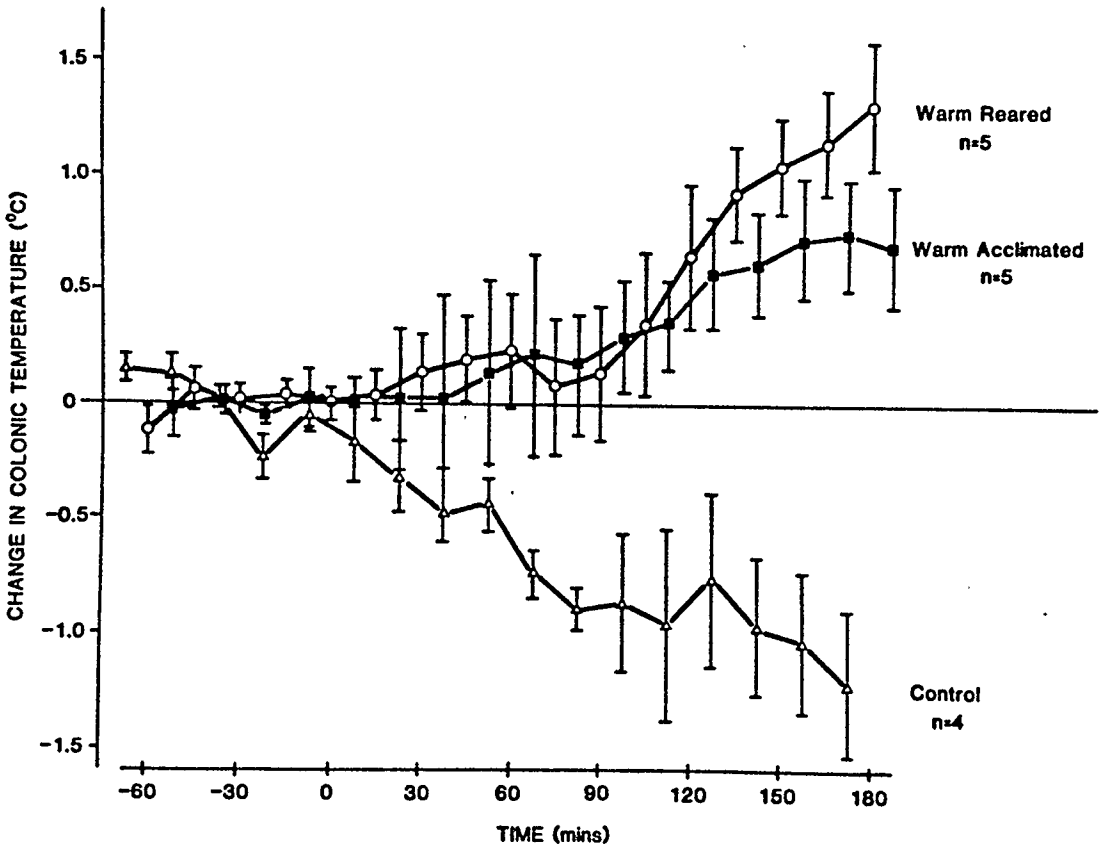


Figure 20: These curves show the effects of dopamine (20.0 µg/side) microinjected into the AH/POA, at time = 0, on the body temperature of warm-reared, warm-acclimated and control rats. Each point represents the mean (± SEM).

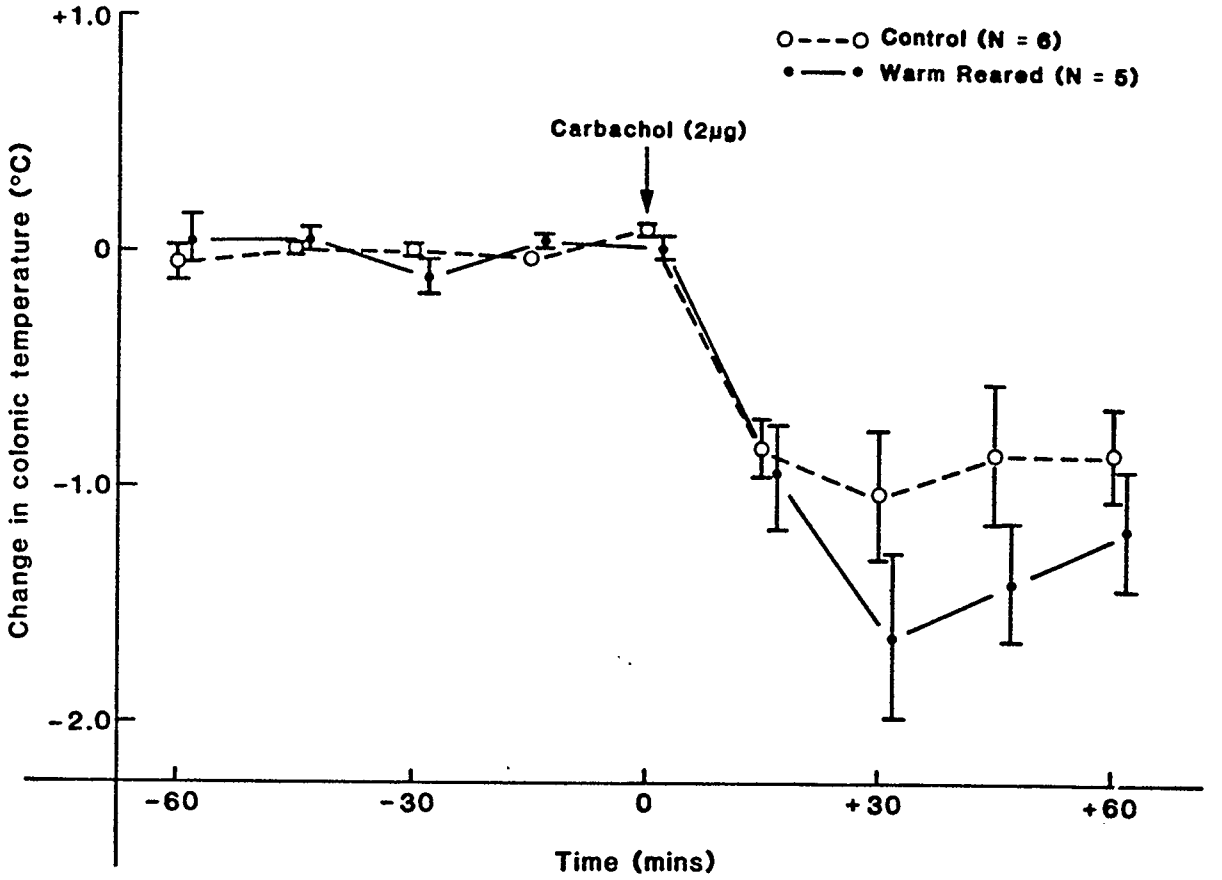


Figure 21: This graph illustrates the changes in colonic temperature which were observed in control and warm-reared animals after intrahypothalamic administration of carbachol (2 µg/side) at time = 0. Each point represents the mean (± SEM).

warm-reared animals by intrahypothalamic microinjection of 5.0 µg serotonin/side are shown in Fig 18. No significant changes in temperature were observed in warm-reared animals, while a short latency hyperthermia followed drug administration in the warm-acclimated group. The changes in temperature of the two groups, 15 mins after microinjection, were found to be statistically significantly different ($p < 0.05$). When 10.0 µg/side serotonin was administered to these two groups of animals, hypothermia occurred in warm-reared animals but no changes in colonic temperature were seen in the warm-acclimated group (Fig 19). When the changes in temperature of these two groups, 15 mins after serotonin treatment, were compared, they were shown to be significantly different ($p < 0.01$).

c) Dopamine

The changes in colonic temperature recorded following intrahypothalamic administration of dopamine are illustrated in Fig 20. Hypothermia was observed in control animals while, in contrast, a longer latency rise in body temperature was found to occur in both warm-acclimated and warm-reared rats. The temperature changes of these latter two groups were both significantly different to controls when measured over 180 mins ($p < 0.01$) but were not significantly different to each other ($p > 0.1$).

d) Carbachol

No statistically significant differences between the changes in colonic temperature of warm-reared and control animals were observed following administration into the AH/POA of the cholinergic agonist carbachol ($p > 0.1$). Rapid onset hypothermia was shown to occur in both groups of rats (Fig 21). As no differences were found between

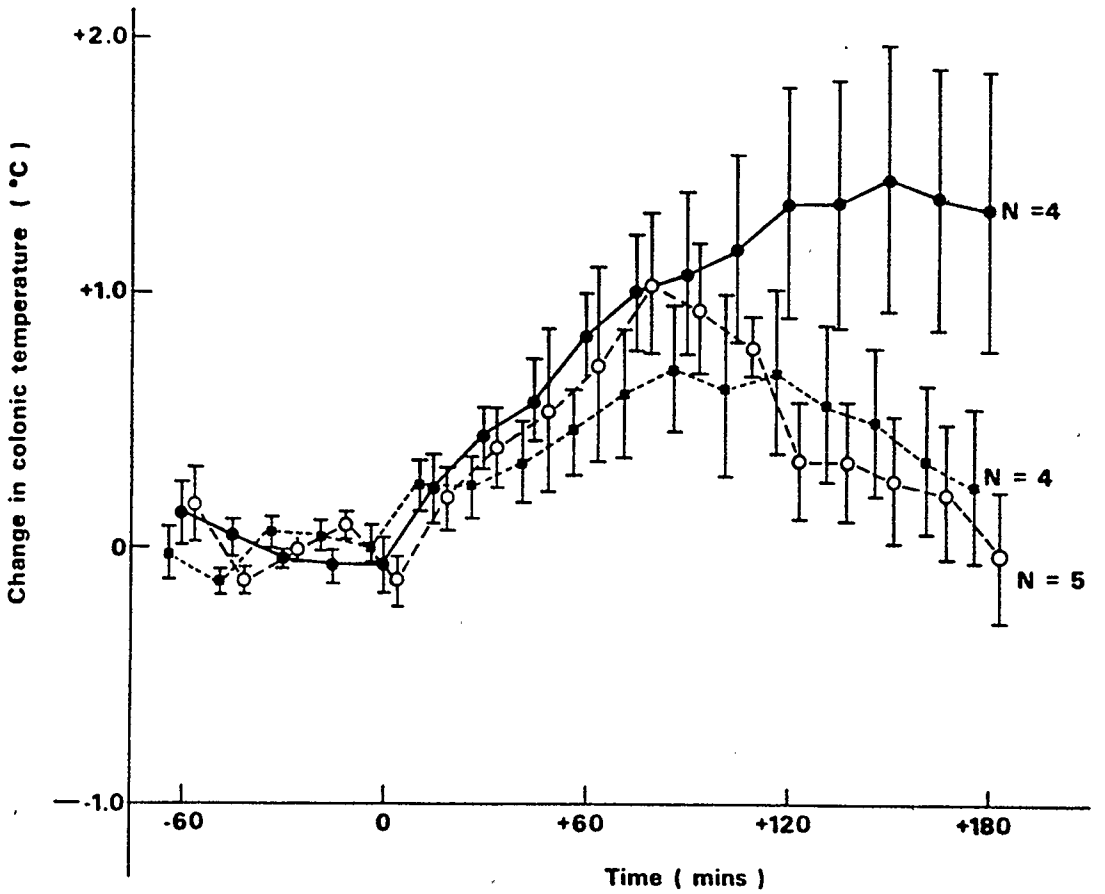


Figure 22: Graph showing the mean (± SEM) changes in colonic temperature which were measured after intrahypothalamic microinjection of endotoxin (5.0 µg/side), at time = 0, in control (■----■), warm-acclimated (○——○), and warm-reared (●——●) Sprague Dawley rats.

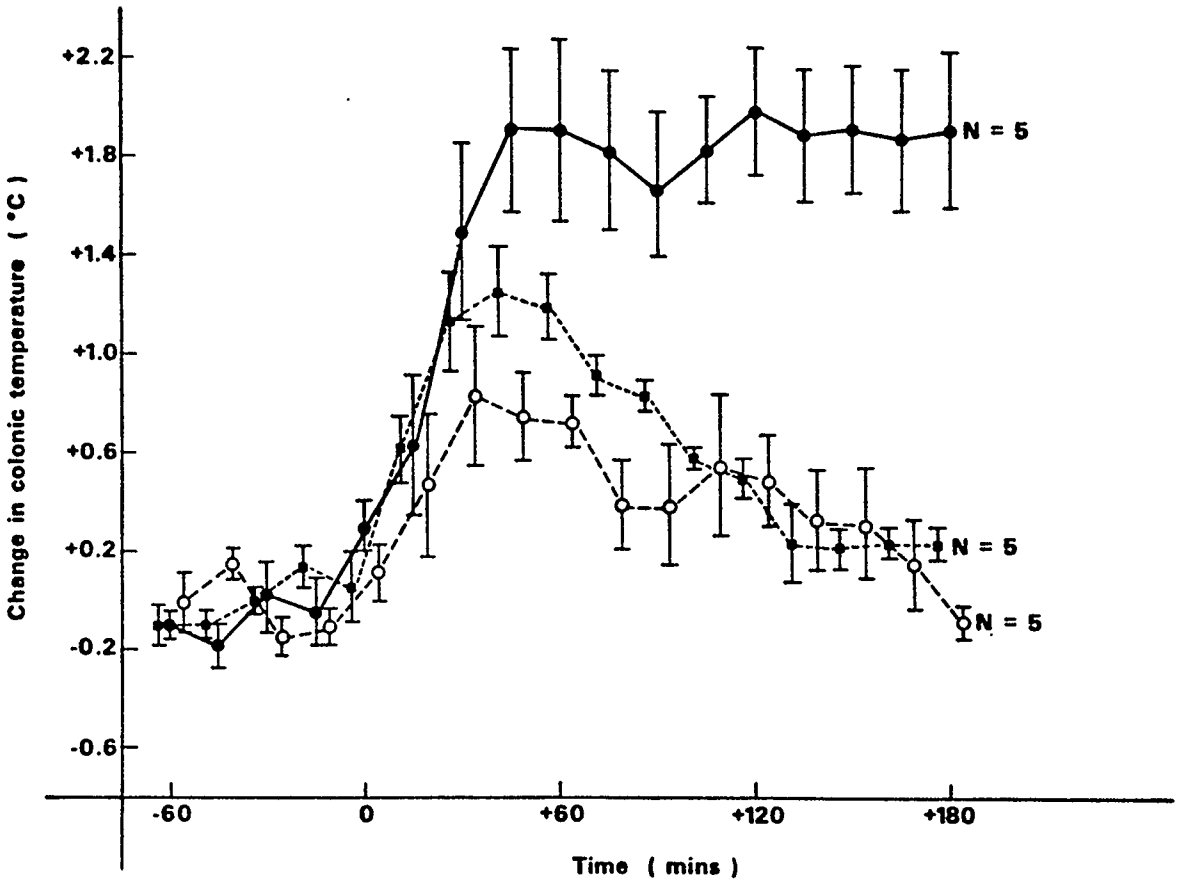
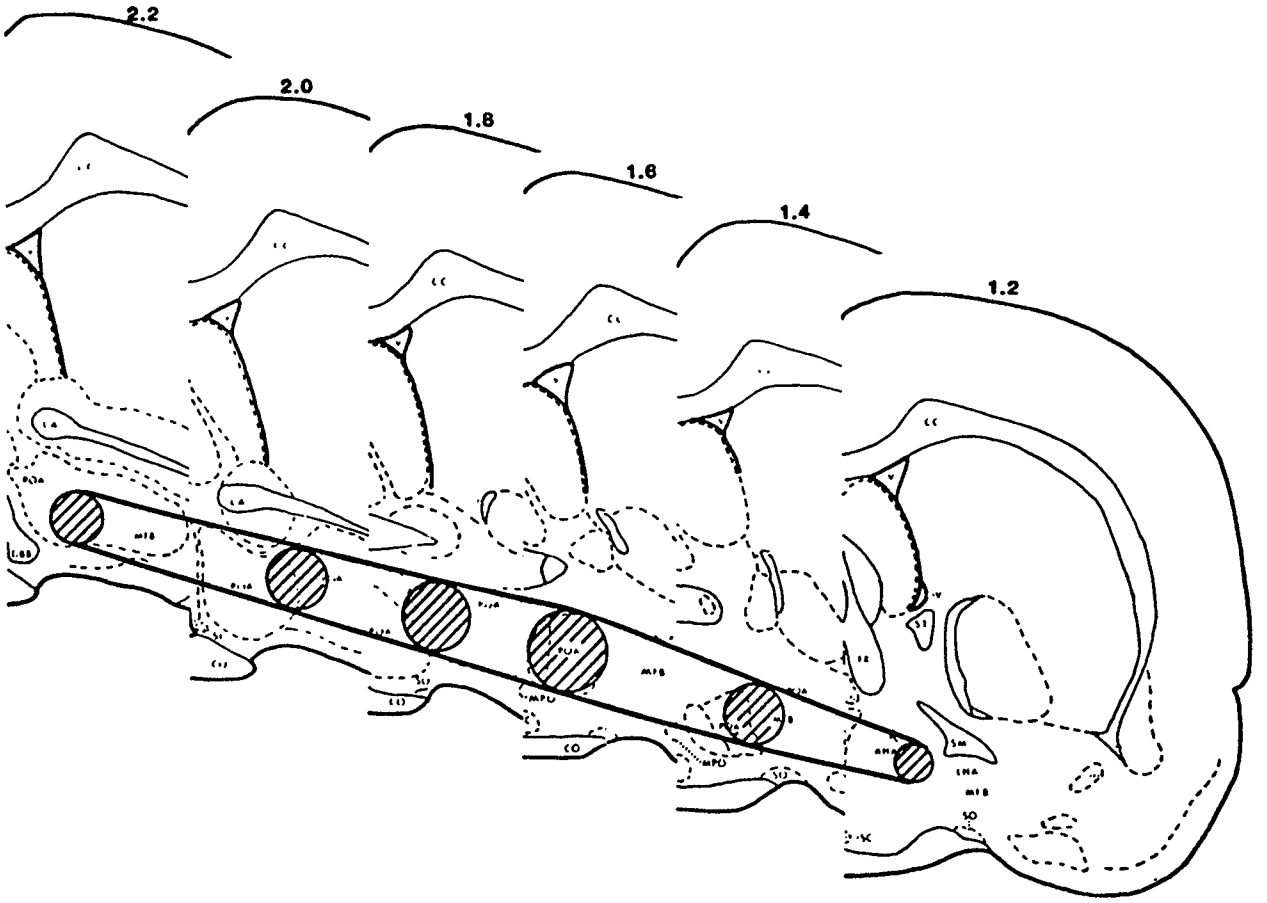


Figure 23: Colonic temperature changes in control (○—○), warm-acclimated (■---■), and warm-reared (●——●) rats in response to microinjection of PGE_2 (100 ng/side), at time = 0, into the AH/POA. Each point represents the mean ± SEM).

Figure 24: A diagramatic representation of brain sections showing the extent of the anatomical locations which were considered to be in the AH/POA of the brain. The number above each section is the distance anterior to bregma of that section, according to the stereotaxic atlas of Pellegrino et al. (1979).

Abbreviations:

AHA - anterior hypothalamic area
CA - anterior commissure
CC - corpus callosum
CO - optic chiasm
DBB - diagonal band of Broca
FX - fornix
LHA - lateral hypothalamic area
MFB - medial forebrain bundle
MPO - medial preoptic area
POA - preoptic area
SC - suprachiasmatic nucleus
SM - stria medullaris
SO - supraoptic nucleus
ST - stria terminalis
V - ventricle


2.2
2.0
1.8
1.6
1.4
1.2

warm-reared and control groups, the comparison with warm-acclimated rats was not made in this group of experiments.

As a second part of this series of experiments, the pyrogenic effects of endotoxin and PGE_2 microinjected into the AH/POA were examined.

a) Endotoxin

Hyperthermia was observed in all groups of animals following intrahypothalamic microinjection of endotoxin (Fig 22). However, there were no statistically significant differences between the fevers recorded from the different groups ($p > 0.1$).

b) Prostaglandin (PGE_2)

Fever was shown in all groups following administration of PGE_2 into the AH/POA (Fig 23). While no significant differences were found between the changes in colonic temperature of control and warm-acclimated rats ($p > 0.1$), the duration of the hyperthermia was significantly reduced in these two groups when compared to warm-reared animals ($p < 0.01$).

The anatomical locations within the brain which were considered to lie within the boundaries of the AH/POA are illustrated in Fig 24.

DISCUSSION

A considerable amount of variability was observed within individual groups in all experiments where microinjections were made into the AH/POA of the brain. Such variability is not surprising and is probably explained by the relative heterogeneity of infusion sites in terms of their precise anatomical location, i.e. although all sites were in the AH/POA, this does not mean that they were all in the most sensitive

region of this particular area of the brain in terms of the thermoregulatory responses elicited by the various drugs administered . Despite this variability, the thermoregulatory effects of these putative neurotransmitters in control animals correspond well to the actions reported in previous studies (see Introduction). The results obtained from warm-reared and warm-acclimated animals suggest that both the processes of warm-rearing and warm-acclimation lead to changes in the way in which the hypothalami of these different groups of animals control body temperature. The experimental results fall into three basic categories:

i) Drugs which, when administered into the AH/POA. have the same effects on the body temperature of both control and warm-reared animals. The warm-acclimated group becomes an unnecessary control in such cases as warm-rearing has not affected the hypothalamic thermoregulatory response to the drug. The effects of carbachol and endotoxin both fall into this category.

ii) Drugs which produce significantly different temperature responses in both warm-acclimated and warm-reared animals, when compared to controls, following intrahypothalamic microinjection. Dopamine has such effects on the three groups. The fact that there were no differences between the responses of warm-reared and warm-acclimated animals indicates that such changes are a part of the normal acclimation process.

iii) The most significant drug treatments in terms of this discussion are those in which thermoregulatory responses of warm-reared animals are statistically different to both warm-acclimated and control groups. The effects of intrahypothalamic injection of noradrenaline,

serotonin, and PGE_2 all fall into this category. These data suggest that rearing animals at 33.0°C alters the role which these putative thermoregulatory neurotransmitters play in the hypothalamic control of body temperature.

Many different neuronal models of temperature regulation have been proposed (Hammel, 1965; Wyndham & Atkins, 1968; Bligh & Cottle, 1969; Hardy & Guieu, 1971; Bligh, 1973; Bligh, 1974; Hellon, 1975; Myers, 1975; Frens, 1980), although a unified theory appears to be no closer today than it was ten years ago. Thus, it would appear to be a great over simplification to discuss how the results presented in this chapter relate to any one specific model; more significant is the fact that changes were observed. However, some of the changes found in warm-reared animals will be discussed in terms of a few of the more consistent features of these neuronal models.

Many of the above models of temperature regulation have postulated that noradrenaline acts as an inhibitory neurotransmitter in neuronal pathways which control both heat production and heat loss. Which of these two pathways is inhibited thus would depend on the relative activity of the cold and warm receptors throughout the body. According to such a model, the decrease in body temperature observed following intrahypothalamic infusion of noradrenaline would be due to an inhibition of heat production. This effect was observed in both control and warm-acclimated animals. Obviously, in the dose used in the present studies, noradrenaline does not fulfill this role in the hypothalami of warm-reared animals. The observed rises in temperature suggest that, in warm-reared rats, this monoamine may be inhibiting heat loss only, an effect which would be masked normally by a stronger inhibitory influence

of the amine on heat production effector mechanisms. Such changes in hypothalamic reactivity to noradrenaline could be explained in several different ways, i.e. a hyposensitivity of the AH/POA to this transmitter or significant changes in the neuronal circuitry of this area of the brain. The exact nature of this change has not yet been experimentally examined. However, it should be mentioned that the noradrenergic inhibition of heat production, according to many neuronal models of thermoregulation, acts on the direct pathway between cold sensors and heat production.

Much controversy still exists as to the role which serotonin plays in the hypothalamic control of body temperature (compare reviews by Jacob & Girault, 1979 and Myers, 1981), although it has been suggested that this substance may act to stimulate both heat conservation and heat loss (Komiskey & Rudy, 1977). However, the point of major importance to this discussion is the observation that serotonin has different thermoregulatory effects when microinjected into to the hypothalami of warm-reared as compared to warm-acclimated rats. It would appear that stimulation of heat loss by serotonin may be potentiated in warm-reared animals. Alternatively, the observed results may represent a decreased serotonergic drive to heat conservation.

Dopamine has been implicated as a neurotransmitter which plays a role in the control of peripheral vasomotor tone (Cox, 1979; Frens, 1980). The results reported in this section show that the thermoregulatory role of this particular substance in the hypothalamus is modified during the normal warm-acclimation process. These data support the concept that some degree of plasticity exists in the central nervous systems' control of body temperature in adult animals, and also

raise the possibility that functional plastic changes within the nervous system may be an essential part of the normal acclimation process. A second possibility is that the modified response to dopamine may be due to changes in the peripheral effector mechanisms of temperature regulation.

The majority of studies in which acetylcholine or carbachol are microinjected into the AH/POA of the rat indicate that this neurotransmitter increases heat loss (Crawshaw, 1979). In the studies reported here, carbachol was utilized as a cholinergic agonist owing to its longer duration of action. The observation that there were no significant differences between the thermoregulatory effects of this substance in warm-reared, as compared to control animals, following intrahypothalamic administration fits well with the earlier observation that, during heat stress, when this mechanism would be activated, there were no differences between colonic temperature changes which occurred in the two groups of rats.

The increased febrile response of warm-reared, as compared to warm-acclimated and control rats, following intrahypothalamic administration of PGE_2 is surprising in view of the observation that fever in response to intravenous injection of endotoxin was reduced in the warm-reared rabbit. This paradox may represent either a simple species difference or a change which is related to the route of administration and the pyrogen used.

In summary, these studies show differences in the hypothalamic thermoregulatory role of certain putative neurotransmitters in warm-reared, as compared to control and warm-acclimated animals. Such changes suggest that the early environment of these rats may have

affected the hypothalamic neuronal circuitry involved in the control of body temperature.

VI. Investigations to Define a "Critical Period" of Plasticity of the Thermoregulatory System

This series of experiments were designed to examine the time period during early postnatal life during which the thermal environment influences the development of temperature regulation. This question was approached by moving rats from the normal 20.0°C environment to the high temperature (33.0°C) environmental chamber at a variety of ages and then measuring in which groups the thermoregulatory deficits of warm- reared animals were observed.

MATERIALS & METHODS

The specific strategy of this series of experiments was to deprive animals of cold stimulation for differing time periods during early development. As already described, warm-reared animals were born into a 33.0°C environment. Three other groups of animals were used in this work, consisting of Sprague Dawley rats which were placed in the 33.0°C environmental chamber at 2, 30, or 60 days of age. The thermoregulatory responses of these groups of animals were then examined between 150 and 240 days of age in order to determine in which, if any, of these groups the deficits found in warm-reared animals occurred.

RESULTS

The effect of a 4.0 hr cold exposure at 2.0°C on the four groups of animals was first examined. The data obtained are illustrated in Fig 25. This figure represents the mean falls in colonic temperature during the final 60 mins of each group of animals' first exposure to the cold.

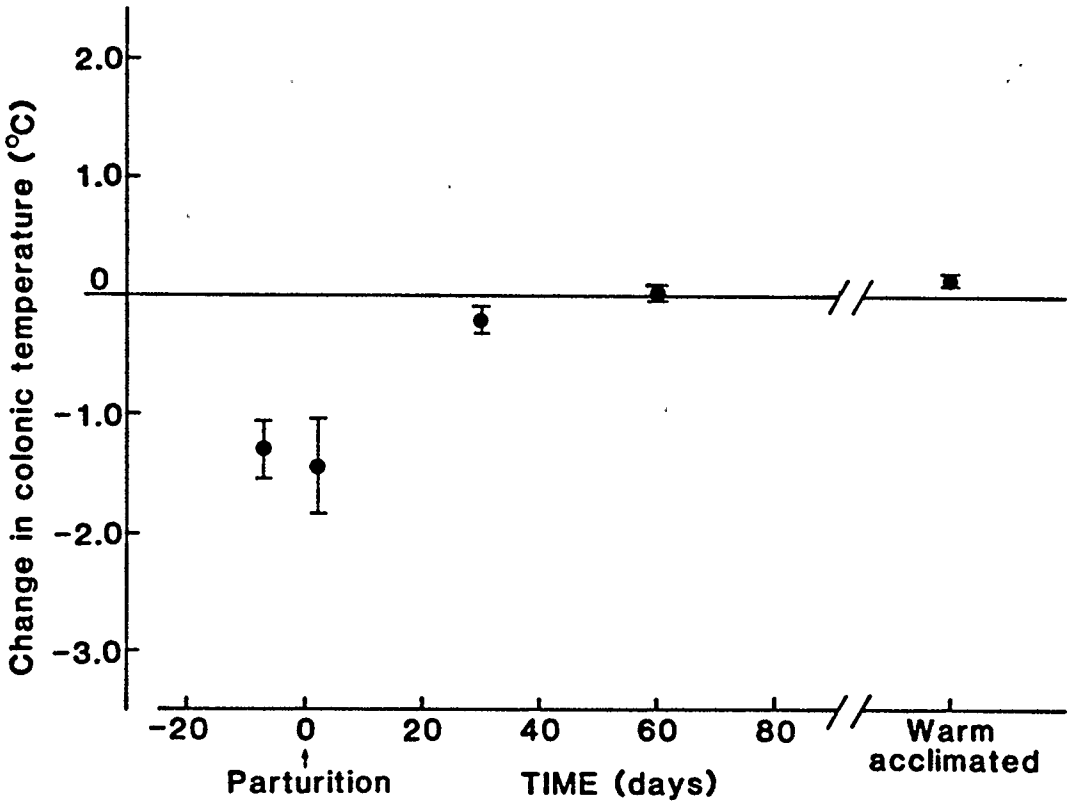


Figure 25: This figure illustrates the mean (± SEM) falls in colonic temperature which occurred during the final 60 minutes of cold exposure in rats born at 33.0°C (N = 8); in animals moved to this environment 2 days (N = 6), 30 days (N = 8), and 60 days (N = 6) after birth; and also in warm-acclimated rats (N = 8).

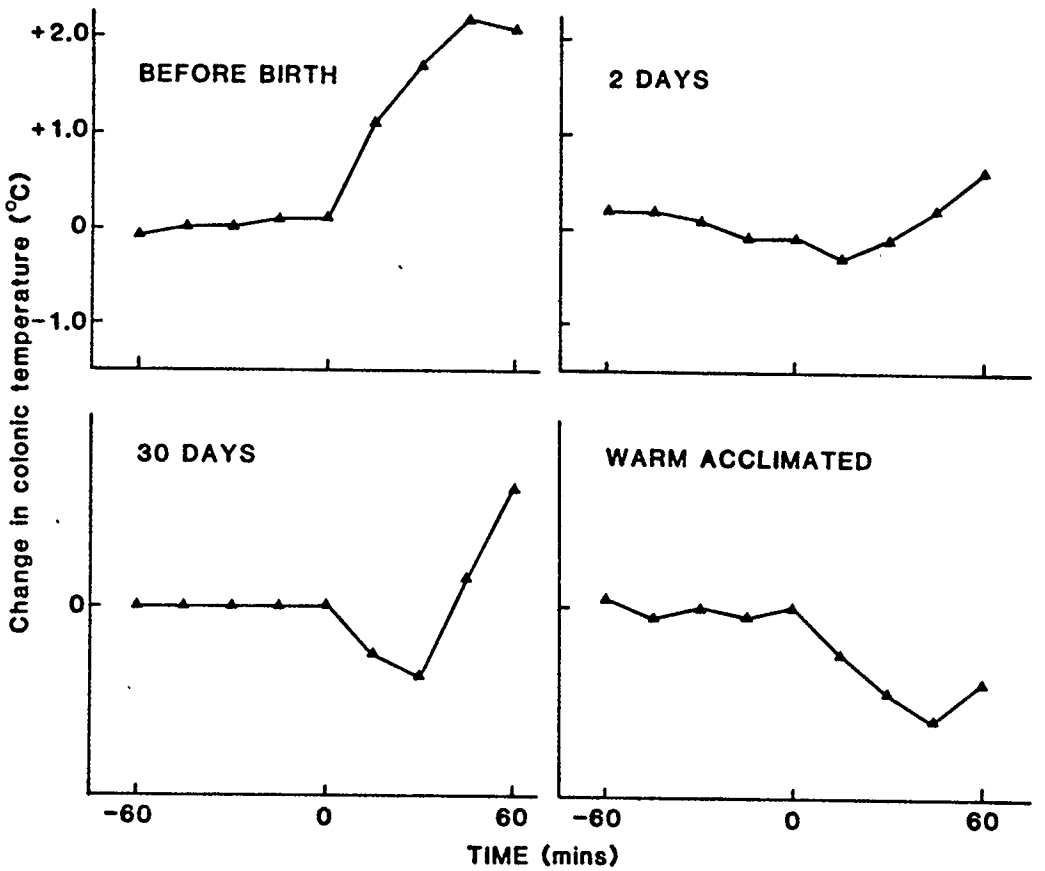


Figure 26: This graph shows the changes in colonic temperature of rats born at 33.0°C; of warm-acclimated animals; and of animals moved to this environment at 2 and 30 days of age, in response to intrahypothalamic administration of noradrenaline (10.0 µg/side). The same temperature scale is used in all four graphs.

These results show that during this time period the falls in colonic temperature of rats placed in the environmental chamber at 2 days of age were not significantly different to the original warm-reared group. In contrast, no significant falls in colonic temperature occurred during the final 60 mins of cold exposure in animals placed at 33.0^{o}C at either 30 or 60 days of age.

The effects of intrahypothalamic microinjection of noradrenaline were examined also in a limited number of animals moved to the 33.0^{o}C environment at 2 (N=2) and 30 (N=3) days of age. Representative examples of the responses observed in these two groups, as well as typical examples already obtained from warm-reared and warm-acclimated animals are presented in Fig 26. These data suggest that the hyperthermia which occurred in the group of rats moved to 33.0^{o}C at 2 days of age was less pronounced than that which occurred in the warm-reared group. In the group placed in the environmental chamber at 30 days of age, a short latency hypothermia was observed as in the warm-acclimated animals. This hypothermia was less pronounced than in the warm acclimated group and was followed rapidly by a rise in temperature. All microinjection sites were shown to be in the AH/POA as described previously.

DISCUSSION

Although it was believed for some time that the "critical period" for visual plasticity in the cat was a clearly defined time period during the first six weeks of neonatal life (Hubel & Wiesel, 1970; Blakemore & Van Sluyters, 1975), there is now considerable evidence

which demonstrates that visual experience during other periods of life also may influence the functional characteristics of visual cortical neurons (Cynader & Mitchell, 1980; Cynader et al., 1980; Juraska et al., 1980).

Many studies in which the responses of the adult, central nervous system to injury have been examined show that, even at this stage of "development", a considerable degree of axonal regeneration and synaptic remodelling occur (Westrum & Black, 1971; Hamori, 1973; Steward et al., 1974). However, whether such morphological changes contribute to a functional physiological recovery is not known. Despite the evidence above, which indicates that plasticity of the central nervous system is not an all or nothing effect which occurs only in early life, there is general agreement that a much greater potential for plastic change exists in the early stages of development.

The data presented in this chapter indicate that the thermal experience of an animal may be of most significance to the development of temperature regulation during the first 30 days of life, at least in the Sprague Dawley rat. Whether all of the changes in temperature regulation observed in warm-reared animals are a result of environmental deprivation during this specific time period is not known. The fact that noradrenergic and serotonergic neurons make functional synaptic connections in the brain at different times during development (Kasamatsu & Pettigrew, 1976) raises the possibility that the influence of warm-rearing on the physiological actions of these two neurotransmitters within the hypothalamus may have critical periods related to the specific ontogeny of these neurochemical networks.

In summary, the observation that animals first placed at 33.0^{o}C

when 30 days of age are able to maintain colonic temperature throughout the final 60 mins of cold exposure, suggests that the "critical period" occurs before the animals reach 30 days of age. The data on intrahypothalamic noradrenaline microinjection support such an hypothesis, although a note of caution should be added in that the interpretation of this latter group of data is difficult, as true comparisons cannot be made from one animal to another unless the anatomical location of microinjection sites is precisely the same in all animals. Despite the fact that all sites were in the AH/POA, they did not fulfill this criterion.

VII. Experimental Evidence Demonstrating Age-Related Changes in the Febrile Response

The evidence so far presented in this thesis has suggested that thermoregulatory function can be changed by the thermal experiences of an animal in early life. There is now some experimental support for the concept that the ability of the body to regulate its temperature may be impaired with advancing age (see introduction). The studies reported in this chapter were designed to investigate the febrile response of New Zealand White rabbits of different ages and, thus, to establish whether the thermoregulatory mechanisms involved in this response change with increasing age.

MATERIALS AND METHODS

New Zealand White rabbits were used in these studies and were split into two different age groups, one consisting of animals less than 1 yr old, while the second included all animals greater than 3 yrs of age. For convenience, these two groups were named "young" and "old", respectively. Fever was induced in experimental animals by intravenous injection of either endotoxin derived from Salmonella abortus equi (1.5 μg/kg) or live Pasteurella multicida (6×10^9 organisms/animal).

In order to measure plasma catecholamines during fever, blood samples were collected at 60 min intervals, both before and after intravenous injection of endotoxin. Each sample (0.5 ml) was drawn through a cannula (PE 60 Intramedic) attached to a 20 gauge, sterile, stainless steel needle which was inserted into the central ear artery on

the day of experimentation. Samples were kept on ice until centrifugation, the plasma was retained and stored at -20°C until later determination of both plasma adrenaline and noradrenaline levels, as previously described.

Changes in plasma catecholamine concentrations were expressed as a percentage of baseline levels, these were calculated for each animal by taking the average of two catecholamine measurements during the 60 mins prior to endotoxin administration. Comparison of "baseline" and "during fever" concentrations within groups was achieved by use of the paired Student's "t" test, while evaluation of the differences between young and old groups utilized the unpaired students' "t" test.

Blockade of the α or β adrenergic receptors was achieved by intravenous injection of either phenoxybenzamine (Smith, Kline & French, 1.0 mg/kg) or propranol (Sigma HCl, 1.0 mg/kg plus 0.25 mg/kg every 30 mins), respectively, and the effect of such pharmacological treatments on the febrile response of young animals was examined.

The effects of intravenous infusion of both adrenaline (Adrenaline-Bitartrate, K & K Lab.) and noradrenaline (HCl, Sigma), dissolved in sterile saline, were investigated. The catecholamines were infused through acutely implanted 23 gauge sterile, stainless steel needles attached to PE 50 (Intramedic) polyethylene tubing, at a rate of 2.0 µg/kg/min for 60 mins by use of a Harvard infusion pump.

RESULTS

Animals less than 1 yr of age developed a typical biphasic fever in response to intravenous injection of endotoxin. The febrile response of rabbits greater than 3 yrs of age was significantly reduced in the first

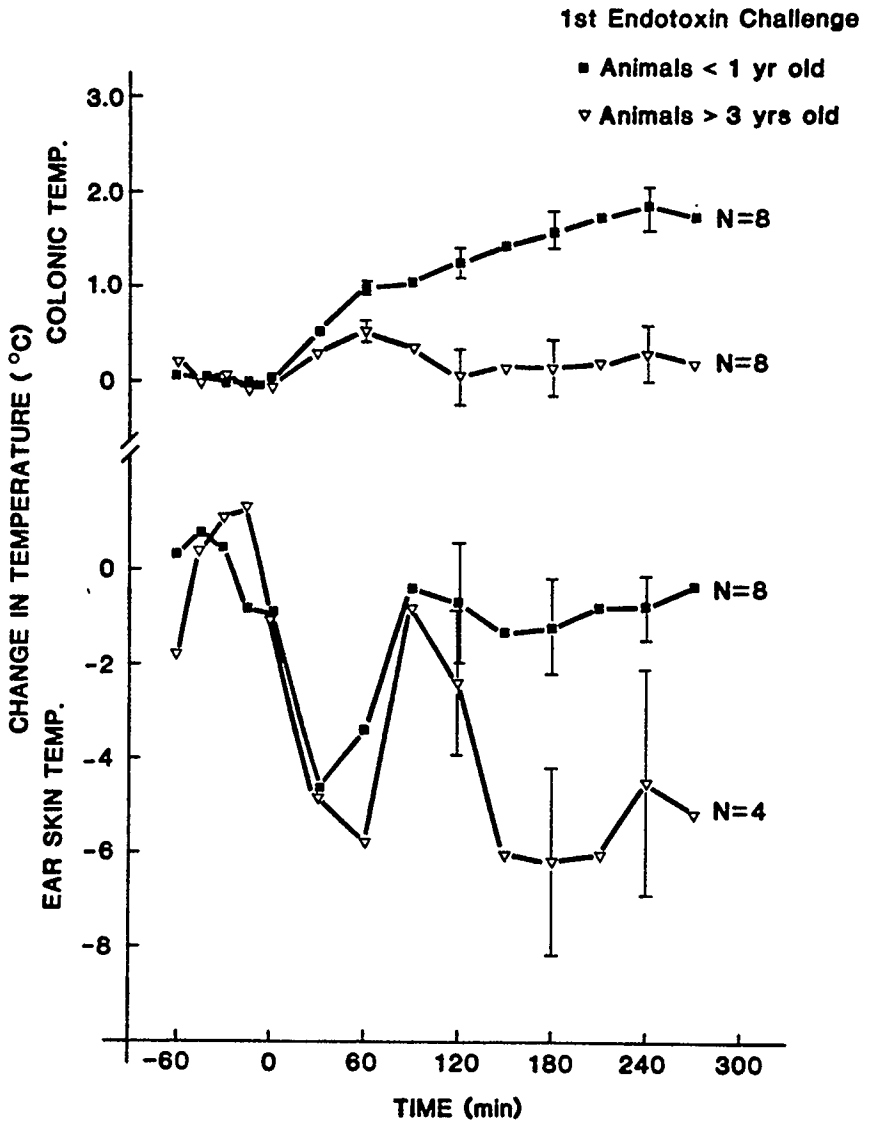


Figure 27: Graphs showing the mean (± SEM) changes in colonic (upper panel) and ear skin (lower panel) temperatures following intravenous injection of endotoxin at time = 0.

peak ($p < 0.01$), while the second peak was absent (Fig 27). Also, it was observed that peripheral vasoconstriction occurs in both groups of animals during the first peak of fever (as assessed by changes in ear-skin temperature) but only in the older group of animals during the time period when the second peak of fever occurred in young rabbits (Fig 27). No second phase of ear vessel vasoconstriction was observed in young animals during the second peak of fever.

The biphasic nature of the febrile response was not observed following intravenous injection of Pasteurella multicida and there were no significant differences between the fevers of the two groups in the two hours immediately following injection as shown in Fig 28 ($p > 0.05$). However, the old animals' fevers were found to be significantly reduced after this time period (Fig 28, $p < 0.01$).

The changes observed in peripheral vasoconstriction suggested that the plasma catecholamine levels may be significantly higher in the old, as compared to the young group of animals, during fever. The data presented in Fig 29 show that plasma noradrenaline increased significantly in both young (+116 ± 24%; $p < 0.05$) and old (+125 ± 25%; $p < 0.05$) animals during fever. However, there were no significant differences between the two groups ($p > 0.05$). Similarly, plasma adrenaline levels were significantly elevated during fever in both young (+114 ± 42%; $p < 0.05$), and old (+1890 ± 560%; $p < 0.01$) animals (Fig 30), but, in this case, the increase observed in the old animals was significantly greater than that observed in the young group ($p < 0.01$). There were no differences between the baseline levels of catecholamines in the two groups, as shown in Table 5. Other controls demonstrate that saline injections in old animals did not lead to increases in plasma

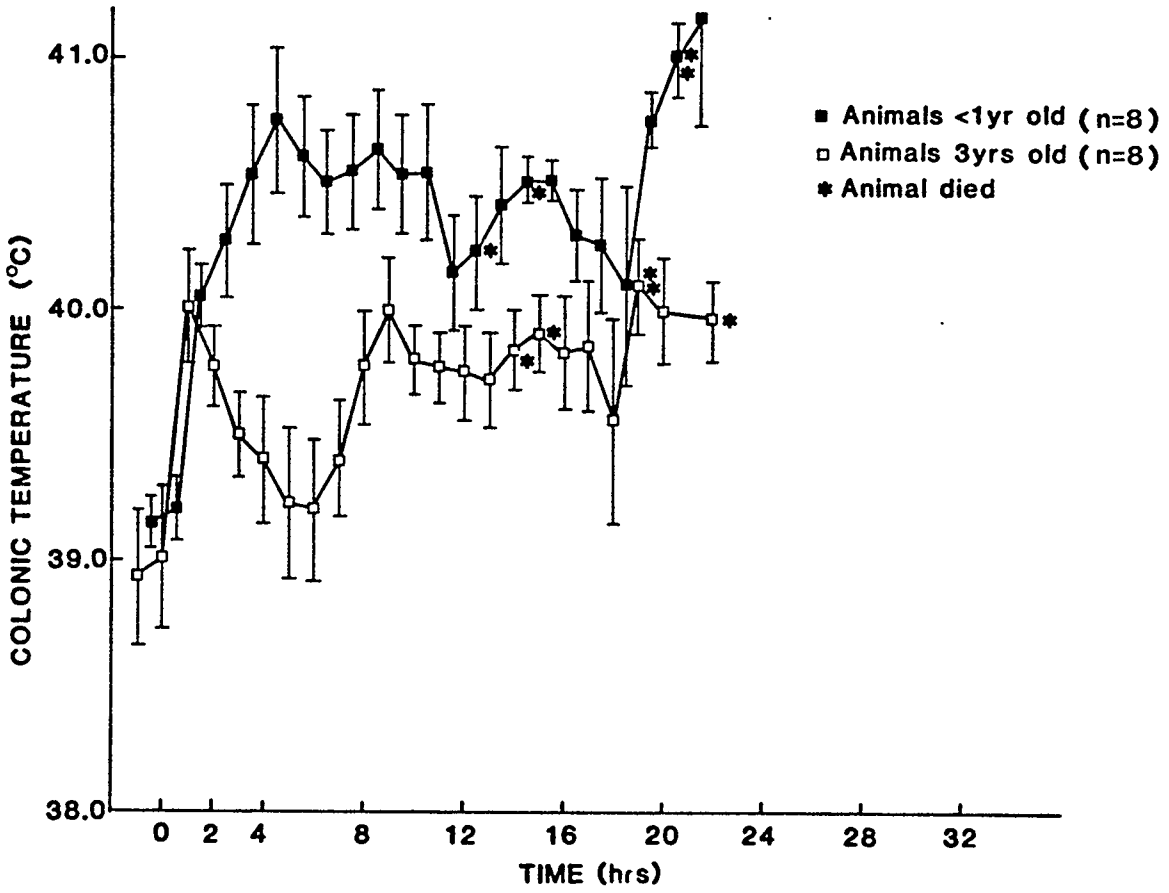


Figure 28: This graph shows the mean (± SEM) colonic temperatures of New Zealand White rabbits following intravenous injection of live Pasteurella multicida (9 x 10^6 organisms/animal) at time = 0.

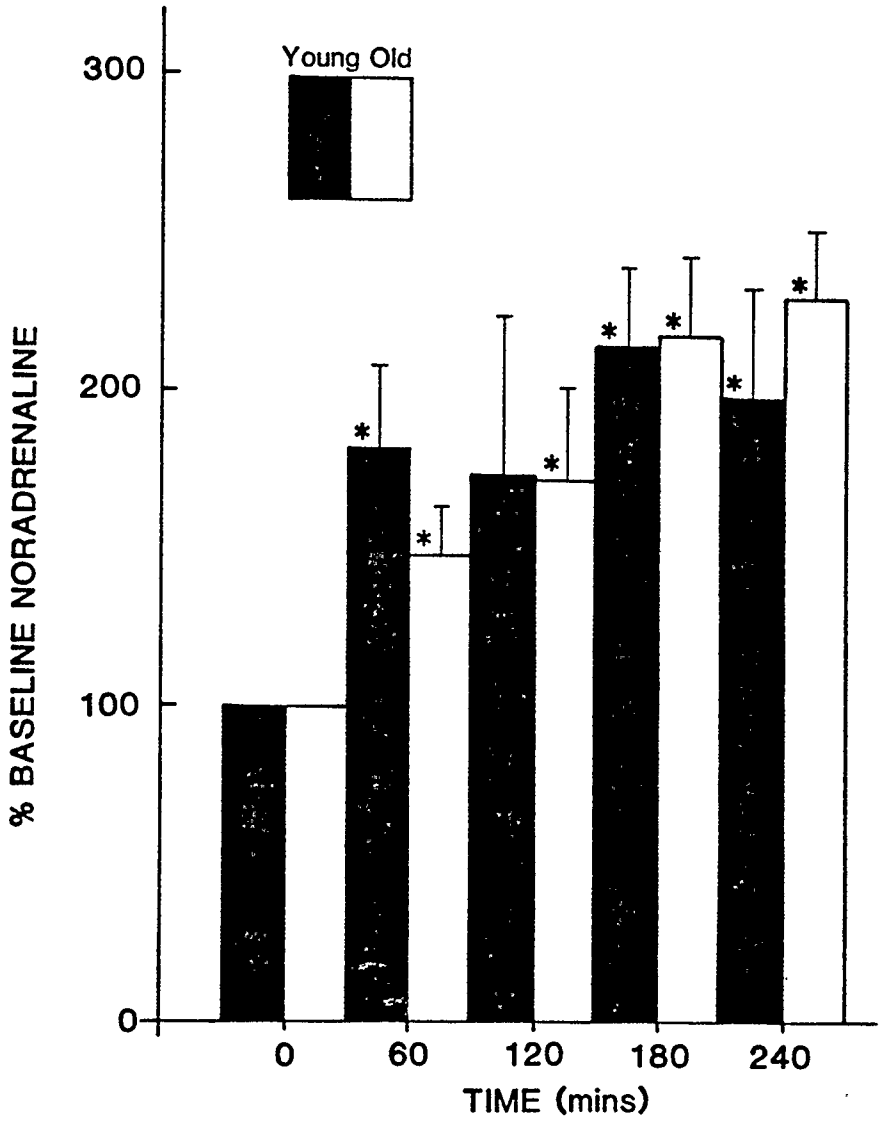


Figure 29: Histogram demonstrating the changes in plasma noradrenaline concentrations during endotoxin fever in rabbits < 1 yr of age (N = 8) and > 3 yrs old (N = 11). Values are presented as a percentage of baseline concentrations. Each bar represents the mean ± SEM. * $p < 0.05$ compared to baseline.

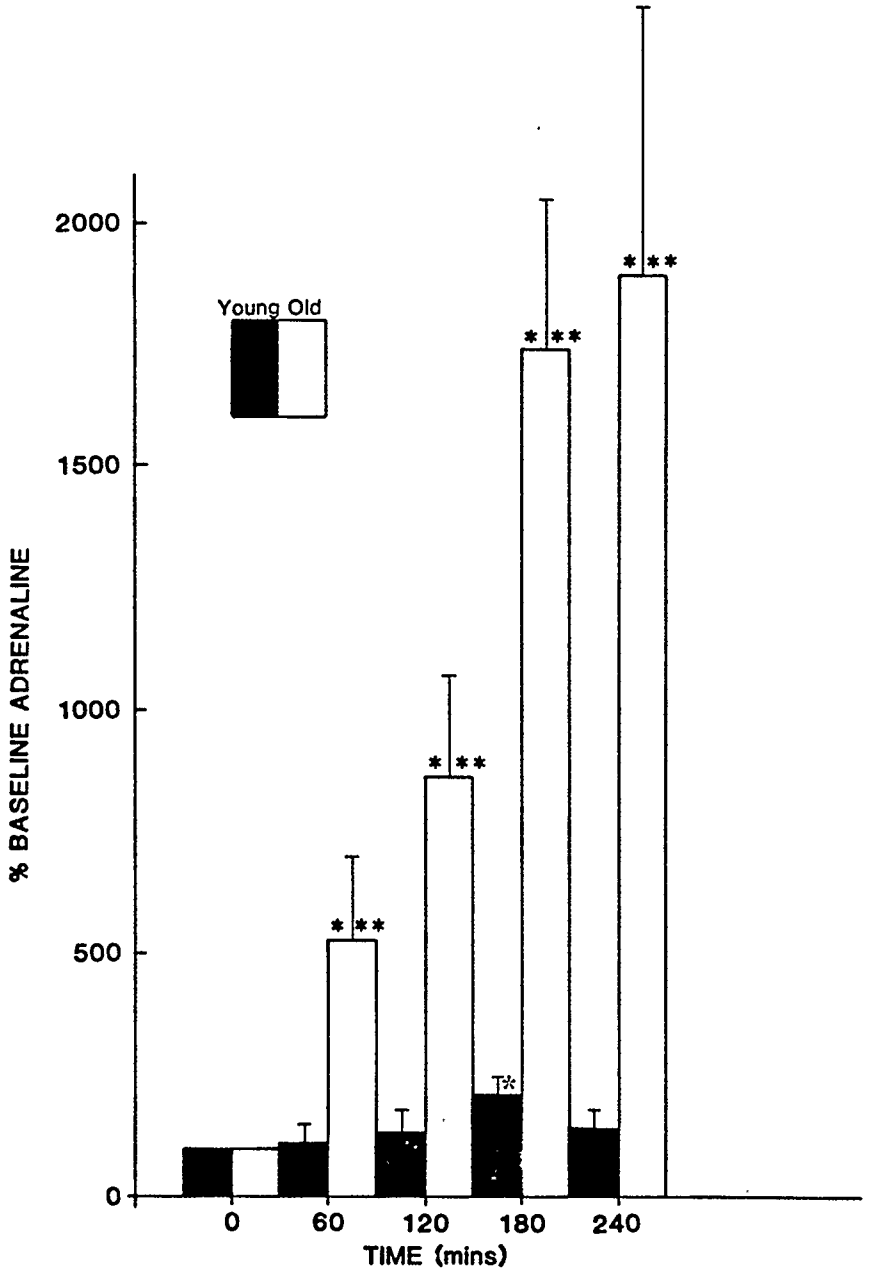


Figure 30: Histogram showing % changes in plasma adrenaline levels during fever in animals < 1 yr of age (N = 8), and > 3 yrs of age (N = 11). Each point represents the mean ± SEM. * $p < 0.05$ compared to baseline. ** $p < 0.01$ compared to animals < 1 yr of age.

TABLE 5: Basal catecholamine levels

Age	Catecholamines (pg/ml)	
	Adrenaline	Noradrenaline
< 1 year (N = 8)	54 ± 17	259 ± 31
> 3 years (N = 11)	69 ± 20 *	371 ± 59 *

* $p > 0.1$ compared to < 1 yr old group

catecholamines (Table 6). In order to establish if these rises in plasma adrenaline and noradrenaline were a non-specific response to increases in colonic temperature in old animals, plasma catecholamines were measured during hyperthermia induced by heat stress. No significant changes in plasma adrenaline or noradrenaline were observed following a 1.0°C rise in colonic temperature (Table 7).

The observed increases in plasma catecholamines with no subsequent increase in body temperature observed in old animals suggested a deficit in non-shivering thermogenesis in this group of rabbits. Thus, the effects of adrenaline and noradrenaline infusion on body temperature was examined in both groups of rabbits. Noradrenaline infusion was found to cause a rise in body temperature in young animals (+0.85 ± 0.16°C), while a slight fall in temperature was observed in the old group (-0.34 ± 0.10°C) as shown in Fig 31. Similarly, adrenaline infusion caused significant rises in body temperature in young animals ($P < 0.05$) but no significant rise above baseline temperature in old rabbits (Fig 32).

Finally, the effect of adrenergic receptor blocking agents on the febrile response of young animals was examined. Treatment with the α-adrenergic blocking agent, phenoxybenzamine, resulted in significantly reduced fevers in young animals (Fig 33), while the β-adrenergic blocking agent, propranolol, had no significant effects on the changes in body temperature following intravenous injection of endotoxin in rabbits less than 1 year old (Fig 16).

TABLE 6: Plasma catecholamine levels in 3 yr old rabbits (N = 4) following intravenous injection of sterile saline at time 0.

Time (mins)	Catecholamines (pg/ml)	
	Adrenaline	Noradrenaline
0	30 ± 18	232 ± 18
120	38 ± 2 *	256 ± 39 *
240	26 ± 16 *	262 ± 33 *

* $p > 0.1$ compared to time 0

Table 7: Plasma catecholamine levels during hyperthermia induced by heat stress in 3 yr old rabbits (N = 4).

Increase in body temperature (^{o}C)	Catecholamines (pg/ml)	
	Adrenaline	Noradrenaline
0	50 ± 16	372 ± 86
1.0	42 ± 20*	407 ± 116*

* $p > 0.1$ compared to baseline temp ($\Delta T = 0$)

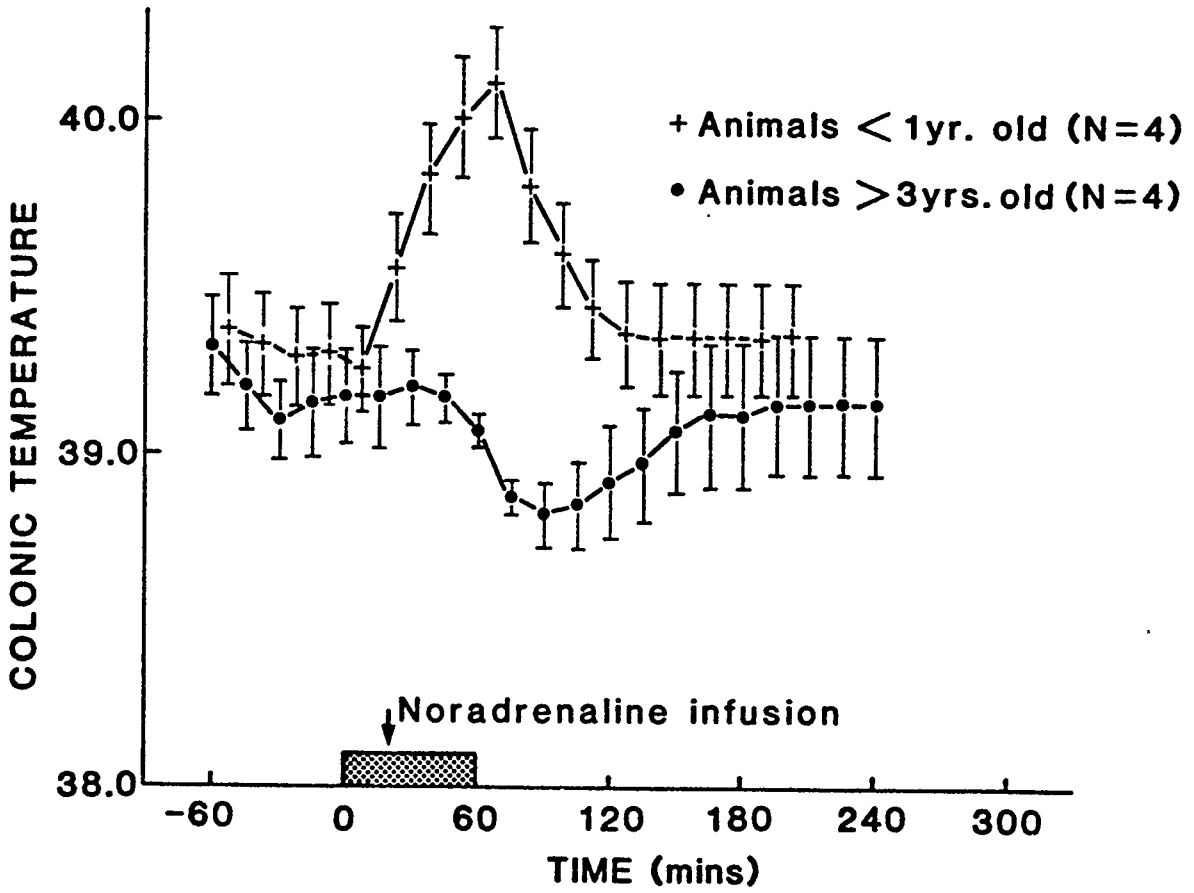


Figure 31: Graph showing the mean ± SEM colonic temperature changes in response to intravenous infusion of noradrenaline for 60 minutes.

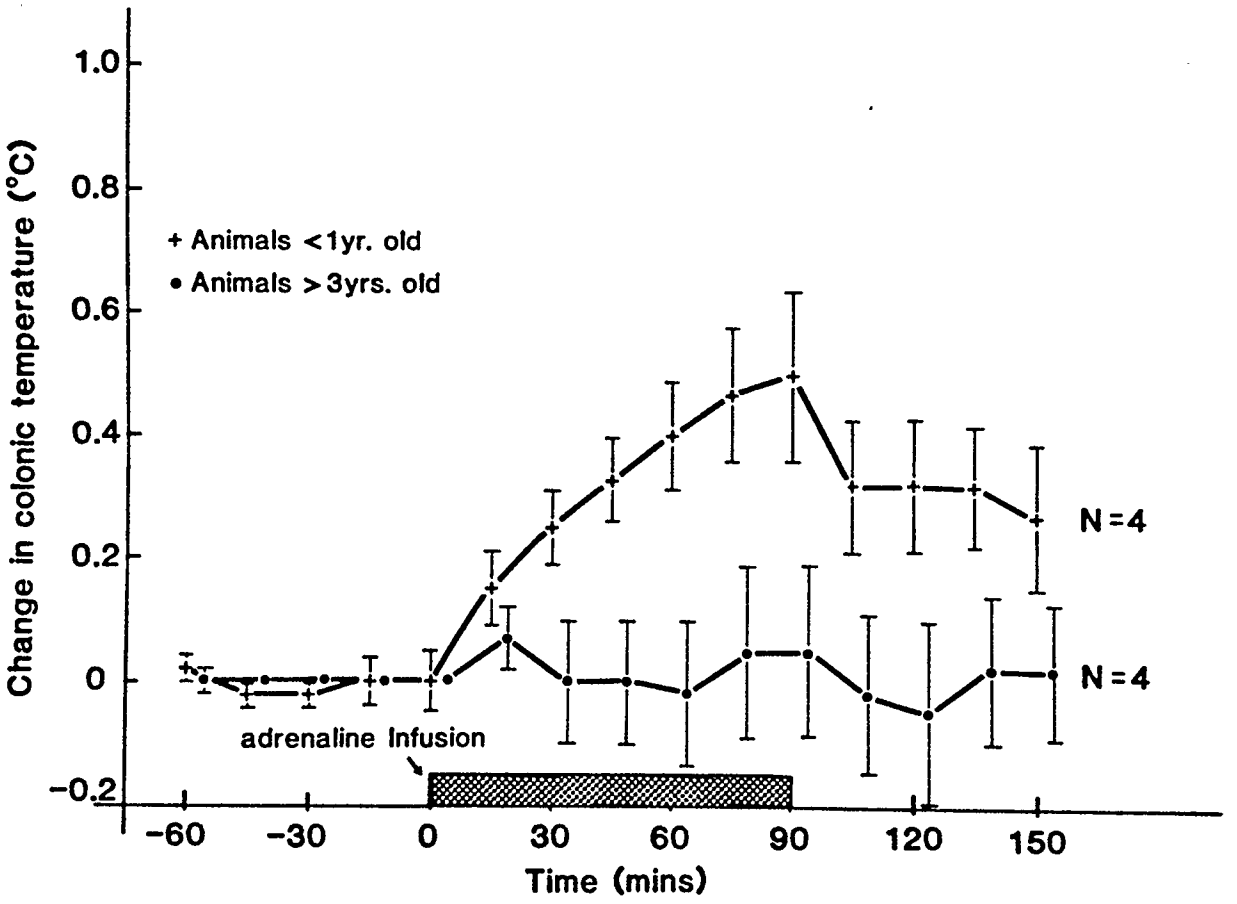


Figure 32: Graph showing the mean ± SEM colonic temperature changes in response to intravenous infusion of adrenaline for 90 minutes.

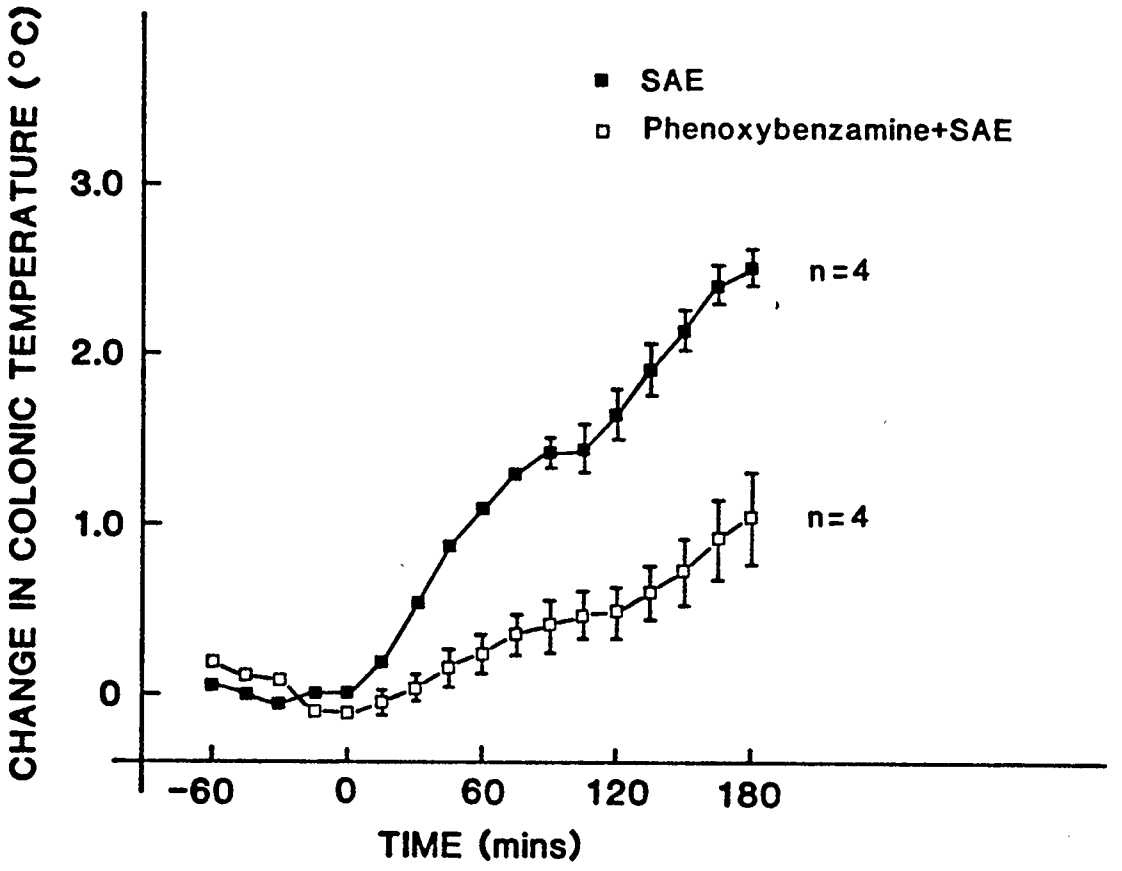


Figure 33: Graph showing the effect of phenoxybenzamine treatment on the febrile response of young animals to endotoxin given at time = 0.

DISCUSSION

The differences between the ages of the two groups of animals used in these experiments are not ideal from the standpoint of aging research. Reliable data on the lifespan of the New Zealand White rabbit are hard to find but it would appear that laboratory rabbits may live up to 5 years of age (Fox, 1980). Thus, it must be stressed that the "old" group of animals in this study are not truly aged in the geriatric sense. However, the differences observed show clearly that increasing age in the rabbit is accompanied by significant alterations in the febrile response to pyrogens. Interestingly, the febrile response of 3 yr old animals to endotoxin has similar characteristics to the fevers which were observed in warm-reared animals, that is, when compared to young control animals, the first peak is reduced while the second peak is absent.

The changes in ear skin temperature recorded during fever indicate that, in both young and old animals, peripheral vasoconstriction plays a role in the normal development of the first peak of fever. The finding that ear-skin temperature decreases again in old animals, but not in the young group during the time period when the second peak of fever normally occurs, is surprising. However, these data may be interpreted as suggesting that the mechanism normally responsible for the secondary rise in body temperature during endotoxin fever is not functional in the old group of animals. In contrast, the drive to increase body temperature remains, and thus a secondary effector mechanism, that of heat conservation by peripheral vasoconstriction, is activated in an attempt to elevate body temperature.

The observed differences in peripheral vasoconstriction were indicative of differences in the pyrogen induced release of catecholamines in the two age groups investigated.

The direct measurement of both plasma adrenaline and noradrenaline in young animals demonstrates for the first time that these catecholamines are both significantly increased in the plasma following intravenous injection of endotoxin. Previous work demonstrating that the urinary excretion of catecholamines is increased 24 hours after administration of endotoxin (Serafimov, 1962) and that total adrenal venous catecholamines increase after large doses of endotoxin (Egdahl, 1959) suggested that plasma catecholamines released from the adrenal medulla may play a significant role in the development of fever. Also, the latter report showed that spinal cord section between C-7 and T-1 abolished this increase in catecholamines and thus they concluded that it was under the direct control of the central nervous system. The finding that the only difference between the young and old animals' plasma catecholamines during fever is in the adrenaline levels suggests that these large increases may be due to activation of the adrenal medulla, which, in the rabbit, secretes 99% adrenaline (West, 1955). Also, the significantly greater increases in plasma adrenaline concentrations of old, as compared to young, rabbits during fever may explain the second period of vasoconstriction which was observed only in the former group of animals.

Apart from vasoconstriction, the increases in plasma adrenaline observed in the old group of animals would be expected to stimulate non-shivering thermogenesis, thus elevating body temperature. However, these mechanisms appear to be non-functional in this group of animals,

as demonstrated by the facts that neither adrenaline nor noradrenaline infusion cause significant rises in temperature in 3 yr old animals in contrast to the hyperthermia which occurs in young animals during infusion of either of these amines. Another possibility which must be considered is that the large increases in plasma adrenaline which occur in the old animals may be the cause of the reduction in fever. In support of such a hypothesis, large doses of adrenaline, given intravenously, have been shown to result in an inhibition of shivering in the anaesthetized cat (Hall & Goldstone, 1940). However, the fact that shivering was not normally observed during the development of fever in young animals at 20.0^{o}C indicates that such a deficit alone would not result in a reduction in fever.

The observations that plasma catecholamines did not change significantly following injection of sterile saline or during a heat stress which raised body temperature 1.0^{o}C demonstrated that the rises in plasma catecholamines, which occurred after intravenous injection of endotoxin, were specific to the febrile response.

The above discussion suggests that the reduced fevers observed in 3 yr old animals may be due to a deficit in the catecholaminergic activation of non-shivering thermogenic mechanisms. However, evidence against such a hypothesis is provided by the observation that pharmacological blockade of this system with the β-adrenergic receptor blocking agent, propranolol, does not affect the febrile response of young animals. This indicates that catecholamine stimulated non-shivering thermogenesis does not play an essential role in the genesis of fever in young animals. In order to determine if the catecholamines played a functional role in the increased body

temperature which occurred following administration of endotoxin by an action on the α-adrenergic receptor, the effects on fever of α-adrenergic receptor blockade were examined. The reductions in fever which were observed following phenoxybenzamine treatment suggest that the catecholamines do play a functional role in the elevation of body temperature. It is possible that the effect of this drug, when administered systemically, may be due to its entry into the central nervous system and action on the AH/POA, or, due to a direct action on the peripheral effector mechanisms of temperature regulation. The work of Laburn et al. (1975) which showed that phenoxybenzamine, when administered into the AH/POA caused similar reductions in fever, lends support to the former hypothesis.

The evidence presented in this section shows a reduced febrile response in the 3 yr old New Zealand White rabbit as compared to animals less than 1 year of age and also demonstrates that the normal mechanisms by which the catecholamines activate non-shivering thermogenesis are non-functional in the older group of animals. These data suggest that this latter deficit may be responsible for the reduction in fever. However, the observation that pharmacological blockade of non-shivering thermogenic mechanisms in young animals does not affect the febrile response provides evidence against such a hypothesis. Thus, it may be that a second thermoregulatory deficit exists in older animals which is primarily responsible for the reduction in fever. The exact nature of this deficit is not known; one possibility is that there may be a reduced ability to feel the cold in older animals, as has already been demonstrated in the aged human population (McMillan et al., 1967; Collins et al., 1977). The increased levels of adrenaline in the older

animals during fever may represent the animals' recruitment of secondary thermogenic mechanism in an attempt to develop the normal second peak of fever. Such an activation of the adrenal medulla, which normally plays no significant role in the body temperature rises associated with fever (Grant & Hirsch, 1950), would be expected, in young animals, to cause hyperthermia as a result of both vasoconstriction and the stimulation of non-shivering thermogenesis.

The reduction in fever which was observed in old animals in response to intravenous administration of live bacteria shows that, in this group, the febrile response to a more natural infection is diminished. Also, it was found that despite the differences in the fevers of the two groups of animals, the survival rate was similar in both young (50%) and old (38%) rabbits. These data suggest that fever may not play a significant role in survival during infection. However, it is possible that the other physiological changes which take place during fever, e.g. decreases in serum iron, may still occur and thus may be the significant factors which enhance survival during infection. Further experimental work is necessary to examine these possibilities.

VIII. Conclusions and General Discussion

The research reported in this thesis was designed to investigate how the thermal experience, in early life, and, increasing age, affect the functional characteristics of temperature regulation in both the New Zealand White rabbit and the Sprague Dawley rat.

Raising animals from birth at an ambient temperature of 33.0^{o}C has been shown to influence the development of the thermoregulatory system. Significant falls in colonic temperature during cold exposure and a reduction in the febrile response to intravenous injection of endotoxin were observed in animals in which the early sensory input was manipulated in this way. Similar deficits did not occur in either warm-acclimated or control groups of animals. As already discussed, both of these challenges to the thermoregulatory system normally result in an increase in the cold receptor activity. In contrast, during a thermoregulatory challenge such as heat stress, when the cold receptors would normally be silent, no differences between the colonic temperature changes of warm-reared and control animals were observed. The critical period for these effects of warm-rearing appears to be during the first 30 days of life.

Investigation of the heat production side of the effector mechanisms of temperature regulation in warm-reared animals showed no deficits which would explain the altered thermoregulatory responses. Shivering was normal. Oxygen consumption was increased during fever. Plasma concentrations of catecholamines were elevated during falls in colonic temperature. The only change which was observed was a reduction in the calorigenic actions of intravenously administered noradrenaline

which occurred in both warm-reared and warm-acclimated animals. However, this change does not explain the deficit of warm-reared animals as control groups did not demonstrate similarly modified thermoregulatory responses when the thermogenic actions of noradrenaline were blocked by treatment with propranolol.

The effects on body temperature of various neurotransmitters proposed to be involved in the hypothalamic control of body temperature, when administered directly into the hypothalamus, were examined to establish whether warm-rearing affected the role of these substances within the brain. In warm-reared animals, the effects of noradrenaline, serotonin, and prostaglandin E_2 were all changed in comparison to warm-acclimated and control animals. Dopamine had the same differential effects in both warm-reared and warm acclimated rats, while carbachol and endotoxin caused similar changes in temperature in all three groups. These data suggest that, in warm-reared animals, the functional neuronal circuitry is changed in terms of the role which these putative hypothalamic neurotransmitters play in the control of body temperature. The exact nature of these changes in warm-reared animals is not known, although several lines of evidence point to these modifications being a function of a deficit in the cold receptor input. Thermoregulatory deficits were observed during challenges which normally result in the activation of cold receptors, e.g. cold exposure and fever, while normal responses were observed during heat stress when cold receptors are normally inactive. Also, Dawson and co-workers have approached this same problem neurophysiologically and, despite finding no significant differences in the number of neurons driven by cold receptors in the caudal trigeminal nucleus of warm-reared rats (Dawson et al., 1981),

they have found differences between the dynamic response characteristics of such cold receptor afferents in warm-reared as compared to control animals (personal communication).

Further circumstantial evidence in support of a deficit in the cold receptor input of warm-reared animals comes from experiments utilizing the neurotoxin, capsaicin. It has been demonstrated that this substance has a neurotoxic action on spinal substance P neurons (Nagy et al., 1980) and that it specifically affects warm-sensitive neurons of the hypothalamus (Szolcsanyi et al., 1971). Thus, it may be that this neurotoxin specifically influences the warm-receptor input in treated animals. Relevant to this particular discussion is the observation that the long term effects of capsaicin treatment on thermoregulation appear to be the complete opposite of the effects of warm-rearing, i.e. when compared to controls, capsaicin treated animals develop significantly larger fevers (Szekely & Szolcsanyi, 1979), are unable to maintain temperature during heat stress, but have a normal ability to maintain temperature during cold exposure (Jancso-Gabor & Szolcsanyi, 1970). These data suggest that the warm and cold receptor inputs may have very different roles in the normal control of body temperature.

The reduction in fever observed in 3 yr old rabbits closely parallels the changes found in the febrile response of warm-reared animals. The absence of the calorigenic response to catecholamines is another thermoregulatory modification common to both warm-reared and 3 yr old rabbits. Thus, one may speculate that the underlying changes in the mechanisms of temperature regulation in these two groups of animals, as compared to young rabbits raised at 20.0^{o}C, may be the same. As already discussed, the available evidence suggests that the

thermoregulatory deficits of warm-reared animals may be specifically related to the cold receptor input, and circumstantial evidence indicates that a similar deficit in the ability to feel the cold may exist in the aged human population (Horvath et al., 1955; Watts, 1972; Collins et al., 1977).

In conclusion, the modification of thermoregulation in warm-reared animals by the early thermal environment provides one of the first clear demonstrations of developmental plasticity at the final output level of a physiological system, i.e. the temperature at which the body is maintained. Also, it provides an animal model which may prove valuable in future thermoregulatory research. Finally, this plasticity of the developing temperature regulating system may be of direct clinical significance in that it suggests that the fine control of environmental factors in early life influences the development of experimental animals. The question arises as to what effects the closely controlled environment of a hospital incubator, for example, may have on the development of newborn infants maintained in these machines during early life.

The reduction in fever observed in old animals, and the physiological changes associated with it, provide clear evidence that age related changes in temperature regulation do occur in New Zealand White rabbits. If reduced fevers occur in the human aged as well, such change would be of significance in terms of the physicians' ability to diagnose infection in this particular section of the population. Further investigation of age-related changes in temperature regulation in experimental animals also may provide an indication as to the exact

mechanisms underlying the increased incidence of hypothermia in the aged.

IX. References

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