Developmental changes in the human infant:
Patterns of endocrine excretion, body temperature
and sweating between 1 and 4 months of age.

Thesis submitted for the degree of
Doctor of Philosophy
at the University of Leicester.

by
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April 1995
Dedication.

To my Parents:

My Dad who knew of my ambition but never saw it realised and my Mum who has always believed in me. Without their love and support I would have never been able to complete a PhD.

Thankyou.
Acknowledgements.

As with all PhD’s I am indebted to a whole army of people without whom I would not have succeeded:-

I’d like to thank Stewart and Mike for finding, ‘room at the inn,’ their encouragement, ideas and academic support.

For their insight into life, their friendship and data collection services I thank, ‘The Health Visitors,’ Ann, Carol, Di, Jenny and by no means least Rachel.

For their urine, patience, biochemical expertise and ideas I must thank Tim, Richard and Dave in the steroid laboratory (LRI), without whom growth hormone certainly would not be measurable!

Thanks go to Liz who apart from sharing a birthday, has shared her own recent PhD experience and friendship with me.

To Fatima and Craig, thanks for all those useful computer hints. And to Barbara for getting all those vital orders and information, thanks.

Thanks to the staff of all four childrens wards for their time and the rather pleasant title of ‘The Sweat Lady’. Similarly, to the maternity staff who were always busy but always ready to help, thank-you.

A special thanks go to my friends and family: To John who has faced tears and tantrums in the difficult bits and to Sarah for sharing some of the best and worst accommodation with me; to my mum, for being a mum; to Sylvia and Trevor for all those letters they didn’t write and to all those friend who I owe letters - I promise no more excuses!

Finally, these acknowledgements could never be complete without thanking all those families who welcomed me with open arms. How could I forget the efforts exerted collecting urine and the time spent getting baby to sleep? Without their interest and perseverance none of this would have been possible.

    Thanks to the MRC and FSID for financial assistance.
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CHAPTER 1

INTRODUCING

THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

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INTRODUCTION

The metamorphosis changing infant into adult takes many years to reach completion. Certain stages of this process are very rapid and occur over weeks, days or even hours. The most rapid changes in the body occur during foetal and early postnatal life. As soon as we are born survival necessitates that we make almost instantaneous changes in several important physiological systems. In the subsequent weeks and months of early life many more adjustments are made so that by 6 months of age not only has the brain doubled in size but many of the adult patterns of physiological regulation appear to be established.

Despite a fairly good knowledge of the first four weeks of life outside the womb, the events during the subsequent weeks and months have until recently eluded investigation. It is therefore not surprising that we are unable to describe in sufficient detail when, how or why these changes occur, nor understand the implications they might have on infant well-being. What we do know is that during these early months as an infant adjusts to life outside the womb the mortality due to inherent physiological problems and disease are higher than at any other time of life until old age. As medical technology and social standards have improved, the mortality rate of the population as a whole has decreased, this is particularly evident in the mortality and morbidity statistics for the first year of life. Now in the Western world the death of infants under one year of age is at the lowest ever recorded.

Deaths occurring in the first month of life are mostly due to problems during gestation and labour. Those infants with fatal abnormalities or severe problems related to birth trauma generally do not survive the neonatal period (birth to 4 weeks). The post-neonatal (4-52 weeks of age) mortalities have also fallen over the past decades so that now only 1 - 2 per 1000 live births fail to survive this period. A small proportion of these deaths are due to abnormalities, overwhelming infection or other untreatable diseases. However, we remain unable to give good reason as to why the majority of infants in this age group die. This group of unexpected and unexplained infant deaths has remained resistant to reduction over recent years so that now they are the largest sole contributor to post-neonatal mortality.
If we have not described the 'normal', how can we identify the 'abnormal'?

Why should mortality and morbidity be so high in the very young? The answer probably lies hidden in our lack of understanding of the development and physiology of early life. It seems likely that immature physiology may confer vulnerability particularly should the maturation process be delayed for some reason. Similarly, changing systems of any kind whether they be mechanical or living are inherently unstable. If, as in the infant changes are occurring not only in one system but several, over the same short time period, this may lead to instability and vulnerability, particularly if one system fails to change, changes in an inappropriate manner or the body is challenged (stressed) in some way at a crucial time when the appropriate responses are impossible. As almost all infants survive this period of life there must be a carefully orchestrated 'master plan' which prevents such disasters. Exactly what it is or how it works is unclear but perhaps the most likely candidate to fulfil the role as co-ordinator is the hypothalamus-pituitary-axis (HPA). In adults this neuroendocrine system regulates and co-ordinates systemic changes.

Is this true for the infant?

Little is known about the development of the HPA nor how it co-ordinates and regulates physiological responses in the infant. Few studies have examined the development of the physiological systems it controls and fewer still have related developmental changes in one system to similar changes in others or the function of the HPA. This study attempts to examine some aspects of the development of the hypothalamically controlled functions. As a neuroendocrine organ there are many systems which could be examined which relate to the function of the hypothalamus. There are some studies which examine the cardiovascular system, the respiratory system and the thermoregulatory system but few examine endocrine function. However, the endocrine function of the hypothalamus plays an essential role in survival. In its role as part of the HPA, the hypothalamus plays a vital role in the mechanisms for coping with stressful events, be they missed meals or charging bulls!
By examining the maturity of the stress response mechanisms, the ability of the infant to cope with everyday events can be established. The three endocrine glands which form the HPA elicit effects on every cell of the body in order to prepare for and survive everyday events. This results in the HPA being implicated in a wide variety of illness and even deaths in the adult. It is possible that as in adults the HPA plays a role in infant morbidity and mortality but that our knowledge of the system is insufficient to perceive its involvement. After all, the stress response for which the HPA is a major contributor, has been suggested to play a role in sudden and unexplained deaths in adults (Kelly, 1980) so why not in infants whose ability to cope may be compromised by immaturity, or rapid development? Stress hormones have been found in the serum and cerebral spinal fluids of some unexplained deaths in infancy. This is thought to be as result of the final agonies of death but it may also be as a part of the cause of death (Goumont, Coquerel, Pfaff, Basset, Voz, Taylor & Proust, 1993).

What follows is a brief summary of the HPA with particular reference to the pituitary hormone, growth hormone (GH) and the adrenal hormone cortisol, together with our current understanding of temperature regulation, the stress response and the role of the hypothalamus in the co-ordination and regulation of these systems. Investigating the HPA could yield an abundance of questions that could never be completely answered in one small project. The hormones cortisol and GH are focused on in this project as they are produced by the adrenals and pituitary respectively and therefore not only represent the function of each of these glands but also provide information about the functionality of the HPA and the body's ability to cope. By relating the hormonal observations to the already well defined developmental pattern of temperature regulation the overall picture of development of some of the neuroendocrine functions of the hypothalamus can be inferred and related to each other.
THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS.

The hypothalamus is located below the third ventricle of the brain above the median eminence which gives rise to the pituitary. It has a rich blood supply which arises from the cerebral artery and the posterior communi artery.

In 1929 Cushing described the hypothalamus as, "the very main-spring of existence." This statement is becoming increasingly true with continuing research adding further support to the claim that the hypothalamus integrates all the higher physiological functions. The hypothalamus can be regarded as a connection between the electrical and the chemical communication systems employed to adjust to the continually changing conditions. The hypothalamus receives feedback from the conditions the body is experiencing via the central nervous system in addition to feedback via the concentrations of circulating hormones and metabolites. The hypothalamus assimilates the information initiating a cascade of hormone release and neuronal action potentials to other parts of the brain thereby allowing the body to work at its most efficient in any given conditions.

The functions of the hypothalamus can be divided into those of neural origin and those of endocrine origin. These functions are all intimately related and affect the same target organs one generally for immediate responses (neural) and the other for preparatory responses (endocrine).

The neural hypothalamus.

In addition to the autonomic functions involving thermoregulation, cardiovascular, respiratory and gastrointestinal systems the neurocrine hypothalamus also effects sleep-wake behaviour, cognition and memory, emotional behaviour, energy and water balance and the immune system (Reichlin, 1993). Stimuli necessitating immediate responses stimulate the appropriate neuronal (and endocrine) responses which require the preparatory responses of the endocrine hypothalamus to have been fulfilled. If the endocrine hypothalamus fails then the neurocrine
hypothalamus function will also fail. The preparatory role of the hypothalamus is essential for life maintenance.

The endocrine hypothalamus

The endocrine systems play a vital role in the maintenance of the body during 'mundane' daily events but also in the preparation of the body for a 'crisis' situation. This system utilises chemical messengers called hormones. These are blood borne chemicals produced and secreted by one type of cells which exert their effect on a different type of target cell which is generally distant to the secretor cells. In order to produce the most efficient system the hormonal, along with the neuronal, effects must be organised to avoid chaos. This organisational role is fulfilled by the hypothalamus. As part of the endocrine system the hypothalamus produces many hormones which are mainly small peptides with very short plasma half lives. These hormones fall into two categories: the hormones which stimulate their target organ to produce hormone - the releasing hormones; and the hormones which inhibit the production and/or secretion of the target organ hormones - the release inhibiting hormones. Out of a myriad of hypothalamic hormones the three which are of direct relevance to this study are corticotrophin releasing hormone (CRH), GH releasing hormone (GRH) and GH release inhibiting hormone or somatostatin (GRIH). It is these three hormones which have been examined indirectly by assessment of the urinary metabolites of the end product hormones cortisol and GH.

CRH, GRH and GRIH all act at the level of the pituitary. CRH stimulates the production and secretion of adrenocorticotrophin (ACTH) which in turn stimulates the production and secretion of cortisol from the adrenal cortex. GRH stimulates the production of GH itself a biologically active hormone which in turn stimulate the production of insulin-like growth factors (IGFs) which are also biologically active. GRIH inhibits the production of GH and thereby IGFs. The production of these hormones and their regulation will be considered in turn.
THE PITUITARY

The pituitary is the next component of the HPA. It is formed from two distinct embryological
tissues, Rathke's pouch and the infundibular process from the diencephalon. It is located in
the stella turcica of the sphenoid bone at the base of the skull and weighs approximately 0.5 g
of which 75 % is the anterior pituitary and the remaining 25 % the posterior pituitary in the
adult.

As can be expected of an endocrine organ, the pituitary has a rich blood supply to enable the
distribution of the secreted hormones to the appropriate target organ. The hypothalamus is
intimately connected with the function of the pituitary. The anterior pituitary receives
hormonal signals from the pituitary via the portal blood flow while the posterior pituitary has a
neural connection with the hypothalamus and acts as a storer and secretor of hormones
produced by the hypothalamus.

Anterior Pituitary

The anterior pituitary produces both polypeptides and glycoproteins. The polypeptides include
corticotrophin (CRH), prolactin (PL) and GH while the glycoproteins include thyrotrophin
(TSH), follicle stimulating hormone (FSH) and lutenizing hormone (LH). In this section the
polypeptide hormone GH will be examined in greater detail while corticotrophin will be
considered in the section on the control of the adrenal hormone, cortisol.

GROWTH HORMONE.

GH as indicated earlier, has the prime function of stimulating growth and repair of the body.
It is secreted from the anterior pituitary under the influence of releasing- and release-inhibiting
factors from the hypothalamus, direct feedback from the plasma concentration of GH itself and
the insulin-like growth factors as well as other neural mechanisms.

There are two forms of human GH of different molecular weights (20 kD and 22 kD) which
may have different biological activities. Each consist of single polypeptide chain of about 188
amino acid residues. Once secreted into the blood stream GH circulates either bound to one of the two specific binding proteins (50 %) or in the unbound (free) form (50 %). The 20 kD form of GH is only found in the pituitary while the 22 kD form circulates bound to one of the binding proteins (Kelly, 1990; Baumann & Abramson 1988; Baumann, Amburn & Shaw, 1988; Baumann, 1991; Herrington, Tiong, Ymer et al, 1991). By circulating as a bound complex of protein, GH increases its size to about 80-85 kD and thereby restricts its distribution to the intervascular spaces and increases the clearance time. Access to the extracellular spaces and the cellular receptor sites is only possible in the free, unbound form suggesting that the free hormone is the biologically active component of plasma GH (Baumann, Stolar, Amburn, Barsano & DeVries, 1986).

**Actions.**

GH acts directly and indirectly via the insulin-like growth factors (IGF's) on cellular activity and metabolism, particularly in muscle, adipose tissue, cartilage and connective tissue. Other sites of action including the liver, the immune system and the reproductive organs are also documented. GH and the IGF's modulate the somatic growth of bone and cartilage (Isaksson, Lindahl, Nilsson & Isgaard, 1987) in addition to bone mineral density (O'Halloran, Tsatsoulis, Whitehouse et al, 1993). In adipose tissue GH modulates the differentiation and growth of fat cells. GH decreases the deposits in the adipose tissues and increases lean body mass by mobilising free fatty acids and glycerol (Hauner, 1992). In the liver GH regulates the expression of certain proteins (Olivecrona, Ericsson, Berglund & Angelin, 1993). The effects of GH on the immune system are of current interest. There is evidence of GH and IGF's leading to cellular proliferation in lymphoid tissues in rats (Gelato, 1993) and T-cells in humans (Geffner, Berch, Lippe et al, 1990). Finally, GH is essential for the development of the gonads in puberty. A deficiency of GH results in delayed puberty (Cristman and Halme, 1992; Stanhope, Albanese, Hindmarsh & Brook, 1992).
Regulators.

GH is primarily regulated by the hypothalamic hormones, growth hormone releasing hormone (GHRH) and growth hormone release inhibiting hormone (GRIH) or somatostatin. GHRH is secreted from the arcuate nucleus of the basal hypothalamus (Bloch, Gallard, Brazeau et al, 1984) into the portal circulation and stimulates both GH production and secretion (Barinaga, Yamamoto, Rivier et al, 1983). GRIH is synthesised in the periventricular region but other areas such as the preoptic area and lateral hypothalamus have also been implied (Gabriel, Marshall & Martin, 1987). GRIH suppresses GHRH secretion and decreases the release of GH. An increase in circulating GH results in negative feedback reducing the secretion of GHRH (Kelijman & Frohman, 1991). IGF-I and IGF-II also play a role in GH regulation. The IGF's may inhibit gene transcription or secretion of GH from the pituitary or it may actually affect secretion at the level of the hypothalamus.

In addition to these two direct regulators GH interacts with a variety of other factors which act to either reduce or enhance the action of GH at the target organ, while other factors modulate the production and release of GH by the pituitary. These factors include metabolic and hormonal regulators which provide feedback resulting in the retention of nitrogen and increase in lean body mass, lipolysis and the preservation of adequate blood glucose at the expense of body fat.

Metabolic modulators.

The effects of the plasma levels of glucose, amino acids and free fatty acids feedback on the production and secretion of GH. Included with these metabolites is stress as it effects the nutritional status of the body resulting in changes in blood glucose, amino acids and free fatty acids and hence GH secretion. The effect of these modulators is described below:-

1. High blood glucose results in a marked suppression of GH secretion mediated by an increase in somatostatin. The effect of glucose is believed to act on glucoreceptors in the hypothalamus (Valcavi, Zini, Davoli & Portioli, 1992)
2. Amino acids and proteins result in elevated plasma GH levels. Presumably this is to preserve the lean body mass of the body (Pimstone, Wittman, Hansen, 1966).

3. Free fatty acids reduce GH secretion in humans. This is probably mediated by direct suppression of GH secretion from the pituitary (Imaki, Shibaski, Shizume et al, 1985).

4. Stress (physical and emotional) can stimulate GH secretion. Stimuli as diverse as a visit to a dentist and exercise (Merimee, Fineberg & Tyson, 1969; Weltman, Weltman, Schurter et al, 1992) have been shown to elevate plasma GH levels in adults. Some such events result in rapid increases in plasma GH concentrations within a matter of minutes (Baylis, Greenwood, James et al 1968). Conversely, prolonged stress in some children results in growth failure due to a decreased GH secretion (Brierley, 1992).

Hormonal modulators.

As with changes in nutritional status the circulating levels of other hormones modulate both the secretion and effects of GH. Hormones such as the sex hormones and the glucocorticoids affect the production and secretion of GH by the pituitary while hormones such as the thyroid hormones have a permissive role on the effects GH exerts on the target tissues. The role of some hormonal modulators is described below:

1. Thyroid hormone:- The thyroid hormones seem to exert their action by means of allowing GH to exert its action on tissues. In the absence of thyroid hormone (e.g. after thyroidectomy) growth failure occurs which GH administration alone cannot reverse. However on administration of thyroid hormone normal growth is exhibited. There is also evidence which indicates that GH production is inhibited by the lack of thyroid hormones (MacGillivray, Aceto & Frohman, 1968).

2. Oestrogens: Oestrogens potentiate the secretion of GH in humans. This results in a greater plasma concentration in women than in men. Oestrogens sensitise the GH releasing mechanism to the effects of exercise and amino acids resulting in greater GH release in response to exercise in women (Merimee et al, 1969). However, high oestrogen
concentrations also inhibits the effect of GH on the bones resulting in the achievement of a shorter stature in women than men (Zachmann, Ferrandez, Murset and Prader, 1975).

3. Androgens: Androgens also appear to increase the basal levels of GH secretion. Pre­pubertal children show less GH secretion in response to hypoglycaemia than do adult men and women (Martin, Clark, Connor, 1968). Androgens increases the pulse amplitude of GH secretion and the circulating levels of both GH and IGF-I (Parker, Johanson, Rogol, Kaiser & Blizzard, 1984).

4. Progestins: Progestins inhibit the secretion of GH. The mechanism of this action is unknown (Lawrence & Kirsteins, 1970).

5. Adrenal corticoids: Glucocorticoids increase levels of mRNA by increasing gene transcription. However, high circulating levels of glucocorticoid may result in growth retardation in children the mechanism of which is unclear (Hartog, Graafar, Fraser, 1964).

Neural mechanisms.

In addition to the metabolic and hormonal regulators there are also neuronal mechanisms of GH regulation. Both cholinergic and adrenergic pathways along with a variety of neuropeptides also play a role in the regulation of GH secretion.

Metabolism and urinary excretion.

In order to exert its metabolic effects GH must first bind to receptor sites located in the cell membranes of various tissues. At each of these sites some metabolism of GH occurs. The rate at which a substance is cleared from plasma is dependent on the rapidity of the metabolism by the tissues. The half-life of GH is about 20-30 minutes in humans (Plotnick, Thompson, Kowarski et al, 1975; Finkelstein, Roffwarg, Boyar et al, 1972). In both patients with cirrhosis of the liver (Daughaday , Trivedi & Andrews, 1987; Taylor, Lipman, Salam, Mintz, 1972) and renal insufficiency (Hattori, Shimatsu, Kato et al, 1989) there is an increase in the
half-life of GH from the plasma, indicating that both the renal and hepatic tissues play a role in the clearance of GH from the body.

The role of the kidney in the metabolism of GH is the most significant and most relevant to this study as the concentrations of GH found in urine is as a direct result of the effect of the kidney. The results of many investigations has revealed that GH is filtered by the glomeruli, absorbed at the proximal tubules and catabolised within the renal tubule cells (Maack, Johnson, Kau et al, 1979). The rate limiting step for this process is the initial filtration which is determined by the molecular size (only molecules less than 25 kD are filtered) and the charge of the molecule. This means that GH bound to binding protein is not filtered. Once the free GH has entered the tubule it binds to the receptor sites on the apical borders of the tubular cells. Endocytotic vesicles form and gradually internalise the GH for digestion in the lysosomes. The resultant amino acids are released into the extracellular space and circulation for reuse. Under normal conditions only minor amounts of GH are excreted because of the extensive reabsorption of the small molecules (Maack et al, 1979; Tsukahara, Tsuchida, Nakamura et al, 1992). This explains why in renal insufficiency the amounts of GH found in urine increases as urinary GH is dependent, firstly, on the filtration rate and secondly, and more importantly on the reabsorption (Tsukahara et al, 1992). In normal children and adult subjects urinary GH concentrations are between 0.003 and 0.025 % of those found in plasma (Hourd & Edwards, 1989; Hattorri et al 1989; Baumann & Abramson, 1983). Despite these small levels of GH in urine, urinary GH still reflects plasma GH levels and production (Edge, Hourd, Edwards & Dunger, 1989; Albini, Soto, Sherman et al, 1991).

In infants the kidneys are not fully mature and so this may effect how growth hormone is excreted in urine. The infantile pattern of excretion and the effect of kidney maturation are discussed in, 'Pattern of Urinary Excretion.'
Patterns of secretion.

Ontogeny of GH secretion.

GH first appears in the foetus at about 12 weeks (20 ng.ml\(^{-1}\)) after conception (Kaplan & Grumbach, Shepherd, 1972). There is a continuous rise in the amount of GH in the foetus until about 22 weeks (80 ng.ml\(^{-1}\)) gestation after which there is a progressive decline. At birth plasma GH levels for term infants (10 ng.ml\(^{-1}\)) remain very much higher than those seen in adults (Cornblath, Parker, Reisner et al, 1965). Premature infants showing higher GH secretion than their fullterm counter parts (Wright, Northington, Miller et al, 1992). During the first few weeks after birth there is a rapid decline in plasma GH followed by a more gradual decline over the next few months to reach the childhood (pre-pubertal) level of secretion (Finklestein, Roffwurg, Boyar et al, 1972). At puberty there is an overall increase in secretion which corresponds with the overall growth velocity of the period (Garnier, Nahoul, Grenier et al, 1990). After puberty there is a continuous decline in GH secretion with age (Zadik, Chalew, McCarter et al, 1985).

Adult pattern of secretion.

This pulsatile secretion of GH is vital for the normal regulation of growth (Brook & Hindmarsh, 1992; Issaksson, Jansson, Clark & Robinson, 1986). It consists of a basal continuous level of secretion with periods of increased activity superimposed. In adults, children and neonates (Goji, 1993), GH is secreted in a pulsatile manner with bursts of activity occurring at 1-4 hourly intervals (Holl, Hartman, Veldhuis et al, 1991; Casanueva, 1992) throughout the 24 hour period (Winer, Shaw, Baumann et al, 1990; Plotnick, Thompson, Kowarski et al, 1975). In general any increase in the mean 24 hour plasma GH is as a result, of and increase in, the amplitude of the secretory burst of GH and not an increase in secretory frequency. This pulsatile secretion is dependent upon the two hypothalamic hormones GHRH and GRIH. GHRH stimulates the secretion of GH when GRIH levels are
low (Plotsky & Vale, 1985). It is probably GRIH that controls the timing of secretion as there is a change in the sensitivity of the GRIH cells (Thomer, Vance, Hartman et al, 1990).

Circadian pattern of secretion.

This pulsatile secretion is overlaid on a circadian pattern of secretion where night-time plasma concentrations reach higher levels than those of day-time (Snow, Shaw, Winer, Baumann et al, 1990). GH binding protein does not appear to play a role in this circadian rhythm as there does not appear to be a circadian variation in either circulating levels of the protein or the binding affinity for GH (Snow et al, 1990). GH has been linked to the onset of sleep with maximal secretory bursts occurring during the first 90 minutes of sleep particularly slow wave sleep (Holl et al, 1991). Sleep deprivation experiments have lead to the conclusion that sleep is essential for the maximal GH bursts to occur but that slow wave sleep is not essential for GH secretion. (Sassin, Parker, Mace et al, 1969; Davidson, Moldofsky, Lue, 1991). The magnitude of the release of GH at the onset of sleep is important in the biological action of the hormone. The amount of GH released at this time correlates well with the growth velocity of pubertal children (Albertsson-Wiklan & Rosberg, 1988).

It has recently been suggested that the cortical centres of the brain regulating the onset of slow wave sleep also stimulate the secretion of GH from the pituitary directly (Holl et al, 1991). Conversely it may also be that GHRH and or GH actually mediate the onset of sleep (Kerkhofs, Van Cauter, Van Onderbergen, et al, 1993).

Ontogeny of circadian pattern

The circadian rhythm of GH secretion has only briefly been examined in infants. Most studies point to sometime within the first 1-2 years of life as the time when GH rhythm is discernible (Finkelstein, Anders, Sacher et al, 1971; Shaywitz, Finkelstein Hellman, et al, 1971). One study did suggest that a circadian rhythm was present in infants after 3 months of age and that the first appearance of brain activity similar to that seen in slow wave sleep marked the ontogeny of the circadian pattern (Vigner & D'Agata, 1971).
Pattern of urinary excretion.

Improving techniques have allowed the examination of urinary GH (reviewed by Hourd & Edwards, 1994). These techniques are less invasive and GH levels in urine although small have been shown to correlate well to the plasma levels of GH (Hattori, Shimatsu, Kato, et al, 1990; Albini et al, 1991).

The studies of urinary GH assumes that there is normal renal function as functional impairment to the kidneys leads to higher or lower GH levels than would be expected for a given plasma concentration. In the neonate and infant the kidney function can be regarded as impaired as the renal tissues have not completed maturation. Studies have shown that in infants both glomerular filtration rate and tubular function are maturing at the same rate as urinary GH is declining (Tsukahara, Kikuchi, Nakamura, et al 1990). These suggest that a small portion of the changes in urinary GH seen in young infants may be due to kidney maturation. In addition there are studies indicating that the levels of GH-binding protein is low in young infants and that this may also contribute to changes in excretion by increasing the amount of GH being filtered by the glomeruli (Wright et al, 1992). However, further studies on plasma concentrations of GH indicate that GH secretion is higher in premature infants than fullterm infants and that this decreases with age. So it is likely that the observed changes in urine are predominantly as a result of change in secretion (Daughaday et al, 1987; Fus, Nemoto, Wakae et al, 1993). There are no studies on the secretion of GH in urine in infants.

**SUMMARY.**

1. GH is needed for adequate growth and repair of tissues from birth. It stimulates protein synthesis, mobilises free fatty acids and partially regulates carbohydrate metabolism.

2. GH is produced and released from the pituitary under the direct influence of the hypothalamus. The secretion and production is regulated by a number of mechanisms including hormonal and neuronal which receive input from the nutritional status and activity of the body.
3. The overall production of GH decreases with age with a period of increased activity during puberty.

4. GH is secreted in a circadian pattern with more GH secreted during night-time sleep than during waking day-time hours. This pattern has been observed in infants from 3 months of age.

THE ADRENAL GLANDS.

The adrenal glands form the final part of the HPA axis and are generally accepted to be vital for survival. The corticosteroids are probably one of the most widely investigated groups of hormones because of the obvious clinical effects the lack, or excess, of these hormones cause.

The adrenals are found at the upper most edge of the superior-medial pole of each kidney. Each gland consist of two distinct parts the cortex and the medulla. Like the pituitary each part is derived from different embryological tissue and has distinct functions. In the adult each gland weighs between 3 and 5 grams. The adrenal cortex is almost 80% of the total weight and volume of each gland and is composed of three consecutive layers forming the zona glomerulosa, the outer most area and producer of the mineralocorticoids; the zona fasciculata, the medial layer sole producer of cortisol and the glucocorticoids; and the zona reticularis, the innermost layer adjacent to the adrenal medulla and predominant producer of the androgens and oestrogens (Bravo, 1989). However, at birth the adrenal cortex is not fully matured and is referred to as the foetal adrenal cortex (described in the following section, Ontogeny of secretion and the foetal adrenal cortex pp19). The adrenal medulla is principally responsible for the secretion of the catecholamines, adrenalin and noradrenaline.

The glucocorticoids are arguably the most important hormones for the maintenance of life. Under-production of cortisol, as seen in Addison’s disease, leads to an inability to cope with both physical and emotionally stressful situations without replacement therapy. In this section
a brief review of the glucocorticoids their metabolism, function and pattern of secretion will be examined. It is the glucocorticoids, specifically cortisol which is relevant to this study.

THE GLUCOCORTICOID.

Cortisol and corticosterone are steroid hormones known as glucocorticoids. In man, cortisol is the most predominant while corticosterone acts more like a mineralocorticoid. All cortisol production is carried out in the zona fasciculata of the adrenal cortex as outlined in appendix 1 (illustrating the pathways of steroid productions). Once released from the adrenal cortex the steroid hormone circulates in three forms; bound to transcortin (a binding protein specific for corticosteroids more commonly known as corticosteroid binding globulin (CGB)) (Nelson 1980); bound to albumin or in the unbound (free) biologically active form. Transcortin has a high affinity but low capacity for cortisol; albumin has a high capacity but low affinity for binding of cortisol. In a resting state approximately 10 % of cortisol circulates in the unbound form and the remaining 90 % bound to either albumin (10 %) or transcortin (80 %) (Daughaday, 1958; Hadjian, Chedin, Cochet & Chambraz, 1975). The half-life for the clearance of cortisol from plasma is about 90 minutes (Norman & Litwack, 1987; Schulster, Bernstein & Cooke, 1976).

Actions.

In man, most has been learnt about the action of cortisol from diseases such as Cushing's and Addison's, where abnormal amounts of cortisol are produced. These observations have been further examined by the use of animal tissues for more detailed studies so that our knowledge of this vital hormone has expanded greatly. (Greep, Astwood et al, 1974).

Cortisol is probably capable of affecting every tissue of the body as indicated by the vast array of receptor sites located on the lungs, liver, cartilage, mammary glands, bone cells, lenses and brain tissues to name but a few. However the most well known sites of action are the liver, skeletal muscle, adipose tissue and lymphoid tissues (Nelson, 1980).
Metabolism.

The major actions of cortisol on energy balance are as a result of its presence enhancing the action of other hormones, e.g. cortisol has a permissive effect on the actions of GH (Nelson, 1980). Cortisol enhances gluconeogenesis, glycogenesis and lipolysis resulting in an increase in glucagon storage in the liver and muscle tissues and maintaining blood glucose concentrations. This is carried out firstly by enhancing the mobilisation of protein from muscle, increasing protein degradation and decreasing protein synthesis; secondly by increasing enzyme activity in the liver which increases glucagon synthesis; thirdly, by inhibiting glucose uptake by the muscles and adipose tissues and decreasing the suppression of glucose synthesis by insulin; fourthly, by enhancing the mobilisation of fatty acids and glycerol from the adipose tissues (Greep, Astwood et al, 1974). In excess, cortisol has a diabetogenic effect, antagonising the effect of insulin, it leads to muscle wastage and fat re-distribution as seen in Cushing's disease. However, in its absence the glucose stores of the liver and muscle become depleted, and the body becomes unable to cope with a stress and the rapid requirement of glucose needed on such occasions, which can ultimately lead to death.

Immune system (reviewed by McK.Jefferies, 1991).

The overall effect of cortisol on both the early and late responses to an immunological challenge is to suppress the response. Cortisol decreases the local immune response to tissue damage by inhibiting the production of prostaglandins, thromboxanes and leukotrienes. There is a reduction in the recruitment of leukocytes to the trauma site, an increase in the number of circulating neutrophils but a decrease in their activity, a decrease in the number of eosinophils and a decrease in the proliferation of fibroblasts and hence slowing of the healing process. In infections there is generally a decrease in the febrile response probably due to a decrease in the number of lymphocytes and the production of the interleukins and the gamma interferons by the macrophages and the lymphocytes (Berne & Levy, 1993; Greep, Astwood et al, 1974).
In addition to the effects on the metabolism and immune system, cortisol exerts mild mineralocorticoid actions on the kidney increasing water and potassium loss; suppresses bone formation by reducing the protein matrix of bone and enhancing reabsorption, as well as reducing calcium absorption from the gut and increasing its excretion in the urine. Due to cortisol's catabolic actions on protein it can result in thinning of blood vessels and skin by reducing collagen synthesis. The responsiveness of the vascular system requires cortisol to maintain blood pressure. Cortisol also has effect on the central nervous system and is necessary to enable an organised response to stressful situations despite reducing sensory acuity. Finally cortisol is essential in foetal development having been found to facilitate the maturation of the central nervous system, retina, skin, gastrointestinal tract and lung tissue in late pregnancy. (Berne & Levy, 1993; Greep, Astwood et al, 1974).

**Regulation of secretion.**

Cortisol is excreted in response to ACTH release from the pituitary. ACTH is a polypeptide secreted in response to stimulation by CRH from the hypothalamus (Nelson, 1980). ACTH stimulates the rapid synthesis of cortisol in the adrenal cortex in addition to regulating the growth and size of the gland (Schulster et al, 1976). The circulating level of cortisol regulates the further production and release of more cortisol. High levels of cortisol suppresses the secretion of ACTH at both the level of the pituitary and the hypothalamus. This feedback mechanism for control of cortisol production (see figure 1.1) can be overcome by various factors including stress (Bravo, 1989). Other factors or diseases which effect the clearance of cortisol from the circulation can also alter the feedback control of cortisol secretion, e.g. an increase in the thyroid hormone enhances the conversion of cortisol to cortisone and thereby reducing circulating levels of cortisol and reducing the feedback to the pituitary and hypothalamus (Schulster, Bernstein & Cooke, 1976; Vestergaard, 1978 as cited Honour, Kelman & Brook, 1991).
Figure 1.1: The feedback mechanisms involved in the regulation of cortisol production (Gunnar 1989).

PATTERNS OF CORTISOL SECRETION

Ontogeny of secretion and the Foetal adrenal cortex.

During the first part of development, adrenal growth is more rapid than the growth of the rest of the body. Their weight relative to the rest of the foetus increases, reaching a maximum between the 12th and 17th weeks of gestation (Ekholm & Niemineva, 1950). This is followed by a decrease in relative size of the adrenals, although at birth the adrenals remain larger (4-5 g) than the adults (3-4 g) (Winter, 1985). 80% of the adrenals at birth constitute the foetal zone. Postpartum, the foetal zone involutes rapidly so that by 1 month the weight of the adrenals is reduced by half, by 6 months there is almost no foetal zone remaining and by 1 year the adrenal gland weight is about 1 g. Early anatomical studies on human tissues suggested that this involution was as a result of massive necrosis and haemorrhage of the foetal zone tissue. More detailed studies in baboons, macquac and rhesus monkeys indicate that the involution of the foetal zone progresses as the foetal zone cells are gradually remodelled into adult, zona fasciculata cells (Ducsay, Hess, McClellan, et al, 1991). These
later observations would appear to be the most likely processes and are supported by studies carried out by Sucheston & Cannon, (1968).

The anatomical changes occurring in the adrenals from conception to infancy appear to correspond to functional changes occurring over these months. The levels of plasma cortisol are low at mid-gestation but increase rapidly prior to and during birth becoming higher but remaining lower than those seen in the mother (Donaldson, Nicolini, Symes, et al, 1991). About 60-70% of foetal cortisol is produced by the foetal adrenals yet this is despite a deficiency in 3β-hydroxysteroid dehydrogenase activity. This enzyme is responsible for the rate-limiting step in the conversion of cholesterol to cortisol. Foetal cortisol is either of maternal origin or synthesised from placental precursors. Most maternal cortisol passes to the placenta and is converted to cortisone before entering the foetal circulation suggesting that the majority of foetal cortisol is synthesised from placental progesterone or by the conversion of cortisone by the foetus.

As in the adult the pituitary hormone ACTH is an essential regulator of cortisol production. ACTH first appears in the foetus at about 5 weeks post-conception, by mid-gestation a negative feedback regulatory system of ACTH can be demonstrated. Not only does ACTH regulate cortisol production but it also stimulates the growth of the foetal zone of the adrenal cortex and inhibits the 3β-hydroxysteroid dehydrogenase activity.

At term adrenal steroidogenesis is higher than at any other time. Total plasma and free cortisol levels are high. The 3β-hydroxysteroid dehydrogenase activity increases, probably due to the removal of uterine inhibitory factors. This in turn increases the production of both cortisol and aldosterone from cholesterol enabling the foetus to survive independently of the maternal circulation. The high plasma levels of cortisol feedback at the level of the pituitary and the hypothalamus reducing the circulating levels of ACTH and thereby rapidly reducing the production of cortisol. Plasma concentrations of the neonate reach a nadir at about 5 days postpartum (Rokicki, Forest, Loras, et al, 1990).
Although the adrenal glands are immature at birth they are capable of responding to ACTH stimulation and therefore presumably stress. ACTH stimulation in the first week of life results in a 3-4 fold increase in plasma cortisol. Between 1 and 4 months this response has increased massively so that a 30 fold increase in plasma cortisol is initiated by the same stimulus. By 1 year the response has decreased to levels expected in adults (Winter, 1985). It appears that between 1 and 4 months the adrenocortical axis is 'super responsive' to stimulation during this period. This is also supported by work examining the plasma concentration of cortisol after ACTH stimulation. In these experiments infants under 1 year showed greater cortisol elevation than at any other time until at least late puberty (Tanner stage V) (Lashansky, Saenger, Fishman et al, 1991).

**Adult pattern of secretion.**

Adrenocortical function follows a circadian pattern first described in 1943 (Pincus, 1943 as cited in Greep, Astwood et al, 1974). Plasma concentrations of cortisol rapidly increase after midnight before waking (Kreiger, Alan, Rizzo, et al, 1971) to reach a peak between 6 and 8 am before gradually declining throughout the day (Nichols, 1967). This pattern of 6 hours minimal secretory activity (4 hours before and 2 hours after lights out); preliminary secretory phase (3-5 hours after sleep onset); 4 hours main secretory phase (6-8 hours after sleep onset and 1 hour after rousing) followed by 11 hours of intermittent secretory activity (Weitzman, Fukushima et al, 1974) has been confirmed many times (DeGroot et al 1979) in both adults and children. With improved techniques and more frequent blood sampling the secretory activity of the adrenal cortex has been shown to be pulsatile with the largest peak amplitude of cortisol secretion coinciding with the highest plasma concentrations (Veldhuis, Iranmanesh, Lizarralde, Johnston, 1989 as cited Van Cauter, 1990). Suggesting that it is the pulse amplitude and not the pulse frequency of secretory bursts that is responsible for the generation of a circadian pattern.

By measuring the plasma concentrations of ACTH simultaneous with cortisol concentrations it has also been shown that the circadian rhythm of ACTH is virtually identical to that of cortisol.
but with the peaks of pituitary activity occurring about 10 minutes prior to that of the adrenal cortex (Follenius, Simon, Brandenberg & Lenzi, 1987). The plasma levels of cortisol and ACTH oscillate at regular intervals in individuals at about 130 and 110 minutes respectively (Follenius et al., 1987). Similarly, CRH is released in a pulsatile and circadian pattern (Kreiger, Allen, Rizzo & Kreiger, 1969) suggesting that the circadian rhythm of cortisol is initiated in the hypothalamus or higher brain centres and is not due to variation in the removal of cortisol from the circulation (DeLacerda, Kowarski, Migeon, 1973) and/or variation in the binding of hormone in the circulation. The circadian pattern of secretion of cortisol is enhanced by the fact that the adrenal gland is less responsive to ACTH simulation in the evening and during the early period of sleep than in the morning (Kreiger, 1980).

The pattern of free cortisol i.e. the unbound hormone, generally correlates well with the total amount of cortisol in the circulation i.e. the sum of both the albumin and transcortin bound cortisol and the free cortisol.

The circadian periodicity of cortisol is influenced by various factors such as the timing of meals and sleep-wake periods. Alteration of these factors does not abolish the pattern of secretion but it does modify it, e.g. in sleep deprivation the amplitude of deviation of cortisol peak and trough levels from the mean are reduced from about 75% to 55-65% (Van Cauter, 1990).

Pattern of urinary excretion.

In adults and children the circadian variation in plasma cortisol is reflected in the excretion of cortisol and its metabolites in urine (Honor, Kelnar & Brook, 1991). Approximately 90% of all cortisol is metabolised in the liver and to a lesser extent in the kidney (Schulster et al, 1976). The metabolites of cortisol are filtered; reabsorption does not take place and they enter the urine directly. The remaining 10% of cortisol in plasma (free cortisol) is filtered by the glomerulus. Once in the kidney tubules most of the cortisol is reabsorbed so that only a small fraction of the cortisol filtered passes into the urine. Measurement of free cortisol in the plasma is seen as a direct measure of the biological activity of cortisol. Urine free cortisol
reflects plasma free cortisol and therefore can be used as an indirect measure of biological activity (Nelson, 1980). The excretion of free cortisol is dependent upon the renal function so that like GH the excretion of cortisol is increased in diseases that increase glomerular filtration and disturb tubular reabsorption.

Development of a circadian pattern.

Reports have suggested that the circadian pattern of secretion of cortisol is not observed in the newborn infant. Martin-Du-Pan and Vollenweider (1967) (as cited in Price et al, 1983) suggested that this rhythm was first established by the 4th week of life; Price et al (1983), Vermes, Dohanics, Toth, Pongracz, (1980) and Spangler (1991) suggested 3 months of age, Onishi, Miyazawa, Nishimura et al (1983) suggest 6 months and both Franks (1967) and Zurbruger (1976) indicated some time between 1 and 3 years of age. The data from the Vermes et al (1980) study actually show a diurnal variation of cortisol at four weeks of age although the timing of the peaks and troughs of secretion were not synchronised to the sleep wake periods and other zeitgebers as seen in adults until 3 months of age. Once established the cortisol rhythm appears to remain virtually unchanged throughout childhood.

SUMMARY

1. Cortisol is needed to prepare the body for adequate responses to stressful situations prior to their occurrence and at the start of crisis.

2. Cortisol is produced from the adrenal cortex and is regulated by ACTH from the pituitary which in turn is regulated by CRH and the circulating levels of cortisol.

3. Cortisol is secreted in a circadian fashion with most cortisol secreted in the early hours of the morning before waking and the least during the late evening and onset of sleep. This pattern of secretion has generally not been reported before 4 weeks of age in the infant.

4. Under production of cortisol is associated with the inability to cope with stress, while overproduction reduces immunity and the healing process.
**BODY TEMPERATURE.**

Measurement of body temperature is probably the most frequently made observations for both experimental and clinical assessment. The advantage of such measurements is that they are simple to make and offer information about the balance of heat loss and heat gain. Changes in body temperature and hormonal status are intimately linked in adults. Both are believed to be organised by the hypothalamus, the former a result of metabolism and the latter a control of metabolism. Therefore, it is not surprising that, at least in adults, changes in temperature are often reflected by changes in hormonal status and vice versa.

Temperature regulation in infants is fairly well understood. We know that infants at birth are capable of maintaining their body temperature remarkably well in 'average' temperatures but that they do not display the characteristic adult circadian pattern until several weeks after birth. The following section reviews the concept of thermoregulation and the developmental changes seen in the infant with special reference to water loss from the skin, a method of heat loss which is partially uncontrolled diffusion and partially regulated by the parasympathetic nervous system and hypothalamus.

**THERMOREGULATION**

Mammals, including man, maintain their body temperature within a narrow range of temperature, this is called thermoregulation. Thermoregulation is the process which integrates input from both the external and internal environments of the body before adjusting the regulatory mechanisms thereby maintaining the body temperature at its most efficient for any given set of conditions. All animals are influenced by the same physical factors effecting heat exchange with the ambient conditions. These four factors are radiation, conduction, convection and evaporation. In order to maintain the body temperature within a narrow range the sum of heat lost by these processes must be equal to the amount of heat produced by metabolic processes.
Heat exchange is dependent upon the surface area available for heat exchange and the thermal insulation of the tissues and clothing through which the heat exchange is to take place. The larger the surface area to mass ratio the greater the heat loss, therefore infants who are small need to have a higher metabolic rate than adults who are large in order to maintain their body temperature at any given ambient temperature. The insulation of the tissues is the reciprocal of conductivity defined as the ability of heat to transfer from the core to the surface. If the conductivity of the skin is increased by increasing blood flow to the skin surface the insulating ability of the skin is reduced. In man clothing also has an effect on insulation not only by decreasing the total conduction of heat from the core of the body to the surface of the clothes but also by trapping air next to the skin and thereby decreasing evaporative water loss.

Radiation is dependent upon the surface temperature of the body, the surface area exposed and the radiant temperature of the surroundings. In man it is estimated that 60% of the total heat loss is by radiation.

Convection is dependent on the surface temperature, air temperature and air movement. Natural convection occurs as a result of the air in immediate contact with the skin being warmed, rising from the skin and being replaced by cooler denser air. Forced convection by increased air movements i.e. use of a fan increases the loss of heat by convection.

Conduction is dependent upon the amount of surface area in direct contact with another surface. If the surface is cooler then heat will be lost, if the surface is warmer then heat will be gained.

Evaporation is dependent on the skin temperature, air movement and the ambient humidity. For every gram of water that evaporates from the surface approximately 2.51kJ (0.6 kCals) of heat are lost making evaporation a very efficient means of increasing heat loss. Evaporation from the skin occurs by both the passive diffusion of water through the skin and the active process of sweating.
Physical heat conservation

Heat can only be conserved by increasing the thermal insulation of the tissues or behaviourally by decreasing the amount of surface area available for heat exchange by changing posture. The body can increase or decrease the thermal insulation by means of a counter-current heat exchange mechanism. In cold temperatures the superficial arteries in the skin of the limbs constrict, increasing the peripheral resistance to blood flow and a diversion of blood away from the surface to deeper vessels laying in close apposition to the arteries. In this fashion blood flowing to the extremities is cooled and blood returning is warmed. Conversely in warm environments the dilation of the vessels allows more blood to the skin and an increase in heat loss (Mount, 1966).

If heat conservation is insufficient to maintain an adequate body temperature the body must increase the heat production by increasing metabolism. There are two physiological mechanisms employed by the body to increase metabolism, these are shivering thermogenesis and non-shivering thermogenesis.

Non-shivering thermogenesis.

Non-shivering thermogenesis is the function of brown adipose tissue which is well defined in infants but unknown in adults (Lean, 1989; Hull, 1966; Rothwell, 1989). Brown adipose tissue is a yellowish tissue full of mitochondria accounting for its ability to increase the metabolic rate. In the infant it surrounds the major organs of the body and is well innervated by the sympathetic nervous system. Stimulation of the tissue is by both endocrine and neural mechanisms which are mediated by the hypothalamus.

Shivering thermogenesis.

Shivering thermogenesis uses no specific organ to increase heat production, instead it utilises the skeletal muscles of the body. Prior to the onset of shivering muscular tone increases in an attempt to increase heat production, if this is insufficient shivering begins. As shivering
commences involuntary uncoordinated contractions of the skeletal muscles occur which increase the metabolic rate. Shivering can only increase the basal metabolic rate 2-3 times whereas maximal voluntary work can increase metabolism 20 fold (Hammel, 1968; Hemingway, 1963). In the presence of voluntary muscular activity shivering ceases as the two cannot occur simultaneously. In infants it is non-shivering thermogenesis which is the predominant means of heat generation, over the first year this becomes of less value as shivering thermogenesis takes over.

Heat Loss.

In recent years the ability to lose heat adequately in infants has been questioned. Several authors suggest that overheating may be a more important factor in the older infant than hypothermia (Bacon, 1983; Bacon, Scott, Jones, 1979; Stanton, Scott, Downham, 1980; Waddington, Tucker, Fly, Greene, 1976) In adults and infants the most efficient method of heat loss is sweating. This section will examine the development of skin, its function and the ability to control water loss from its surface.

SKIN (reviewed by Sato, Kang, Saga & Sato, 1989 & Quinton, 1983)

Development

Before 28 weeks of gestation the skin is immature although the sweat glands, sebaceous gland, hair follicles and nerve endings are fully formed by this time. The epidermis is only 2-3 cell layers thick, the stratum corneum is not fully keratinised and the hypodermal layer of adipose tissue is not present. By 32 weeks the epidermis is virtually indistinguishable from that of the term foetus or adult (Rutter, 1988).

Function.

One of the main functions of the skin is to act as a barrier protecting the body's contents from the adverse physical and chemical environment. Body water is conserved while bacteria and potentially harmful chemicals are kept out. The barrier properties of the skin are not fully
developed in the preterm infant younger than 28 weeks of gestation. However, the skin rapidly matures in premature infants so that within 2 weeks of birth the properties of the skin are comparable with those of the term infant and adult (Rutter, 1988).

**Conservation of body water.**

Body fluids cross the barrier of the skin by either diffusion or by the active secretion of fluids onto the skin surface. Diffusion through the epidermis is a passive process which depends upon the water pressure gradient between the body and the environment. This transepidermal water loss is not under physiological control but is determined by the effectiveness of the epidermis as a barrier to water diffusion and environmental conditions such as temperature, humidity and air speed. Active secretion of fluid onto the surface of the skin can occur from sebaceous, apocrine, and eccrine glands. Sebaceous fluids are waxy and oily helping to reduce the loss of water by diffusion. The eccrine glands are involved in the physiological control of body temperature and therefore can contribute the greatest to water loss in warm environmental conditions and at high body temperatures (Sato et al., 1989).

**SWEATING.**

Sweating is the active secretion of fluid onto the surface of the skin. It is an important physiological mechanism in the control of body temperature. Although they are not the only secretory glands of the skin the eccrine glands are the most important in temperature regulation and contribute the most to transepidermal water loss. In order to understand the mechanism of sweating the eccrine gland and its control mechanisms must be understood.

**ECCRINE GLANDS.**

Eccrine sweat glands cover the entire body it is only the oral, anal and genital areas that do not have sweat glands. The density of their distribution varies from 10. cm\(^{-2}\) to 700. cm\(^{-2}\) (Sato, Kang, Saga & Sato, 1989). Normal adult individuals when sweating maximally can lose 2-4l of hypotonic fluid per hour dissipating 18 kCal.min\(^{-1}\) during the evaporation process. Unacclimatised adults begin to sweat before their body temperature has risen by 1 °C, while in
acclimatised individuals sweating can begin soon after a heat load commences (van Beaumont & Bullard, 1963).

The intrauterine development of sweat glands commences at the end of the first trimester on the palms of the hands and soles of the feet. By 5 months they have started to appear on the rest of the body; initially on the face and head, so that by 8 months their distribution is similar to that of the adult (Sato et al, 1989)

**Sweat gland structure.**

The eccrine gland consists of a single unbranched tubule coiled into a bolus of about 300 μm diameter. The bolus of the gland is found about 2-5 mm below the surface of the epidermis. The proximal tubule of the gland is composed of a blind-end secretory tubule coiled in the bolus of the gland. Following on from the secretory tubule is the reabsorptive duct which is also located in the bolus of the gland. The rest of the gland consists of a straight segment of reabsorptive tubule connecting the bolus of the gland with the skin surface.

The secretory tubule of the sweat gland is a simple epithelium made of three distinct cell types; the light cells which are large and peripheral and thought to be the fluid secreting cells; the dark cells that are located near the lumen of the tubule and responsible for the secretion of macromolecules into the lumen, and the myoepithelium cells which provide mechanical support for the secretory coil against the luminal hydrostatic pressure. The reabsorptive duct consists of two cell types; the luminal and basal duct cells.

**Production of sweat.**

**Secretion:**

The production of sweat takes place in two stages. The first stage is the secretion of primary sweat which is isotonic with plasma and the second stage is the reabsorption of important ions. The process of secretion involves the active transport of electrolytes by means of the sodium/potassium pump located in the basal-lateral membranes of the secretory cells. This
active process sets up an electrochemical gradient for sodium causing the movement of sodium into the secretory cells. Chloride ions move into the cells against the electrochemical gradient via the sodium/chloride carrier. The basal membrane of the cells are impermeable to chloride therefore chloride can only leave the intracellular space via the luminal membrane thereby creating a movement of negative charge into the lumen. The negative charge increases in the lumen and the positively charge sodium ions cross into the lumen. Water and small permeable solutes move into the lumen down the osmotic gradient increasing the hydrostatic pressure in the lumen and forcing the primary sweat down the lumen to the reabsorptive ducts. The tight junctions between the basal cells do not allow the movement of chloride back into the extracellular spaces and so maintain the continuous flow of fluid into the lumen.

**Reabsorption:**

The distal part of the sweat gland reabsorbs sodium, chloride and bicarbonate along with glucose and other small solutes from the primary sweat. This reabsorptive capacity seems to be most highly developed in man conferring the advantage of being able to cope with heat stress while conserving the major solutes of the extracellular fluid. The sweat duct is impermeable to water thereby allowing the movement of the solutes out of the lumen without water following.

**CONTROL OF SWEATING.**

The hypothalamus integrates input from both the body and brain before adjusting output to the sweat glands.

Thermal inputs from sensors located in the skin, muscles and central nervous system are integrated at various levels of the nervous system, the final consequence being the stimulation of the hypothalamus and so too stimulation of sweat secretion via the parasympathetic nervous system (Hori, 1981). The hypothalamus itself has thermal sensors which provide important information about the change in temperature in the brain. In addition to stimuli from the hypothalamus the local conditions around the sweat glands are important. Local blood flow,
wetness of the skin and humidity of the surrounding air can all influence the rate of sweating (Hensel, 1973).

Not only is sweating stimulated in response to thermal conditions, but in areas such as the palms of the hand and the soles of the feet sweating is determined by emotional factors. In adults sweating from the palms of the hands and the soles of the feet is independent of the thermal stimuli and is instead determined by emotional factors such as pain, fear, anxiety, concentration, relaxation and sleep. Emotional sweating has been documented in infants by 36-37 weeks post-conception and by 43 weeks of age regardless of the gestation age at birth. The emotional sweating from the palms of the hands during crying is comparable to that of an anxious adult (Harpin & Rutter, 1982 a and b). In addition emotional sweating can be exhibited in extremely stressful situations as generalised sweating often described as a 'cold sweat' or clamminess.

TRANSEPIDERMAL WATER LOSS.

The sum of the water lost by diffusion and by active secretion (sweating) can be defined as total transepidermal water loss (TEWL). TEWL has been extensively studied in the adult and the newborn infant as it can give valuable information about the barrier properties of the skin and the activity of the eccrine sweating. In the newborn infant this can be very important as much energy and water may be lost, particularly in the preterm infant, causing dehydration or hypothermia. Conversely inadequate TEWL may result in overheating and hyperthermia.

In early studies of water loss, it was difficult to separate water losses from the respiratory tract and those from the skin. It is only in the past 20 years that an instrument which measures the water loss directly from the skin has become available. The evaporimeter, as it is known, makes use of the fact that within 1cm of the surface of the skin the diffusion gradient for water is linear and therefore can be used to calculate the amount of water lost.

TEWL studies have shown that in adults and infants different areas of the skin surface contribute to the total water loss from the body by different proportions. In adults basal water
losses are in the region of 500 g·24h⁻¹ but this can increase to 2-4 l·h⁻¹ (but is not sustainable for long) during activity or thermal stimuli. In adults the highest TEWLs are observed on the palms of the hands (90 g·m⁻²·h⁻¹), soles of the feet (45 g·m⁻²·h⁻¹) and head (34 g·m⁻²·h⁻¹) while the rest of the body have mean TEWL of about 10 g·m⁻²·h⁻¹. This means that approximately half of the total TEWL is from an area that is only 15% of the total body surface area (Lamke, Nilsson & Reithner, 1977). Similarly, in infant up to 1 month after birth TEWL from the palms of the hands (19 g·m⁻²·h⁻¹) soles of the feet (10 g·m⁻²·h⁻¹) and head (18 g·m⁻²·h⁻¹) are higher than the TEWL from the rest of the body but not of the same magnitude as those of the adult (Hammarlund, Nilsson, Oberg & Sedin, 1977).

Further studies by Hammarlund and coworkers have investigated TEWL in newborn infants in greater detail. The effect of activity (Hammarlund, Nilsson, Oberg & Sedin, 1979), both gestational (Hammarlund et al, 1979) and post-natal age (Hammarlund, Sedin & Stromberg, 1983), nutritional status at birth and skin blood flow (Stromberg, Hammarlund, Oberg & Sedin, 1983) have all been investigated in these neonates. This work has shown firstly that TEWL in all infants increases appreciably with activity and that TEWL in resting infants increases when rectal temperature rises above 37.1°C. Secondly, preterm infants have higher TEWL than term infants and this water loss decreases in an exponential fashion with age so that by 4 weeks after birth there is little difference between term and preterm infants. Thirdly, that small-for-date infants have lower TEWL than age matched appropriate-for-date infants. Fourthly, that TEWL in infants seems to be independent of skin blood flow.

**Circadian pattern of TEWL.**

In adults TEWL, in common with body temperature, skin temperature and hormones shows a circadian pattern. At night sweat rates and evaporation have been shown to be lower than during the day (Parmeggiani, 1980; Shapiro et al, 1974). In infants, TEWL has been estimated by a variety of means but the circadian pattern of low TEWL at night with higher during the day has only been examined using the electrical resistance of skin. Hellbriggge (1960) indicated that a diurnal pattern of electrical resistance was present virtually from birth
becoming more marked during the first year of life but never quite reaching the adult pattern during childhood.

In addition to a circadian pattern, TEWL appears to be linked to the activity during sleep. Measurements of TEWL during periods of night-time sleep in adults and in infants indicates that TEWL is lower during rapid eye movement (REM) sleep than during non rapid eye movement sleep (NREM) (Khan, Rebuffat & Blum et al, 1987). Sleep structure in the neonate is not as distinct as that seen in the adult. For the first year of life REM (active sleep), and not NREM as in adults, marks the boundary between waking and sleeping. The periodicity of NREM-REM sleep is present but the cycle length is about 50-60 minutes compared with 90 minutes in the adult. The amount of time in REM at birth is 50 %, it is not until 4 years of age that this has reduced to 20 %, as seen in young adults (Mills, 1973,). In adults REM sleep is associated with a period of down-regulation where responses to environmental changes are not as effective as during waking or NREM sleep. The greater amount of time spent in REM by infants is therefore probably significant in the body's regulatory system.

**DEVELOPMENT OF THERMOREGULATION.**

It is well established that the human infant is very capable of making physiological responses to thermal stimuli (Hey, 1969; Hill & Rahimtulla, 1965; Lodemore, 1993). The problem that it faces is that it is unable to make the necessary behavioural responses and is reliant on carers to provide adequate temperatures and clothing to remain at thermal neutrality (Hey, 1975). At birth the full-term infant is able to sweat (although ambient conditions are rarely sufficient to elicit this response) (Hull, 1988; Hey & Katz, 1970) and increase the metabolic rate by means of non-shivering thermogenesis (Lean, 1989, Hey, 1969). Over the ensuing months the infant becomes larger and the need for heat conservation becomes less important as the surface area to mass ratio decreases and the ability to make adequate behavioural responses in response to over-heating increases.
In adults there is a well defined circadian variation in body temperature with high body temperatures during the active day period and low body temperatures during the night-time sleep period (Minors & Waterhouse, 1981). Lodemore, (1993) characterised the patterns of change occurring in the circadian pattern of body temperature in the infant. This work followed the work of many people, such as, Hellbrügge (1960) who had suggested that the infantile pattern of body temperature matured sometime in the first 1-2 years of life to become adult-like. Lodemore’s study described a process which was all but complete by about 3 months of age but which was comprised of three basic stages; the neonatal, the pre-rhythm and the post-rhythm stages. The neonatal state was denoted by day-time and night-time rectal temperatures being little different. In the pre-rhythm state a day-time / night-time difference in rectal temperatures was becoming evident, but the night-time rectal temperature never fell below 36.7°C. The transition from the pre- to the post-rhythm state was a rapid one occurring within one week in individual infants. The appearance of a rectal temperature minimum of 36.5°C or below was taken as the achievement of post-rhythm status. Once achieved the night-time rectal temperature altered little throughout the rest of infancy, childhood and adulthood. Both infections and immunisations were found to elevate the temperature achieved overnight by an infant.

In addition to identifying a period of dramatic change in the night-time rectal temperature other observations were made which identified factors associated with the early or late attainment of an adult-like temperature pattern by an individual. Being male, bottle fed, with a smoking mother, of lower social status and higher birth order all tended to delay the first appearance of a mature temperature pattern. Once established the mature temperature pattern did not change unless the infant was immunised or had an infection. Both the timing of the development of the maturation of the night-time rectal temperature and the factors which effected this development shared characteristics with the epidemiology of SIDS (described in the section

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1The word 'rhythm' is not used in this study although the criteria on which Lodemore made these characterisations of night-time rectal temperature patterns are used. Instead the notation of 'Maturation week' is used to indicate the first appearance of an adult-like temperature pattern where a minimum night-time rectal temperature of 36.5°C or below is achieved. All weeks prior to this week become pre-maturation and all weeks subsequent to this week post-maturation or mature.
below, 'Sudden Infant Death Syndrome'). The coincidence of a developmental change, which may mark changes in other systems and the peak incidence of SIDS suggested that there may be hidden answers to explain these mortalities.

**SUMMARY.**

1. Studies on the water loss from the skin indicate that in adults and infants the rate of water loss varies depending upon the skin site being measured.

2. Water loss from the skin and temperature appear to be linked to activity during sleep with TEWL being lowest during REM sleep.

3. TEWL is linked to a circadian pattern of activity with the lowest values found during the night-time.

4. TEWL may increase in response to nervous stimulation i.e. emotional sweating.

5. Infants are capable of regulating the body temperature efficiently. However, the pattern of basal temperature changes at about 3 months of age to a more adult-like day-night pattern linked to the onset of sleep.

6. The onset of these changes in night-time rectal temperature at 3 months of age are affected by a variety of child care practices and social factors.

7. Once established the mature rectal temperature pattern with a minimum night-time temperature of 36.5°C does not change unless the infant is suffering from an infection or has been immunised.
STRESS (reviewed by Standard, Salmon & Gray, 1993).

Stress has many different definitions and many actions on the body which unites every endocrine and physiological system already described above. In the context of this study it is taken to be either a physical or psychological stimuli resulting in a characteristic set of physiological responses. The stress response was first described by Seyle in 1936 before he subsequently expanded it to become the General Adaptive Syndrome (1946). The main focus of his theory was the adrenal secretion of cortisol in response to stressful stimuli. It is now known that the body's response to stress is multifaceted and co-ordinated by the hypothalamus. Input from the higher centres of the brain, peripheral sensors and feedback from the circulating levels of hormones converge on the hypothalamus which then assesses the situation before initiating a vastly diverging array of responses via neuronal and hormonal systems. The immediate stress responses are mediated by sympathetic discharge and catecholamine release into the blood leading to increased heart rate, blood pressure and respiratory rate, pupil dilation, perspiration and pallor as well as mobilising the bodies resources (Weissman, 1990). It is not until 10 minutes later that cortisol secretion is increased. These changes occur in response to both physical tissue trauma (Jeffries & Vance, 1992) and to psychological stimuli such as maternal loss and frustration (Levine & Ursin, 1991). The result is a system which drives an organism to eliminate the stress while protecting against injury and infection.

In adults we understand some of the aspects of the stress response. In both acutely and chronically stressful situations this adaptive mechanism has been related to both morbidity and mortality (Dohrenwend & Dohrenwend, 1974), although when working optimally this system protects against both. Increased susceptibility to the common cold (Cohen, Tyrrell, Russel et al, 1991; Klein, 1993), peptic ulcers (Anand, 1993), psychological disorders (Brown, Bifulco, Harris & Bridge, 1986), multiple sclerosis (Grant, McDonalt, Patterson, et al, 1989) and menstrual disorders (Harris, 1989) have been related to life stress in adults. In children we know that stress may result in growth disorders (Breirley, 1992), psychological disorders
and behavioural problems, increased illness (Johnson, 1989, Boyce, Jensen, Cassel et al, 1977) and may increase the incidence of morbidity in adulthood (Johnson, 1989). However, in infants although we know that they are capable of responding to stress from birth (Schmeling, Coran, 1991) we do not know how mature these responses are, nor the detrimental effects that stress has on an infants well-being. Some evidence does exist suggesting that protracted periods of stress in infancy or even just temporary separation from the mother can be detrimental at other times of life (Gunnar, 1989). Some animal studies further this by suggesting that at certain stages of development critical periods occur in which stressful stimuli can make the adult less able to cope with stresses (Levine, 1969). This has important implications for our child care practices.

Stressful situations in animal models have been seen to alter circadian rhythms, reducing the amplitude of daily body temperature rhythm (Kant, Baumann & Pastel, 1991). In infants a similar reduction in amplitude of circadian rhythm have been reported following immunisation (Rawson, Petersen, Wailoo, 1990) and illness (Jackson, Petersen, Wailoo, 1994) suggesting that this is similar to that observed by Kant et al (1991) and therefore probably resultant of some stress. Other studies have examined the immediate hormonal response (within 1 hour) of infants to immunisation (Lewis & Thomas, 1991) but none have examined the longer term effect of immunisation.

**SUDDEN INFANT DEATH SYNDROME**

Infants have been dying suddenly and unexpectedly since records began and probably earlier. The death rate in the first year of life is now at its lowest ever. However, there remains a fairly constant mortality rate of about 7 per 1000 live births of which 0.7 per 1000 (1993) are sudden, unexpected and unexplained deaths. These unexpected deaths generally occur during the post-neonatal period i.e. after 4 but before 52 weeks of age, and are currently known as 'cot death', 'crib death' or as, 'sudden infant death syndrome - SIDS'. The definition of SIDS is one of exclusion so it is only deaths where thorough post-mortem provides no sufficient explanation of cause that are classified as SIDS (Beckwith, 1970).
To date the cause or causes of SIDS continue to elude both the scientific and medical professions with most information about the nature of SIDS emerging as a result of epidemiology and pathology. Such studies have shown that there is a fairly unique age distribution for SIDS with 93% of all attributed deaths occurring between 4 weeks and 1 year, with most occurring between 1 and 4 months. Victims are more likely to be male, of lower social status, have younger mothers, larger numbers of siblings, be small for date, be preterm births or be one of a multiple birth. Other factors including aspects of child care also appear to play a role in SIDS. Infants with smoking mothers, who were bottle fed, placed prone to sleep or who are highly insulated are at further risk of SIDS (Golding, Limerick, Macfarlane, 1985). Other studies have suggested a role for infection which ties in with the greater number of deaths during the winter months of the year (Berry, 1992).

Explanations of the occurrence of SIDS have included hypermetraemia, nutritional deficiencies, breathing obstructions, anaphylactic reactions and various respiratory and cardiac reflexes left over from prenatal life. However, no single factor nor satisfactory combination of such factors has been described which can fully explain the pathology and epidemiology of SIDS. Theories behind the cause of SIDS are many fold and as can be seen from above, include almost all the physiological systems. The cardiovascular, the respiratory, the central nervous system and the immune system have all been suggested to play a role in SIDS (Guntheroth, 1982). One underlying theory is that a variety of unusual circumstances must be achieved before a trigger event can become the 'last straw' for an already vulnerable system (Filiano & Kinney, 1994; Hoppenbrouwers & Hodgeman, 1992). All the physiological systems implicated in SIDS share a set of characteristics which may interact to create the 'unusual circumstances' and explain why some infants may be more vulnerable than others (Filiano & Kinney, 1994; Hoppenbrouwers & Hodgeman, 1992). Firstly, each of the above systems are immature at birth; secondly they are all believed to change rapidly within the first months of life to become more adult-like; thirdly, it is vital that responses in each system are co-ordinated with responses in other systems in order to avoid systemic collapse and fourthly, at least in the adult, each of these systems are regulated and co-ordinated by the hypothalamus and the HPA.
How might the development of the HPA relate to SIDS? If the HPA of the infant is not fully developed at birth, then as it develops there may be a time period when it may not be able to respond appropriately to certain stimuli. The pathology of deaths due to a generalised system failure may not be easily recognised as being sufficient to cause fatalities and perhaps would not have caused death had the individual not been put under an additional unknown stress. At least some of the functions of the HPA are changing over a time period when SIDS victims are most likely to die. As already stated changing systems are unstable and vulnerable so perhaps the peak incidence of SIDS is a marker of changing HPA systems, high vulnerability and stress.

As discussed in the previous section ('Stress'), stress is a very potent stimulus having far reaching effects on all systems of the body via the HPA and nervous system. Unfortunately, infant physiology is such that avoidance behaviour is limited leaving them to rely on the parent to exhibit appropriate behavioural responses to stress. This reliance on the adult is a far from perfect system as the only means of communication for the infant are crying and posture changes which can lead to mis-interpretations and inappropriate adjustments. Inappropriate adjustments and mis-interpretations could increase the stress of an event until it becomes fatal, perhaps explaining why certain child care practices and social factors carry an increased risk for SIDS. However, most of the population survives infancy, so it is unlikely that inappropriate parenting is the sole cause of SIDS. Instead, environmental factors suggested by epidemiological studies of SIDS probably interact with infant physiology to increase vulnerability by either hindering responses to stress or disrupting development in some way, thus allowing an ordinary every day event to become a fatal one.

In order to investigate the role of the HPA and stress in infant well-being and its possible role in SIDS, a greater understanding of the changes occurring in this system in the infant over the first months of life is essential.
CONCLUSIONS.

To date knowledge of the developmental process in general is limited and HPA function is one of the most rarely investigated systems. In this study an attempt to examine some of the physiological systems under the control of the HPA is made. The chosen age range of study was taken as between 4 and 20 weeks of age, a period coinciding with great physiological change and the peak incidence of SIDS. Not only has the control of body temperature been described as changing at this time but adjustment to respiratory, cardiovascular and sleep physiology, changes in endocrine activity and immunity are also believed to occur over this crucial 16 week period.

There has been much time and energy expended by the scientific and medical communities describing body temperature, cardiovascular, respiratory and sleep physiology over the early months of life. Due to unsuitable methodology few studies have examined endocrine secretion or the development of sweating ability in this age group, so it is unclear if or how infants differ from adults in these aspects. In order to study infants the means of data collection has to be simple, non-invasive and acceptable to both parents and infant. Hormones that are present in urine provide the simplest method for assessment of endocrine aspects of the HPA. Cortisol and GH, as described earlier, are essential parts of the HPA system dealing with body maintenance, coping and the stress responses. Both these hormones are excreted into the urine, providing an avenue for non-invasive assessment of the HPA. However, novel experiences and changed environment have been shown to alter endocrine secretion in previous studies, this effect can be minimised by changing the daily routine as little as possible. In order to establish a basal reference data collection from the home environment is the simplest and most effective method. Similarly, to investigate the effect of a stress, it is more ethical and convenient to utilise the scheduled immunisation of infants at specific ages as uniform stressors thereby allowing comparison of responses between individuals, than establishing a specific stress response experiment.
Like many other physiological systems, TEWL is widely investigated in the neonate, child and adult but not well described for infants between 4 and 20 weeks. Changes in the ability to lose heat by evaporation may be an important feature of the development of a mature pattern of thermoregulation. Investigation of the ability to lose heat by evaporative water loss from the skin also provides insight into a third aspect of HPA function. By studying a group of infants in the home environment a range of child care practices and thermal loads and their effect on TEWL can be examined.
Aim:

To characterise certain aspects of HPA function by utilising urinalysis, body temperature and TEWL measurements.

Objectives:

1. Describe the pattern of cortisol and GH excretion establishing reference ranges and the presence or absence of circadian periodicity. Development of circadian periodicity may be indicative of the stage of general maturity.

2. Relate changes in the excretory rates of cortisol and GH to the maturation of the rectal temperature pattern. Changes in HPA function controlling hormone production may occur over the same period as changes in HPA function controlling body temperature.

3. Examine the effect of different aspects of child care, family health and social status on the excretion of cortisol and GH. These factors have already been shown to be associated with an increased risk of SIDS and delayed appearance of a mature rectal temperature and have been postulated to effect stress in individuals.

4. Describe the effect of immunisation on the excretory rate of both cortisol and GH. Cortisol and GH are essential parts of the stress response. Immunisation has already been shown to be a stressful event disturbing rectal temperature. The effect of immunisation on cortisol and GH excretion further characterises the infant’s ability to cope.

5. Measure TEWL from the surface of the skin establishing the effect of site of measurement, age and activity of the subject on TEWL rates. TEWL regulation is another function of the HPA. Changes in the ability to lose heat via water loss from the skin may play a vital role in the maturation of the rectal temperature pattern.
METHODOLOGY
CHAPTER 2

METHODS OF DATA COLLECTION AND ANALYSIS
STUDY DESIGN

This study was divided into two distinct sections; the first to examine hormonal changes and the second to examine sweating and water loss from the skin in human infants between 1 and 6 months of age. Each section was designed to enable the longitudinal data to be related to rectal temperature patterns by simultaneously collecting this data.

1. Investigation to examine the excretion of urinary metabolites.

A pilot study designed to assess the feasibility of collecting urine specimens and to clarify that both cortisol and GH were present in the urine in sufficient quantities to be measureable was undertaken. Urine samples were collected from a cross-section of infants of all ages already participating in ongoing research in Leicester. This confirmed that it would be possible to collect adequate supplies of urine from this age group to examine creatinine, free cortisol and growth hormone in urine. Once established the investigation of these three metabolites was extended from a cross-sectional study to a more longitudinal study following infants over several weeks. This allowed the temperature pattern maturity to be assessed along with the effect of age on the excretion of these metabolites. Furthermore, by annotating each urine collection and temperature recording with data about family health and child care practices the effects of more social factors could be investigated.

2. Investigation to examine sweating and water loss from the skin.

The aim of the first phase of this section of the study was to collect evaporation measurements from a large group of individual infants from skin sites during various activities. Information gained from this first phase of data collection was used to adjust the technique for use in the second phase of the study. This second stage of data collection enabled the longitudinal collection of data from a smaller number of sleeping infants during a period similar to the longitudinal urine collection. In fact a small subset of the infants participating in the longitudinal urine collection were used.
RECRUITMENT OF INFANTS.

Cross-sectional study of TEWL:

Daily visits were made to the children's ward of Leicester Royal Infirmary between October 1991 and August 1992. At each visit staff were asked about the past days admissions and their suitability for the study. The staff of the children's wards advised as to which infants they felt were considered to be well enough to take part in the study and whose families were least distressed by their child's admission to hospital. Those parents who were coping well and whose child was well enough were approached and asked if they would like to take part in the study taking place that afternoon. Most infants that took part in this study were waiting to be discharged later the same day and had been admitted for either a scheduled minor investigation or for observation.

Longitudinal study of temperature, urine metabolites and TEWL.

Recruitment took place between October 1992 and January 1994. Subjects were recruited from a random sample of infants born at Leicester Royal Infirmary maternity hospital whose mothers lived in the postal districts LE2 and LE3. An introductory letter (see Appendix 2 for example) was sent out to these families outlining the project and requesting to visit to explain further and discuss the possibility of taking part in the study.

Visits were arranged on an opt-out basis, i.e., if no contact was made it was assumed that the suggested appointment was acceptable and the visit made. Consequently, visits were often made at the appointed time only to find the family out. This was taken to be a negative reply and no further attempt to contact was made. Further families did not make contact but declined to take part on their arrival.

Each recruitment visit took between 30 and 40 minutes and consisted of a demonstration of the equipment used in the project and explanation of what the study would involve. At these initial visits mothers were often the only parent present and were encouraged to discuss the
project with the fathers before responding. Occasionally, a second visit was made to explain everything to the fathers of the babies before a decision was made.

Appointments for the first monitoring session were made either provisionally on the day of the recruitment visit or within a week of the initial visit. It was stressed that participants could withdraw their consent to take part at any time they felt appropriate.

At the same time a poster was place in the ante natal clinic inviting families to request further information. Families who responded to this advertisement were sent letters and went through the above recruitment process. (See appendix 2 for examples).

Total compliance was 1:5.

Collection of urine:

In addition to the infants recruited and monitored by myself, urine specimens were collected along with night-time temperature recordings by a team of 4 Health Visitors employed as research assistants on a larger project. Infants were again recruited from Leicester Royal Infirmary maternity hospital whose families lived in any Leicester postal district. The recruitment method employed by each member of the team was similar to that outlined above. Recruitment of infants to this part of the study took place between June 1992 and February 1993.

Each Health Visitors had different rates of volunteer compliance but was on average 1:4.
RECORDING SCHEDULE

Cross-sectional collection of TEWL.

Infants recruited to participate in this part of the study were between 1 and 6 months of age. A single set of evaporation measurements were made on each infant while they were patients on the childrens wards of Leicester Royal Infirmary.

Evaporation measurements were taken from as many of the 12 sites shown below (figure 2.1) as possible. Each measurement took between 30 and 60 seconds to make. Measurements from each site were made in triplicate allowing a 5 minute interval between readings at any given site.

There was no attempt made to standardise the activity of the infants, measurements were taken during quiet and active periods of sleeping and waking.

Figure 2.1. Sites of measurement for TEWL

**Longitudinal collection of temperature, urine and TEWL.**

Each infant was monitored at weekly intervals from 6 weeks of age for about 8 weeks or until one week after the appearance of a mature adult-like temperature pattern, whichever was the earlier.

On a given evening a night-time sleeping body temperature record was made which lasted approximately 10 hours from the last nappy change of the evening until waking in the morning.

Weekly recordings of evaporation rates and associated temperature changes were also made. Generally evaporation measurements were made during a period of sleep at sometime the day after night-time temperature recording. However, a small proportion of recordings were made either the day before night-time temperature recordings or during the early period of night-time sleep. Each recording of sleeping evaporation rates lasted between 15 and 60 minutes depending on the length of sleep.

**Collection of urine samples:**

Urine samples were collected from the above group of infants simultaneously with the recording of sleeping night-time body temperature. Samples were timed so as to collect a night-time specimen (from the last nappy change of the evening until the first feed of the morning) and a morning specimen (for 2-4 hours following the first feed of the morning).

Most urine samples were collected as part of a larger study by a team of health visitors employed as research assistants. Urine samples were collected in a similar manner to that described above at the same time as night-time temperature recordings. The success rate for the collection of urine was about 50%.
TEWL measurement:

The evaporimeter was used to measure transepidermal water loss from the surface of the skin (see appendix 3 for theory and calibration). After allowing the evaporimeter to reach equilibrium with the environment the head of the measuring probe was held gently at the skin surface for between 30 and 60 seconds after any movements. Once a stable reading had been reached this was noted.

In the second phase of data collection the longitudinal collection from the home environment the evaporimeter was connected to a Grant Squirrel data logger as described later in this chapter. This allowed evaporimeter readings to be logged simultaneously with readings of rectal, skin and ambient temperatures over a continuous period. The logger was set to sample at 5 second intervals.

In both phases of data collection the amount of insulation covering the infant was estimated (total togs). In the longitudinal study logging the readings during a recording allowed the notation of the activity of the infant.

Temperature recordings:

Grant Squirrel data loggers were chosen for their convenient size, portable nature and adequate number of recording channels (up to 12 different channels). These data loggers have proven to be sufficient for the recording of temperature data from infants in several studies carried out by a Leicester based research team (see appendix 4 for the calibration of the data logger probes).

The logger measures 18 x 12 x 6 cm and weighs 0.98 Kg including the battery supply. When used to record temperatures between -25 and +105°C or DC voltage between -20 and +20 V the resolution of the logger is 0.05°C or 1mV respectively. This is both sufficient for the logging of rectal, skin, and ambient temperature and evaporation rate measurements from the evaporimeter as voltage measurements.
Whilst in use the logger was placed inside an anti-static bag enclosed within a canvas shoulder bag designed to allow the simultaneous movement of infant and logger. In this fashion the control buttons of the logger were protected from tampering. Tampering with the controls could result in the loss of data or the termination of data logging.

During the night-time monitoring period the data logger was set to sample readings once every minute. Day-time evaporation rate measurements were sampled more frequently at 5 second intervals.

The temperature readings were logged from the probes as true values in degrees centigrade. Evaporation rate measurements logged from the evaporimeter were expressed as voltages. In order to convert the evaporation rates each value was multiplied by a factor of 10.

Record of social and health data:

Data about the health, care and social conditions of both family and infant were collected either directly from the family or from maternity records. Perinatal and social data were collected by asking the mothers about their recollection of events happening throughout pregnancy and the birth. Further details were collected from the maternity notes.

Summary of the information collected (for details of the forms used see appendix 2):

- Postcode of family home
- Marital status
- Occupation of parents (later used to determine the social class as defined by the Registrar Generals Classification (Office of Population and Surveys, 1991)
- Age of parents
- Parental smoking
- Parental health
- Family history of illness
- Number of siblings
- Complications of pregnancy
- Complications of labour and delivery
- Apgar score and onset of respiration
- Admittance to the special care baby unit (SCBU)
- Date of birth
- Gestation age
- Birth weight
- Method of feeding at birth
- Sleeping position during first week
- Problems or illness during first week
Further information was collected each week at the time of monitoring about the general health
of the family and infant care practices:-

- Age and weight of infant
- Feeding regime
- Age of weaning
- Sleeping position
- Date of immunizations
- Type of heating
- Position and type of cot

From the infant health diary completed each week other factors were identified:-

- Time and type of illness (ie symptoms)
- Age of infant at time of illness
- Seen by GP
- Admission to hospital
- Prescribed medicines
- Reaction to immunization

Finally the event diary completed during each monitoring provided data on:-

- Timing and length of sleeps
- Nappy and clothing changes
- Periods of crying and wakefulness
- Time of placement into cot
- Time and type of feeds
- Time of bowel movements
- Time and duration of each urine collections

**Tog meter values:**

An estimation of the togmeter values of all clothing and bedding used to insulate the infant was
made by noting the type and amount of wrapping and using the average values provided by the
Shirley Institute Manchester (table 2.1).
Table 2.1: Estimated tog values for common articles of clothing and bedding.

<table>
<thead>
<tr>
<th>Item of clothing</th>
<th>Togmeter value</th>
<th>Item of clothing</th>
<th>Togmeter value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nappy</td>
<td>2.0</td>
<td>Socks</td>
<td>0.2</td>
</tr>
<tr>
<td>Vest</td>
<td>0.2</td>
<td>Mittens</td>
<td>0.2</td>
</tr>
<tr>
<td>T. shirt</td>
<td>0.3</td>
<td>Cotton sheet</td>
<td>0.2</td>
</tr>
<tr>
<td>Babygro</td>
<td>1.0</td>
<td>Flannelette sheet</td>
<td>0.5</td>
</tr>
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<td>Velour babygro</td>
<td>1.5</td>
<td>Old blanket</td>
<td>1.5</td>
</tr>
<tr>
<td>Night gown</td>
<td>0.6</td>
<td>Thermal blanket</td>
<td>2.0</td>
</tr>
<tr>
<td>Pyjamas</td>
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<td>Thin quilt</td>
<td>2.5</td>
</tr>
<tr>
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<td>Medium quilt</td>
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</tr>
<tr>
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<td>Cot quilt</td>
<td>9.0</td>
</tr>
<tr>
<td>Trousers</td>
<td>2.0</td>
<td>Baby nest</td>
<td>4.0</td>
</tr>
<tr>
<td>Tights</td>
<td>0.2</td>
<td>Sleep suit</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Method for collecting urine samples:

Each urine specimen was collected using a paediatric 24 hour U-bag, urine collector (Hollister). The bags were attached to the infants in the manner prescribed by the manufacturers. The infant was placed supine and its legs were separated and any powders, oils or lotions in the pubic and perineal area were removed and the area dried thoroughly to ensure that the U-bag could be attached securely.

The protective paper was removed from the U-bag to reveal the hypoallergenic adhesive flaps designed to attach the U-bag to both male and female infant.

Attachment to the female: The perineum was stretched to remove skin folds. By starting at the bridge of skin separating the anus from the external vulva and working upwards the
adhesive could be firmly attached to the skin around the external vulva. This was the most effective way of ensuring that no creasing of the flaps which might lead to urine leakage occurred.

Attachment to the male: The U-bag was fitted over the penis and the adhesive flaps pressed firmly to the perineum ensuring that no puckering of the flaps occurred to avoid leakage of urine.

The 24 hour U-bags are fitted with a drainage tube. This was trimmed to the preferred length and anchored by means of a rubber stopper into a collection pot. To aid the free flow of urine into the collection pot a second hole was made in the rubber stopper. Leakage from this second hole was limited by means of a short piece of drainage tubing placed in this hole.

The infant was then clothed as usual for the evening e.g. nappy, vest and babygro. The U-bag remained attached to the infant throughout the night until the following morning unless the nappy became soiled necessitating the removal of the bag. If this was the case the infant was cleaned and a second U-bag attached. The parents of the infants were much better at collecting urine from their own child than any one of the health visitors or myself.

Storage of sample:

Samples were labelled with the name of the infant, date and time of collection before transportation to the laboratory. The urine samples reached the laboratory within 2 hours of the end of the morning urine collection. Only urines that were not contaminated with fecal matter were allocated a unique number before being placed in a 4 °C store, all others were discarded. Urines were kept in this manner until analysed for creatinine and cortisol which usually took place within 7 days of collection. Following estimation of creatinine and cortisol the samples were transferred to a -20 °C store where they were stored until analysis of growth hormone was carried out. This method of storage minimised the number of freeze-thaw cycles occurring before analysis for growth hormone. No preservatives were added to the urine samples throughout collection or storage.
CHEMICAL ANALYSIS OF URINES.

ESTIMATION OF CREATININE

Creatinine was estimated by a Jaffé rate reaction method using a centrifugal analyser, Cobas Bio (Roche Diagnostic Products), using commercially available reagents (picric acid and sodium hydroxide). The controls used were Biorad Lyphocheck urine controls I.I and I.II and the calibration standard used was IL 508 urine standard.

Procedure:

1. Any turbid urines were centrifuged to remove particulate matter.

2. The working reagent was freshly made up for each run as instructed by the manufacturers.

   800 ml of the 2.5 mol.L⁻¹ sodium hydroxide (alkaline reagent) were added to 10 ml of 13 mmol.L⁻¹ picric acid reagent.

3. 0.5 ml of each urine were pipetted neat in the Cobas Bio sample vials as directed by the rotor run sheet.

4. 350 μl of the reagent were added into the primary wells of the reagent boat and 100 μl of the standard were added to the three small wells of the reagent boat.

5. The reagent boat and samples were placed into the spectrophotometer with the parameters set as below.
Parameters:

1. units = mmol.l⁻¹
2. calibration factor = 0
3. standard 1 concentration = 17.7 mmol.l⁻¹
4. standard 2 concentration = 17.7 mmol.l⁻¹
5. standard 3 concentration = 17.7 mmol.l⁻¹
6. limit = 35 mmol.l⁻¹
7. temperature = 25°C
8. type of analysis 4 = initial rate determination
9. wavelength = 500 nm (absorption)
10. sample volume = 5 ml
11. dilution volume = 30 ml
12. reagent volume = 350 ml
13. incubation time = 10 seconds
14. starting reagent volume = 0 ml
15. time of first reading = 0.5 seconds
16. time of interval = 10 seconds
17. number of readings = 3
18. blanking mode = 1
19. printout mode = 1

The results were printed out as concentrations of creatinine in mmol.l⁻¹.

**ESTIMATION OF URINARY CORTISOL.**

Cortisol was estimated using Coat-a-count (Diagnostic Products Corporation) extractive radioimmunoassay modified to allow the estimation of the small amounts of cortisol found in some paediatric samples. This assay consists of antibody coated tubes normally used for the assessment of cortisol excess in adult patients. The preparation of samples and the radioimmunoassay procedure was similar to that specified by the manufacturer varying only in the addition of 200 µl of dichloromethane extract instead of the specified 50 µl of dichloromethane extract as used in the adult, into the assay tubes.
Extraction of urine samples.

1. Any samples that were cloudy or where a precipitate had formed were first centrifuged.

2. 0.5 ml of each urine were placed in a polypropylene tube to which 1 ml of dichloromethane was added. The tubes were securely capped and gently mixed on an inversion mixer for 5-10 minutes.

3. Each sample was then rested until the two liquid phases separated.

4. 200 µl of the lower dichloromethane phase were then placed into a labelled antibody coated tube as set out by the run sheet and evaporated to complete dryness using a gentle stream of nitrogen at room temperature. Each sample was analysed in duplicate sample volume allowing.

Radioimmunoassay procedure.

Tubes labelled 1 and 2 were plain polypropylene tubes (dimensions 12x75 mm) which were not coated with cortisol antibodies. These tubes were the total count (TC) tubes to which only 1 mL of $^{125}$I labelled cortisol was added.

The remaining tubes in the run were either part of the standard curve, non-specific binding (NSB) tubes, controls or samples. These tubes were antibody coated tubes.

1. 25 µl of the zero calibrator A was placed into each of the NSB tubes and into each of the antibody tubes containing dried urine samples.

2. A further 25 µl of calibrator A through to F were placed into the appropriate tubes as indicated by the run sheet.

3. 1 ml of $^{125}$I labelled cortisol was added to every tube and vortex mixed.

4. The samples were covered and incubated at room temperature overnight. The TC tubes were capped and kept separately until counting.

5. All the tubes were decanted thoroughly and allowed to drain for 2-3 minutes before removing all residual droplets by striking the tubes sharply on absorbent paper.
6. Each tube was then counted for 1 minute using a gamma counter (Gamma Master, Wallac).

**Typical assay run sheet.**

- Tubes 1, 2 = TC
- Tubes 3, 4 = NSB
- Tubes 5, 6 = A, 0 nmol.l⁻¹
- Tubes 7, 8 = B, 27.6 nmol.l⁻¹
- Tubes 9, 10 = C, 138 nmol.l⁻¹
- Tubes 11, 12 = D, 276 nmol.l⁻¹
- Tubes 13, 14 = E, 552 nmol.l⁻¹
- Tubes 15, 16 = F, 1380 nmol.l⁻¹
- Tubes 17, 18 = CONTROLS
- Tubes 19, 20 = SAMPLE 1
- Tubes 21, 22 = SAMPLE 2 .... etc.

**Calculation of results.**

The counts per minute for each of the first 16 tubes for each run were used to create a standard curve (figure 2.2). The concentration of cortisol in each of the unknown samples was calculated by estimating the values from the standard curve, and dividing by four to account for the addition of 200 µl instead of 50 µl of dichloromethane extract.

**Validation of modification.**

An investigation was undertaken to examine the effect, if any, the increase in volume of extraction medium (ie dichloromethane) may have on the assay.

The urine samples were extracted as normal before following the standard assay procedure. 50 µl, 100 µl, 200 µl and 400 µl of dichloromethane were placed into assay tubes and the assay procedure followed as normal. The results were plotted and examined for a deviation from the expected values (figure 2.3).

Figure 2.3 below shows the amount of cortisol recovered using each of the volumes above. An almost linear increment in the amount of cortisol recovered was obtained with the increase in dichloromethane volume. This does not deviate significantly from the expected values and therefore the increase in volume did not affect the assay detrimentally. By using 200 µl of the
**Figure 2.2:**
Typical standard curve used to calculate the concentration of cortisol in each sample.

**Figure 2.3:**
Recovery of cortisol from samples containing increased concentrations of dichloromethane.
extraction medium instead of 50 µl the sensitivity of the assay has effectively increased four fold. After correction for this volumetric change (i.e. division by 4) values obtained by the use of 200 µl can be compared with those obtained by the use of 50 µl.

ESTIMATION OF URINARY GROWTH HORMONE.

Growth hormone was estimated using an immunoradiometric assay (IRMA) designed for use on serum (IDS). Before this could be used to measure urinary growth hormone the assay was modified. Chapters 3 and 4 present the method of modification and the validation of the newly modified assay. The standard protocol used for the measurement of growth hormone in serum was altered very little and full details of the final procedure are present in chapter 3.
CHAPTER 4

VALIDATION OF THE MODIFIED GROWTH HORMONE ASSAY
MODIFICATION OF GROWTH HORMONE ASSAY FOR USE WITH PAEDIATRIC URINE

The aim was to modify the available radioimmunoassay for measurement of serum growth hormone (IDS) for use with urine samples collected from infants in the 2-6 month age range. Adult urine has an osmolality of 50-140 mOsm/Kg and the major constituents are urea (110-387 mmol.l\(^{-1}\)), phosphate (11-32 mmol.l\(^{-1}\)), sodium (3.3 mmol.l\(^{-1}\)), potassium (23.3-60 mmol.l\(^{-1}\)) and chloride (about 67 mmol.l\(^{-1}\)). The concentrations of these constituents are different and more variable than those of plasma even in the paediatric population. Therefore in the process of modification the effect of these urinary constituents on the binding of growth hormone to the assay antibody had to be examined. In addition the urinary growth hormone concentrations are estimated to be only 5% of those of plasma and so if the assay is to be usable for the measurement of this level of growth hormone it must be sufficiently sensitive at the lower end of the standard curve to allow accurate measurement. The modification of the assay was completed over several weeks. After the adjustments had been made to the assay reagents and the protocol, the full precision profile of the assay could not be calculated until many samples and quality controls had been repeated.

MATERIALS

**Bovine serum albumin**: Sera-Laboratories, Crawley Down, Sussex.

**Sodium hydrogen orthophosphate** (\(\text{Na}_2\text{HPO}_4\) anhydrous):- Fisons Scientific Equipment, Bishops Meadow Road, Loughborough.

**Sodium dihydrogen orthophosphate** (\(\text{NaH}_2\text{PO}_4\cdot2\text{H}_2\text{O}\)): Fisons Scientific Equipment, Bishops Meadow Road, Loughborough.

**Urea**: BDH, Poole, England

**Sodium chloride** (\(\text{NaCl}\)): - Fisons Scientific Equipment, Bishops Meadow Road, Loughborough.

**Growth hormone kit** (serum):- IDS plc, Bolden Business Park, Boldon, Tyne and Wear.

**Pool urine 140892**: waking male urine collected from presumed healthy volunteers.

**Pool urine 021293**: waking male urine collected from presumed healthy volunteers collected on a different occasion to pool 140892.
EXPERIMENTAL REAGENTS

1. Bovine serum albumin (BSA) solution A 5% solution of BSA was made up by dissolving 2.5 g of freeze dried BSA in 50 ml of distilled water. Care was taken to avoid too much foaming of the protein solution as denaturing of the protein can occur.

2. High phosphate buffer (1.25 mol.l⁻¹, pH 8.1). 2.23 g of NaH₂PO₄ and 15.72 g of Na₂HPO₄ in 100 ml of distilled water. The phosphate tended to precipitate out of solution and could only be redissolved by sonication.

3. Low phosphate buffer (0.25 mol.l⁻¹, pH 8.1). This was a 1:5 dilution of the high phosphate solution described above. 20 ml of high phosphate solution were made up to 100 ml with distilled water and the solution thoroughly mixed.

4. High urea solution (1.25 mol.l⁻¹) 7.5 g of urea were dissolved in a volumetric flask in 100 ml of distilled water and mixed.

5. Low urea solution (0.625 mol.l⁻¹) This was a 1:10 dilution of the high urea solution. 10 ml of the high urea solution were made up to 100 ml in a volumetric flask with distilled water.

6. Saline 1 (0.16 mol·l⁻¹) 1:10 dilution of Saline 4. 10 ml of Saline 4 were made up to 100 ml with distilled water.

7. Saline 2 (0.31 mol·l⁻¹) 1:5 dilution of Saline 4. 20 ml of Saline 4 were made up to 100 ml with distilled water.

8. Saline 3 (0.61 mol·l⁻¹) 2:5 dilution of Saline 4. 40 ml of Saline 4 were pipetted into a volumetric flask and made up to 100 ml with distilled water.

9. Saline 4 (1.56 mol·l⁻¹). 18.281 g of NaCl were dissolved in 200 ml of distilled water.

10. Growth hormone spike 1 (8.6 μU.l⁻¹) 1:5 dilution of spike 2. 2 ml of phosphate buffer and 500 μl of spike 2.

11. Growth hormone spike 2 (43 μU.l⁻¹) 1:2 dilution of spike 3. 1 ml of phosphate buffer and 1 ml of spike 3.
12. **Growth hormone spike 3** (86 μU.l⁻¹) - 1:5 dilution of spike 4. 2 ml of phosphate buffer and 500 μl of spike 4.

13. **Growth hormone spike 4** (430 μU.l⁻¹) - 1:11 dilution of standard G (4725 μU.l⁻¹). 1.5 ml of phosphate buffer and 150 μl of standard G provided by the manufacturers.

14. **Modified assay buffer 1** (0.25 mol.l⁻¹) - 1:2 dilution of modified buffer 2. 10 ml of modified buffer 2 were made up to 20 ml using assay buffer.

15. **Modified assay buffer 2** (0.5 mol.l⁻¹) 0.6 g of sodium chloride in 20 ml of assay buffer.

**FINAL ASSAY REAGENTS**

1. **Phosphate buffer** (0.5 mol.l⁻¹, pH 8.1). 8.892 g of NaH₂PO₄ and 62.888 g of Na₂HPO₄ dissolved in 1 l of water as described for the making of the high phosphate solution reagent 2 (Experimental Reagents).

2. **Assay buffer** 5 g of BSA were dissolved in 100 ml of the phosphate buffer. The BSA was dissolved in phosphate buffer as for the making of the BSA solution reagent 1 experimental reagents replacing the distilled water with the phosphate buffer.

3. **Extra Growth hormone standard B/4** (7.5 μU.l⁻¹) - 1:20 dilution of standard D. 50 μl of standard D and 950 ml of phosphate buffer.

4. **Extra Growth hormone standard B/2** (15.0 μU.l⁻¹) - 1:10 dilution of standard D. 100 μl of standard D and 900 ml of phosphate buffer.

5. **Quality control 1. Growth hormone concentration** 0.151 μU.l⁻¹ 200 μl of standard G were added to 250 ml of pool urine.

6. **Quality control 2. Growth hormone concentration** 0.076 μU.l⁻¹ 100 μl of standard G were added to 250 ml of pool urine.

7. **Quality control 3. Growth hormone concentration** 22 μU.l⁻¹, 30 μl of standard G were added to 250 ml of pool urine.

8. **Wash solution.** The wash solution provided in the assay kit was made up to 1 l with distilled water as described by the manufacturer.
STANDARD PROCEDURE

The assay protocol set out below varied little from that for the serum assay. An additional immune extraction (step 5) and wash step (step 6) were used. Further changes to this protocol were not needed.

1. Any urine samples, pools and QCs were removed from the -20 °C store and allow to thaw to room temperature.

2. An assay run-sheet identifying the sample in each tube was created. Each sample was assayed in duplicate unless the sample size prohibited.

3. The required reagents were made up.

4. Following the run-sheet the required reagents were placed into each tube. The standards were added after the 2 ml volume of urine, or model and before the addition of the assay buffer.

5. The tubes were securely capped and placed on a drum mixer set to invert the tubes once every minute during the overnight incubation at room temperature.

6. The caps were removed and the contents decanted. The tubes were allowed to drain for several minutes before removing residual droplets by striking sharply on absorbent paper.

7. 4 ml of the wash solution was pipetted into each tube.

8. The contents were decanted as in step 6.

9. 200 µl of ¹²⁵I labelled anti-growth hormone antibody were added to each tube and vortex mix. The two total count tubes were capped and put to one side until counting.

10. The tubes were incubated for 2 hours at room temperature.

11. The contents were decanted as in step 6.
12. Each tube was washed as in step 7.

13. The contents were decanted as in step 6 for the final time.

14. Each tube was counted for 1 minute using a gamma counter.

15. The standard curve was calculated using the counts per minute.

A. The effect of phosphate and urea on antibody binding.

The effect of phosphate and urea on the standard curve of the assay was investigated by using five model systems (A-E) designed to look at a combination of two different concentrations of phosphate and urea. A serum curve was set up as described in the assay insert for comparison. Each of these standard curves are described in tables 3.1-3.3. Table 3.1. shows the additions made for a typical serum standard curve; Table 3.2 the additions made for a typical urine model (urine) standard curve with concentrations of growth hormone in the assay tube and the equivalent in 2ml of urine model. Table 3.3 describes the additions necessary to create model systems A-E.

Reagents used:-

   Experimental reagents: 1, 2, 3, 4 and 5
   Final assay reagents: 8.

Protocol: Standard assay procedure as described.
TABLE 3.1: Serum standard curve showing growth hormone concentration in the tubes.

<table>
<thead>
<tr>
<th>Tube number</th>
<th>Tube contents</th>
<th>Tube concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A &amp; B</td>
<td>200μl 125 anti-growth hormone antibody</td>
<td>Total counts</td>
</tr>
<tr>
<td>1 &amp; 2</td>
<td>50μl Growth hormone standard A (0mU.l(^{-1}))</td>
<td>0</td>
</tr>
<tr>
<td>3 &amp; 4</td>
<td>50μl Growth hormone standard B (1.3mU.l(^{-1}))</td>
<td>32.5μU.l(^{-1})</td>
</tr>
<tr>
<td>5 &amp; 6</td>
<td>50μl Growth hormone standard C (3.8mU.l(^{-1}))</td>
<td>95μU.l(^{-1})</td>
</tr>
<tr>
<td>7 &amp; 8</td>
<td>50μl Growth hormone standard D (6.0mU.l(^{-1}))</td>
<td>150μU.l(^{-1})</td>
</tr>
<tr>
<td>9 &amp; 10</td>
<td>50μl Growth hormone standard E (17.7mU.l(^{-1}))</td>
<td>442.5μU.l(^{-1})</td>
</tr>
<tr>
<td>11 &amp; 12</td>
<td>50μl Growth hormone standard F (63.0mU.l(^{-1}))</td>
<td>1575μU.l(^{-1})</td>
</tr>
<tr>
<td>13 &amp; 14</td>
<td>50μl Growth hormone standard G (189.0mU.l(^{-1}))</td>
<td>4725μU.l(^{-1})</td>
</tr>
</tbody>
</table>
TABLE 3.2: Urine model standard curve showing growth hormone concentration in tube and equivalent in urine.

<table>
<thead>
<tr>
<th>Tube number</th>
<th>Tube contents</th>
<th>Concentration in tube (vol = 2.5ml)</th>
<th>Equivalent concentration in urine (vol = 2ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A &amp; B</td>
<td>200ml $^{125}$ anti growth hormone antibody</td>
<td>Total counts</td>
<td></td>
</tr>
<tr>
<td>1 &amp; 2</td>
<td>2ml of model system A, B, C, D, or E 50μl Growth hormone standard A (0μU.l$^{-1}$)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 &amp; 4</td>
<td>2ml of model system A, B, C, D, or E 50μl Growth hormone standard B/4 (0.3μU.l$^{-1}$)</td>
<td>6x10$^{-3}$ μU.l$^{-1}$</td>
<td>7.5x10$^{-3}$ μU.l$^{-1}$</td>
</tr>
<tr>
<td>5 &amp; 6</td>
<td>2ml of model system A, B, C, D, or E 50μl Growth hormone standard B/2 (0.6μU.l$^{-1}$)</td>
<td>12 μU.l$^{-1}$</td>
<td>15 μU.l$^{-1}$</td>
</tr>
<tr>
<td>7 &amp; 8</td>
<td>2ml of model system A, B, C, D, or E 50μl Growth hormone standard B (1.3μU.l$^{-1}$)</td>
<td>26 μU.l$^{-1}$</td>
<td>32.5 μU.l$^{-1}$</td>
</tr>
<tr>
<td>9 &amp; 10</td>
<td>2ml of model system A, B, C, D, or E 50μl Growth hormone standard C (3.8μU.l$^{-1}$)</td>
<td>76 μU.l$^{-1}$</td>
<td>95 μU.l$^{-1}$</td>
</tr>
<tr>
<td>11 &amp; 12</td>
<td>2ml of model system A, B, C, D, or E 50μl Growth hormone standard D (6.0μU.l$^{-1}$)</td>
<td>120 μU.l$^{-1}$</td>
<td>150 μU.l$^{-1}$</td>
</tr>
<tr>
<td>13 &amp; 14</td>
<td>2ml of model system A, B, C, D, or E 50μl Growth hormone standard E (17.7μU.l$^{-1}$)</td>
<td>354 μU.l$^{-1}$</td>
<td>443 μU.l$^{-1}$</td>
</tr>
<tr>
<td>15 &amp; 16</td>
<td>2ml of model system A, B, C, D, or E 50μl Growth hormone standard F (63.0μU.l$^{-1}$)</td>
<td>1260 μU.l$^{-1}$</td>
<td>1575 μU.l$^{-1}$</td>
</tr>
<tr>
<td>17 &amp; 18</td>
<td>2ml of model system A, B, C, D, or E 50μl Growth hormone standard G (189.0μU.l$^{-1}$)</td>
<td>3780 μU.l$^{-1}$</td>
<td>4725 μU.l$^{-1}$</td>
</tr>
</tbody>
</table>

* Standards B/4 and B/2 were omitted in parts A and B of the modification.
5 Standard G was only included in parts AS and B of the modification and not the final assay.
TABLE 3.3: Model systems A-E for urinary phosphate and urea showing the concentrations of phosphate and urea in the tube and equivalent in urine.

<table>
<thead>
<tr>
<th>Standard curve</th>
<th>Tube contents</th>
<th>Concentration in tube (vol = 2.5ml)</th>
<th>Equivalent concentration in urine (vol = 2ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1ml distilled water, 1ml low phosphate solution, 500μl BSA solution, 50μl standard A, B, C, D, E, F or G</td>
<td>0.1mol.l⁻¹</td>
<td>0.13mol.l⁻¹</td>
</tr>
<tr>
<td>B</td>
<td>1ml low urea solution, 1ml low phosphate solution, 500μl BSA solution, 50μl standard A, B, C, D, E, F or G</td>
<td>0.05mol.l⁻¹, 0.1mol.l⁻¹</td>
<td>0.63mol.l⁻¹, 0.13mol.l⁻¹</td>
</tr>
<tr>
<td>C</td>
<td>1ml low urea solution, 1ml high phosphate solution, 500μl BSA solution, 50μl standard A, B, C, D, E, F or G</td>
<td>0.05mol.l⁻¹, 0.5mol.l⁻¹</td>
<td>0.063mol.l⁻¹, 0.63mol.l⁻¹</td>
</tr>
<tr>
<td>D</td>
<td>1ml high urea solution, 1ml low phosphate solution, 500μl BSA solution, 50μl standard A, B, C, D, E, F or G</td>
<td>0.5mol.l⁻¹, 0.1mol.l⁻¹</td>
<td>0.63mol.l⁻¹, 0.13mol.l⁻¹</td>
</tr>
<tr>
<td>E</td>
<td>1ml high urea solution, 1ml high phosphate solution, 500μl BSA solution, 50μl standard A, B, C, D, E, F or G</td>
<td>0.5mol.l⁻¹, 0.5mol.l⁻¹</td>
<td>0.63mol.l⁻¹, 0.63mol.l⁻¹</td>
</tr>
</tbody>
</table>
B. The effect of sodium chloride on antibody binding

Using a phosphate buffer (0.1 mol.l\(^{-1}\), pH 8.1) in the assay tubes, variation of sample phosphate and pH were both stabilized allowing the investigation of sodium chloride concentrations on the standard curve of the assay.

Four standard curves were created each modelling different concentrations of sodium chloride (A-D) as described in table 3.4. These standard curves were then compared with an aqueous standard curve identical to that described in table 3.2 replacing the model system with 2ml of distilled water and the BSA solution (experimental reagent) with Assay buffer (final assay reagent).

Reagents needed:-

Experimental reagents: 6, 7, 8, 9
Final assay reagents: 1, 2, 8.


**TABLE 3.4: Model systems A-D for sodium chloride showing concentrations of saline in tube and equivalent in urine.**

<table>
<thead>
<tr>
<th>Standard curve</th>
<th>Tube contents</th>
<th>Concentration in tube (vol =2.5ml)</th>
<th>Equivalent concentration in urine (vol =2ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2ml saline 1</td>
<td>0.125mol.l(^{-1})</td>
<td>0.16mol.l(^{-1})</td>
</tr>
<tr>
<td></td>
<td>500(\mu)l Assay buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50(\mu)l standard A, B, C, D, E, F or G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2ml saline 2</td>
<td>0.25mol.l(^{-1})</td>
<td>0.31mol.l(^{-1})</td>
</tr>
<tr>
<td></td>
<td>500(\mu)l Assay buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50(\mu)l standard A, B, C, D, E, F or G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2ml saline 3</td>
<td>0.5mol.l(^{-1})</td>
<td>0.61mol.l(^{-1})</td>
</tr>
<tr>
<td></td>
<td>500(\mu)l Assay buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50(\mu)l standard A, B, C, D, E, F or G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2ml saline 4</td>
<td>1.25mol.l(^{-1})</td>
<td>1.56mol.l(^{-1})</td>
</tr>
<tr>
<td></td>
<td>500(\mu)l Assay buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50(\mu)l standard A, B, C, D, E, F or G</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C. Recovery of growth hormone from urine.

Urine contains many constituents other than those investigated in the model systems, which although minor may have some unforeseen effect on the assay. Adult male urine collected during waking hours from healthy volunteers contains little if any growth hormone and is well below the detection limit of this assay. Therefore, by adding known amounts of growth hormone to adult male urine the recovery of growth hormone can be calculated. The recovery of growth hormone from paediatric urines may be different to that of adult male urine. However, in order to conserve the paediatric samples most of the initial recovery investigations were carried out on adult pool urine 140892. Only when recovery of growth hormone from adult urine was seen to be adequate did we examine a small number of paediatric samples.

Recovery of growth hormone was measured at four different levels in both the adult urine and paediatric urines using an aqueous standard curve for calculation of concentrations.

Reagents needed: -
Experimental reagents: 10, 11, 12, 13, 14, 15
Final Assay reagents: 1, 2, 3, 4, 8

Protocol:
Adult male pool urine 140892 was diluted using distilled water to give:
A 1:8 dilution - 3 ml of urine made up to 24 ml
B 1:4 dilution - 6 ml of urine made up to 24 ml
C 1:2 dilution - 15 ml of urine made up to 30 ml
D 1:1 dilution - no addition of water.

Using pool 140892 dilutions described above the standard protocol was followed substituting modified assay buffers 1 and 2 to model further urine concentrations as described in Table 3.5. Each of these models and a series of 6 paediatric urines of low, medium and high creatinine concentrations (different urine concentrations) were used to assess recovery of growth hormone at each of the spike levels 1-4 as described in Table 3.6.
TABLE 3.5: Model systems for investigation of recovery of growth hormone from urine showing the concentrations of chlorine in the tube and urine achieved using the modified buffers.

<table>
<thead>
<tr>
<th>Model system</th>
<th>Tube contents</th>
<th>Concentration in tube (vol = 2.5ml)</th>
<th>Equivalent concentration in urine (vol = 2ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2ml urine pool 140892 1:8 dilution</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500μl Assay buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50μl growth hormone spike 1, 2, 3 or 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2ml urine pool 140892 1:4 dilution</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500μl Assay buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50μl growth hormone spike 1, 2, 3 or 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2ml urine pool 140892 1:2 dilution</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500μl Assay buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50μl growth hormone spike 1, 2, 3 or 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2ml urine pool 140892 1:1 dilution</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500μl Assay buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50μl growth hormone spike 1, 2, 3 or 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>2ml urine pool 140892 1:1 dilution</td>
<td>additional NaCl 0.1mol.l⁻¹</td>
<td>0.125mol.l⁻¹</td>
</tr>
<tr>
<td></td>
<td>500μl Modified Assay buffer 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50μl growth hormone spike 1, 2, 3 or 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>2ml urine pool 140892 1:1 dilution</td>
<td>additional NaCl 0.05mol.l⁻¹</td>
<td>0.065mol.l⁻¹</td>
</tr>
<tr>
<td></td>
<td>500μl Modified Assay buffer 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50μl growth hormone spike 1, 2, 3 or 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>2ml paediatric urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500μl Assay buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50μl growth hormone spike 1, 2, 3 or 4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 3.6: Growth hormone concentrations at each of the spike levels 1-4.

<table>
<thead>
<tr>
<th>Growth hormone spike</th>
<th>Tube contents</th>
<th>Concentration in tube (vol=2.5ml)</th>
<th>Equivalent concentration in urine (vol=2ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2ml Model system A, B, C, D, E, F or paediatric urine. 500µl Assay buffer 50µl spike 1 (8.6mU.l⁻¹)</td>
<td>0.172U.l⁻¹</td>
<td>0.25µU.l⁻¹</td>
</tr>
<tr>
<td>2</td>
<td>2ml Model system A, B, C, D, E, F or paediatric urine. 500µl Assay buffer 50µl spike 2 (43mU.l⁻¹)</td>
<td>0.86µU.l⁻¹</td>
<td>1.075µU.l⁻¹</td>
</tr>
<tr>
<td>3</td>
<td>2ml Model system A, B, C, D, E, F or paediatric urine. 500µl Assay buffer 50µl spike 3 (86mU.l⁻¹)</td>
<td>1.72µU.l⁻¹</td>
<td>2.10µU.l⁻¹</td>
</tr>
<tr>
<td>4</td>
<td>2ml Model system A, B, C, D, E, F or paediatric urine. 500µl Assay buffer 50µl spike 4 (430mU.l⁻¹)</td>
<td>8.6µU.l⁻¹</td>
<td>10.75µU.l⁻¹</td>
</tr>
</tbody>
</table>

D. The effect of dialysis on the recovery of growth hormone from urine.

The recovery of growth hormone from urine was sufficient for the assay to be used in the measurement of growth hormone. However, there have been few attempts to measure growth hormone that have not included dialysis of the samples (for review of current common methodology, Hourd & Edwards, 1994). To complete our investigation we examined the effect of dialysis on the recovery of growth hormone and the accuracy of measurement in both adult male pool 140892 and a series of paediatric urines. A comparison was made between the use of an aqueous standard curve and pool urine 021293 standard curve for calculation of growth hormone concentration in the samples.
Dialysis:

1. Four 10 ml aliquotes of each urine to be assayed were measured out into labelled pots.
2. 200 μl of growth hormone spike 1, 2, 3 or 4 were added to each of the urines.
3. 10 cm lengths of dialysis tubing (diameter 1 cm) were cut and washed in warm distilled water. A tight knot was tied in one end of each of the lengths to form sacks.
4. 5 ml of each of spiked sample were placed into a dialysis sack and the open end secured.
5. The closed sacks were tied to an identification tag before anchoring in a large beaker under running water for dialysis overnight. The remaining 5 ml of each spiked sample were undialysed controls, kept overnight in a 4°C store.
6. Each of the dialysis sacks were carefully emptied into a pre-weighed container which was then labelled with the spike number and the weight of the sample recovered from the sack. Thus allowing the estimation of the volumetric changes that occurred during dialysis.

Reagents:

- Experimental reagents: 10, 11, 12, 13
- Final assay reagents 1, 2, 3, 4, 5, 8

Protocol: After dialysis the standard assay protocol was followed.

Pool 140892 was repeated in duplicate five times (eg 10 tubes with spike level 1 after dialysis, 10 tubes with spike level 1 without dialysis) at each of the four spike levels to evaluate the reproducibility of the effect of dialysis (see table 3.7). There was only sufficient paediatric urine to duplicate each of the first two spike levels in 1 of the paediatric urines, the first three spike levels in two and all four of the spike levels in a further 2. Both pool 140892 and each of the paediatric urines were also assayed without any growth hormone spike. An aqueous standard curve and a urine standard curve were set up as described in table 2 substituting 2ml of male pool urine 021293 for distilled water.
TABLE 3.7: Concentrations of growth hormone in the spikes used for dialysis.

<table>
<thead>
<tr>
<th>Growth hormone spike</th>
<th>Tube contents</th>
<th>Concentration before dialysis (vol = 5ml)</th>
<th>Concentration in tube (vol = 2.5ml)</th>
<th>Equivalent concentration in urine (vol = 2ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10ml urine pool 140892 or paediatric sample</td>
<td>0.17μU.L⁻¹</td>
<td>0.14μU.L⁻¹</td>
<td>0.17μU.L⁻¹</td>
</tr>
<tr>
<td></td>
<td>200μl spike 1 (8.6mU.L⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10ml urine pool 140892 or paediatric sample</td>
<td>0.84μU.L⁻¹</td>
<td>0.67μU.L⁻¹</td>
<td>0.84μU.L⁻¹</td>
</tr>
<tr>
<td></td>
<td>200μl spike 2 (43mU.L⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10ml urine pool 140892 or paediatric sample</td>
<td>1.68μU.L⁻¹</td>
<td>1.34μU.L⁻¹</td>
<td>1.68μU.L⁻¹</td>
</tr>
<tr>
<td></td>
<td>200μl spike 3 (86mU.L⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10ml urine pool 140892 or paediatric sample</td>
<td>8.4μU.L⁻¹</td>
<td>6.72μU.L⁻¹</td>
<td>8.4μU.L⁻¹</td>
</tr>
<tr>
<td></td>
<td>200μl spike 4 (430mU.L⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E. The precision profile of the modified assay.

This part of the assessment investigated the reproducibility of the assay both within an assay run and between several assay runs. In order to obtain adequate data to thoroughly investigate this, many paediatric samples were repeated in duplicate and additional quality controls were placed in duplicate at the beginning and end of every assay run.
CHAPTER 3

MODIFICATION OF THE GROWTH HORMONE ASSAY
VALIDATION OF MODIFIED GROWTH HORMONE ASSAY

The assessment of the pituitary secretion of GH is most commonly carried out on children to evaluate growth retardation. Currently, there are no commonly used methods of measuring urinary GH. Existing urinary assay kits for GH are expensive and inappropriate for research purposes as measurement of plasma GH is more commonly used. However, the collection of plasma is invasive and difficult to approve ethically when studying healthy infants. Measurement of urinary GH would be a more acceptable way of studying this hormone in infants.

The main problem of measuring GH is that the concentrations found in urine are very low and estimated to be about 0.03 % or less than those found in plasma. As part of this study a plasma GH assay was adapted to measure urinary GH in the infant population. The assay adapted had been found to be highly sensitive at the lower end of the standard curve when used with serum and therefore had the potential for use with urine. During the adaptation of the assay several investigations were carried out to ensure that the constituents of urine did not detrimentally affect the assay. If the concentrations of phosphate, urea, sodium chloride or an unspecified constituent of urine altered the binding of GH by the assay antibody it may have rendered the assay useless for use with urine.

The following chapter presents the results of several investigations carried out to validate the serum GH assay after adaptation for use with urine.

A comparison of the typical standard curves obtained for serum, an aqueous based and an urine based system is shown in figure 4.1. The antibody binding of GH is greater in both the aqueous based and urine based standard curves.

The aqueous based standard curve consists of a phosphate buffer of tubular concentration 0.1 mol.L⁻¹ for stabilisation of urinary pH.
Figure 4.1a:
Comparison of the typical standard curves obtained when using serum, aqueous and urine matrices.

Figure 4.1b:
Comparison of typical standard curves obtained when using serum, aqueous and urine matrices. (Expanded at origin)
The urine based standard curve was identical to the aqueous standard curve except that adult male pool urine was used as the dilutant rather than distilled water.

A: The effect of phosphate, urea and sodium chloride on antibody binding of GH.

The effect of these three major constituents of urine was assessed to establish whether the serum assay for GH could be successfully modified for use on urine.

The aqueous based standard curve was used as the baseline for this series of experiments.

The reduction in baseline binding with increasing phosphate concentration is illustrated in figure 4.2. At phosphate concentrations of 0.5 mol.l⁻¹ the antibody binding was reduced by as much as 42.8%. However, within the adult range of phosphate (0.011-0.032 mol.l⁻¹) and paediatric range (up to 0.0144 mol.l⁻¹) the reduction in antibody binding is less than 5%.

Figure 4.3 shows a linear graph with a reduction in binding at all concentrations never greater than 24.2% at all levels of urea investigated. Within the adult and childhood range of urinary urea concentrations the deviation from 100% binding is insignificant (0.11-0.387 mol.l⁻¹).

The effect of sodium chloride on antibody binding is less than the effect of phosphate (figure 4.4). In adult urine the chloride concentration of urine is about 0.067 mol.l⁻¹ and in children about 0.15 mol.l⁻¹. This investigation covers a range of chloride concentrations between 0 and 1.25 mol.l⁻¹. The general effect of increasing sodium chloride concentration was to reduce the antibody binding. Below 0.5 mol.l⁻¹ sodium chloride the reduction in antibody binding is less than 23.6%. The variation in effect of sodium chloride particularly at low concentrations of GH is probably due to technical error e.g. inaccurate pipetting rather than an actual effect of sodium chloride.
Figure 4.2: The effect of phosphate on antibody binding of growth hormone at concentrations of 0.5 mol/L and 0.1 mol/L of urea.

Key for figures 4.2 and 4.3:
- 25 M/1 growth hormone
- 75 M/1 growth hormone
- 125 M/1 growth hormone
- 750 M/1 growth hormone

Figure 4.3: The effect of urea on the antibody binding of growth hormone.

Figure 4.4: The effect of sodium chloride (NaCl) on the antibody binding of growth hormone.

Key for figure 4.4:
- 0.05 M/1 growth hormone
- 0.5 M/1 growth hormone
- 1.0 M/1 growth hormone
- 4.0 M/1 growth hormone
- 12.5 M/1 growth hormone
B: Recovery of GH from urine.

Having established that the effect of phosphate, urea and sodium chloride were minimal within the expected range of values in urine the recovery of GH from urine was investigated.

These experiments were carried out using an aqueous baseline.

Recovery of GH from adult urine at different dilutions is illustrated in figure 4.5. The graph shows a linear increase in the amount of GH recovered with an increase in amount of GH added to the urine. There is no significant difference in the recovery of GH from any of the dilutions examined. It is only the addition of 0.1 mol.L\(^{-1}\) of sodium chloride that reduces antibody binding at higher GH concentrations.

By presenting the data to show the amount of GH added against the amount of GH recovered from urine as in figure 4.6 it can be seen that at all dilutions the recovery is linear indicating that there is no urine matrix interaction with the assay. The regression coefficient for recovery of GH from every dilution of adult pool is greater than 0.9. The regression line crosses the y-axis at about 20 µU.L\(^{-1}\). This is probably due to the recovery of GH at higher levels biasing the regression line fit.

Antibody binding of GH is greater in urine than in an aqueous solution as shown in figure 4.7.

The above recovery experiments carried out using male pool urine were repeated on a small number of paediatric urines to ensure that paediatric urine was no different to adult urine.

Figure 4.8 shows a linear relationship existing between the amount of GH added to each of the urines and the amount recovered. Correcting for the amount of GH present in the sample prior to spiking shows that the percentage recovery is reduced by less than 30 % at GH concentrations greater than 43 µU.L\(^{-1}\) (figure 4.9).
Figure 4.8: The recovery of growth hormone from pediatric urines with low, medium and high creatinine contents.

Figure 4.9: Percentage recovery of growth hormone from pediatric urines after correction for individual variation in growth hormone content.

Figure 4.10: The recovery of growth hormone from 5 pediatric urines after 3-fold dilution of urines.
Finally using data from 5 paediatric urines diluted 2-fold using adult male pool as the dilutant it can be seen that there is a linear increase in the amount of GH recovered from diluted urine with increasing amounts of GH present in the undiluted sample (figure 4.10). The regression coefficient for figure 4.10 is 0.44 indicating a close match between the expected and actual values of GH obtained in the 2-fold dilutions. As in figure 4.6 the intersection with the y-axis is not zero.

The results from paediatric urine confirm that GH recovery corresponds well with the level of GH present in both adult and paediatric urines. On the basis of these experiments the assay protocol was established. The standard procedure was as set out in the methodology using a urine based standard curve as the baseline for measurement of GH.

C: The effect of dialysis on the assay system.

Many of the assays available for measurement of urinary GH use dialysis as part of the protocol (Hourd & Edwards, 1994). It was therefore decided that investigation of the effect of dialysis on the assay system was necessary.

In these experiments an aqueous standard curve was used for dialysed urine and an urine based standard curve was used for undialysed urine.

Figure 4.11 compares the amount of GH recovered from dialysed pool urine (after correction for the volumetric changes) with recovery from undialysed pool urine. There is no significant difference between the recovery of GH before or after dialysis. However, examining the recovery of GH from paediatric urine (figure 4.12) shows that there is a significant reduction in recovery of GH from dialysed paediatric urines. The %CV of the assay was also reduced by dialysis making the assay less precise.
Figure 4.11:

Comparison of the recovery of growth hormone from dialysed pool urine after correction for volumetric changes, with undialysed pool urine.

Figure 4.12:

Comparison of the recovery of growth hormone from a typical paediatric urine before and after dialysis.
D: Validation of the final assay protocol.

The standard curve of the final assay system was urine based as this gave the best baseline for undialysed paediatric urines. In order to calculate the variation occurring within any single assay run and between all the assay runs the data from three quality controls was used. Figure 4.13 illustrates the %CV. The intra-assay CV is below 9.68% at GH levels greater than 22 μU.l⁻¹ while the inter-assay variation is slightly higher but below 14.32%.

The variation for each sample was calculated. The variations are highest at the lowest GH levels. Figure 4.14 shows the pooled variance for paediatric urine samples in groups of about 50 pairs. At GH concentrations below the %CV is less than 40%. The variance declines as the detectable level of GH increases.
Figure 4.13:
Comparison of the assay variance both within an assay run and between assay runs.

Figure 4.14:
The pooled variance for the sample duplicates of all paediatric urines assayed.
SUMMARY

1. Antibody binding in the presence of urine is greater than in the serum standard curve.

2. A phosphate buffer (0.1 mol.l⁻¹) does not effect the antibody binding, stabilises the pH and reduces the significance of individual variation of urinary phosphate. Phosphate only reduces antibody binding significantly at levels greater than those found in urine.

3. Urea does not effect antibody binding.

4. Sodium chloride reduces antibody binding but not significantly within the expected urinary range of concentrations.

5. Growth hormone can be recovered from urine. The correlation between the amount of GH present in the urine and the amount recovered from the urine is good.

6. Dialysis is not necessary for this assay. Dialysis makes the assay less precise without improving the accuracy of the assay.

7. Growth hormone can be detected accurately to levels of about 10 μU.l⁻¹ where the CV of the assay is about 20 %. At values lower than 10 μU.l⁻¹ the accuracy is reduced as the %CV increases to about 40 %.

8. The assay is adequate for measurement of paediatric urinary GH.
RESULTS
CHAPTER 5

DESCRIPTION OF THE MATURATION OF THE RECTAL TEMPERATURE PATTERN FOR THE SAMPLE POPULATION
SAMPLE POPULATION USED IN THE TEMPERATURE, COR TISOL AND GROWTH HORMONE STUDIES.

In order to assess the excretion of both GH and cortisol in infant urine, their relationship to body temperature and the effect of child care practices on these hormones, a sufficiently large number of infants were recruited and monitored to enable analysis to be carried out. Infants were monitored at weekly intervals from six weeks postnatal age until the attainment of an adult-like night-time temperature pattern (mature pattern see footnote on page 34) was observed. At each visit the collection of a night-time rectal temperature recording, a pair of urine samples and a record of family health and child care practices was attempted. The following description is of the total number of infants who took part in the study of night-time rectal temperature patterns, cortisol and GH excretion.

77 infants were monitored at weekly intervals from 6 weeks postnatal age. This gave a total of 515 visits, each infant being visited between 2 and 11 times, an average of about 6 visits per infant. On 259 (50 %) of these occasions at least one successful urine collection was made generally in conjunction with a recording of night-time rectal temperature. On 39 (15 %) out of the 259 successful monitorings night-time rectal temperature recordings were lost due to equipment failure. However, as successful recordings of rectal temperature had been made both immediately prior and after this week the exact age and the temperature maturation status could be estimated fairly accurately. On none of the 33 occasions where rectal temperature recordings were unavailable were there any visible signs of illness in the baby or family. All rectal temperature recordings collected within 24 hours of any immunisation was analysed separately in a subsequent chapter.

34 (44 %) of the infants were female while 43 (56 %) were male. The mean (SEM) gestation age of the infants was 39.60 (0.25) weeks with a mean (SEM) birth weight of 3.38 (0.07) Kg. 38 (49 %) infants continued to receive breast milk in their diet at six weeks of age, 1 had commenced weaning and 7 received an occasional bottle feed. 39 (51 %) were bottle fed, six of which had commenced weaning. Approximately half (40 infants) were first or second
infants and the remaining 37 were third or subsequent children in a family. Parental smoking
was found in 39 (51%) households. In 10 (13%) the mother was the sole smoker; in 12 (16%
the father the sole smoker and in 14 (22%) households both parents smoked. The
remaining 38 (49%) households had no parental smoking.

Each family was classified according to the major wage earner of the family as indicated by
government classifications (OPCS). 45 (58%) families were classified as belonging to social
classes 1, 2 or 3. The remaining 32 (42%) were in the lower social classifications 4, 5, 6 or
7. 66 of the infants slept in the same room as at least one parent while the remaining 11 slept
either in their own room or shared a room with a sibling. The majority of the infants slept
either lateral (36 (47%)) or supine (38 (49%)), only 3 (4%) regularly slept in the prone
position.

Table 1 illustrates the distribution of age, maximum and minimum room temperature, the total
tog value for the night-time coverings of the infant and the thermal score for all infants
included in this study. The thermal score is an index of the amount of covering and the
minimum night-time environmental temperature which has previously been used as a simple
measure of thermal environment (Lodemore, 1993).

Thermal score = Total tog + Minimum room temperature
TABLE 5.1: Thermal environment of study infants.

<table>
<thead>
<tr>
<th>Age (weeks) (number of monitorings)</th>
<th>Total tog mean(SEM)</th>
<th>Maximum room temperature mean(SEM)°C</th>
<th>Minimum room temperature mean(SEM)°C</th>
<th>Thermal score mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-7 weeks (n=45)</td>
<td>10.43(0.51)</td>
<td>21.59(0.61)</td>
<td>17.88(0.42)</td>
<td>28.55(0.63)</td>
</tr>
<tr>
<td>8-9 weeks (n=62)</td>
<td>10.32(0.46)</td>
<td>22.01(0.39)</td>
<td>17.35(0.39)</td>
<td>26.87(0.66)</td>
</tr>
<tr>
<td>10-11 weeks (n=77)</td>
<td>10.17(0.42)</td>
<td>21.29(0.25)</td>
<td>17.66(0.34)</td>
<td>26.80(0.50)</td>
</tr>
<tr>
<td>12-13 weeks (n=46)</td>
<td>9.42(0.37)</td>
<td>21.34(0.43)</td>
<td>17.57(0.44)</td>
<td>25.95(0.54)</td>
</tr>
<tr>
<td>14-15 weeks (n=19)</td>
<td>9.71(1.00)</td>
<td>20.09(0.53)</td>
<td>17.10(0.47)</td>
<td>24.72(0.59)</td>
</tr>
<tr>
<td>16-17 weeks (n=10)</td>
<td>10.61(1.07)</td>
<td>21.41(0.54)</td>
<td>18.19(0.95)</td>
<td>27.90(1.64)</td>
</tr>
</tbody>
</table>

Insulation covering infants at night-time and ambient temperature changes little over the age range examined by this study. Most infants were covered by about 10 togs of insulation which generally included 3.2 togs of clothing (i.e. nappy, vest and babygro) and approximately 7 togs of bedding generally made up of blankets and sheets rather than quilts. There were no significant differences between the total amount of insulation infants of different age groups. In addition the ambient temperature remained between 17 and 22°C. Room temperatures were significantly lower in the 14-15 week age band than in the 8-9 weeks age band (Student’s unpaired t-test, p=0.01). Similarly, when comparing thermal scores the older age group (14-15 weeks) were lower than those of the youngest infants (6-7 weeks), (Student’s unpaired t-test, p<0.05) but not at 16-17 weeks due to the small sample size. It appears that parents tend to keep the rooms of younger infants warmer than those of older infants. However, these differences may be as a result of seasonal temperature changes.
and subsequent alteration of parental insulation choice. These values correspond well with the guidelines issued by both the government and the Foundation for the Study of Infant Deaths (FSID).

Characterisation of rectal temperature pattern.

Until recently it was only known that at sometime during the first six months of life a circadian rhythm of body temperature appears. Earlier studies in Leicester found that the emergence of a circadian rhythm of body temperature is probably marked by the first appearance of a minimum night-time rectal temperature of 36.5°C or below (Lodemore, 1993). They also found that the appearance of a minimum night-time rectal temperature below 36.5°C occurred abruptly in any given individual sometime between 2 and 3 months after birth and coincided with the largest change in minimum rectal temperature occurring between weeks. Once this lowering of night-time body temperature was achieved it did not revert to levels seen prior to the emergence of a rhythm unless an illness or some other event affecting body temperature such as immunisation occurred.

This chapter examines the emergence of the temperature rhythm or more accurately the maturation of the night-time rectal temperature pattern in the group of infants I have studied to confirm that the pattern of development already described is true for this particular group of subjects.

Only temperature recordings made at the same time as a urine collection are analysed here. However, each infant was monitored at a sufficient frequency and for an adequate number of weeks for an accurate determination of changes in the rectal temperature pattern.

Individual changes in night-time rectal temperature

Figure 5.1 illustrates the minimum night-time rectal temperature achieved at various ages by 4 infants who were part of this study. All four show a similar pattern of change, with a number of weeks where the lowest the night-time rectal temperature reaches being between 36.60°C and 36.80°C. Then within a short period of approximately one week there is a rapid lowering
Figure 5.1:
Minimum rectal temperature achieved by four individuals over several weeks showing each infant developing a mature temperature pattern at different ages.

Figure 5.2:
Distribution of the age of attainment of an adult-like temperature pattern showing the normal distribution with a mean of about 10-11 weeks.
of the minimum rectal temperature achieved to about 36.50°C or below. The timing of the most rapid change in the rectal temperature coincides with the first time a rectal temperature of 36.50°C or below is achieved. This week of change from one developmental state to another has been identified as, "maturation week". The weeks prior to this change thus become those of "pre-maturation" and those subsequent become those of "post-maturation" or "mature". The timing of maturation week varies between individuals, some achieve a minimum nighttime temperature of 36.50°C or below earlier and others later.

**Timing of the appearance a mature rectal temperature pattern.**

Figure 5.2 illustrates the timing of this event in this sample population. The distribution of the timing of maturation week in these infants is approximately normal with the average (SEM) age for maturation week to occur being 11.16 (0.32) weeks. In this group of infants maturation week occurred at some time between 6 and 20 weeks of age. The modal value of achieving an adult-like temperature pattern was 8 weeks. 95% achieved an adult-like pattern by 15 weeks and 50% by 9 weeks of age.

**Cross-sectional analysis**

Figures 5.3a and 5.3b group the data collected from every infant into 2 week age bands. Figure 5.3a is the result of analysing the data with respect to chronological age. This graph indicates a more gradual change in rectal temperature than that seen in individuals. At 6-7 weeks of age the rectal temperature falls from about 37.10°C to a minimum of about 36.7°C at about 2.5 hours after bedtime. By 10-11 weeks of age the rectal temperature is falling from about 36.90°C to a minimum of 36.55°C, 2.5 hours later. It is only in the 16-17 week age band that a minimum rectal temperature of 36.5°C is achieved. This underestimation of the timing and magnitude of the changes occurring in an individual is a direct result of grouping the data in this fashion. This is not surprising as individuals have already been shown to achieve maturity at different age. Maturation week in most infants occurs between 8 and 13 weeks and therefore accounts for the dramatic change occurring in the 10-11 week age band.
Figure 5.3a:
Averaged 30 minute recordings of sleeping rectal temperature on the study group displaying the gradual change of the population rectal temperature with age.

Figure 5.3b:
Averaged 30 minute recordings of rectal temperature on the study group displaying the abrupt and irreversible change from the pre- to the post-mature temperature state.
However, as some infants do not reach maturity until much later it is to be expected that the
average change is an under estimation of that occurring in an individual.

In order to overcome this all the data must first be normalised to maturation week as illustrated
in figure 5.3b. This ensures that infants are grouped together in similar developmental states,
i.e. those that have yet to dramatically lower the night-time temperature they achieve, the pre-
maturity infants, and those that have undergone this change and are in the post-maturation
state. Normalising the data to maturation week is a simple procedure which conveniently
divides the data into two groups accurately reflecting the different developmental stage an
individual goes through. Each of the two week age bands in the pre-maturation state show a
fall from about 37.1°C to about 36.7°C, 2.5 hours later. There is no significant difference
between temperature patterns achieved in these age bands. At maturation week the pattern of
temperature dramatically changes so that the rectal temperature falls from 36.9°C at bedtime to
a minimum of 36.45°C at 2.5 hours later. All data from subsequent age band i.e. the post-
maturity infants show a similar pattern. There is no significant difference between rectal
temperatures in the weeks up to 3-4 weeks post-maturation. However, comparison of the pre-
maturity temperatures with those of post-maturation indicates a highly significant difference
between the two states.

Factors affecting the timing of maturation week

Previous studies have shown that there are a variety of differences between those infants who
develop a mature rectal temperature of 36.5°C or below, at an early age, when compared with
those infants who develop a mature rectal temperature later. Of the 77 infants studied here, 32
attained maturity at 10 weeks of age or earlier, 12 at the average age of 11 weeks and 34 at 12
weeks or later. Table 5.2 examines some of the differences between these two groups.

There were approximately equal numbers of males and females in both the group of early and
late developers. Infants continuing to receive breast milk in their diet at 6 weeks were slightly
over-represented in the early developers and under-represented in the late developers. The
average age for temperature pattern maturation in male and female infants were identical. The
converse was true of the bottle fed infants, with fewer bottle feeders developing a mature temperature pattern in the early group ($\chi^2=3.03$, $p=0.086$).

**TABLE 5.2 Differences between early and late development of a mature temperature pattern.**

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>Early developers (&lt;10 weeks)</th>
<th>Late developers (&gt;12 weeks)</th>
<th>Attainment of mature temperature pattern, Mean (SEM) weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALE</td>
<td>N=17</td>
<td>N=16</td>
<td>11.40(0.42)</td>
</tr>
<tr>
<td>FEMALE</td>
<td>N=14</td>
<td>N=17</td>
<td>11.41(0.45)</td>
</tr>
<tr>
<td>BREAST FED AT 6 WEEKS</td>
<td>N=18</td>
<td>N=12</td>
<td>10.35(0.39)</td>
</tr>
<tr>
<td>BOTTLE FED AT 6 WEEKS</td>
<td>N=13</td>
<td>N=21</td>
<td>11.98(0.38)</td>
</tr>
<tr>
<td>1ST OR 2ND INFANTS</td>
<td>N=17</td>
<td>N=17</td>
<td>11.82(0.44)</td>
</tr>
<tr>
<td>3RD OR SUBSEQUENT</td>
<td>N=16</td>
<td>N=16</td>
<td>10.88(0.37)</td>
</tr>
<tr>
<td>SMOKING HOUSEHOLDS</td>
<td>N=12</td>
<td>N=19</td>
<td>11.60(0.42)</td>
</tr>
<tr>
<td>NON-SMOKING HOUSEHOLDS</td>
<td>N=19</td>
<td>N=14</td>
<td>11.10(0.42)</td>
</tr>
<tr>
<td>SOCIAL CLASSES 1, 2 OR 3</td>
<td>N=22</td>
<td>N=17</td>
<td>11.00(0.39)</td>
</tr>
<tr>
<td>SOCIAL CLASSES 4, 5, 6 OR 7</td>
<td>N=10</td>
<td>N=16</td>
<td>12.13(0.47)</td>
</tr>
<tr>
<td>MATERNAL AGE MEAN(SEM)</td>
<td>29.43(0.90)years</td>
<td>26.39(0.91)years</td>
<td></td>
</tr>
<tr>
<td>GESTATION AGE MEAN(SEM)</td>
<td>39.83(0.35)weeks</td>
<td>39.35(0.45)weeks</td>
<td></td>
</tr>
<tr>
<td>BIRTH WEIGHT MEAN(SEM)</td>
<td>3.43(0.12)Kg</td>
<td>3.20(0.13)Kg</td>
<td></td>
</tr>
<tr>
<td>TOTAL TOG MEAN(SEM)</td>
<td>10.52(0.72)</td>
<td>9.20(0.45)</td>
<td></td>
</tr>
<tr>
<td>THERMAL SCORE MEAN(SEM)</td>
<td>27.24(0.68)</td>
<td>25.46(0.58)</td>
<td></td>
</tr>
</tbody>
</table>

This was more obvious when comparing the average age of temperature maturation in those infants who were breast fed with those who were not. Bottle fed infants in general attained their mature temperature pattern about 1.5 weeks later than breast fed infants. Similarly when
examining the numbers of infants living in homes where parental smoking occurred with those in smoke free homes, indicated that those infants in smoking households tended to develop their mature temperature pattern later than those from non-smoking households ($\chi^2=2.28$, p=0.140). However, comparing the average age of maturation revealed no significant difference between the two groups. Comparison of early and late developers with respect to social status indicates that those infants in the higher social categories are more likely to be early developers. This was further supported by the fact that the average age of maturation for those infants of lower social class was approximately 1 week later than those of the higher social categories. This probably reflects a combination of several factors including smoking and method of feeding habits. Both smoking and bottle feeding tend to occur in those families of lower social status. Therefore, it is not too surprising that the early developers show an over-representation of social classes 1, 2 and 3 and an under-representation of social classes 4, 5, 6 and 7 ($\chi^2=2.35$, p=0.132).

Examining other factors such as maternal age show that early developers tend to have older mothers and those of the later developers younger mothers (Student's unpaired t-test, p=0.02). The gestation age of both early and late developers is virtually identical (Student's unpaired t-test, p=0.41) but the birth weight is marginally greater in the early developers when compared to the late developers (Student's unpaired t-test, p=0.19). Both mean Tog covering for the early developers is slightly larger than that of the later developers but not significantly so (Student's unpaired t-test, p=0.18). The thermal environment of early developers is significantly greater than the later developers (Student's unpaired t-test, p=0.05).

Summary

1. There was no significant change in either the amount of togs or the thermal environment of the infants from 6-17 weeks of age.

2. The average tog ranged from 9.42 to 10.61; the average maximum room temperature was always 22°C or below and the average minimum temperature was greater than 17°C indicating
that in this sample the general environmental conditions recommended by FSID and the Department of Health (Chief medical officer's report, 1993) were adhered to.

3. All infants achieved a minimum rectal temperature of 36.5°C or below at a given age. This occurs abruptly, i.e. within 1 week and at a variety of ages but generally after 8 weeks but before 13 weeks of age.

4. The average age at which the minimum night-time rectal temperature fell below 36.5°C was 11.16 (0.32) weeks but varied between 6 and 20 weeks of age in the individual.

5. The data can be normalised to the appearance of a night-time rectal temperature below 36.5°C. This ensures that the changes seen in the individual are not lost by the grouping of data. Rectal temperature minima before the appearance of a mature temperature pattern are alike in all infants. Rectal temperatures in the post-maturation state are similarly alike.

6. Once temperature maturation is established it is consistently achieved each week in all infants unless events such as infection or immunisations occur. Only then may the night-time rectal temperature pattern be disturbed.

7. Early maturing individuals (less than or equal to 10 weeks of age) tended to be breast fed until at least 6 weeks, from non-smoking households in social classes 1, 2 or 3, be slightly heavier at birth and have older mothers than the later developers. The later developers (maturation week was 12 weeks or later) tended to be bottle fed from birth or soon after, lived in smoking households of social class 4, 5, 6 or 7, have younger mothers and be slightly smaller at birth. These are similar characteristics attributed to greater risk of becoming a SID (Golding, Limerick & Macfarlane 1985).

8. The infants in this study develop in an identical fashion to those previously described.
CHAPTER 6

DESCRIPTION OF THE URINARY EXCRETION OF CORTISOL AND GROWTH HORMONE
ANALYSIS OF URINE FOR CREATININE, CORTISOL AND GROWTH HORMONE.

This chapter presents the data collected from urinalysis for the hormones GH and cortisol in addition to the metabolite creatinine. The results of urinalysis for creatinine are presented here for completeness as both cortisol and GH concentrations are expressed relative to the concentration of creatinine in each urine sample.

By comparing the levels of analyte in morning and night-time urine collections over several weeks not only can the normal range of values be established for the age group but also the presence or absence of a circadian pattern can be inferred. By combining the urinalysis data with the rectal temperature observations made on these infants or by simply organising the data using chronological age, can provide further information about the effect of age and the development of a rectal temperature maturity. By examining this data, the method to be used for data organisation (i.e. whether the data was organised by age or by rectal temperature maturity) was decided.

A total of 419 urine samples were collected from the 77 infants described in the rectal temperature chapter. Any samples collected within 24 hours of any immunisations were omitted from this analysis. 176 (42 %) of the samples were collected during the first four hours after the morning feed, usually a period of wakefulness. The remaining 240 (58 %) of samples were collected over the period from the last nappy change before bedtime, over the night-time period immediately prior to the morning sample collection. All samples had a corresponding night-time rectal temperature recording or at least the age and the maturity status of the infant for that urine collection could be estimated to within 1 week. Those urines which had no corresponding temperature recording were only used if successful recordings of temperature had been made the weeks before and after the failed recording and if the infant and the rest of the family showed no sign of illness.
The number of metabolites measured was limited by the volume of each sample collected. Cortisol and GH were measured relative to the amount of creatinine per litre of urine. Therefore, creatinine was measured in every sample collected (100%). Cortisol was measured in 160 (91%) of all morning samples and 233 (97%) of the night-time samples. Growth hormone was measured in 123 (70%) of morning and 190 (79%) of night-time samples.

For purposes of analysis the data for each metabolite were firstly divided into 2 week age bands from 6-17 weeks postnatal age. Table 1 shows the distribution of urine samples analysed for each age band and urinary constituent. The second stage of analysis used the same data as described above normalised to temperature maturation week (i.e. the first appearance of a night-time rectal temperature of 36.5°C or below). Table 2 shows the distribution of urines for each of the age bands and urinary constituents for this second stage of analysis. By comparing the data represented with respect to chronological age with the data presented relative to temperature maturation the most appropriate method for further analysis was decided.
As can be seen from Table 6.1, there were adequate samples in both morning and night-time populations to allow the comparison of the two in all age bands and for all metabolites except for the 16-17 week group. Comparison of the levels of creatinine, cortisol, and GH were not carried out in this group as sample numbers below 10 were felt to be inadequate for analysis.

### Table 6.1: The number of urines analysed in 2 week age bands between 6 and 17 weeks of age.

<table>
<thead>
<tr>
<th>Chronological age</th>
<th>Number of samples analysed in each age band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning (Night-time)</td>
</tr>
<tr>
<td>6-7 weeks</td>
<td>Creatinine n=27(41)</td>
</tr>
<tr>
<td></td>
<td>Cortisol n=26(40)</td>
</tr>
<tr>
<td></td>
<td>Growth hormone n=18(32)</td>
</tr>
<tr>
<td>8-9 weeks</td>
<td>Creatinine n=46(57)</td>
</tr>
<tr>
<td></td>
<td>Cortisol n=41(54)</td>
</tr>
<tr>
<td></td>
<td>Growth hormone n=35(46)</td>
</tr>
<tr>
<td>10-11 weeks</td>
<td>Creatinine n=51(72)</td>
</tr>
<tr>
<td></td>
<td>Cortisol n=44(72)</td>
</tr>
<tr>
<td></td>
<td>Growth hormone n=35(59)</td>
</tr>
<tr>
<td>12-13 weeks</td>
<td>Creatinine n=30(44)</td>
</tr>
<tr>
<td></td>
<td>Cortisol n=28(41)</td>
</tr>
<tr>
<td></td>
<td>Growth hormone n=22(31)</td>
</tr>
<tr>
<td>14-15 weeks</td>
<td>Creatinine n=14(19)</td>
</tr>
<tr>
<td></td>
<td>Cortisol n=14(10)</td>
</tr>
<tr>
<td></td>
<td>Growth hormone n=10(16)</td>
</tr>
<tr>
<td>16-17 weeks</td>
<td>Creatinine n=8(7)</td>
</tr>
<tr>
<td></td>
<td>Cortisol n=7(7)</td>
</tr>
<tr>
<td></td>
<td>Growth hormone n=3(6)</td>
</tr>
</tbody>
</table>
TABLE 6.2: The number of urines analysed in 2 week age bands from 6 weeks prior to maturation until 4 weeks after maturation.

<table>
<thead>
<tr>
<th>Age normalised to the appearance of a mature temperature pattern</th>
<th>Number of samples analysed in each age band Morning(Night-time)</th>
</tr>
</thead>
</table>
| 5-6 weeks pre-maturation                                         | Creatinine n = 16 (20)  
Cortisol n= 14 (18)  
Growth hormone n = 10 (14) |
| 3-4 weeks pre-maturation                                         | Creatinine n = 42 (43)  
Cortisol n= 38 (43)  
Growth hormone n = 31 (35) |
| 1-2 weeks pre-maturation                                         | Creatinine n = 42 (62)  
Cortisol n= 36 (59)  
Growth hormone n = 32 (49) |
| Maturation week                                                  | Creatinine n = 37 (52)  
Cortisol n= 35 (50)  
Growth hormone n = 24 (38) |
| 1-2 weeks post-maturation                                        | Creatinine n = 25 (38)  
Cortisol n= 23 (38)  
Growth hormone n = 15 (31) |
| 3-4 weeks post maturity                                          | Creatinine n = 8 (15)  
Cortisol n= 8 (15)  
Growth hormone n = 6 (13) |

As in the chronological analysis of the data there were sufficient samples up to and including the 1-2 week post-maturation band to allow comparison of morning and night-time data. The number of morning urines in the 3-4 week post-maturation age band were below 10 for all three metabolites. Therefore, a good comparison of morning and night-time was impossible in this age band.
Creatinine: (i) Relative to chronological age.

Figure 6.1a illustrates the mean (SEM) levels of creatinine (mmol.l⁻¹) in 2 week age bands between 6 and 15 weeks of age. Between these ages the creatinine content of both morning and night-time urine increases. Morning values increase from 1.44 (0.17) mmol.l⁻¹ at 6-7 weeks to 1.91 (0.51) mmol.l⁻¹ at 14-15 weeks, a significant increase in excretion (Student's unpaired t-test, p<0.01). This increase in creatinine shows a step like incline between the age bands of 10-11 weeks (1.43 (0.13) mmol.l⁻¹) and 12-13 weeks (2.01 (0.21) mmol.l⁻¹), (Student's unpaired t-test, p=0.008). Night-time creatinine levels show a more gradual increase with age from 1.21 (0.09) mmol.l⁻¹ at 6-7 weeks to 1.88 (0.43) mmol.l⁻¹ at 13-14 weeks, (Student's unpaired t-test, p=0.009). A significant difference between morning and night-time creatinine is never achieved over the period of observation.

Creatinine: (ii) Relative to temperature maturation.

Figure 6.1b illustrates the mean (SEM) results of the analysis for creatinine after normalisation to maturation of the temperature pattern. Creatinine generally increases in both morning and night-time urines. There is no significant difference between morning and night-time levels throughout the age range studied. However, morning creatinine levels tend to be marginally greater than those found in night-time urines. Both morning and night-time creatinine oscillate from week to week.

Morning creatinine excretion falls from 1.69 (0.21) mmol.l⁻¹ (5-6 weeks pre-maturation) to 1.35 (0.13) mmol.l⁻¹ (3-4 weeks pre-maturation) before increasing to 1.67 (0.17) mmol.l⁻¹ (1-2 weeks pre-maturation) then declining to 1.51 (0.16) mmol.l⁻¹ at maturation week and finally increasing to 1.89 (0.35) mmol.l⁻¹ at 1-2 weeks post-maturation.

Night-time creatinine levels run almost parallel with those levels seen in morning urine. Starting at 1.50 (0.15) mmol.l⁻¹ at 5-6 weeks pre-maturation reaching a low of 1.15 (0.09) mmol.l⁻¹ at 3-4 weeks pre-maturation before steadily increasing to 1.78 (0.24) mmol.l⁻¹ at 1-2 weeks post-maturation.

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Figure 6.1a:
Averaged concentrations of creatinine found in morning and night-time over several weeks. Data normalised to chronological age.

Figure 6.1b:
Averaged concentrations of creatinine found in morning and night-time urines over several weeks. Data normalised to the first appearance of a minimum rectal temperature of 36.5 deg C.
When normalised to the appearance of a mature adult-like temperature pattern changes in neither the morning nor night-time excretion of creatinine reach the significance observed when normalised to chronological age.

Comparison of the two methods of analysis reveal that there does not appear to be any abrupt changes in creatinine excretion associated with the development of a mature temperature pattern. It was decided that further analysis of the factors that may effect creatinine would be hindered if the data were firstly analysed to the appearance of mature temperature pattern.

**Cortisol: Relative to chronological age**

Figure 6.2a illustrates the mean (SEM) cortisol concentrations (nmol.mmol⁻¹ creatinine) observed between 6 and 15 weeks of age. After 6 weeks of age there is a highly significant difference between morning and night-time levels of cortisol observable throughout the entire age range examined (Student's unpaired t-test, p<0.05). The significance of the difference increases at 14-15 weeks when morning cortisol concentrations increase and night-time concentrations decrease. At 6-7 weeks of age morning concentrations are at about 22.32 (2.98) nmol.mmol⁻¹ creatinine. This increases gradually to 25.44 (2.12) nmol.mmol⁻¹ creatinine before decreasing to 23.37 (2.22) nmol.mmol⁻¹ creatinine 1-2 weeks later at 12-13 weeks of age. The cortisol concentration then increases again to 26.99 (2.60) at 14-15 weeks. The difference between 6-7 weeks (22.32 (2.98) nmol.mmol⁻¹ creatinine) and 14-15 weeks(26.99 (2.60) nmol.mmol⁻¹ creatinine) is not significant (Student's unpaired t-test, p=0.35). The change in morning cortisol excretion between 6-7 weeks and 14-15 weeks is not significant (1-way ANOVA F=0.91 with 1df, p=0.35) Night-time cortisol remains at about 17.5 nmol.mmol⁻¹ creatinine from 6 to 9 weeks after which it declines over several weeks to reach 12.71 (2.34) nmol.mmol⁻¹ creatinine at 14-15 weeks of age (Student's unpaired t-test, p=0.71). The decrease in night-time cortisol between 6-7 weeks and 14-15 weeks is not quite significant (1-way ANOVA F=3.16 with 1df, p=0.08)

By subtracting night-time cortisol levels from morning concentrations a graph of the mean (SEM) differences between morning and night-time excretion can be examined as shown in
Figure 6.2a:
Changes in urinary cortisol excretion in morning and night-time urines, between 6 and 15 weeks of age.

Figure 6.2b:
Changes in the difference between morning and night-time cortisol levels between 6 and 17 weeks of age.
figure 6.2b. This indicates that between the 6-7 week and 8-9 week age band there is an increase in the difference between morning and night-time excretion which is not quite significant (Student's unpaired t-test, $p=0.1$). There is a further increase in the difference between morning and night-time excretion between 8-9 and 10-11 weeks which is significant (Student's unpaired t-test, $p<0.05$). There are no significant changes in the difference between morning and night-time over the following weeks.

Cortisol (ii) Relative to temperature maturation.

Figure 6.3a illustrates the mean (SEM) nmol.mmol$^{-1}$ creatinine of urinary concentrations of cortisol in both morning and night-time urines. There is a significant difference between morning and night-time cortisol from 5-6 weeks pre-maturation similar to that observed when the data was analysed relative to age (Student's unpaired t-test, $p<0.05$).

Morning cortisol falls from 24.32 (3.98) nmol.mmol$^{-1}$ creatinine to 22.62 (1.89) nmol.mmol$^{-1}$ creatinine between 5-6 weeks pre-maturation and 1-2 weeks pre-maturation this is not significant and the cortisol level is very stable at about 22.70 nmol.mmol$^{-1}$ creatinine. At maturation week there is an increase to 24.05 (2.13) nmol.mmol$^{-1}$ creatinine. This is then followed by a further increase reaching 26.73 (2.99) nmol.mmol$^{-1}$ creatinine by 1-2 weeks post-maturation. The increase in cortisol excretion from 1-2 weeks pre-maturation until 1-2 weeks post-maturation is not significant (Student's unpaired t-test, $p=0.17$). The increase in cortisol excretion in morning urine between 5-6 weeks pre-maturation and 3-4 weeks post-maturation is not significant (1-way ANOVA $f=0.67$, $p=0.36$).

Night-time cortisol levels decrease steadily from 17.79 (3.54) nmol.mmol$^{-1}$ creatinine at 5-6 weeks pre-maturation to 15.53 (1.70) nmol.mmol$^{-1}$ creatinine at 1-2 weeks pre-maturation. There is then a step-like decrease to 13.76 (1.50) nmol.mmol$^{-1}$ creatinine at 1-2 weeks post-maturation. This fall in night-time cortisol excretion does not reach significance (Student's unpaired t-test, $p=0.27$). The fall in night-time cortisol excretion between 5-6 pre- and 3-4 post-maturation is not significant (1-way ANOVA $f=1.28$, $p=0.26$)
Figure 6.3a:
Changes in morning and night-time excretion of cortisol between 6 weeks prior- and 2 weeks after the development of a mature temperature pattern.

Figure 6.3b:
Changes in the difference between morning and night-time cortisol levels presented relative to the attainment of an adult-like temperature pattern.
Figure 6.3b illustrates the mean (SEM) difference between morning and night-time cortisol excretion. Between 1 and 6 weeks pre-maturation the difference between morning and night-time excretion of cortisol (about 8 nmol.mmol⁻¹ creatinine) does not change significantly. At the same time as rectal temperature maturity is achieved there is an increase in this difference between morning and night-time concentrations making the difference between 1-2 weeks pre- and 1-2 weeks post-maturation significant (Student's unpaired t-test, p= 0.02). In the following weeks there is a gradual increase in the difference between morning and night-time concentrations of cortisol. These changes are not significant.

Analysis of the urinary excretion of cortisol with respect to chronological age and temperature maturation show remarkable similarity. Normalising the data to the first appearance of a night-time rectal temperature of 36.5°C or less removes any interference temperature may have on cortisol excretion by grouping infants achieving similar night-time rectal temperatures together. Those infants in the pre-maturation state appear to have similar differences between morning and night-time cortisol excretion. These are smaller differences than those seen in the post-maturation infants. Infants up to 4 weeks post-maturation have similar morning/night-time differences in cortisol concentrations. Further analysis of cortisol excretion was therefore carried out after the data was normalised to temperature maturation.
Growth hormone (i) Relative to chronological age.

Figure 6.4a illustrates the mean (SEM) GH concentrations (μU.mmol⁻¹ creatinine) in each of the two week age bands from 6 to 15 weeks. The levels of GH in both morning and nighttime urines tend to fluctuate over the observation period with no clear pattern of change appearing. Morning GH increases sharply from 9.67 (3.36) μU.mmol⁻¹ creatinine at 6-7 weeks to 16.59 (2.84) μU.mmol⁻¹ creatinine in the following age band before decreasing to 11.22 (2.19) μU.mmol⁻¹ creatinine at 10-11 weeks of age. A steady increase occurs subsequently until a level of 16.03 (2.36) μU.mmol⁻¹ creatinine is reach at 14-15 weeks of age. However the overall change with age is not significant and the average morning concentration is about 12 μU.mmol⁻¹ creatinine.

Night-time GH shows a general decline with age. Growth hormone falls from 13.09 (3.16) μU.mmol⁻¹ creatinine at 6-7 weeks to 12.05 (1.57) μU.mmol⁻¹ creatinine at 10-11 weeks of age before increasing the following week to 15.10 (2.37) μU.mmol⁻¹ creatinine. Again the overall change is not a significant one. There is no significant difference between the morning and night-time values of GH in any age band.

Growth Hormone (ii) Relative to the development of a mature rectal temperature pattern.

The mean (SEM) GH data previously analysed relative to chronological age were normalised to the appearance of a mature temperature pattern. Figure 6.4b shows the results of this analysis from 4 weeks pre-maturation to 2 weeks post-maturation. There is no significant difference between concentrations observed in morning and those of night-time urines. However, between 2 weeks pre-maturation and maturation week morning GH tends to be slightly higher than those of night-time urines.

Morning urines have levels of GH of 12.98 (2.57) μU.mmol⁻¹ creatinine at 3-4 weeks pre-maturation. This increases slightly to 15.42 (2.83) μU.mmol⁻¹ creatinine by 1-2 weeks pre-
Figure 6.4a:
Changes in urinary growth hormone in morning and night-time urines, between 6 and 15 weeks of age.

Figure 6.4b:
Changes in morning and night-time excretion of growth hormone between 6 weeks prior to and after the development of a mature rectal temperature pattern.
maturation and remains steady until 1-2 weeks post-maturation development when the GH falls to 12.10 (2.08) μU.mmol⁻¹ creatinine.

Night-time GH mirrors that of morning. At 3-4 weeks pre-maturation and 1-2 weeks post-maturation morning and night-time levels of GH are almost identical. Between these two periods morning GH tends to be marginally greater than that of night-time (steady at about 12 μU.mmol⁻¹ creatinine). However this never reaches significance.

By normalising the data to the appearance of a mature temperature pattern some of the oscillations observed on the chronological analysis are lost although there does not appear to be any clear relationship between the patterns of GH excretion and temperature. Further analysis of GH comparing different groups of infants will use data organised by chronological age.
Summary.

1. There are no significant differences between morning and night-time levels of creatinine when analysed relative to chronological age nor when analysed relative to the development of a mature temperature pattern. Creatinine in both morning and night-time urines is usually between 1.0 and 2.5 mmol.l\(^{-1}\).

2. Both morning and night-time creatinine tend to increase with age. This is a significant increase in the data normalised to chronological age but not when normalised to temperature maturation age.

3. There is no relationship between the excretion of creatinine and the development of a mature rectal temperature pattern.

4. There is a significant difference between morning and night-time cortisol concentrations after 6-7 weeks of age when analysed by chronological age and after 5-6 weeks pre-maturation when normalised to temperature maturation.

5. Cortisol excretion increases in morning urines and decreases in night-time urines with age. When analysed relative to the appearance of a night-time rectal temperature of 36.5°C or below this divergence begins at about rectal temperature maturation week. The average content of cortisol in morning urines was between 20 and 30 nmol.mmol\(^{-1}\) creatinine of cortisol while night-time urines generally averaged 12-20 nmol.mmol\(^{-1}\) creatinine. Cortisol excretion was never usually above 70 nmol.mmol\(^{-1}\) creatinine.

6. There is no significant difference between morning and night-time levels of GH. The average level of GH in morning and night-time urine is about 10-20 µU.mmol\(^{-1}\) creatinine.

7. There is no definite pattern of excretion observable in the excretion of GH that could be linked to the appearance of the mature rectal temperature pattern.
CHAPTER 7

DESCRIPTION OF THE FACTORS AFFECTING THE URINARY EXCRETION OF CORTISOL AND GROWTH HORMONE
FACTORS AFFECTING THE EXCRETION OF CORTISOL AND GROWTH HORMONE.

Previous studies have linked some child care practices and social factors with increased incidence of SIDS and delayed development of rectal temperature maturity (Lodemore, 1993). The effect of several of these factors on cortisol and GH excretion are examined in this chapter. A series of comparisons of the urinary excretion of these analytes in addition to creatinine were made between several groups of infants. These groups included male and female infants, breast and bottle feeding until 6 weeks, smoking and non-smoking parents, thermal environment, birth order and social class.

In the case of creatinine and GH these comparisons were made on data normalised to chronological age. Cortisol, because of its association with rectal temperature maturity, was analysed after normalisation to maturation week therefore comparisons were made between pre- and post-maturation states.

None of the factors examined had any significant effect on the excretion of creatinine.

FACTORS AFFECTING THE EXCRETION OF URINARY CORTISOL.

SEX: Figures 7.1a and 7.1b compare the mean (SEM) levels of cortisol excreted by male and female infants in morning and night-time samples respectively. The data was divided to examined those infants who were in the three or more weeks pre-maturation stage and those who were in the one or more weeks post-maturation stage. In both male and female infants cortisol excretion is higher in morning urines than in night-time urines. This is true for samples collected from infants in the pre- and post-maturation states.

Morning: Female cortisol excretion in morning urine is generally higher than those in male infants. In the pre-maturation state female cortisol (29.74 (3.34) nmol.mmol⁻¹ creatinine) is significantly higher than male cortisol (19.25 (1.72) nmol.mmol⁻¹ creatinine)(Student's unpaired t-test, p<0.01). Similarly, in the post-maturation state female
Figure 7.1a:
Comparison of the excretion of cortisol in morning urines collected from male and female infants in the pre- and post- temperature maturity states.

Figure 7.1b:
Comparison of the night-time excretion of cortisol from male and female infants in the pre- and post-mature temperature states.
Cortisol (33.41 (3.56) nmol.mmol⁻¹ creatinine) is higher than male (24.80 (3.26) nmol.mmol⁻¹ creatinine) but is not quite significant (Student's unpaired t-test, p = 0.1). Both male and female cortisol increases from pre-to post-maturation but not significantly so.

Night-time: In the pre-maturation state female cortisol (20.86 (2.31) nmol.mmol⁻¹ creatinine) is significantly higher than male cortisol (12.58 (1.27) nmol.mmol⁻¹ creatinine), (Student's unpaired t-test, p<0.01). The fall in female cortisol from pre- to post-maturation is highly significant (Student's unpaired t-test, p<0.01). However the increase in male cortisol from pre- to post-maturation is not quite significant (Student's unpaired t-test, p=0.08).

METHOD OF FEEDING AT 6 WEEKS AFTER BIRTH: Figures 7.2a and 7.2b compare the mean (SEM) morning and night-time cortisol levels in groups of infants who were either breast fed until at least 6 weeks of age or those who were bottle fed from birth or soon after.

Morning: There is no significant difference between the excretion of cortisol in breast and bottle fed infants in either the pre- or post-maturation infants, although there was a tendency for the bottle fed infants to excrete more cortisol. Both breast and bottle fed infants show an increase in cortisol excretion as they move from the pre- to the post-maturation stage.

Night-time: Cortisol excretion in both breast and bottle fed infants in night-time urine is less than that seen in morning urines (Student's unpaired t-test, p<0.01 for each). In the pre-maturation state breast fed infants excrete marginally more cortisol than the bottle fed infants but not significantly so. In the post-maturation state the bottle feeders excrete more cortisol than the breast feeders, this is not quite significant (Student's unpaired t-test, p= 0.07). The cortisol excretion of the bottle fed infants does not change from pre- to post-maturation, while the excretion of the breast fed infants falls (Student's unpaired t-test, p= 0.02).
Figure 7.2a:
Comparison of the excretion of cortisol in morning urines collected from breast and bottle fed infants in the pre- and post- temperature maturation states.

Figure 7.2b:
Comparison of the night-time excretion of cortisol in breast and bottle fed infants in both the pre- and post- temperature maturation states.
PARENTAL SMOKING: Figures 7.3a and 7.3b compare those infants in smoking households with those in non-smoking households. Values are given as mean (SEM) nmol.mmol⁻¹ creatinine.

Morning: In the pre-maturation state those infants in smoking households (18.07 (1.91) nmol.mmol⁻¹ creatinine) have significantly lower cortisol excretions than those of non-smoking households (27.92 (2.60) nmol.mmol⁻¹ creatinine), (Student's unpaired t-test, p<0.01). In the post-maturation state cortisol excretion in the smoking households has increased to (26.96 (3.78) nmol.mmol⁻¹ creatinine), (Student's unpaired t-test, p<0.05) while that of those from non-smoking households (29.37 (3.40) nmol.mmol⁻¹ creatinine) remained static. There is no significant difference between the excretion of cortisol from infants in smoking and non-smoking households in the post-maturation group.

Night-time: Infants in the non-smoking household had night-time cortisol excretions that were significantly lower than morning excretion in both the pre- (17.55 (2.16) nmol.mmol⁻¹ creatinine) and post-maturation states (12.67 (1.19) nmol.mmol⁻¹ creatinine), (Student's unpaired t-test, p<0.05). Infants in smoking households only had significantly lower cortisol excretion in night-time urines in the post-maturation state (15.72 (2.23) nmol.mmol⁻¹ creatinine), (Student's unpaired t-test, p<0.05).

There was no significant difference between the night-time levels of cortisol between infants with smoking parents and those with non-smoking parents in either the pre- or post-maturation state.
Figure 7.3a:
Comparison of the morning excretion of cortisol from infants with smoking and non-smoking parents in both the pre- and the post-mature temperature states.

Figure 7.3b:
Comparison of the night-time excretion of cortisol of infants with smoking and non-smoking parents in both the pre- and post-mature temperature states.
THERMAL ENVIRONMENT AND INSULATION: Figures 7.4a and 7.4b examine the total amount of coverings as estimated by tog values. Comparison was made between those under high insulation (10 or more togs) and those under low insulation (under 10 togs). Similar comparisons were made using the thermal score as defined previously. All values given as mean (SEM) nmol.mmol⁻¹ creatinine.

Morning urines: In the pre-maturation state those infants covered by less than 10 togs of insulation (17.47 (1.91) nmol.mmol⁻¹ creatinine) excreted significantly less cortisol than those infants insulated by 10 or more togs (27.15 (2.46) nmol.mmol⁻¹ creatinine), (Student's unpaired t-test, p<0.05). In the post-maturation state although those under higher togs (26.63 (3.60) nmol.mmol⁻¹ creatinine) tend to excrete less cortisol than those under lower togs (30.19 (3.50) nmol.mmol⁻¹ creatinine) there is no significant difference between these infants. This is because those infants under less tog in the post-maturation state have significantly higher morning cortisol than when in the pre-maturation state (Student's unpaired t-test, p<0.05) while the cortisol excretion of those infants under higher togs have identical pre- and post-maturation rates of excretion.

Night-time: Night-time cortisol excretion of infants covered by more than 10 togs and less than 10 togs is lower than that in the morning. In the pre-maturation state those infants under less than 10 togs (12.38 (1.04) nmol.mmol⁻¹ creatinine) have significantly lower cortisol excretion than those under greater insulation (20.12 (2.29) nmol.mmol⁻¹ creatinine), (Student's unpaired t-test, p<0.05). The level of cortisol excretion of the highly insulated infants (14.35 (2.44) nmol.mmol⁻¹ creatinine) falls in the post-maturation state to reach an identical levels and that seen in those infants under less insulation (13.85 (1.16) nmol.mmol⁻¹ creatinine).

By analysing the same data described above but using the thermal score (combination of room temperature and insulation) a similar pattern of relationships are observed between the groups. However, the significance of the differences between those with high thermal scores and those with lower scores are not significant.
Figure 7.4a:
Comparison of the morning cortisol excretion from those infants under low and high insulation in both the pre- and post-mature temperature states.

Figure 7.4b:
Comparison of the night-time excretion of cortisol from infants under high insulation with those under low insulation in both the pre- and post-mature temperature states.
**SUMMARY**

1. Females tend to excrete more cortisol in morning urines than male infants in morning urines in both the pre-and post-maturation states. In night-time urines females again have significantly higher cortisol excretion than males in the pre-maturation state. However, in the post-maturation state the male excretion of cortisol at night is higher than that of the female.

2. Bottle fed infants tend to excrete more cortisol in both morning urines than breast fed infants but this is not significant. In night-time urine the pre-maturation breast fed infants tend to excrete more cortisol (not significant) but on the post-maturation state they have significantly lower cortisol excretion than bottle fed infants.

3. Infants from smoking households excrete significantly less cortisol in morning urines in the pre-maturation state than those in non-smoking households. There are no significant differences between the night-time excretion rates of cortisol in these two groups of infants.

4. Birth order and social class have no significant effect on the excretion of cortisol in morning or night-time urines.
FACTORS AFFECTING THE EXCRETION OF URINARY GH

The same factors examined for affect on creatinine and cortisol excretion were examined for effect on the excretion of GH. Unlike the cortisol data it was felt that the most appropriate way of analysis was to normalise to birth i.e. organise the data with respect to age rather than to the development of a rectal temperature maturity as the pattern of GH excretion was not altered by the maturation of the rectal temperature pattern. Comparisons were made between young (8 weeks or younger) and older (12 weeks or older) groups of infants.

SEX: Figures 7.5a and 7.5b illustrate the mean (SEM) excretion of GH by male and female infants. It would appear that in general male infants excrete more GH than their female counterparts particularly in the morning urines of younger subjects. Statistical significance between male and female GH excretion is never reached.

METHOD OF FEEDING AT 6 WEEKS OF AGE: There were no differences between the patterns of excretion of GH in those infants who were breast fed and those who were not. This was true for both morning and night-time urines.

PARENTAL SMOKING: Figures 7.6a and 7.6b compare the mean (SEM) urinary GH from infants in smoking and non-smoking households. Those infants in non-smoking households tended to excrete more GH than those in smoking households. This never reached significance.

THERMAL ENVIRONMENT AND INSULATION: Figures 7.7a and 7.7b compare the mean (SEM) GH from those infants wrapped in less than 10 togs of insulation with those wrapped in 10 togs or more. Generally there is no significant difference between these 2 groups of infants. There is a tendency for the younger infants under less than 10 togs of insulation to excrete more GH than those under greater insulation. This is not significant in morning or night-time samples. Those infants under low insulation excrete a steady level of GH which does not change in either morning or night-time or young or older age groups. Those infants under more than 10 togs tend to have higher morning excretion of GH in the
Figure 7.5a:
Comparison of the morning excretion of growth hormone by young (<8 weeks) and old (>12 weeks), male and female infants.

Figure 7.5b:
Comparison of the night-time growth hormone excretion from young (<8 weeks) and older (>12 weeks) male and female infants.
Figure 7.6a:
Comparison of the morning excretion of growth hormone by both young (<8 weeks) and older (>12 weeks) infants with smoking and non-smoking parents.

Figure 7.6b:
Comparison of the night-time excretion of growth hormone from young (<8 weeks) and older (>12 weeks) infants with smoking and non-smoking parents.
Figure 7.7a:
Comparison of the morning excretion of growth hormone from both young (<8 weeks) and older (>12 weeks) infants under higher and lower levels of insulation.

Figure 7.7b:
Comparison of the growth hormone excretion in night-time urines of young (<8 weeks) and older (>12 weeks) infants under high and low levels of insulation.
older age group than the younger age group morning excretion. This is not significant. Night-time excretion is identical in both young and older infants and this is identical to that of younger age group morning excretion but less than the older age group morning excretion.

**BIRTH ORDER:** Comparison of those infants who were first or second born infants in a family with those who were third or subsequent infants are illustrated in figures 7.8a and 7.8b. All values are given as mean (SEM) μU.mmol⁻¹ creatinine. Growth hormone excretion in morning and night-time samples collected from first or second infants tends to be constant at about 15 μU.mmol⁻¹ creatinine in both the young and old infants. This level of excretion was generally higher than GH excretion of third or subsequent children. In morning urines the third or subsequent infants in the younger age group excreted significantly less GH than the first or second borns (Student's unpaired t-test, p<0.05). Growth hormone excretion in morning urines increased in this group to reach a level almost identical to that seen in the first or second infants in the older age group (15.31 (3.65) μU.mmol⁻¹ creatinine). In night-time urines GH excretion of the third or subsequent infants was lower in both the young and older age groups of infants. This difference between first and second born infants and third or subsequent infants was significant in the younger age group (Student's unpaired t-test, p<0.05) but not in the older.
**Figure 7.8a:**
Comparison of the morning excretion of growth hormone from young (<8 weeks) and older (>12 weeks) infants who were first or second born with those born subsequently in a family.

**Figure 7.8b:**
Comparison of night-time excretion of young (<8 weeks) and older (>12 weeks) infants who were either first or second born or subsequent infants.
SOCIAL CLASS: Figures 7.9a and 7.9b compare the mean (SEM) GH from those infants in families from the higher social classifications (1, 2 and 3) with those from the lower groups (4, 5, 6, and 7). The patterns of GH excretion is almost identical to that observed in the comparison of infants of different birth order.

Growth hormone in morning and night-time urines from those infants of the higher social group was steady at about 15 μU.mmol⁻¹ creatinine. Growth hormone excretion in this group of infants tended to be greater than those of the lower social group. In morning urines there is a highly significant difference between the higher (16.61 (3.42) μU.mmol⁻¹ creatinine) and lower social groupings (4.01 (1.88) μU.mmol⁻¹ creatinine) in the younger age range (Student's unpaired t-test, p<0.05). The GH excretion of the lower social group increases significantly with age to reach 13.83 (2.71) μU.mmol⁻¹ creatinine in the morning samples (Student's unpaired t-test, p<0.05) to reach a level not significantly different from that of the higher social group.

In night-time urines the GH excretion in the lower social group (6.82 (2.25) μU.mmol⁻¹ creatinine) are significantly less than those infants from the higher social group (14.03 (2.79) μU.mmol⁻¹ creatinine), (Student's unpaired t-test, p<0.05) but only in the younger age group. The difference between lower (10.61 (2.44) μU.mmol⁻¹ creatinine) and higher (14.63 (1.91) μU.mmol⁻¹ creatinine) social categories are not significant in the night-time excretion of cortisol in the older infants.
Figure 7.9a:

Comparison of the morning excretion of growth hormone from young (<8 weeks) and older (>12 weeks) infants of higher and lower social categories.

Figure 7.9b:

Comparison of the night-time excretion of growth hormone of young (<8 weeks) and older (>12 weeks) infants of higher and lower social categories.
SUMMARY

1. Males tend to excrete more GH in morning and night-time urines in all but the night-time urines collected from older infants. This does not reach statistical significance.

2. The method of feeding employed until six weeks of age does not significantly affect GH excretion.

3. Infants with non-smoking parents tend to excrete more GH than those with smoking parents this never reaches statistical significance.

4. Those infants insulated by more than 10 togs tended to excreted less GH than those under less than 10 togs. This does not reach statistical significance.

5. Those infants who were first or second born infants tended to excrete more GH than those who were third or subsequent infants. This was significant in the younger but not older groups.

6. Social class shows an almost identical pattern to that for birth order. Those from higher social categories tended to excrete more GH than those from the lower groups. This was significant in both morning and night-time urines of the younger age group only.
CHAPTER 8

THE EFFECT OF IMMUNISATION ON THE URINARY EXCRETION OF CORTISOL AND GROWTH HORMONE
THE EFFECT OF IMMUNISATION ON RECTAL TEMPERATURE AND THE EXCRETION OF CORTISOL AND GROWTH HORMONE.

Introduction.

As part of the normal health care practises of this country virtually all infants are immunised against Diptheria, Pertussis, Polio, Tetanus and Hemophilus Influenzae B at the specific ages of 8, 12 and 16 weeks. Previous studies have shown that the effect of immunisations can be compared with the effect of naturally occurring infections (Jackson et al, 1994). The effect of immunisations and infections on the average night-time rectal temperature are already documented (Rawson, et al, 1990). These studies show that during the prodromal (i.e. Pre-symptomatic) stage of an infection and on the night immediately after an immunisation the night-time rectal temperature of infants is elevated. The effect of immunisations on other systems of the body have not been examined in infants and so the response of both cortisol and GH excreting systems is unknown. However, it is widely accepted that cortisol is excreted in response to psychologically stressful events such as the actual immunisation process (Lewis & Thomas, 1991), tissue trauma and infection (Jeffries & Vance, 1991). In adults it is believed that corticosteroid secretion is stimulated as an early response to acute infection and that glucocorticoids increase in the period of early symptoms and varies in magnitude with the clinical severity of the illness (Beisel & Rapaport, 1969). However, there do not appear to be any actual studies reporting changes in endogenous cortisol with infection and findings are based on patient recovery in response to exogenous cortisol.

Similarly in the case of GH, although it is known to increase in response to cell trauma and chronic illness (Strobl & Thomas, 1994), how it responds in response to immunological challenge is not understood in neither adults nor children. Current research is examining the affects of GH on the immune system (reviewed by Gelato, 1993) but this a new area of research.
Sample

During the collection of urines from infants in their home environment, in addition to urines collected on typical days there were some urines collected within 24 hours of an infants' immunisation against Diptheria, Pertussis, Polio, Tetanus and Hemophilus Influenzae B. The data collected on these nights was used to assess the infantile response to a standard physical stress. Supplementary data collected as part of a continuous project were used to increase the sample size for analysis. A small fraction of the data presented in this chapter was collected out side the main study period.

There were a total of 56 collections of urine made prior to the development of a mature adult-like pattern of night-time temperature, 32 successful attempts in the morning and 35 attempts successful in the evening. 64 immunisation collections were made after the development of a mature temperature pattern, 25 morning collections and 31 night-time collections. As with the analysis of the non-immunisation data the analysis for cortisol and GH were limited by the sample size. Again for completeness the excretion of creatinine was also examined. In both morning and night-time urines collected before and after (i.e. pre- and post-) maturation of the temperature pattern creatinine and cortisol was measured in all the samples (100 %). Analysis for GH was only possible in 15 (47 %) of the pre-temperature maturation morning samples, 12 (34 %) of the pre-temperature maturation night-time samples, 10 (48 %) of the post-temperature maturation morning samples and 12 (39 %) of the post-temperature maturation night-time samples. Night-time temperature recordings were available for all but 14 of the immunisation collections however both the age and the temperature maturity status of the infant was know to within 1 week. This group of infants was made up of primarily male subjects (70 %), who differed little from the main group of infants already described.

It was postulated that immunisation as an obviously stressful challenge may, not only affect the rectal temperature pattern (Jackson et al, 1994) but also affect the urinary excretion of creatinine, GH and in particular cortisol. As there were insufficient data to group into two week age bands for analysis the data were separated into two groups; those pre-temperature
maturation and those post-temperature maturation. This data was then compared with those from a typical day.

The effect of immunisation on night-time rectal temperature

Figures 8.1a and 8.1b compare the mean (SEM) night-time rectal temperatures recorded after immunisation with those from a typical day in infants in both the pre-maturation (i.e. before the adult-like temperature pattern appearance) and post-maturation (i.e. after the appearance of an adult-like temperature pattern) state respectively. In the pre-maturation state (figure 8.1a) the night-time rectal temperature on a typical non-immunisation night falls from 37.12 (0.03) °C at bedtime to a minimum of 36.75 (0.03) °C, 2.5 hours later. On the night immediately after an infant had received a immunisation the night-time temperature pattern was completely changed. Rectal temperature at bedtime was 37.38 (0.09) °C this remained virtually unchanged throughout the entire night-time period. The minimum night-time temperature achieved on immunisation night occurred at about 3.5 hours after bedtime when rectal temperature reached 37.06 (0.08) °C. The night-time temperatures recorded from infants on immunisation night were significantly and consistently higher than those recorded on non-immunisation nights. This was true for the entire night-time period.

In the pre-maturation state (figure 8.1a) rectal temperature on a non-immunisation night typically falls to a minimum of about 36.80 °C while on an immunisation night a minimum of 37.00 °C is achieved. Immunisation occurring in the pre-maturation state significantly elevates rectal temperature (2-way ANOVA on repeated measures, F=1224.08, 1 df, P<0.001). In the post-maturation state (figure 8.1b) the night-time rectal temperature on a typical non-immunisation night falls from 36.92 (0.03) °C at bedtime to a minimum of 36.45 (0.02) °C approximately 2.5 hours later. This is significantly different to the temperature pattern on an immunisation night in the same post-maturation state. The rectal temperatures recorded on post-temperature maturation immunisation nights are significantly higher than those observed on a non-immunisation night (2-way ANOVA on repeated measures, F=190.37, 1 df, P<0.001) in the same temperature maturity state. Rectal temperature on these post-maturation immunisation
Figure 8.1a:
Comparison of night-time rectal temperature recorded on normal pre-maturation control nights and pre-maturation post-immunisation nights.

Figure 8.1b:
Comparison of normal non-immunisation, post-maturation temperature with post-maturation, post-immunisation night-time rectal temperature.
nights falls from 37.17 (0.09) °C at bedtime to a minimum of 36.92 (0.13) °C about 1 hour later before increasing and plateauing at about 37.00 (0.14) °C from 2.5 hours after bedtime for the rest of the night.

Night-time rectal temperatures recorded from infants in the pre-maturation state were greater than those recorded from infants in the post-maturation state. However, the magnitude of the increase in rectal temperature in the post-maturation state after an immunisation is greater than that seen in the pre-maturation state. The variation in the effect of a immunisation on night-time temperature in the post-maturation state also appears to be more variable, with some infants displaying no apparent change in rectal temperatures and others showing very large changes. This is not seen to the same extent in pre-maturation infants.

The effect of immunisation on the excretion of creatinine.

There was little difference between creatinine excretion in urine collected after immunisations in either the morning or urine samples when compared to non-immunisation samples. This was true for both pre- and post-maturation state infants. The morning after a immunisation tended to have smaller concentrations of creatinine than those of non-immunisation mornings but this did not reach significance. Night-time urines immediately after immunisations and on typical non-immunisation nights were identical. The control values used for comparison were taken from age and maturation status matched values obtained from infants taking part in previous parts of this study.

The effect of immunisation on the excretion of cortisol.

Figures 8.2a and 8.2b compare the mean (SEM) excretion of cortisol in urine collected within 24 hours of a immunisation with those on a typical non-immunisation day. In common with the creatinine comparison, control values were matched so that individual infants who were in the same temperature maturation state and of the same age were compared.
Figure 8.2a:
Comparison of cortisol excretion within 24h of an immunisation with normal pre-maturation, non-immunisation excretion.

Figure 8.2b:
Comparison of post-maturation cortisol excretion after an immunisation and on normal post-maturation, non-immunisation.
Pre-temperature maturation.

Prior to the maturation of the temperature pattern (figure 8.2a) cortisol excretion after an immunisation is significantly higher in morning (37.21(3.71) nmol.mmol⁻¹.creatinine) than the excretion seen on non-immunisation mornings (25.38(2.23) nmol.mmol⁻¹ creatinine), (Student's unpaired t-test, p<0.01). The night-time excretion of cortisol (34.55(4.91) nmol.mmol⁻¹ creatinine) is also elevated after immunisation when compared to the non-immunisation excretion (19.42(2.61) nmol.mmol⁻¹ creatinine). This is a significant elevation (Student's unpaired t-test, p<0.01). After immunisation night-time and morning excretion of cortisol are approximately equal and elevated above the average excretion levels seen on non-immunisation days.

Post-temperature maturation

After the maturation of the temperature pattern the excretion of cortisol in morning urines (43.54(8.17) nmol.mmol⁻¹ creatinine) after an immunisation is not significantly elevated above non-immunisation levels (33.02(3.30) nmol.mmol⁻¹ creatinine), (Student's unpaired t-test, p=0.24). In night-time urines (18.00(2.06) nmol.mmol⁻¹ creatinine) the excretion of cortisol after an immunisation is not elevated significantly above the average on non-immunisation nights (14.52(1.87) nmol.mmol⁻¹ creatinine), (Student's unpaired t-test, p=0.22).

As with the changes seen to occur in temperature the changes occurring in cortisol excretion varies between individuals. Some infants excrete cortisol at the typical non-immunisation level on the morning following a immunisation (i.e. about 24 nmol.mmol⁻¹ creatinine) while others excrete levels several fold greater than normal levels. The highest morning excretion of cortisol measured in a morning urine was 235 nmol.mmol⁻¹ creatinine and this value was so extreme that it was regarded as an outlier and not used in the analysis. Usually the concentrations of cortisol measured were never more than about 100-200 nmol.mmol⁻¹ creatinine.
Night-time cortisol excretion above 65 nmol.mmol⁻¹ creatinine or below were never observed after an immunisation. There were a few concentrations of below 15 nmol.mmol⁻¹ creatinine but most infants excreted cortisol in night-time urine at 15-30 nmol.mmol⁻¹ creatinine.

The large changes in cortisol excretion in morning urine and to a lesser extent in night-time urine are not surprising. High plasma levels of cortisol in adults is seen as an indication of stress. It would appear that infants are also capable of mounting an increase in the production of cortisol, and thereby excretion, in response to a stressful challenge as indicated by their response to immunisation. The young infants i.e. those in the pre-maturation state seem equally capable of increasing their cortisol excretion and therefore presumably production as the older infants studied. The older infants appear to be less stressed the night after an immunisation than the younger infants.

**The effect of immunisation on the excretion of GH.**

Figures 8.3a and 8.3b examine the mean (SEM) GH excretion in the pre- and post-temperature maturation state both after a immunisation and on a typical non-immunisation night. Control comparisons are provided by the total normal population.

**Pre-temperature maturation.**

Pre-temperature maturation excretion of GH in morning urines is greater in those urines collected after an immunisation (19.02 (6.24) μU.mmol⁻¹ creatinine) than on a typical day (15.22 (3.48) μU.mmol⁻¹ creatinine). This does not reach significance. Night-time excretion of GH after an immunisation (15.09 (4.22) μU.mmol⁻¹ creatinine) is identical to that on a non-immunisation night (15.27 (2.56) μU.mmol⁻¹ creatinine).

**Post-temperature maturation**

Post-temperature maturation excretion of GH after a immunisation is virtually identical to that for normal sample collection. Morning urine GH excretion after an immunisation is 13.18 (4.51) μU.mmol⁻¹ creatinine and that for night-time is 11.03 (2.16) μU.mmol⁻¹ creatinine.
Figure 8.3a:
Comparison of growth hormone excretion within 24h of an immunisation with normal pre-maturation, non-immunisation excretion.

Figure 8.3b:
Comparison of growth hormone excretion within 24h of an immunisation with normal post-mature, non-immunisation excretion.
Excretion on non-immunisation mornings is 9.70 (2.13) μU.mmol⁻¹ creatinine and on non-immunisation nights 13.81(2.17) μU.mmol⁻¹ creatinine.

Growth hormone excretion is not significantly affected by immunisation.

The different effects of first and second immunisations on cortisol excretion.

The previous results suggested that cortisol excretion was elevated after immunisation. However, the above analysis combined the data from first and second immunisations and the response to a second challenge may be different to that of a first challenge. This analysis was only carried out on cortisol as it was the only hormone which appeared to be significantly affected by immunisation. There was enough data to allow the separation of the first and second immunisation events in both the pre- and post-temperature maturation states. This analysis is presented below.

TABLE 8.1: Number of urines collected from infants in the pre- and post-mature temperature states after first and second immunisations.

<table>
<thead>
<tr>
<th>Time of urine collection</th>
<th>Pre-maturation immunisation 1</th>
<th>Pre-maturation immunisation 2</th>
<th>Post-maturation immunisation 1</th>
<th>Post-maturation immunisation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>morning</td>
<td>n=25</td>
<td>n=5</td>
<td>n=9</td>
<td>n=16</td>
</tr>
<tr>
<td>night-time</td>
<td>n=29</td>
<td>n=5</td>
<td>n=10</td>
<td>n=21</td>
</tr>
</tbody>
</table>

The numbers of urines collected after second immunisations from infants while still in the pre-temperature maturation state are small, as are the number of urines collected after first immunisations in the post-temperature maturation state. Nonetheless it is possible to get some indication of the response trends after each immunisation. The rectal temperature pattern after first and second immunisations in both infants with pre-mature and mature temperature patterns were not significantly different and closely resembled figures 8.1a and 8.1b.
Pre-temperature maturation (figure 8.4a)

In the pre-temperature pattern maturation the mean (SEM) levels of cortisol excretion after both the first and second immunisations is greater than that seen on normal non-immunisation nights. After the first immunisation morning excretion is 35.63(3.90) nmol.mmol\(^{-1}\) creatinine, while control values are 27.61(2.34) nmol.mmol\(^{-1}\) creatinine. This is a highly significant elevation (Student's unpaired t-test, \(p=0.05\)). Similarly, night-time excretion after the first immunisation is elevated significantly (Student's unpaired t-test, \(p=0.03\)). After the second immunisation cortisol excretion is elevated to 45.73(11.22) nmol.mmol\(^{-1}\) creatinine (morning) and 37.50(9.26) nmol.mmol\(^{-1}\) creatinine (night-time). Both of these excretion levels are significantly elevated above non-immunisation levels (Student's unpaired t-test, \(p=0.02\) and \(p=0.05\) respectively).

Post-temperature maturation (figure 8.4b)

In the post-maturation temperature state immunisation generally tends to elevate the mean (SEM) cortisol excretion but not significantly. After the first immunisation in the post-maturation state cortisol excretion is 28.62 (4.44) nmol.mmol\(^{-1}\) creatinine (morning) and 23.63 (4.14) nmol.mmol\(^{-1}\) creatinine (night-time). These are not significantly elevated above the normal excretion rate (Student's unpaired t-test, \(p=0.7\) and \(p=0.26\) respectively). After the second immunisation the cortisol excretion during both morning and night-time periods are not significantly elevated above normal, (Student's unpaired t-test, \(p=0.18\) and \(p=0.54\) respectively).
**Figure 8.4a:**
Comparison of cortisol excretion within 24h of the first and second immunisation in the pre-maturation temperature state with normal pre-maturation, non-immunisation excretion.

**Figure 8.4b:**
Comparison of cortisol excretion within 24 hours of the first and second immunisation, in the post-mature temperature state with post-maturation, non-immunisation excretion.
Summary

1. Rectal temperatures recorded on the night immediately following an immunisation are significantly higher for most of the night than those recorded on a normal non-immunisation night. This is true for both pre-maturation of the rectal temperature pattern and post-temperature pattern maturation after both first and second immunisations.

2. Creatinine excretion is not affected by immunisation events.

3. The excretion of GH remains almost unchanged after immunisation.

4. There is a large variation in the individual response to immunisation with some infants showing rectal temperatures and cortisol excretions little different to those of non-immunisation nights while others demonstrate significant elevation of temperature and cortisol excretion.

5. There is no correlation between the maximum or minimum sleeping night-time temperature achieved on an immunisation night and the excretion of cortisol or GH.

6. Pre-temperature pattern maturation cortisol excretion is elevated in response to both the first and second immunisations.

7. Post-temperature pattern maturation cortisol excretion after the first immunisation and second immunisations is not elevated.

9. Although numbers are small there is suggestion that a difference between the responses displayed after first and second immunisations exists. By grouping the data from the first and second immunisations these differences are masked.
CHAPTER 9

DESCRIPTION OF TRANSEPIDERMAL WATER LOSS FROM THE SURFACE OF THE SKIN
TRANSEPidermal WATER LOSS MEASUREMENTS.

Cross sectional analysis of evaporation measurements.

Evaporation measurements were made from several sites (identified in figure 9.1) from a total of 95 infants between the ages of 0 and 24 weeks. Of these infants 61 (64 %) were male and 44 (46 %) female all of the infants were felt to be well enough by both the hospital staff and the parents to take part in the study. The majority (32 (34 %)) of the infants had been admitted due to bronchiolitis or upper respiratory infections; about 7 (7 %) were admitted for scheduled operations; 15 (16 %) were admitted due to either diarrhoea or vomiting; 9 (9 %) had high temperatures for no apparent reason; a further 11 (12 %) had infections and were receiving antibiotic treatment; 1 had benign meningitis; 1 had asthma; 1 was having a blood transfusion due to low haemoglobin count; 1 was in due to feeding difficulties caused by a cleft palate and 3 infants had survived near miss events. The remaining 14 (15 %) infants were in for observations for a variety of reasons.

Table 9.1 shows the numbers of infants from which evaporation measurements could be made for each of the listed sites. This gives an indication of which were the most accessible for measurement. As can be seen the site 9 (back) was very inaccessible mainly due to the infants laying supine rather than prone. Those infants from which back measurements were made were sitting on their parents' lap. By far the most accessible sites were those on the head and neck.
Figure 9.1: Sites of measurement for TEWL


Difficulties often arose in taking measurements from all infants and from every site when movements occurred. Even in infants who were asleep the act of placing the probe on the surface of the skin often disturbed the infant sufficiently to illicit a movement. This increased the time needed to get a stable reading as well as making it more difficult to note the reading. Figure 9.2 illustrates the mean (SEM) of the evaporation rates from each of the above sites for infants between 0 and 24 weeks. The highest values were measured from site 3 (the neck area) 13.33 (1.13) g/m^2h. The other head sites had evaporation rates of about 10.3 g/m^2h as did the abdomen. The extremities had slightly lower evaporation rates the more distal they were with the lowest measurements being made from the shin (6.17 (0.51) g/m^2h) and calf (7.58 (0.56) g/m^2h). Measurements were not taken from the palms of the hands or the soles of the feet.
Figure 9.2:
Mean TEWL (SEM) measured from each of the sites illustrated in figure 9.1.

Figure 9.3:
Changes in TEWL measurements from the head and face sites with postnatal age.
TABLE 9.1: The number of recordings taken from each of the 14 sites.

<table>
<thead>
<tr>
<th>Skin site</th>
<th>Number of measurements</th>
<th>Skin site</th>
<th>Number of measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. forehead</td>
<td>92</td>
<td>8. chest</td>
<td>55</td>
</tr>
<tr>
<td>2. cheek</td>
<td>92</td>
<td>9. back</td>
<td>7</td>
</tr>
<tr>
<td>3. behind ear (neck)</td>
<td>85</td>
<td>10. abdomen</td>
<td>54</td>
</tr>
<tr>
<td>4. inner upper arm</td>
<td>19</td>
<td>11. inner thigh</td>
<td>42</td>
</tr>
<tr>
<td>5. outer upper arm</td>
<td>31</td>
<td>12. outer thigh</td>
<td>50</td>
</tr>
<tr>
<td>6. inner lower arm</td>
<td>44</td>
<td>13. shin</td>
<td>57</td>
</tr>
<tr>
<td>7. outer lower arm</td>
<td>54</td>
<td>14. calf</td>
<td>48</td>
</tr>
</tbody>
</table>

Analysis of the evaporation measurements.

Six age bands were created each containing between 10 and 20 infants. Table 9.2 show the distribution of infants in each age band; the mean (SEM) ambient temperature measured using a mercury thermometer; the relative humidity as measured by the evaporimeter and the average coverings (togs). As might be expected there are no significant differences between the temperature, humidity or total togs of covering each group of infants was found. Only sites 1, 2 and 3 (forehead, cheek and neck) had sufficient readings in each age band for analysis. Every other site generally had fewer than 10 infants per age band. The evaporation rates from the forehead, cheek and neck with respect to age are illustrated in figure 9.3. The average evaporation rate from all three tends to be higher after the age of 14 weeks. There also appears to be an increase in the range of evaporation rates measured in these older infants. These differences never reach significance. Although these differences may be due to changes in the ability to lose heat with age it is possible that these differences are nothing more than artefact due to increased activity in the older infants.
TABLE 9.2: Thermal environment of hospitalised infant subjects.

<table>
<thead>
<tr>
<th>Age band</th>
<th>Number of infant</th>
<th>Total togs of coverings mean(SEM)</th>
<th>Ambient temperature °C mean(SEM)</th>
<th>Relative humidity of air % mean(SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5 weeks</td>
<td>20</td>
<td>4.00 (0.29)</td>
<td>25.35 (0.45)</td>
<td>28.00 (1.59)</td>
</tr>
<tr>
<td>6-9 weeks</td>
<td>11</td>
<td>3.52 (0.46)</td>
<td>24.45 (0.41)</td>
<td>32.00 (1.82)</td>
</tr>
<tr>
<td>10-13 weeks</td>
<td>19</td>
<td>3.02 (0.28)</td>
<td>24.60 (0.33)</td>
<td>31.65 (1.53)</td>
</tr>
<tr>
<td>14-17 weeks</td>
<td>15</td>
<td>3.86 (0.48)</td>
<td>25.25 (0.50)</td>
<td>31.69 (1.72)</td>
</tr>
<tr>
<td>18-21 weeks</td>
<td>16</td>
<td>3.29 (0.30)</td>
<td>25.71 (1.05)</td>
<td>31.29 (1.77)</td>
</tr>
<tr>
<td>22-25 weeks</td>
<td>11</td>
<td>4.25 (0.46)</td>
<td>24.64 (0.45)</td>
<td>30.64 (2.02)</td>
</tr>
</tbody>
</table>

Summary

1. The room temperature and the relative humidity of the atmosphere varied little in the childrens’ wards of LRI.

2. The infants taking part in this study had between 3 and 4.5 togs of insulation covering them when averaged in age bands.

3. The most accessible sites for measurement of evaporation rates are those of the head and neck. The back is the least accessible.

4. Evaporation rate measurements are generally higher when measured from the surfaces of the head neck and torso. Evaporation rate measurements taken from the more distal surfaces of the limbs are the lowest.

5. Evaporation rates tend to increase slightly with age and have a greater range in older infants. These tendencies do not reach significance.

6. Collection of spot evaporation measurements are difficult to make from moving infants using the evaporimeter.
Longitudinal analysis of evaporation rates.

Infants in this part of the study were recruited and monitored at weekly intervals during a period of sleep over several weeks in order to observe any possible changes in the evaporation rates from the surface of the skin. In addition several changes in the recording of evaporation rates were implemented as suggested by the cross-sectional study. Evaporation measurements were continuously logged at 5 second intervals simultaneously with rectal, skin and ambient temperature thus also allowing the relationship of transepidermal water loss with these changes to be examined. Continuous logging of readings allowed more attention to be given to ensure that the probe remained in close contact with the surface of the face and to the notation of the activity of the infant.

Successful measurements of evaporation rates were made from 23 individuals; 13 (57 %) female and 10 (43 %) male. Between 1 and 8 recordings of evaporation rates were made from each infant during a period of sleep. For means of analysis data collected from infants during day-time sleeps were separated from those collected during night-time sleeps. Night-time sleep data are not presented here as the numbers were too few. This reduced the number of infants to 22, as recordings could only be made from one male infant at the start of night-time sleeps. The collection of night-time recordings of evaporation rates was generally inconvenient as these measurements required that I should be present at a time when often not only the infant but the entire household were retiring to bed for the evening.

My presence and attention for the entire recording period did allowed a brief diary of events to be made. This included such things as the timing of body and eye movements and any events that may have caused artefactual recordings of evaporation rates. From this diary of events time periods were classified simply as either periods of active or quiet sleep. This is not accurate sleep staging but a simple measure of difference in activity. For these purposes quiet sleep is a description of a period of sleep when no eye or body movements were observed. Similarly, active sleep describes a period of sleep when movements occurred. The artefactual
readings corresponding to movement events were removed and the remaining readings averaged every 3 minutes for each recording.

<table>
<thead>
<tr>
<th>Age band</th>
<th>Number of infant</th>
<th>Total togs of coverings mean(SEM)</th>
<th>Ambient temperature °C mean(SEM)</th>
<th>Relative humidity of air % mean(SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-7 weeks</td>
<td>10</td>
<td>9.67 (1.13)</td>
<td>min 17.54(0.88) max. 18.12(0.92)</td>
<td>52.00(4.74)</td>
</tr>
<tr>
<td>8-9 weeks</td>
<td>15</td>
<td>9.43 (1.18)</td>
<td>min 18.57(0.38) max. 19.92(0.43)</td>
<td>52.45(3.81)</td>
</tr>
<tr>
<td>10-11 weeks</td>
<td>16</td>
<td>8.99 (0.93)</td>
<td>min 17.77(0.42) max. 19.23(0.35)</td>
<td>54.14(2.71)</td>
</tr>
<tr>
<td>12-13 weeks</td>
<td>9</td>
<td>8.13 (1.01)</td>
<td>min 18.35(0.67) max. 19.83(0.75)</td>
<td>60.88(4.99)</td>
</tr>
<tr>
<td>14-15 weeks</td>
<td>9</td>
<td>7.79 (0.73)</td>
<td>min 17.16(0.58) max. 18.96(0.55)</td>
<td>54.11(4.96)</td>
</tr>
</tbody>
</table>

Table 9.3 illustrates the distribution of recordings in each age band: the mean (SEM) ambient temperature, the relative humidity and the average coverings (togs). There were fewer infants in the older age band (12-13 and 14-15 weeks) as most infants in this group had ceased to be monitored by this time. There is a tendency for the amount of insulation covering infants during a daytime to decrease with age. The ambient temperature and the humidity of these homes remained fairly constant throughout the time of monitoring although this covered periods during both summer and winter. The amount of insulation used to cover these infants was approximately twice that used for infants in hospital while the room temperatures were lower by up to 8 °C. The humidity of the parental homes of these infants was also greater than those observed in the hospital.

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Transepidermal water loss, skin and rectal temperature changes and percentage of quiet sleep

Figure 9.4 illustrates the mean (SEM) changes in the facial evaporation rate, rectal temperature, forehead skin temperature and the percentage of quiet sleep from the start of a daytime sleep for a duration of 30 minutes. The evaporation rate increases gradually with time from 13.95 (0.51) g/m$^2$h at t=0-3 minutes to reach 16.12 (0.90) g/m$^2$h at t=28-30 minutes. Rectal temperature simultaneously decreases over the same period from 37.07 (0.03) °C (t=0-3 min) to 36.93 (0.04) °C (t=28-30 min). The skin temperature of the forehead increases from 31.74 (0.26) °C, t=0-3 min to 32.39 (0.23) °C, t= 28-30 min. Similarly the percentage of quiet sleep periods increased with time from 26 % at t=0-3 min to 70 % at t=28-30 min.

There are good positive correlations between the changes in evaporation rate measurements, skin temperature and the percentage of quiet sleep over time and a negative correlation between changes in evaporation rate and rectal temperature with time (table 1) so that as rectal temperature and the number of movements fall, skin temperature and TEWL rise.

**TABLE 9.4:** Correlation of changes in the average, evaporation rate, skin and rectal temperatures and the percentage of quiet sleep, over time for the group of infants studied.

<table>
<thead>
<tr>
<th>Correlation coefficients</th>
<th>Evaporation rate</th>
<th>Rectal temperature</th>
<th>Skin temperature</th>
<th>Percentage of quiet sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaporation rate</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectal temperature</td>
<td>-0.93</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin temperature</td>
<td>0.89</td>
<td>-0.89</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Percentage of quiet sleep</td>
<td>0.91</td>
<td>-0.96</td>
<td>0.88</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 9.4:
Changes in TEWL rate, rectal and skin temperatures and the percentage of quiet sleep periods observed during the first 30 minutes of daytime sleep.
Comparison of young and old infants

Figure 9.5 illustrates the same mean (SEM) data divided into two age groups, those infants of 10 weeks or under and those of 11 weeks or over. There is no significant difference between either the skin temperature, the rectal temperature or the percentage of time spent in quiet sleep. Those infants under 10 weeks of age do tend to have lower forehead skin temperatures with larger standard errors from \( t = 0-3 \) minutes to \( t = 19-21 \) minutes. Similarly there is a tendency for the younger infants to have lower evaporation rates than the older infants.

As illustrated in table 9.5 the young infants show strong correlations between changes in skin temperature and quiet sleep; changes in evaporation rate and quiet sleep and changes in skin temperature and rectal temperature over time. The correlation between the changes in the facial skin temperature and the evaporation rate and rectal temperature and evaporation rate with time are not quite as strong as the correlations with time in older infants. The changes in evaporation rate over time is more strongly related to the increase in quiet sleep periods than either changes in rectal or skin temperatures.
Figure 9.5:
Changes in TEWL rate, rectal and skin temperatures and the percentage of quiet sleep periods observed during the first 30 minutes of daytime sleep, young and old infants.
TABLE 9.5: Correlation of the changes in the average evaporation rates, skin and rectal temperatures and percentage of quiet sleep, over time for young infants.

<table>
<thead>
<tr>
<th>Correlation coefficients</th>
<th>Evaporation rate</th>
<th>Rectal temperature</th>
<th>Skin temperature</th>
<th>Percentage of quiet sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaporation rate</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectal temperature</td>
<td>-0.61</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin temperature</td>
<td>0.66</td>
<td>-0.73</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Percentage of quiet sleep</td>
<td>0.73</td>
<td>-0.67</td>
<td>0.87</td>
<td>1</td>
</tr>
</tbody>
</table>

In the older infants there are very strong correlations between the changes in rectal and skin temperature, the changes in the amount of quiet sleep and the evaporation rates observed taking place over time. The correlation between changes over time of rectal temperature and evaporation rate, skin temperature and percentage of quiet sleep are negative and much stronger than those observed in the younger age group. This seems to suggest that those infants of 11 weeks or older increase the amount of heat lost via facial evaporation in response to changes in temperature over time better than infants of 10 weeks or less.
Table 9.6: Correlation of the changes in evaporation rates, skin and rectal temperatures and percentage of quiet sleep, over time for old infants.

<table>
<thead>
<tr>
<th>Correlation coefficients</th>
<th>Evaporation rate</th>
<th>Rectal temperature</th>
<th>Skin temperature</th>
<th>Percentage of quiet sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaporation rate</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectal temperature</td>
<td>-0.94</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin temperature</td>
<td>0.87</td>
<td>-0.86</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Percentage of quiet sleep</td>
<td>0.88</td>
<td>-0.96</td>
<td>0.80</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 9.6 compares the mean (SEM) evaporation during active sleep periods in both young and old infants with those obtained during quiet sleep periods at t = 13-15 minutes. The younger infants have lower evaporation rates in active (student's unpaired t-test, p=0.04) but not quiet sleep periods (student's unpaired t-test, p=0.21), than the older infants. There is no significant difference between the average amount of active and quiet sleep periods in both young and old infants at t = 13-15 mins. In young infants the evaporation rates observed in active sleep tend to be marginally less than those during quiet sleep but this does not reach significance.
Summary

1. Water loss from the face increases with time from the start of sleep. This correlates well with the increase in skin temperature, and amount of quiet sleep. At the same time as these increases rectal temperature decreases.

2. Younger infants (<10 weeks old) tend to lose less heat by evaporation from the face than older infants (>11 weeks old) at t = 13-15 min.

3. The changes in evaporation rates overtime from the face in younger infants is not as strongly correlated to changes in rectal and skin temperature as those found in older infants.
Figure 9.6:
Average TEWL from the face of young and old infants during sleep and during both active and quiet periods of sleep.
DISCUSSION
CHAPTER 10

DISCUSSION OF THE RESULTS
DISCUSSION.

The main aim of this study was to describe the normal pattern of neuroendocrine development in the human infant, with particular reference to the hypothalamic-pituitary-adrenal axis, over a period of time already identified as one of dramatic change. Few studies have examined this important system which in adults at least, maintains and prepares every other organ of the body for life. Furthermore, because of its widespread effects the development of the HPA may hold vital clues to one of the most frightening and common causes of infant death in the Western world - SIDS. Investigation of a system with such vast arrays of possible effects is difficult. However, as so little is known it was decided that fairly simple, direct aims could be set.

Objectives

1. Describe the pattern of urinary excretion for cortisol and GH establishing reference ranges and the presence or absence of circadian periodicity. Development of circadian periodicity may be indicative of the stage of general maturity.

2. Relate changes in the urinary excretory rates of cortisol and GH to the maturation of the rectal temperature pattern. Changes in hypothalamic function controlling hormone production may occur over the same period as changes in HPA function controlling body temperature.

3. Examine the effect of different aspects of child care, family health and social status on the excretion of cortisol and GH. These factors have already been shown to be associated with an increased risk of SIDS and delayed appearance of a mature rectal temperature and have been postulated to effect stress in individuals.

4. Describe the effect of immunisation on the excretory rate of both cortisol and GH. Cortisol and GH are essential parts of the stress response. Immunisation has already been shown to be a stressful event disturbing rectal temperature. The effect of immunisation on cortisol and GH excretion further characterises the infant's ability to cope.
5. Measure TEWL from the surface of the skin establishing the effect of site of measurement, age and activity of the subject on TEWL rates. TEWL regulation is another function of the HPA. Changes in the ability to lose heat via water loss from the skin may play a vital role in the maturation of the rectal temperature pattern.

In order to address these 5 objectives it was necessary to use the most appropriate means of data collection possible. The first part of the discussion addresses the suitability of each method used to study temperature, TEWL measurement, urinary excretion of cortisol, growth hormone and creatinine. The second part discusses the development of the rectal temperature pattern in this group. The third part of the discussion examines the effect kidney maturation and the excretion of creatinine may have on the urinary excretion of cortisol and GH. The fourth part of the discussion reflects on the success of this study in addressing each of the 5 aims described above, what the results tell us about the HPA, and how it might be implicated in SIDS. The final section summarises this study and proposes further areas of work.

**Part 1: Appropriate means of data collection?**

**Temperature**

The measurement of core body temperature was made using the rectal probe. This method of continuous measurement of deep core body temperature in the home was generally accepted by parents and infants alike and continues to be extensively used for research purposes in Leicester. However, in both scientific and clinical communities there has been much debate about the most suitable method and situation of body temperature measurement. The oesophagus at cardiac level (Aikas, Karvonem, Pironen, Ruosteenoja, 1962) and the external auditory meatus (Straton, 1977) have been suggested to be more ideal locations. Poole & Stephenson (1977) evaluated rectal temperature measurements in rats in a similar manner to
both of the above methods concluding that this was a reliable location for continuous measurement of body temperature.

In man the rectal site for temperature measurement has been further clouded by safety. Early use of mercury glass thermometers in the newborn occasionally lead to rectal perforation and pneumoperitoneum (Buntain, Pregler, O'Brien & Lynn, 1977). However, the use of slim, soft rectal probes correctly inserted to within 5 cm of the anal margin make the possibility of perforation negligible (Morley, Hewson, Thornton & Cole, 1992).

No other suitably accurate, safe and acceptable site of measurement for core body temperature is available for routine use.

Urine

Urine samples were collected using a paediatric 24h U-bag (Hollister). These were tolerated well by parents and infants but were not without some problems. This method of collection, at least in Leicester Royal Infirmary, is infamously unpredictable and on the childrens wards of LRI the volumes of collection are rarely above 5mls. With perseverance and by allowing the parents to contribute to the method and timing of use, a success rate of about 50% was achieved over the collection period. (The success of urine collection in Leicester research team has improved so that now this is increased to about 70% of all attempts at urine collection being successful). A successful urine collection by this method provided urine volumes generally in excess of 10mls and up to 200 ml. Unfortunately, due to the design of both urine collector and female infants, urine collection from females was less successful than from the experimentally well designed male! Other researchers have overcome some of the problems of infant urine collection by using more complex systems including suspension nets and collection trays, filter paper pads and extracting urine from nappies (Beeram & Dharureddy, 1991). These methods were either inappropriate for home use or inadequate for the required chemical analysis as they either increased contamination of the sample or did not allow a sample of adequate volume to be collected. For this study approximately 10 ml of urine was adequate for urinalysis for creatinine, cortisol and growth hormone to be carried
out. A minimum of 70% of the successful urine collections allowed analysis to be carried out for all three analytes (i.e. at least 35% of all attempts at urine collection) and at least 91% of all successful collections allowed analysis for creatinine and cortisol.

A few infants reacted to the self-adhesive flaps attaching the urine collector necessitating withdrawal from the study. The main difficulties with urine collection with the U-bag were those of initially getting the collector attached securely and then getting it to drain adequately. Often, in the early stages of collection parents would report that their child and cot had been completely soaked with urine but that the urine collection pot was empty. It was found that these parents had attached the bag adequately to collect urine, urine had collected but not drained and the weight of the fluid had pulled the U-bag off. Modern nappies appear to be capable of consuming large quantities of urine but only over long periods of time and so were unable to cope with copious quantities released from the full U-bag over a relatively short time period, hence soaking the child and wetting the bed. By encouraging drainage (allowing air displacement and the cunning use of gravity) this problem was overcome so that now such reports are rare.

**TEWL measurement.**

The measurement of TEWL was made using an evaporimeter. This method of measurement was well tolerated but very sensitive to movement and environmental conditions. One of the major disadvantages of this method of measurement was that the probe unlike those for temperature could not be left attached to the baby. It is well known that the presence of an investigator disrupts observations and that a probe that can be monitored in a remote fashion without being too intrusive is preferable. However, measurement of evaporative water loss from the skin has always been difficult to make. Use of whole body plethesmography makes it difficult to separate water loss from the skin from those from the lungs, urine and faeces (Azaz, Fleming, Levine et al 1994), similar problems are encountered when the loss of body weight is assessed over time (Day, 19389). Other methods of study have utilised the electrical conductivity of the skin (Curzi-Dascalova, Pajot & Dreyfus-Brisac, 1974), dew point hygrometers (Sagot, Amoros, Candas & Libert, 1987) and moisture collection techniques.
(Brisson, Boivet et al 1991; Phillips 1980). After consideration each of these techniques proved to be inappropriate for use in the home.

Many studies have been carried out on hospitalised infants utilising the evaporimeter which have been successful (Khan & coworkers, 1987; Hammarlund and co-workers 1977-1983; Rutter, 1985, to name but a few). These studies along with those of Pinnagoda, Tupker Coenraads and Nater (1989) found the evaporimeter to be easy to use and to give reproducible measurements. My own experience of the evaporimeter in both the hospital and home environment led me to the conclusion that they are far from ideal for measurement of TEWL but that they are adequate for the estimation of TEWL from quiet, preferably inactive, sleeping infants. In order to get the best readings of TEWL possible, from the evaporimeter continuous recording of TEWL should be made rather than spot readings. Spot readings, especially on active infants can be artefactual because of movements and difficulty in choosing the most appropriate screen reading. Continual logging of the measurements allows a value to be obtained by averaging the readings over a period of time so reducing the artefact.

Many families with young infants also have small curious children who are drawn to a new person, the new baby and a 'box with flashing lights'! The increased air movements caused by such intrigue and the normal household movements cause problems because of the need to firstly hold the measuring probe in position next to the skin surface without disturbing the infant and secondly because of the probes sensitivity. Furthermore, the measuring probe of the evaporimeter is easily damaged by contamination as mundane as sweat and water. Infants and young families hold many hazards for such a probe which are more easily avoided in the laboratory than in a household.

Although the evaporimeter was adequate for this study a more detailed study would necessitate a measuring probe that, like the temperature probes, could be attached to the preferred skin site and left to record the data over a period of time. In addition the preferred probe would be sensitive to TEWL but not as sensitive to movement and environmental conditions or to contamination.
Chemical analysis of urine.

The techniques used for analysis of urine for creatinine and cortisol are well proven, standard and widely used methods in the clinical setting. These techniques proved to be adequate for the study of the infant population although the cortisol assay needed minor modification as described in the methodology.

The technique used for the analysis of growth hormone was a newly developed technique which appears to be adequate to measure urinary growth hormone in the infant population without necessitating pre-treatment or dialysis of the urine. The adaptation of the serum assay by ourselves, was being considered by the assay manufacturer (IDS) but who had more pressing development priorities. However, using our preliminary modifications they intend to investigate development of this assay for commercial markets. The largest advantages attributable to this method of growth hormone estimation was the low cost, the reasonable run schedule and above all the lack of pre-treatment or dialysis.

Dialysis of the subject urines would have required larger urine volumes and increased the run schedule length by at least 1 day. Dialysis is not only time consuming but also tedious and introduces the scope for large technical errors in this assay. However, for many years now, the argument about the necessity of dialysis for the accurate measurement of urinary growth hormone has continued so that most if not all assays available for the measurement of urinary growth hormone require dialysis or some other pre-treatment (Hourd & Edwards, 1994). The validation procedure for this assay indicate that dialysis far from improving this particular technique was actually detrimental, adding further fuel to the already great argument over the need for dialysis.

We did benefit from the fact that we were studying an infant population who secrete and therefore excrete large amounts of growth hormone so that although the modified assay was adequate for our subject population it may not be suitable for children or adults who secrete less growth hormone.
Part 2: The development of night-time rectal temperature pattern.

Following on from the studies of Hellbrügge (1960), Mills (1975), and Minors & Waterhouse (1980), Lodemore (1993) further characterised the pattern of night-time rectal temperature. She established that the development of rectal temperature patterns was best described as a 3 stage process:

The neonatal stage lasting from birth to about 2-4 weeks of age. Where night-time temperature remains stable during sleep at a level just below daytime levels;

The pre-rhythm stage between about 4 and 14 weeks of age. Where the rectal temperature is above 37°C in the evening, falling to about 36.8°C with night-time sleep, rising only when the infant wakes for a feed during sleep;

The post- rhythm stage. When the rectal temperature is considered to develop a minimum rectal temperature below 36.5°C with night-time sleep.

Of these three stages the later stages of pre- and post- rhythm were of direct relevance to this study. However, the naming of the transitional period and stages by Lodemore has caused some debate as some researchers believe that circadian rhythms are present in the foetus and neonate (Miram & Kok, 1991) and the fact that full 24 hour recordings were not made. Although the same criteria were used for identification purposes in this study, the word 'rhythm' was replaced by maturation to indicate that a pattern of night-time rectal temperature similar to that of the adult was either absent (pre-maturation) or present (post-maturation). As in the study carried out by Lodemore (1993) the timing of the change from the pre- to the post-mature temperature pattern varied between individuals with the timing of maturation week ranging from 4 to 18 weeks of age with a mean of about 11 weeks. The pattern of development was similar in all infants with only the timing of maturation week differing. The only time that the mature pattern of temperature was altered was when infants were ill or had just received immunisations.
In common with previous studies certain factors affected the timing of maturation. The way in which an infant was cared for and family social factors affected the physiological development of temperature regulation. Infants who developed mature temperature patterns earlier generally tended to be marginally heavier at birth, from families of higher social categories, with older mothers, who were breast feeders and non-smokers. However, breast feeding, non-smoking and being an older mother are all factors associated with the higher social categories. Multivariate analysis would be necessary to investigate the contribution of each of these factors to the physiological effects on temperature pattern development. For such analysis to be carried out looking at about 8 different factors necessitates a very large sample size. Although 77 individuals were included in this study this is not large enough to get sensible relationships from such a complex statistical analysis. Tuffnell (personal communication) has processed the complete data set collected over several years by the Leicester based research team. This has included 1197 infants and has shown that low birth weight, supine sleeping, high environmental temperature, weight at monitoring and bottle feeding in combination with smoking parents all increase rectal temperature. Furthermore, the description of the change in rectal temperature as a step-like transition is not strictly true. Those infants achieving a mature rectal temperature of 36.5 °C or below at an early age are actually undergoing the transition from pre- to post- mature so quickly it appears to be a step while those later developers probably start the transition at the same age as the early developers but take longer to achieve the maturation criteria i.e. the slope of transition is steeper in the earlier developers. Nonetheless the classification of pre- and post- temperature maturation remains a useful tool for grouping individuals.
Part 3: The kidney and creatinine excretion (reviewed by Houston & Oetliker, 1974).

At birth the kidney is both histologically and functionally immature. Over the first 1-2 years of life the function of the kidney gradually changes to reach maturity when the glomerular filtration rate, after correction for height remains constant into adulthood. The initial adaptations to post-natal life are rapid and unlikely to correlate to anatomical differences. The foetal kidney is geared to the production of vast quantities of urine. This does not cause a problem as the placenta provides ample replacement of both water and salt. In the extrautrine environment salt and water must be conserved as these are not in constant supply. Whatever the gestation age, all babies at birth show a low rate of tubular reabsorption of sodium and high urine flow. Within the first 24 hours after birth, sodium retention increases and water loss decreases before gradually increasing with rehydration of the first week. Glomerular filtration follows the same pattern as reabsorption but in a less pronounced manner.

Glomerular filtration in the infant

In the infant glomerular filtration rate (GFR) is disproportionately low in the newborn and then it gradually increases with age (Barnett, 1940). In the first month of life the GFR is about 25% of the expected value, by 3-6 months about 50% and about 75% by 6-12 months. It is possible that this relates to the fact that there is a maturation of the glomeruli over this period and that blood pressure and renal vascular resistance change. Some evidence exists suggesting that the porosity of the glomerular capsule is low and the surface area for each glomeruli is also low.

Tubular function

Saline loads are not well tolerated by the neonate as they are not able to remove the excess salt from the blood, as their prime aim is to conserve salt as it is more likely that salt wastage will occur. In infants the removal of para-aminobipiric acid from plasma is only about 50% of that seen in the mature kidney, showing that the tubular secretion of this substance is
not mature. It is possible that other secretory mechanisms are also reduced. The immaturity of tubular function in infancy can be explained by the fact that both the diameter and the length of the tubules is smaller than those seen in adults.

**Water handling**

The infantile kidney is only capable of concentrating urine to a maximum of 700-800 mosmol/Kg i.e. about half that of an adult or child (1200-1400 mosmol/Kg) and dilute urine to the same level as that of older individuals (50mosmol/Kg). Excess water is not as effectively excreted in the infant as in the adult.

**Creatinine**

Creatinine is an endogenous metabolite of protein which can be used to estimate the glomerular filtration rate of the kidney. It is not as accurate as measuring inulin clearance (an exogenous chemical) as it is also excreted in small amounts by the renal tubules and therefore over estimates GFR. In newborn infants urinary and plasma creatinine is high, it rapidly falls over the first days as the creatinine clearance by the kidney increases (Sertel & Scopes 1973) and the creatinine accumulated during foetal life is removed. After the initial removal of creatinine following birth, plasma creatinine correlates to the lean body mass of the individual and therefore the amount of creatinine in the urine will be dependent on the body composition to a certain extent.

Creatinine clearance has been shown to follow a circadian pattern in some studies (carried out in adults and children) but not others (Borst & de Vries, 1950; de Vries,ten Holt, van Daatselaar et al, 1960). It is does not appear to have a large amplitude rhythm when it is present and this is probably due to the fact that although there is a circadian variation in GFR this is counteracted by the inverse rhythm of tubular secretion (van Acker, Kooman, Koopman et al 1992).
Is the excretion of cortisol or growth hormone affected by the excretion of creatinine? What is the pattern of excretion of creatinine? Is this affected by different child care practices, social factors or immunisation? As summarised above the infant kidney is undergoing functional changes over the period of this study. Similarly, creatinine excretion does not truly reflect glomerular filtration. However, as we were unable to collect the total amount of urine produced over a certain time period expressing cortisol and GH excretion relative to the excretion of creatinine is the best method of standardisation for the concentration of urine available. Creatinine excretion gives some indication of urine concentration although it also depends on the body composition, diet, and kidney function. Body composition and diet in an infant population aged between 4 and 17 weeks varies very little between individuals. Some infants may be bottle fed and some breast fed but as equal numbers of each took part in this study the effect of such should be negligible. Similarly, some infants may have been introduced to solids but these were very few and in general their major food source continued to be milk.

In order to ensure that expressing both cortisol and growth hormone relative to creatinine excretion was not creating artifactual patterns all data obtained for creatinine were analysed in an identical fashion to that of the cortisol and GH.

Firstly, although creatinine excretion increased marginally with age it did not develop a circadian periodicity. The reference range established for the urinary creatinine in the 6 to 17 week old infants in this study is between 1 and 2 mmol.l⁻¹. Secondly, creatinine excretion did not appear to be affected by the maturation of the rectal temperature pattern. Thirdly, none of the factors examined for affect on cortisol and growth hormone excretion affected creatinine excretion. Finally, creatinine excretion was not significantly affected by immunisation.

These four observations of creatinine excretion imply that all observed changes in both cortisol and growth hormone excretion are due to changes in the production of these hormones and the amount of free hormone circulating the infant body rather than any changes in creatinine.
excretion. Expressing the excretion of cortisol and growth hormone relative to the creatinine content of the urine sample does not create artifactual patterns of excretion.

Part 4: Changes in the excretion of cortisol and GH, how they reflect changes in the HPA and how they might be implicated in infant well-being and SIDS.

Each of the 5 objectives outlined at the start of the discussion are addressed in turn below:

1. Describe the pattern of cortisol and GH excretion, establishing reference ranges and the presence or absence of circadian periodicity.

Cortisol:

Early studies of cortisol secretion suggest that a circadian pattern of secretion does not exist until, at the earliest, 4 weeks of age (Martin-Du-Pan & Vollenweider, 1967; Vermes et al, 1980). The study carried out by Vermes et al, suggest that in the sample this was not a clear circadian pattern, synchronised to the sleep-wake cycle until 3 months of age. The pattern of excretion of urinary free cortisol as carried out in this study appears to confirm this. In the very early (5-6) weeks although a morning/night-time difference between excretion rates exist it is not significant until the infant is older. The difference between morning and night-time excretion rates increases as morning excretion increases and night-time excretion decreases with age. This means that the development of cortisol excretion is similar to the development of rectal temperature patterns. Furthermore, when normalised to the first appearance of a mature temperature pattern the pattern of development becomes clearer and similarly to the development of rectal temperature patterns, cortisol can be assigned to a 3 stage process:

Stage One: From 5-6 weeks pre-maturation of temperature pattern (6-7 weeks of age but possibly from birth). A tendency for higher excretion of cortisol in morning urine exists but this is not significantly higher than night-time excretion.
Stage Two: From 1 to about 4 weeks pre-maturation of temperature pattern (8-12 weeks of age). There is a definite and steady significant difference between morning and night-time excretion of cortisol.

Stage Three: From maturation week until at least 4 weeks post-maturation (12-17 weeks of age). The difference between morning and night-time excretion of cortisol increases as the excretion rate in morning urine increases and that in night-time urine decreases.

This three stage classification is almost identical to that created by Lodemore (1993) for the description of temperature maturation. This type of description of a pattern is probably an over simplification which like the rectal temperature pattern would require many more observations to clarify as true or not. It is probable that like rectal temperature maturation, the maturation of cortisol excretion shows a similar transitory period with some infants developing faster than others.

In general the normal excretion rates of cortisol from about 7 weeks of age have to be separated into morning and night-time ranges. Prior to this age the reference ranges for morning and night-time excretion overlap. From this study the average excretion rates for cortisol are 25 - 30 nmol.mmol⁻¹ creatinine (morning) and 12 - 18 nmol.mmol⁻¹ creatinine (night-time). Concentrations of cortisol greater than 70 nmol.mmol⁻¹ creatinine appear to be outside the normal range for excretion. This chosen range corresponds well with the reference ranges calculated (3.4 - 59.4 nmol.mmol⁻¹ creatinine (pre-maturation) and 3.8 - 63.4 nmol.mmol⁻¹ creatinine (post-maturation)) to obtain a 95% confidence interval (personal communication Tim Davies). The younger infants tend to have higher night-time and lower morning excretion rates than older infants.

As in adults the excretion rates of cortisol are very variable in infants. This variability is due to varying responses to environmental cues. Cortisol excretion in an individual may be high one day and low the following, for no apparent reason, making observation of circadian periodicity difficult to identify. By combining data from many individuals such variation can
be smoothed out making the circadian variation more obvious and allowing the suggestion of average reference ranges to be made.

**Growth Hormone.**

There have been few studies on growth hormone secretion and fewer still on growth hormone excretion. Studies which have been undertaken have generally focused on growth retarded children or agromegalic adults, few examine infantile excretion. Signs of circadian periodicity have been reported at about 3 months (Vignen et al, 1971). However, other studies have been unable to identify such a pattern until 1-2 years of age (Finkelstein et al, 1971; Shaywitz et al 1971). Grouping the individual data together, as for cortisol, did not reveal any circadian periodicity whether represented with respect to chronological age or maturation of rectal temperature pattern. However, there was some evidence of circadian periodicity in the older (12 - 15 weeks of age) female infants but not in male infants. This appears to suggest the existence of a further physiological difference between male and female infants adding to other differences such as those observed in heart rate (Pratt, personal communication) as well as suggesting that growth hormone circadian periodicity is either being masked by other factors or does not develop within the 6-15 week age band. However, as a sample population the rate of growth hormone excretion in male and female infants varies little with age and there is no distinct morning/night-time difference as seen in cortisol excretion. The average reference range for growth hormone excretion between 6 and 15 weeks of age is about 10 - 18 μU.mmol⁻¹ creatinine.

To conclude the first objective of this study has been addressed, average reference ranges for the excretion of cortisol and GH are now available for 2-3 month old infants, cortisol has been shown to exhibit circadian periodicity after 5-6 weeks of age, while GH excretion is more complex requiring further investigation to clarify the presence or absence of circadian periodicity.
2. Relate changes in the excretory rates of cortisol and GH to the maturation of the rectal temperature pattern.

Cortisol:

This study suggests that there is an intimate link between the development of a mature rectal temperature pattern and the excretion of cortisol so much so that all cortisol data was analysed after normalisation to the first appearance of a mature rectal temperature pattern. Maturation week appears to mark, not only the first appearance of a pattern of night-time rectal temperature little different to that of the adult but also the week that morning and night-time cortisol excretion rates begin to diverge. This strongly suggests that either one mechanism which contributes to both temperature and cortisol regulation is maturing or synchronising with environmental cues; or that changes in cortisol excretion trigger off a change in rectal temperature pattern or vice versa. The hypothalamus is considered to play equally important roles in both temperature and cortisol control in addition to being vital in the generation of circadian rhythms. Does this change in both cortisol and temperature regulation occur as a result of changes in the hypothalamus?

Growth hormone:

Unlike cortisol, growth hormone does not appear to be significantly effected by the appearance of a mature rectal temperature pattern. It was not felt necessary to normalise this data to maturation week as in cortisol. There are several factors which may account for this difference in development between cortisol and growth hormone excretion. Firstly, growth hormone circulates the body bound to growth hormone binding proteins which are known to be low at birth and which may be increasing at the time of investigation. If this is so then, although the total amount of growth hormone may be increasing in the plasma the amount of free growth hormone may be decreasing. A reduction in the amount of free growth hormone in plasma would reduce the amount of growth hormone in urine. However, this change in binding protein would only account for the absence of circadian periodicity if the production of such protein was out of phase with production of growth hormone. There is no evidence for a
circadian periodicity in neither the production of growth hormone binding protein nor its ability to bind protein in adults (Snow et al, 1990). Secondly, the study period of 6-15 weeks may be too short to observe the ontogeny of a circadian pattern of growth hormone secretion. Thirdly, the grouping of the data may be masking changes that are occurring e.g. a male/female difference. Fourthly, growth hormone excretion may be influenced by kidney function in a different way to cortisol. This would seem unlikely as free cortisol and free growth hormone are both filtered and reabsorbed by the kidney in a similar fashion so both would be expected to be affected in a similar fashion by kidney maturation or malfunction.

Again the general objective of this study has been fulfilled. Cortisol excretory changes have been found to be closely related to changes in rectal temperature regulation. GH excretion does not appear to correlate with changes in rectal temperature. The changes in cortisol excretion discussed above are highly dependent upon the development of a mature pattern of body temperature with the largest increase in the circadian amplitude coinciding with the week rectal temperature falls with night-time sleep to below 36.5°C. Both changes in cortisol excretion and body temperature must confer advantages. Low night-time body temperature allows the conservation of vital energy stores while decreased night-time cortisol reflects or results in the reduced stress of night-time sleep, perhaps due to the reduction in the energy requirements. It may be that the longer an infant has higher night-time rectal temperatures and higher levels of cortisol excretion the longer the period of 'stress' and vulnerability and therefore possible increased risk of illness and SIDS. How GH might fit into this model of vulnerability is not clear.

3. Examine the effect of different aspects of child care, family health and social status on the excretion of cortisol and GH.

Similar factors previously studied for association with delayed development of a mature rectal temperature pattern were examined for influence on the excretion of cortisol and growth hormone. Some of these factors are also associated with an increased risk for SIDS.
Cortisol:

The most important influences on cortisol excretion appear to be sex, method of feeding, parental smoking and the amount of insulation used while birth order and parental social categories were less important. The stage of temperature maturation was also extremely influential of the excretory rate of cortisol.

There were distinct differences between male and female infants. Females excreted more cortisol in morning urines in both the pre- and post-temperature maturation states than did males. In night-time urines this relationship was only true in the pre-maturation state while in the post-maturation state males excreted more cortisol than females. It appears that female infants in the post-maturation stage of temperature development have got a well established circadian periodicity for cortisol excretion while males show evidence of a circadian periodicity but of a smaller magnitude to that of the females. Are females more able to respond to stressful events or are they more stressed? The significance of such sex differences is difficult to understand.

There were no significant differences between breast and bottle fed infants in the pre-maturation temperature developmental stage although bottle fed infants excreted marginally more cortisol in morning urines and significantly more in night-time urine in the post-maturation weeks. This perhaps reflects the fact that bottle fed infants tend to have fewer feeds than do breast fed infants and so the stress of utilising the body's energy stores rather than receiving frequent direct energy supplies may increase the cortisol excretion. This would become particularly obvious in the older infants, who if bottle fed begin to sleep through without a mid-night-time feed while breast fed infants continue with night-time feeds.

Parental smoking threw-up a surprising finding with those infants whose parents smoked and were in the pre-maturation stage of temperature development showing significantly lower cortisol excretion than those infants with non-smoking parents. Those infants from non-smoking households appeared to display evidence for circadian periodicity of cortisol in the pre- and post-maturation weeks while those from smoking households only display
moming/night-time differences in the after temperature pattern maturation. This appears to suggest that those infants in smoking households show delayed development of circadian periodicity and that perhaps cortisol circadian periodicity should appear before temperature pattern maturation as in the non-smoking households. However, it is just as likely that those infants in smoking households are changed and fed at different times in the morning than those in non-smoking households and that results in the differences in morning excretion.

Those infants under less than 10 togs of insulation were compared with those infants under more than 10 togs of insulation. In both morning and night-time urines the excretion of cortisol was higher prior to the development of a mature temperature pattern in urine collected from highly insulated infants when compared with those under less insulation. After the development of a mature temperature pattern the difference between those under high and low levels of insulation was insignificant. This appears to suggest that those infants under high levels of insulation are stressed. Once a mature temperature pattern is developed the ability of the infants to cope with high insulation appears to increase and the need to respond to such a stress by increasing cortisol disappears.

Neither social class nor birth order revealed an effect on cortisol excretion.

Growth Hormone:

Growth hormone excretion again produced some interesting results. Due to the large range of values obtained for growth hormone there were few highly significant differences.

The sex of the infant did appear to influence growth hormone excretion with male infants exhibiting a constant rate of excretion in both morning and night-time samples in both young and old infants. Meanwhile young female infants excreted a lower but constant amount of growth hormone in both mornings and night-time samples. However, in the older infants female growth hormone excretion had increased in morning urines to become approximately the same as male excretion while night-time excretion remained constant. Possibly these tendencies indicate that female infants begin to develop a circadian periodicity of growth hormone excretion and males do not, or at least do so later.
The method of feeding at six weeks did not show any obvious effects on growth hormone excretion.

Parental smoking did not affect growth hormone excretion vastly. However, those infants in non-smoking households tended to excrete more growth hormone than those from smoking households.

Infants under higher levels of insulation appeared to excrete a constant amount of growth hormone in both morning and night-time urine and in young and old infants. Those under more insulation increased the amount of growth hormone excretion in morning urines with age. In the young infants those under higher insulation excreted less growth hormone than those under smaller amounts of insulation suggesting that their energy needs are less than those infants under less insulation. However, in the older infants, those infants under more insulation excreted as much growth hormone as those under less insulation in the morning indicating that they may be expending as much energy as those under less insulation, as they try to keep cool. This increased growth hormone excretion in the older infants under more insulation in morning urine appears to generate a small amplitude circadian rhythm which does not appear to be present in those infants under less insulation.

Both birth order and social class appear to influence growth hormone excretion in similar fashions. Those infants who are first or second born or had families in social categories 1, 2 or 3 showed a constant excretion rate of growth hormone in both morning and night-time samples and in both young and old infants. Those infants who were third or subsequent infants or who had families in the lower social categories demonstrated a pattern of significantly less growth hormone excretion in the younger age groups in both morning and night-time urines. This level of excretion was less than that of the growth hormone excretion of those infants who were first or second born or from the higher social categories. In the older age group the morning excretion of growth hormone was little different between the social categories or the birth order. The night-time excretion of growth hormone was slightly lower in the lower social categories and in the third or subsequent infants than those from the higher social categories or earlier infants. The effects of birth order and social categories
display a high degree of similarity. This is not too surprising as those families of higher social
categories tend to have fewer children. Similarly, a highly significant effect of social category
on the excretion of growth hormone is not too surprising as those families from higher social
categories tend to breast feed, be non-smokers, and have fewer children, all of which have
some effect on GH excretion but which may interact to give a greater effect. It was not
possible to separate out the effect of each of these factors as multivariate analysis of the data
would have resulted in sub-samples of small size.

The third objective of this study has been met, certain factors already established as having an
affect on infant physiology have been shown to exert some affect on the urinary excretion of
cortisol and GH. Some of the effects the factors exert on the urinary excretion of these
hormones are unexpected and may in fact warrant further study. A larger number of subjects
and samples would allow multivariate analysis to unravel some of the interaction between
factors.

The epidemiology of SIDS associates smoking parents, bottle feeding, infant sex, high birth
order, low social status and thermal environment with increased risk of fatalities. The results
discussed above provide some suggestions of possible mechanisms as to why such factors
may increase risk. There are sex differences in cortisol excretion suggesting that there may be
differences in the ability to cope between male and female. Bottle feeding may increase stress
because of the infrequency of feeds at night particularly in older infants who sleep through.
High levels of insulation may increase the stress in younger infants as they may not be as
capable of coping with environments necessitating heat loss (see aim 5). Parental smoking
may subdue the infant or suppress the ability to produce cortisol while birth order and social
class express the interaction of an array of factors which may increase stress.
4. Describe the effect of immunisation on the excretory rate of both cortisol and GH.

In common with the findings of Rawson et al (1990) some of the infants studied exhibited a characteristic night-time rectal temperature pattern the first night after their first Diphtheria, Pertussis, tetanus, polio and Hemophilus Influenzae B immunisation. This pattern of elevated rectal temperature was not altered by the introduction of the Hemophilus Influenzae B immunisation or the new schedule of immunisation for each infant.

**Cortisol:**

Studies have been carried out by Lewis & Thomas (1991) which indicate that with 15 minutes after an immunisation event salivary cortisol is increased. This elevation of cortisol is unlikely to reflect major physiological changes due to the contents of the vaccine and its immunological effect. Instead this probably reflects the psychological trauma of having a painful and unusual stimulus. In this study the excretion of cortisol was studied within 24 hours of an immunisation; the earliest urine collection occurring about 12 hours after the event. In general cortisol excretion was significantly higher in morning and night-time urines but less so in night-time urines. This type of response of elevated cortisol has been seen in 3-6 month infants presenting with bronchiolitis and gastro-enteritis and was correlated to the severity of the illness (Deshpande, Ward-Platt, Aynsley-Green, 1993) so it seems reasonable to assume that it is a result of the stress caused by immunological response. By separating the first and the second immunisation event it became clear that each immunisation occasion results in a slightly different response. In the pre-maturation temperature stage cortisol excretion was significantly elevated after both first and second immunisations in morning and night-time urines alike. After maturation of the temperature pattern neither first nor second immunisations resulted in significantly elevated cortisol excretion. It would seem that immunisation in the pre-maturation state is more stressful than immunisation in the post-maturation state.
Growth hormone:

Our knowledge of the role of GH in the immune and stress responses is far from complete. We do know that plasma GH increases in response to trauma and chronic illness (Jeffries & Vance, 1992) so we might have expected to observe a significant increase in GH excretion after immunisation. Growth hormone excretion after immunisation in the pre-mature temperature state is marginally elevated above control values but not significantly. The elevation of GH excretion is perhaps not as large as expected from observation of the cortisol response. GH production is elevated after injury, burns or surgery to levels which reflect the severity of the trauma. However, GH secretion rapidly returns to normal levels so it may be that the timing of urine collections was too late to observe an increase or it may be that immunisation is not stressful enough to elevate GH excretion. This later suggestion is unlikely to be true. In the post-maturation temperature state growth hormone excretion is not significantly different to that observed on normal non-immunisation nights. The effect of first and second immunisations on growth hormone excretion could not be analysed due to the small sample size.

There did not appear to be a correlation between the maximum rectal temperature achieved and the elevation of either cortisol or growth hormone excretion. Those infants who exhibited high night-time rectal temperatures after immunisations did not necessarily show elevated excretion rates of either cortisol or growth hormone.

The fourth objective of the study to describe the effect of immunisation on the excretion of cortisol and GH has been addressed. The results of this part of the study require further investigation to allow a more detailed description of the response to immunisations on different occasions and in different stages of temperature maturation.

These results suggest that immunisation is a highly stressful event. If like the temperature pattern, cortisol excretion during the prodromal period of an illness changes in a similar fashion to that observed after immunisation how might such a response benefit the body. Does a high cortisol production in the prodromal stage of an illness better prepare the body for
symptoms? Once challenged by a particular infection is the need for an elevated cortisol production at a subsequent challenge reduced as the stress of such an event reduces? Some infectious challenges may provoke excessive or inadequate responses in some infants and hence explain the supposed sub-lethal presence of infection in some SIDS victims.

5. Measure TEWL from the surface of the skin establishing the effect of site of measurement, age and activity of the subject on TEWL rates.

From the cross-sectional analysis of TEWL measurements it appears that in general the more distal the extremity the lower the rate of TEWL. Those sites around the head and abdomen showed higher TEWL rates than those from on the arms and legs. These findings correspond well with those of Hammarlund, Nilsson, Oberg & Sedin (1977), who took TEWL measurements on young infants in the laboratory environment. Those measurement taken from the face and neck of an infant when examined over several weeks indicated that TEWL from older infants tended to be higher than those collected from the younger infants. This could be an actual physiological difference or a result of the older infants being more alert and active when the TEWL measurements were taken.

Observations of TEWL from the face of sleeping infants indicated that TEWL at the onset of sleep increased as rectal temperature fell, skin temperature rose and the number of eye and body movements fell. This pattern of TEWL was similar in both young and old infants. The correlation between changes in TEWL and skin and rectal temperature and percentage of time spent in quiet sleep with time was not as strong in the younger infants as the older infants. Young infants tend to have lower TEWL rates than older infants during both active and quiet periods of sleep.

This objective was not addressed as fully as was hoped. The study was hindered by the unavailability of an appropriate means of measurement. The evaporimeter is an useful tool for the study of inactive subjects in stable conditions. However, this study really required a means of TEWL measurement that allowed remote measurement and attachable probe. As with the other parts of this study the analysis of TEWL from the surface of the skin would
benefit from more data. However, the collection of TEWL measurements is both time consuming (having to wait or time arrival at homes to coincide with the onset of sleep) and labour intensive (having to personally hold the probe in skin contact).

Part 5: Summary, future work and conclusions.

This study has reaffirmed the findings that body temperature is undergoing a major developmental change at about 3 months of age in the human. Furthermore, these changes in the circadian periodicity of body temperature appear to be accompanied by changes in the excretion of free cortisol in urine. The changes in cortisol excretion and therefore presumably, secretion are remarkably similar to those of temperature maturation which has already been shown to be a useful marker for heart rate (Pratt personal communication) and possibly changes in other systems. It may be that these easily observed changes mark changes in the regulatory function of the HPA and hypothalamus. Perhaps this period of change in regulatory function of the HPA is a transition from a vulnerable to a less vulnerable stage of development, or perhaps more feasible, is that the transitional phase itself is the time of vulnerability. If the latter is true and the period at about 2-3 months when the majority of infants are developing mature adult-like temperature patterns and cortisol secretion, in addition to changes in heart rate and respiration, is one of increased risk, this may help us understand why infants are more at risk of becoming SIDS victims at this age. The peak incidence of SIDS is at 3 months of age with virtually all deaths occurring before 6 months. The timing of the transitional phase from infantile to mature and adult-like is such that it coincides with the peak incidence of SIDS. Most if not all infants have undergone this transition by 5 months so that it is the extremely rare infant who would undergo changes after 6 months. If the cause of SIDS is linked to a period of vulnerability, as currently favoured, changes in the central controlling mechanism - the hypothalamus and HPA, could provide a mechanism leading to vulnerability. Added together with a possibly inappropriate stress responses or system overload caused by stress, the function of the HPA could account for some if not all the epidemiology and pathological findings in SIDS victims. It is hoped that the descriptions
produced by this study may, with further work, help to answer some of the questions posed by this theory.

**Future work**

In order to clarify some of the associations between hormone excretion, temperature regulation and child care practices many more observations need to be made to provide a sample set large enough to allow multifactorial statistical analysis to be made. With out such analysis the interaction of various factors such as sex and feeding method or birth order and social status and the true relevance of their effect on infant physiology cannot be established. Precise causes of SIDS still remain unclear. A syndrome with so many associated risk factors is unlikely to be attributed one clear cause. However, because so many physiological systems have been hypothesised to play a role in the final fatal events perhaps a system which is known to play a role in whole body maintenance holds the biggest clues. The neuroendocrine function of the hypothalamus and the HPA is complex and some aspects of its function remains unclear in adults. Unfortunately, this system is more difficult to investigate in infants as most information would be gained from frequent blood sampling but this is difficult to ethically justify for purely research purposes in any infant but perhaps more so in the healthy. This project has utilised advances in technology to find a less invasive method of examining two of the hormones produced by the HPA which are known to play a role in the adult stress response. By using similar methods to examine other hormones a more complete picture could be built-up. Many more observations of hormonal excretion collected simultaneously with measures of cardiovascular, respiratory and thermal physiology would help to assimilate a model of the function of the hypothalamus, the stress response and the kind of events that were particularly stressful to an infant which may provide answers or clues about the mechanisms of survival and their role in the well-being of an infant and possible causes of SIDS. The immune response of an infant and how it is affected by the HPA may also be of great interest.

Such descriptive work is of great value but may not provide the full answers of the development of the HPA and so more detailed studies perhaps relating anatomical and
functional changes are necessary. Obviously, such studies are limited on human subjects so a convenient animal model must be established. Work on the adrenal cortex and the involution of the foetal zone have been carried out on non-human primates but not in conjunction with physiological observations. Such studies could clarify many problems. For example I have hypothesised that it is the hypothalamus controlling both the changes in temperature regulation and cortisol excretion but how do changes in the foetal zone of the adrenal cortex effect the production and ontogeny of a circadian periodicity? Perhaps it is only the responsiveness of the adrenal cortex to hypothalamic stimuli which results in a change in cortisol production and hence temperature maturation. Similarly, the role of growth hormone in the infant requires further attention. Does high GH excretion reflect faster growth, lower or higher blood glucose or more (less) stress? Are changes in the production of GH clouded by changes in the production of growth hormone-binding protein when studying the urinary excretion of this hormone?

In conclusion:

This study has started a momentous task which will provide a research topic for many years to come but provided some information about the infantile excretion of cortisol and GH, confirmed the pattern of temperature maturation and attempted to examine TEWL in infants. Perhaps the most interesting and informative part of the study has been the description of the pattern of urinary excretion of cortisol by the infant, how it changes with age and its association with developmental changes in temperature regulation. The description of urinary GH excretion has been less easy to analyse and explain partly due to our limited understanding of its role in the infant and partly because of the fairly small numbers. However, an assay system adequate for the measurement of GH in infant urine has been established which could prove to be a useful tool in further research or clinical environment. TEWL measurement was not as revealing as had been hoped but it has shown that at least one of the mechanisms of heat loss at the onset of sleep is TEWL. Until an improved technique of measuring TEWL is established revealing observations will be impossible.
.....And finally, until more is known about the developmental changes endured by an infant, true understanding and the ability to intervene in the problems they experience will remain outside our reach.
APPENDICES
APPENDIX 1

NOMENCLATURE AND METABOLISM OF STEROID HORMONES
STEROID NOMENCLATURE AND SYNTHESIS.

Figure A1.1: The numbering of the carbon atoms in the steroid molecule.

The steroid hormones all have a structure based on the above molecular structure where each of the carbon atoms are labelled 1 to 27 (figure A1.1). Ultimately all steroid hormones are synthesised from acetate (a smaller 2 carbon atom molecule) via cholesterol as outlined in figure A1.2. These reactions are catalysed by several enzyme complexes (P450) specific to different areas of the adrenal to produce a wide variety of biologically active steroids.

Figure A1.2: Pathway for the generation of steroid hormones from acetate (Drucker & New, 1987).

\[
\begin{align*}
\text{acetate} & \rightarrow \text{MINERALOCORTICOIDs} & \text{GLUCOCORTICOIDs} & \text{SEX HORMONES} \\
\text{cholesterol} & \rightarrow \text{pregnenolone} & \rightarrow 17\text{-hydroxypregnenolone} & \rightarrow \text{dehydroepiandosterone} \\
& \rightarrow \text{progesterone} & \rightarrow 17\text{-hydroxyprogesterone} & \rightarrow \text{androstenedione} \\
& \rightarrow 11\text{-deoxycorticosterone} & \rightarrow 11\text{-deoxycortisol} & \rightarrow \text{testosterone} \\
& \rightarrow \text{corticosterone} & \rightarrow \text{cortisol} & \rightarrow \text{oestrogens} \\
& \rightarrow 18\text{-hydroxycorticosterone} & \rightarrow \text{aldosterone} & 
\end{align*}
\]
APPENDIX 2

EXAMPLES OF RECRUITMENT LETTER, HEALTH VISITOR NOTIFICATION LETTER, RECRUITMENT POSTER AND DATA RECORD FORMS
Over the past 6 years a team of researchers have been making weekly visits to homes in Leicester, in order to learn how young babies control their body temperature.

Until now we have looked at body temperature and heart rate over night using safe and simple equipment. We now wish to look at when and if possible, why some babies sweat during sleep, while others appear not to.

In order to do this I am looking for families that would be willing to have me visit during the afternoon/early evening, or at a time when baby is likely to be taking a nap or even going to bed for the night. The visits would be arranged each week and continue from about 5 weeks until the baby is about 12 weeks.

However, before this, I would like to visit you to show you the equipment, explain further and answer any questions you may have. May I visit on:

If this is an inconvenient time, or you feel unable to take part in the study, please phone to leave a message for me on any of the following numbers.

Leicester 585682 (ansaphone), Leicester 586078, Leicester 585773

Thank you for your help,

Yours sincerely,

Christine Atkinson
Researcher
Dear 

RE: Thermoregulation Studies in Babies.

I am writing to let you know that:

have kindly agreed to take part in our research project.

We are studying babies between 5 and 12 weeks of age. Once a week a visit is made to measure the baby's body temperature, the temperature of the room it is in and the amount of sweat evaporating from the skin surface, all during a period of sleep. Each time a brief questionnaire is completed.

If you would like to know more about our work, see the equipment or have details of articles published by the group, I can be contacted on:

Leicester 585682

Yours sincerely,

Christine Atkinson

Researcher
CAN YOU HELP?

I am working as part of a team looking at how babies control their body temperature.

By understanding how babies normally control their temperature we hope to answer previously unexplained questions about childhood illness.

Babies are currently being monitored at home using safe, simple equipment during a period of night sleep.

I would like to find some families to help me look at sweating patterns during the daytime or early evening.

If you are interested could you fill in one of the yellow forms and leave it with sister. Alternatively leave a message for;

CHRISTINE ATKINSON
on Leicester 585682

THANKYOU
Family Information

<table>
<thead>
<tr>
<th>Baby’s Name:</th>
<th>D.O.B.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Address:</td>
<td></td>
</tr>
</tbody>
</table>

| G.P.                     |             |
| Address:                 |             |

<table>
<thead>
<tr>
<th>Health Visitor:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers name</td>
<td>Age</td>
</tr>
<tr>
<td>Hospital Number</td>
<td>Health</td>
</tr>
<tr>
<td>Previous Pregnancies</td>
<td>Smokes</td>
</tr>
<tr>
<td>Occupation</td>
<td>Social Class</td>
</tr>
<tr>
<td>Fathers name</td>
<td>Age</td>
</tr>
<tr>
<td>Health</td>
<td>Smokes</td>
</tr>
<tr>
<td>Occupation</td>
<td>Social Class</td>
</tr>
<tr>
<td>Sibling names</td>
<td>D.O.B.</td>
</tr>
</tbody>
</table>

Health of siblings

Medical history of:
Asthma/Diabetes/Renal/Cardiac/Epilepsy/Febrile convulsions

Family members affected:

Family pets:
Baby Information

Name: 
D.O.B. 

Gestational Age

Complications of pregnancy:

Labour:
Induced Yes / No Complications
Foetal distress Yes / No Type:
Stages of labour 1. 2. 3.

Delivery:
Type Placenta
Apgar scores

Measurements:
Birthweight H.C. Length

Illness at birth:
Neonatal Unit Yes / No How long?
Jaundice / Apnea / Convulsions / Feeding / Infection / Other
Congenital anomaly Yes / No

Feeding at birth:
breast / bottle / mixed / nasogastric / other

Sleep position at birth:
prone / supine / lateral

Illness in first 6 weeks following birth
# Home Conditions

## Baby's Name

### File Number

<table>
<thead>
<tr>
<th>Type of House:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detached</td>
</tr>
<tr>
<td>Terrace</td>
</tr>
<tr>
<td>Bedstyt</td>
</tr>
<tr>
<td>Council</td>
</tr>
<tr>
<td>Housing Association</td>
</tr>
<tr>
<td>Bungalow</td>
</tr>
<tr>
<td>Flat</td>
</tr>
<tr>
<td>Semi-Detached</td>
</tr>
<tr>
<td>Owner Occupied</td>
</tr>
</tbody>
</table>

### Heating:

<table>
<thead>
<tr>
<th>Heating Type:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Heating</td>
</tr>
<tr>
<td>Gas Fires</td>
</tr>
<tr>
<td>Electric Fire</td>
</tr>
<tr>
<td>Storage Heaters</td>
</tr>
<tr>
<td>Open Fire</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

### Heating in Room Where Baby Sleeps:

<table>
<thead>
<tr>
<th>Heating Type:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiator</td>
</tr>
<tr>
<td>Fan Heater</td>
</tr>
<tr>
<td>Electric Fire</td>
</tr>
<tr>
<td>Wall Heater</td>
</tr>
</tbody>
</table>

### Room Insulation:

<table>
<thead>
<tr>
<th>Insulation Type:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavity Wall Insulation</td>
</tr>
<tr>
<td>Double Glazing</td>
</tr>
<tr>
<td>Carpet</td>
</tr>
</tbody>
</table>

## Any Problems with Housing

Please draw diagram showing position of cot in room, relationship to heater etc and ventilation:
## Monitoring Data

| **Baby’s name:** |  |
| **Date:** |  |
| **Weight:** |  |
| **Feeding** |  |
| **Breast** | Age of weaning: |
| **Bottle** | Frequency of solids: |
| **Type of bed:** | Type of solids: |
| **Door:** open/closed | Which room?: |
| **Heat:** house: | Window: open/closed |
| **Clothing total togs:** |  |
| Nappy (2.0) | Sheet (0.2) |
| Vest (0.2) | F. Sheet (0.5) |
| Babygro (1.0) | Shawl (2.0) |
| pyjamas (2.0) | Old blanket (1.5) |
| Socks (0.2) | New Blanket (2.0) |
| Mitts (0.2) | Thin quilt (2.5) |
| Cardigan (2.0) | Medium quilt (4.0) |
| Other | Thick quilt (9.0) |
| **Sleep position:** |  |
| Prone to prone |  |
| Supine to supine |  |
| Lateral to Lateral |  |
| **Bedding position:** |  |
| on / off |  |
| **Imm. & Vac. type:** |  |
## Health Record

<table>
<thead>
<tr>
<th>Problem</th>
<th>Comment / Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes -</td>
<td>Watery</td>
</tr>
<tr>
<td></td>
<td>Sticky</td>
</tr>
<tr>
<td>Nose -</td>
<td>Snuffles</td>
</tr>
<tr>
<td></td>
<td>Discharge</td>
</tr>
<tr>
<td>Mouth -</td>
<td>Sore gums</td>
</tr>
<tr>
<td></td>
<td>Thrush</td>
</tr>
<tr>
<td>Throat -</td>
<td>Sore</td>
</tr>
<tr>
<td></td>
<td>Cough</td>
</tr>
<tr>
<td>Chest -</td>
<td>Rib recession</td>
</tr>
<tr>
<td></td>
<td>Wheeze</td>
</tr>
<tr>
<td>Feeds -</td>
<td>Reduced</td>
</tr>
<tr>
<td></td>
<td>Refused</td>
</tr>
<tr>
<td></td>
<td>Vomit</td>
</tr>
<tr>
<td></td>
<td>Colic</td>
</tr>
<tr>
<td>Motions -</td>
<td>Firmer</td>
</tr>
<tr>
<td></td>
<td>Looser</td>
</tr>
<tr>
<td>Urine -</td>
<td>Reduced</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
</tr>
<tr>
<td>Skin -</td>
<td>Hotter / Colder</td>
</tr>
<tr>
<td></td>
<td>Paler / Bluer</td>
</tr>
<tr>
<td></td>
<td>Sweatier (where)</td>
</tr>
<tr>
<td>Rashes -</td>
<td>Nappy</td>
</tr>
<tr>
<td></td>
<td>Eczema</td>
</tr>
<tr>
<td></td>
<td>Sweat</td>
</tr>
<tr>
<td></td>
<td>Other</td>
</tr>
<tr>
<td>Attitude -</td>
<td>Restless / Irritable</td>
</tr>
<tr>
<td></td>
<td>Different cry</td>
</tr>
<tr>
<td></td>
<td>Drowsier</td>
</tr>
<tr>
<td></td>
<td>Floppier</td>
</tr>
</tbody>
</table>

Name:  
Age:  
Date:
Activity Sheet

Baby's name:  Date:

Whilst your baby is attached to any monitors, please note the timing of any of the following activities:

When baby is sleeping
Final bedtime
Feeds
Nappy or clothing changes
Washes
Any medicines including gripe water, infacol and paracetamol

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 3

THEORY AND CALIBRATION OF THE EVAPORIMETER FOR USE IN THE MEASUREMENT OF TRANSEPIDERMAL WATER LOSS

The transport of water by diffusion in the absence of forced convection, assuming that the effect of thermal diffusion is negligible can be expressed by Fick's Law of diffusion.

\[
\frac{1}{A} \cdot \frac{dm}{dt} = -D \frac{dp}{dx}
\]

where
A = surface area (m²)

m = mass of water transported (g)

t = time (hours)

D = diffusion constant 0.0877g.m⁻².h⁻¹.mmHg⁻¹

p = partial pressure of water vapour pressure in the air (mmHg)

x = distance from the surface (m)

This equation predicts a constant vapour-pressure gradient within the 10mm zone directly above the surface from which evaporation is occurring e.g. skin. Within this distance the evaporation rate \( \frac{dm}{dt} \) is proportional to the partial pressure gradient \( \frac{dp}{dx} \). Therefore the evaporation rate can be estimated by calculation of the partial pressure gradient within 10mm of the surface.

Structure

The evaporimeter makes use of the above principle. The measuring probe consists of a pair of transducers at two distances from the surface. Each pair of transducers includes a temperature thermister and a relative humidity sensor. These sensors are protected from large air currents by an open Teflon cylinder. From the signals derived from the pair of transducers the partial pressure of water at the two locations is calculated, from these the partial pressure gradient and finally the evaporation rate is calculated.
Calibration of the sensors.

The main unit and the probe are manufactured and calibrated to a great accuracy. In order to ensure that the calibration of the humidity sensors had not deteriorated to an unacceptable level the calibration was checked at three monthly intervals as suggested by the Operation Handbook. Recalibration of the sensor was not undertaken unless absolutely necessary as the accuracy obtained during the manufacturing process is difficult to match.

Calibration was performed by utilising the fact that in a sealed container the air above a saturated salt solution has a specific humidity depending upon the salt. Saturated solutions of Lithium chloride (LiCl), magnesium nitrate (Mg(NO₃)₂) and potassium sulphate (K₂SO₄) were used to create low, medium and high humidities at a given temperature.

CALIBRATION OF THE EVAPORIMETER (as stated by manufacturer)

Making up the calibration solutions

Lithium chloride

30-33 g of chemically pure Lithium chloride were dissolved in 30 ml of distilled water. To prevent the formation of a hard crust of salt forming small portions were added to the water during continuous stirring. After each addition the flask was allowed to cool until the flask could be held before adding further salt.

Magnesium nitrate

Approximately 65 g of chemically pure Mg(NO₃)₂ were dissolved in 13 ml of distilled water. The flask was sealed using a rubber stopper and stirred for about 30 seconds by performing small circular movements.
Potassium sulphate

Approximately 20 g of K₂SO₄ were dissolved in 40 ml of distilled water, sealed and stirred for 30 seconds as above.

After preparing the solutions the flasks were allowed to stand for at least 24 hours before using them to calibrate for the first-time. This is to ensure that the solutions are saturated and to allow the heat of hydration to dissipate. If the flasks did not contain an obvious salt residue further salt was added and the solutions left to stand for a further 24 hours.

The transducers of the evaporimeter are very sensitive. Contamination of the transducers by means of fluids, dust or oils coming into contact with the sensors creates inaccuracy. Therefore, great care was taken to ensure that the inner surface of the neck of the flask and any surfaces which may come into contact with the probe were free from drops of solution. This also prevented cross contamination of the salt solutions. The salt solutions were stored sealed for up to 1 year after which they were discarded and fresh solutions made up.

CALIBRATION OF THE PROBE.

The resolution of the digital indicator of the evaporimeter is not sufficient to allow transducer circuits A and B to be accurately calibrated to ensure that water transport measurement is precise.

Instead circuit A is calibrated for relative humidity and circuit B is then adjusted in relation to circuit A until zero water evaporation is correctly indicated. This ensures that water evaporation is correct and that circuit B is accurate enough to measure relative humidity.

Procedure

1. The measuring head of the probe was carefully fitted with a stopper to enable a tight seal to be created when placed into the calibration flasks.
2. The evaporimeter was switched on and the measuring head carefully placed into the flask containing the lithium chloride solution. The probe was left in this flask for at least three hours but more usually overnight. The evaporimeter was set to display the relative humidity of the air above the solution. The reading which was obtained in the lithium chloride solution should be 11±1%. If the reading read 10, 11, 12 the evaporimeter was not adjusted otherwise the trim potentiometer marked %RH\text{a} 11.2 on the plug-in box of the measuring head was adjusted until it read 11. This calibrated the A circuit for measurements at low humidities.

3. If adjustments had been made to the trim potentiometer then the following sequence of buttons were pushed. WE, CAL and RANGE followed by a pause of 30 seconds after which the OFFSET control was adjusted until the display read 0.0 g.m\(^{-2}\).h\(^{-1}\). The OFFSET control was not adjusted any further throughout the rest of the calibration procedure.

4. The CAL button was released and 30 seconds allowed before ensuring that the display read 0.0±0 g.m\(^{-2}\).h\(^{-1}\) by adjustment of the %RH\text{b} potentiometer marked 11.2. This calibrated the B circuit relative to the A circuit to enable water transport and humidity to be calculated at low humidities.

5. The measuring head of the probe was removed from the lithium chloride solution and placed into the magnesium nitrate flask and left for 3 hours.

6. The Rh button was pressed and the temperature in the insulating box measured. The RH of the air above the magnesium nitrate solution was read off the chart below. The display of the evaporimeter showed this value ±2 %. If this was not the case the %RH\text{a}54.5 potentiometer was adjusted to calibrate the A circuit for medium humidities.

7. The buttons WE and RANGE were then pushed to check the calibration of the B circuit. If the display did not show 0.0±1.0 g.m\(^{-2}\).h\(^{-1}\) then the %RH\text{b}54.5 potentiometer needed adjustment.

8. Steps 2-7 were then repeated until no further adjustment was necessary as measurements of
humidity at low and medium humidities influence each other.

9. The final step was to calibrate the probe to make measurements at high humidities by placing the measuring head into the flask containing potassium sulphate solution. The RH button was pressed and the and at least 1 hour was waited before checking the calibration. The display showed 97±3%RH. If not the %RH button was adjusted until the display red within the limits.

10. WE and RANGE buttons were depressed and the display adjusted until it read 0.0±2g.m⁻².h⁻¹.

11. Steps 2-10 were repeated to ensure correct calibration.
APPENDIX 4

CALIBRATION OF THE TEMPERATURE PROBES
CALIBRATION OF THE TEMPERATURE PROBES.

Rectal temperature: a soft rectal probe of dimensions 5 cm x 2 mm attached to a data logger by 2-core PVC cable. The manufacturers state the range for recording is -50 to 150°C with a tolerance in the 0 to 70°C range of 0.1°C and a response time of 0.8 seconds. The thermistors are guaranteed for three years.

The probes were calibrated and standardised against a total immersion Emil Goldline mercury thermometer and were correct to 0.15°C for all measurements and correct to 0.05°C for the range 35.5°C to 40°C. The probes were immersed in a Grant Instrument waterbath and heated slowly. Readings were taken from each probe with every 0.5°C increase in temperature, as measured by the mercury thermometer. This was repeated as the probes cooled. In addition the reaction time of the probes was established by rapidly cooling the probes from 38°C to room temperature (21°C) by the addition of ice (see figure A4.1).

Blemished data from monitoring was easily recognised as either apparatus failure or loss of the probe from the infants rectum. Comparison of the rate of temperature fall when the probe drops from the rectum into the nappy and the most rapid fall in the temperature recorded physiologically show that there are easily recognisable differences between the two. The temperature recorded in the nappy is much lower than any rectal temperature recorded (see figure A4.2).

Although skin temperature correlates with rectal temperature buttok skin temperature is about 2°C lower than normal rectal temperature. Therefore the falling of the probe from the rectum would lead to a rapid change in the recorded temperature. Records which demonstrate a fall in temperature greater than 0.5°C/min or show temperatures which fall below 35.8°C were discarded.
Surface probes: Dimensions 3 mm x 8.5 mm epoxy coated copper with a sensor on the back of the disc. During evaporation measurements the probe was placed on the forehead as this gave a close estimate of the surface temperature on the facial skin. During night-time temperature recordings the probe was placed on the buttock for convenience.

These probes were calibrated and standardised against a total immersion mercury thermometer in the same way as the rectal probes. They were found to be correct to 0.05°C and 0.1°C.

Data were excluded when temperature changes greater than 0.5°C/min were recorded as these indicated the probe had become detached.

Ambient temperature probes: Stainless steel probes with a response time of about 3 seconds were used to record the environmental temperature near the baby.

The probes were attached using either micropore or mefix tape whichever was the best tolerated by the infant. Tests carried out on adults indicate the tape did not influence the value of temperature recorded by more than 0.05°C.
Figure A4.1:
The time taken for each of the probes to react to a temperature change.

Figure A4.2:
Comparison of recordings of rectal probe fallen from the rectum into the nappy and the largest physiological fall in rectal temperature.
APPENDIX 5

ASSAY TYPES AND CHARACTERISTICS FOR CREATININE, URINARY FREE CORTISOL AND GROWTH HORMONE
ASSAY TYPES AND CHARACTERISTICS FOR CREATININE, URINARY FREE CORTISOL AND GROWTH HORMONE.

Creatinine

The method of measuring creatinine by determining colorometric change was first described in 1886 by Jaffé and has been in common clinical use for about 70 years (Folin & Wu, 1919) over which period it has changed little. The assay has an established linear standard curve. Using the Cobas Bio instrument and Biorad Lyphocheck, urine controls LI and LII, the lower detection limits for creatinine was 0.1 mmol.l⁻¹. The upper limit for detection was 35 mmol.l⁻¹ which was determined by the concentration of the picric acid reagent. The inter-assay percentage CV for standards at 8 and 20 mmol.l⁻¹ were typically less than 2% for urine.

Cortisol

This is a competitive binding assay in common clinical use, which is taking part in the UK National External Quality Assurance Scheme (UK NEQAS) for assessment of urinary free cortisol (Holder, 1994).

Details published (assay kit insert, Diagnostic Product Corporation, 1994) by the manufacturers indicate that there are low levels of cross-reactivity to a variety of therapeutic drugs and naturally occurring steroids (typically less than 2.3%), with the exception of prednisolone which shows a substantial cross-reactivity (76%). Further details published by the manufacturer gives an intra-assay percentage CV of between 3.0% and 5.1% and an inter-assay percentage CV of 4.0% to 6.4%; with dilution studies showing that the assay has a good linearity and a detection limit of 5nmol.l⁻¹.

In this study most urines were assayed on the end of scheduled hospital runs. This meant that the inter-assay percentage CV was more important than the intra-assay percentage CV. Even with the
small modification of the assay the inter and intra-assay percentage CV's were typically about 8%. The limits of detection of urinary free cortisol in this assay were between 5 and 345nmol.l⁻¹.

**Growth Hormone**

This assay is a sandwich assay. The percentage CV of the unmodified serum growth hormone assay as published by the manufacturers (assay kit insert, IDS, 1990) were typically less than 10% between 1 and 500mU.l⁻¹. Dilution studies on the unmodified assay showed that the assay has good linearity and low cross-reactivity (less than 5%) for human prolactin (hPrL), and human chorionic gonadotrophin (hCG).

After modification, results illustrated in chapter 4, show that the assay continued to behave in a similar manner. The inter-assay percentage CV is typically below 15% at growth hormone levels of 22μU.l⁻¹ or more, while the intra-assay percentage CV is typically below 10%. Dilution studies indicate that there was good linearity of the assay (Chapter 4 figures 4.6 and 4.10). The lower detection limit was taken as 10μU.l⁻¹ as below this growth hormone level the percentage CV rose above the acceptable 20%.

**TABLE A5.1: Summary of the assay characteristics.**

<table>
<thead>
<tr>
<th>Assay System</th>
<th>Linearity</th>
<th>Inter-assay % CV</th>
<th>Intra-assay % CV</th>
<th>Detection limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaffé rate reaction</td>
<td>Good</td>
<td>&lt;2%</td>
<td>&lt;2%</td>
<td>0.1 - 35mmol.l⁻¹</td>
</tr>
<tr>
<td>Urinary free cortisol</td>
<td>Good</td>
<td>8%</td>
<td>&lt;8%</td>
<td>5 - 345mmol.l⁻¹</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>Good</td>
<td>14.3%</td>
<td>9.7%</td>
<td>10μU.l⁻¹ - at least 189mU.l⁻¹</td>
</tr>
</tbody>
</table>
APPENDIX 6

STATISTICAL TESTS USED FOR ANALYSIS
STATISTICAL TESTS USED FOR ANALYSIS OF DATA (see Altman, 1991).

ANALYSIS OF VARIANCE

One way analysis of variance: Allows the comparison of all data without carrying out t-tests on each pair of data points. The principle is for use for examining one experimental variable and one quantitative dependent variable, e.g. changes in cortisol with age.

Two way analysis of variance on repeated measures. This is used to examine data sets where two or more observations of the same variable are taken from the same individual under different circumstances, e.g. half-hourly recordings of body temperature from infants who have and have not received an immunisation. The comparison between sets of observations does not affect the ability to distinguish between the sets of observations.

CHI SQUARED ($X^2$). This evaluates the possible associations between variables. It is defined as the sum of the differences between the observed frequencies ($O$) and the expected frequencies ($E$) all squares and then divided by the expected frequency of the variable.

$$\chi^2 = \sum_{i=1}^{r} \sum_{j=1}^{c} \frac{(O_{ij} - E_{ij})^2}{E_{ij}}$$

where, $i$ and $j$ indicate the number of rows and columns. The degrees of freedom for the test statistic are given by $(r-1)(c-1)$. The expected frequencies are defined as product of the column total and the row total divided by $n$.
CONFIDENCE INTERVALS: A range of values covering a large proportion of the sampling distribution of the statistic of interest, such that, anything inside this range has a high probability of being a true value, e.g. any value outside a 95% confidence limit is probably an untrue value.

CORRELATION: A method of analysis used to study the possible associations between two continuous variables. Calculation of the correlation coefficient (r) gives a measure of the degree of association between variables and can take any value between -1 and +1. A positive correlation coefficient indicates that higher values of one variable are associated with higher values of the second variable, while a negative correlation coefficient indicates that a higher value in one variable is associated with a lower value in the second variable.

MEAN (x̄) The sum of all observations divided by the number of observations.

\[
\bar{x} = \frac{\sum(x_1, \ldots, x_n)}{n}
\]

MODE: The most frequent observation.

PERCENTAGE COEFFICIENT OF VARIATION (%CV): Defined as the standard deviation of the samples divided by the mean and multiplied by 100.

\[
\%CV = \frac{\sigma}{\bar{x}} \times 100
\]

REGRESSION: Describes the relation between two continuous variables, thereby enabling the prediction of one variable from another. The standard method of producing a regression line is called the method of least squares. This produces a line of general equation, \(Y = a + bX\), where, \(X\) and \(Y\) are different variables; \(a\) is the intercept of the line with the Y axis and \(b\) is the slope of the line.
STANDARD ERROR OF MEAN (SEM): Is defined as the standard deviation divided by the square root of the number of samples. It is an indication of the variability among many sample means, i.e. how close a single mean is to the population mean.

\[ \text{SEM} = \frac{\sigma}{\sqrt{n}} \]

STANDARD DEVIATION (σ): The square root of variance and is an index of the variability in the original measurement units.

\[ \sigma = \sqrt{\frac{\sum (x_i - \mu)^2}{n-1}} \]

where, \( x_1 \) is a sample and \( n \) is the number of samples.

STUDENT'S UNPAIRED T-TEST: Compares the mean of two independent groups of observations to determine the probability that observed differences are real and not due to chance. It examines the mean difference between the group but unlike a paired t-test the variability between subjects is important. To carry out any t-test, the sample distributions are assumed to be normal and the variances of the two populations the same. However, the t-test is a robust test and is little affected by moderate failure to meet these assumptions and so can be used on a wide variety of data.

\[ t = \frac{\bar{x}_1 - \bar{x}_2}{\text{SEM}(\bar{x}_1 - \bar{x}_2)} \]

which is then compared with the \( t \) distribution with \( n_1 + n_2 - 2 \) degrees of freedom.

VARIANCE: A means of quantifying the variability of a sample by estimating the average distance of each observation from the mean of the sample.

\[ \text{Var} = \frac{\sum (x_i - \bar{x})^2}{n-1} \]


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