METABOLIC AND ENDOCRINE EFFECTS OF SURGERY AND ANAESTHESIA

IN THE HUMAN NEWBORN INFANT

by

Kanwaljeet Singh Anand

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This thesis is dedicated to

"WAHEGURUJI"

and my parents

who have given me roots, wings and love.
This project was designed to investigate the ability of newborn infants to respond to surgical stress and to consider alternative methods of anaesthetic management in view of their hormonal and metabolic response.

Concentrations of blood metabolites (glucose, lactate, pyruvate, alanine, acetoacetate, 3-hydroxybutyrate, glycerol, non-esterified fatty acids, triglycerides) and plasma hormones (insulin, glucagon, noradrenaline, adrenaline, aldosterone, corticosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, progesterone, 17-hydroxyprogesterone, cortisol) were measured in blood samples drawn before and after surgery, at 6, 12 and 24 hours postoperatively. Urinary total nitrogen and 3-methylhistidine/creatinine ratios were measured for 3 days postoperatively. Peri-operative management was standardised and severity of surgical stress was assessed by a scoring method.

In a preliminary study of 29 neonates, substantial hormonal and metabolic changes demonstrated the ability of neonates to mount a stress response to surgery. Compared to adult responses, the magnitude of these changes was greater but their duration was remarkably short-lived. Significant differences were found between preterm and term neonates, and between neonates given different anaesthetic management.

Randomised controlled trials were designed for studying the effects of:
(1) halothane anaesthesia in 36 neonates undergoing general surgical procedures, (2) fentanyl anaesthesia in 16 preterm neonates undergoing ligation of patent ductus arteriosus, (3) high-dose fentanyl anaesthesia in 13 neonates undergoing cardiac surgery.

On comparing the responses of neonates within each trial, the stress response of neonates given halothane or fentanyl anaesthesia was diminished; their:
(a) catecholamine responses were decreased or abolished,
(b) glucocorticoid responses were suppressed,
(c) changes in blood glucose and gluconeogenic precursors were decreased,
(d) postoperative analgesic requirements were reduced, and
(e) their clinical condition after surgery was more stable.

The neonatal response was related to the severity of surgical stress, as assessed by the scoring method.

Thus, hormonal and metabolic changes following surgery in preterm and term neonates are distinctly different from those of adult patients; the lack of adequate anaesthesia may cause an accentuation of the stress response.
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I would like to state that, apart from the help and guidance which has been acknowledged, the work described in this thesis is my own and that I am responsible for all the deficiencies, mistakes and errors in this work.
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"There is a circumstance attending accidental injury which does not belong to disease, namely, that the injury done, has in all cases a tendency to produce both the disposition and the means of cure...

John Hunter, (1728-1793).

1.1 INTRODUCTION:

The capability of responding to a noxious stimulus is one of the most fundamental characteristics of living matter; the amoeba that releases a number of lysosomal enzymes in response to a noxious chemical is probably operating along similar mechanisms as those of a patient who mounts an endocrine and metabolic response to accidental or surgical trauma. To extend the analogy further, these responses may be essential for the survival of the respective organisms, or if very severe, may even result in their death or disability. It is not surprising, therefore, that the stress response of human subjects has been under investigation for over a century and is a topic for current research as well. In recent times, the study of endocrine and metabolic changes following anaesthesia and surgery has been stimulated by the availability of sensitive and accurate methods for measurement of the hormones and intermediary metabolites concerned in the stress response, the finding that these changes may have an important influence on the morbidity and mortality following major stress, and the therapeutic implications arising from manipulation of the stress response by various methods of anaesthesia and analgesia or by the administration of hormones and substrates.
1.2 HISTORICAL BACKGROUND :-

1.2.1 Early studies :- In 1832, W B O'Shaughnessy in Newcastle-upon-Tyne, carried out a chemical analysis of the blood of cholera patients and observed that large quantities of water, neutral saline ingredients (i.e. sodium and chloride ions), and free alkalis has been lost from it. He also noted that all these constituents of blood were present in the stool of cholera patients, and that blood urea concentrations were raised in those patients who were not passing urine. Soon thereafter, Par M Boussingault (1839) studied the effects of nutrition in lactating cows on the composition of milk produced and later carried out balance studies of carbon, hydrogen, oxygen, nitrogen, and "salts of the earth" in lactating cows and horses.

In 1842, Justus von Leibig based on his knowledge of organic chemistry, defined metabolism as, "...... the sum of chemical changes of materials under the influence of living cells ....". This definition is still valid today. His contemporary was Carl Schmidt, who made the first comparative analysis of normal blood and the blood of cholera patients in 1850. Schmidt was also the first to show that potassium is lost in diarrhoea, but the implications of this finding were not realised until Sydney Ringer, the Professor of Medicine in London, studied the effects of constituents of blood on the ventricle of a frog's heart; and was able to elucidate the role of potassium in the body (Ringer and Murell, 1878; Ringer, 1882 and 1883).

1.2.2 Nitrogen excretion:- Metabolic changes following trauma were first studied by Dr Jos. Bauer in 1872, who found that nitrogen excretion by the body was increased after haemorrhage. In 1893, J D Malcolm found an
increased excretion of urea in the urine of operated patients, and related it to the increased metabolism following abdominal surgery. He also postulated that the shock following trauma is "...more a part of the phenomena caused by injury, surgical or otherwise, than a complication thereof". Wertheimer and his colleagues (1919) studied battle casualties during the First World War and found that the excretion of urea increased markedly and could even be doubled within a few days after major injury. Soon thereafter, Aub and co-workers (1920) made the observation that when traumatic shock was produced in cats by crushing the thigh muscles in both legs, there was a marked fall in the basal metabolic rate and an increase in non-protein nitrogen, urea, creatine and sugar levels in blood.

1.2.3 Hyperglycaemia:- The role of the central nervous system in metabolic regulation was reported by Claude Bernard in 1849, when he found that puncturing the 4th ventricle in dogs was followed by a diabetic state, with the appearance of sugar in the urine. In 1855, he proposed that an internal secretion is produced by the adrenal gland which may be related to the control of blood sugar in the intact animal. In 1878, Claude Bernard proposed the concept of the 'milieu interieur' and, a year later, published the first treatise on peri-operative physiology. Amongst other important observations, he clearly demonstrated an increase in blood sugar and a simultaneous depletion of liver glycogen as a consequence of haemorrhage or trauma. These observations led to the experiments of Brown-Sequard (1889) in dogs which established the presence of adrenaline secretion from the adrenal gland. In 1901, Blum found that injections of a watery extract of the adrenal glands caused glycosuria in dogs and postulated that internal secretions from the adrenal gland were responsible for this effect which was called 'epinephrine glycosuria'. From a review of the evidence in 1917, GM Mackenzie concluded that nervous stimuli cause an increased secretion of
adrenaline, which directly stimulates glycogenolysis in liver cells, and was also responsible for the hyperglycaemia in depancreatized dogs.

The earliest report showing that anaesthesia itself can cause metabolic changes was by Seelig in 1905, who found that diethyl ether anaesthesia stimulated a marked hyperglycaemia in dogs. In 1915, F G Benedict published his classical monograph on fasting, showing that the carbohydrate stores in man were limited and could provide the body's fuel for only a few days; 15% of the energy requirements thereafter came from the breakdown of proteins and the remainder from fats.

In 1915, WB Cannon proposed the neuroendocrine response to "stress" in the form of pain, hunger, fear and rage. In the Shattuck Lecture of 1917, he directed attention, for the first time, to the endocrine response to injury (Cannon, 1917; Cannon, 1918). Amongst other contributions, Cannon described a marked increase in the activity of the sympathetic nervous system which was associated with an output of adrenaline-like substances (sympathin E, sympathin I and sympathin M), and a marked increase in blood sugar during wound shock. In his book, "The Wisdom of the Body" Cannon (1932) introduced the term "homeostasis", that is, the constancy of the cellular environment. This environment, he proposed, was provided by the extra-cellular fluid, and every surgical or non-surgical injury constituted an attack on the 'homeostatic' mechanisms of the body.

While investigating the hyperglycaemic response to surgery in 1934, Weddell and Gale found that surgery under ether anaesthesia caused a marked increase in blood sugar which was greater in males than in females and was greater during intraperitoneal operations as compared to extraperitoneal operations. At the same time, Reid and Banerji (1933) showed that in
experimental animals the hyperglycaemia caused by ether anaesthesia was due to the liberation of adrenaline, and that the severity of hyperglycaemia was quantitatively related to the amount of adrenal medullary tissue.

In 1936, Hans Selye found that the responses of experimental animals to stress were always in a similar and characteristic manner, which he called the "general adaptation syndrome" and postulated that the body's defence mechanism goes through 3 stages:- (1) the alarm reaction of the organism, which was divided into the phases of shock and counter-shock; (2) the 'adaptive' stage in which the organism was capable of resisting the effects of that particular stress but had a lowered resistance to other types of stress; and (3) the stage of exhaustion, which developed after prolonged exposure to stressful stimuli. He showed that this 'adaptive process' was associated with hyperglycaemia, acidosis and a negative nitrogen balance (Selye, 1946).

In 1929, Cuthbertson observed that prolonged rest, even in the absence of trauma, increased the loss of nitrogen, sulphur and phosphorus in the urine and these changes were markedly increased following bony or soft-tissue injury to the limbs (Cuthbertson, 1930). He proposed that the material being catabolised was skeletal muscle and coined the term, 'the catabolic response to injury' (Cuthbertson, 1932). Later, he investigated the changes in plasma proteins following trauma (Cuthbertson and Tompsett, 1935) and the effects of diet on the metabolic response to injury in man and found that diets high in protein and energy content could decrease the loss of protein from body tissues, but could not abolish it completely (Cuthbertson, 1936; Cuthbertson and Munro, 1937). Cuthbertson (1941) also found that injection of an anterior pituitary extract to injured rats was associated with the retention of nitrogen, and injections of metabolic stimulants like thyroid
extract or dinitrophenol caused an accelerated rate of wound healing. These were the first attempts to modify the metabolic changes caused by trauma. In the Arris and Gale lecture of 1942, Cuthbertson introduced the terms 'the ebb phase' and 'the flow phase' of the metabolic response to injury, the former characterised by a decreased metabolic rate immediately after injury and the latter described by hypermetabolism and severe catabolism in the post-traumatic period (Cuthbertson, 1942).

Based on this research, Moore and Ball (1952) carried out extensive studies on surgical metabolism which formed the basis for their classical monograph "The Metabolic Response to Surgery". In 1946, Moore described methods for the measurement of total body water and solids. By combining these measurements with metabolic balance studies he found that surgical stress causes a decrease in the utilization of carbohydrates, markedly increases fat oxidation; and results in a nitrogen loss which is usually in the range of 5 to 7 grams per day but may increase up to 25 grams per day (Moore et al, 1952; Moore, 1958). Moore and Ball described the response to injury in 4 phases: the phase of injury, the corticoid withdrawal phase, the spontaneous anabolic phase and the fat gain phase. Over the next few years, they carried out further studies to substantiate this four-phase sequence of metabolic changes and gained wide acceptance for their model thereafter.

Hayes and Coller (1952) found that the excretion of cations after surgery was determined by the level of adrenocortical activity whereas water excretion was controlled by vasopressin. Sandberg et al (1954) found that 17-hydroxycorticosteroids increased slightly during anaesthesia and markedly during surgery. Engel (1951) found that adrenocortical hormones played a significant role in the regulation of protein metabolism during stressful states. Hayes and Brandt (1952) conducted intravenous glucose
tolerance tests before and after surgery and demonstrated an insulin resistance in the post-operative period as reflected by the presence of abnormal glucose tolerance.

In 1959, Hume and Egdahl reported their classical experiments to show that ACTH responses to injury were not altered by decortication or by sectioning the pituitary stalk; whereas interruption of the afferent sensory pathways (by transection of the peripheral nerve, spinal cord or medulla oblongata above the level of injury) or lesions in the anterior medial eminence of the hypothalamus abolished the response. Since then, the hypothalamus has been accepted as the centre of control for stress responses.

Thus, the hormonal and metabolic stress response to injury had been studied intensively during the first half of this century and the advances made in the past 25 years, with the use of sophisticated methods for measurement of hormonal and metabolic variables, have confirmed several previous findings. In addition, recent work has focused on (1) the neuroendocrine mechanisms responsible for initiation and modulation of the stress response, (2) the metabolic pathways affected by these hormonal changes, and (3) techniques for therapeutic manipulation of the stress response to injury. These recent findings are reviewed in the following sections.
1.3 THE ENDOCRINE RESPONSE TO SURGERY:

1.3.1 The role of the endogenous opioid system:

The discovery of opiate receptors in the brain by Terenius (1973) and Pert and Snyder (1973), which led to the search for an endogenous ligand (Terenius and Wahlstrom, 1975) and its discovery independently by Hughes and Kosterlitz (1977) and by Li and Chung (1976) has greatly clarified the role of the hypothalamus in initiation of the stress response.

Subsequent research has shown that opioid peptides belong to three distinct families: the enkephalins, the dynorphins and the endorphins (Thompson, 1984) which may act on five types of, possibly interconvertible, opioid receptors (Paterson et al, 1983) as short-acting neurotransmitters and co-transmitters, or long-acting neuronal modulators and hormonal mediators (Costa et al, 1980; Hughes and Kosterlitz, 1983). A rich supply of neurones containing all three classes of opioid peptides has been mapped in the hypothalamus as well as the presence of \( \mu \), \( \delta \) and \( k \) receptors which are mainly responsible modulating the responses to noxious stimuli and the control of hormonal secretion through the pituitary gland (Atweh and Kuhar, 1983). Potent analgesic properties have been demonstrated with two similar peptides: \( \beta \)-endorphin and met-enkephalin (Loh et al, 1976; Foley et al, 1979; Morley, 1983). \( \beta \)-lipotropin, the precursor of \( \beta \)-endorphin, and ACTH are derived from a common molecule: pro-opiomelanocortin (Mains, Eipper and Ling, 1977; Terenius, 1978); ACTH and \( \beta \)-endorphin are stored within the same secretory granules in the pituitary gland and are released together during stress (Guillemin et al, 1980).

Effects of anaesthesia: The effect of non-opiate anaesthetic agents on the release of endogenous opioids is currently an unresolved controversy.
In 1976, Berkowitz and co-workers reported that nitrous oxide produced a
dose-related analgesic effect in mice which was significantly antagonised
by an opiate antagonist (naltrexone 5 mg/kg). Furthermore, they found that
found that inhalation anaesthetic agents like halothane, cyclopropane and
enflurane in rats were antagonised by naloxone (10 mg/kg) (Finck et al,
1977). Thus it was proposed that inhalation anaesthetic agents produced
their effects by the release of endogenous opiates in the central nervous
system. Arndt and Freye (1979a and 1979b) found that the cardiovascular and
hypnotic effects of halothane anaesthesia in dogs were inhibited when
naloxone (10 μg/ml) was perfused through the 4th ventricle, and concluded
that opiate receptors in structures bordering the 4th ventricle mediate the
anaesthetic effects of halothane.

Subsequent research has provided contrary evidence to these studies. Harper
et al (1978) found that graded doses of naloxone had no effect on the the
requirement of halothane in rats and thus, concluded that anti-anaesthetic
effects of naloxone were probably due to its analeptic effects on the
central nervous system. Smith et al (1978) found that naloxone failed to
antagonise a loss of the righting reflex in mice caused by nitrous oxide
anaesthesia and Bennett (1978) showed that naloxone did not antagonise the
righting reflex of rats anaesthetised with halothane. Pace and Wong (1979)
also reported that naloxone and naltrexone did not have any effect on the
requirement of halothane anaesthesia in dogs.

In a recent report, Maiewski et al (1984) have shown that the procedures of
intubation, artificial ventilation and anaesthetic induction contribute,
separately and additively, to the increase of plasma β-endorphin
immunoreactivity in rats and these responses were abolished by treatment
with morphine.
Similar conflicting results have been obtained from human subjects, with some studies showing that the analgesic effects of nitrous oxide can be partially reversed by opiate-antagonists (Yang et al, 1980; Chapman and Benedetti, 1979) whereas others have failed to demonstrate any such effects (Duncalf et al, 1978; Levine et al, 1981; Way et al, 1982).

It is not known whether the opiate-antagonist drugs, apart from their blocking action, have any indirect effects on the endogenous analgesic systems. Moreover, if the opiate system is stimulated by some anaesthetic agents as documented by Maiewski et al (1984), \( \beta \)-endorphin or other opioid ligands thus released may bind to opiate receptors which are not recognised by (or blocked by) opiate antagonists like naloxone or naltrexone (Pleuvry, 1983). Furthermore, the binding of \( \beta \)-endorphin to specific non-opiate receptors has been documented, which is not affected by opiate agonists and antagonists, or by enkephalin analogs (Hazum, Chang and Cuatrecasas, 1979). Thus, an explanation of the differential effects of anaesthetic agents on the endogenous opioid system awaits further research on the pharmacology of opiate and non-opiate receptors and their endogenous ligands.

**Effects of surgery:** Dubois et al (1981) were the first to document an increase in plasma \( \beta \)-endoorphin immunoreactivity (PBE(ir)) in patients undergoing abdominal surgery. PBE(ir) and cortisol concentrations did not change during induction of anaesthesia but increased markedly during surgery. The PBE(ir) was raised after awakening from anaesthesia but decreased after the injection of morphine for postoperative analgesia. This group of investigators also found that cortisol and PBE(ir) during surgery were correlated positively with preoperative values of the respective hormones, thus suggesting that levels of arousal before surgical stress may
be important in predicting the stress response. In addition, morphine requirements after surgery were inversely related to the PBE(ir) and cortisol concentrations measured pre-operatively as well as mean PBE(ir) during surgery (Cohen et al, 1982; Pickar et al, 1983). Tamsen et al (1978) reported similar findings in patients undergoing abdominal surgery showing that \( \beta \)-endorphin concentrations in CSF before surgery were inversely related to the amount of analgesia required in the post-operative period. Naber et al (1983) found markedly elevated PBE(ir) in patients undergoing cardiac surgery which were normalised by the first postoperative day.

The effect of anaesthesia on the release of endogenous opioid peptides during surgery has been investigated sparsely. Dubois et al (1982) found that in patients given low-dose fentanyl anaesthesia during abdominal surgery, plasma cortisol and PBE(ir) did not increase during surgery and were raised only when the patients awakened from anaesthesia. A preliminary report has shown that spinal anaesthesia may also block the increase in PBE(ir) during surgery (Finlay et al, 1982). Similarly, Browning et al (1983) found that the increase in PBE(ir) and related peptides during caesarean section or vaginal delivery was obtunded by epidural anaesthesia.

Thus, in adult subjects undergoing surgery, the occupation of opioid receptors by exogenous opiate drugs may prevent the release of endogenous ligands due to stress. It is possible that a negative feed-back mechanism may exist between opioid-receptor occupancy and the endorphin-release mechanisms during stress, either as a result of deep analgesic effects or due to a direct inhibition of endorphin release.

**Endocrine effects of endorphins** :- The various endocrine changes that characterize the stress response may directly or indirectly be related to
the central or peripheral effects of endorphin release during surgery.

Guillemin et al (1977) have demonstrated that ACTH and β-endorphin are released concomitantly from the pituitary gland and that the feed-back inhibition of ACTH secretion also serves to inhibit β-endorphin secretion. Furthermore, experimental data has shown that central administration of human β-endorphin to rats stimulates central sympathetic outflow (Van Loon et al, 1981) to the adrenal medulla and causes the release of adrenaline, noradrenaline and dopamine. Feldman et al (1983) found that injection of β-endorphin to normal subjects stimulates the secretion of glucagon (in low doses) and insulin (in high doses) from the pancreas. In addition, the effect of intravenous and intracerebroventricular β-endorphin injections on the anterior pituitary hormones were studied in human subjects by Foley et al (1979). They found that central and peripheral injection of β-endorphin increased the plasma prolactin concentrations. Plasma growth hormone concentrations were decreased by central injection of β-endorphin, whereas plasma TSH concentrations are unaffected.

Thus, current evidence suggests that endogenous opioids are not only released during surgery but also may be responsible for mediating many of the endocrine responses to surgical stress and thus play a role in initiating the stress response. Further research may further define the role of endogenous opioids in the response to surgical stress.

1.3.2 Catecholamines :-

The methods used currently for the measurement of catecholamines are based on radioenzymatic assay (REA) and high performance liquid chromatography (HPLC) which have a much greater sensitivity (0.01 pmol/L; Hjemdahl, 1979) than the fluorometric methods used previously. It is, therefore, necessary
to rely mainly upon the data obtained from measurement of catecholamines by REA or HPLC techniques (Traynor and Hall, 1981). It is believed currently that the measurement of plasma catecholamines as indices of a ‘response’ (due to activation of the sympathetic nervous system) is of greater value than that of sympathetic activity at rest (Derbyshire and Smith, 1984; Bravo and Tarazi, 1982). Furthermore, the complexities of metabolism and regional uptake that beset the interpretation of plasma noradrenaline concentrations are not applicable to adrenaline; which is metabolically the more important hormone (Christensen et al., 1984; Clutter et al., 1980).

Effects of anaesthesia: Most anaesthetic agents cause a decrease in plasma catecholamines due to a decrease of sympathetic activity that accompanies the onset of unconsciousness. This effect is accentuated in patients who may have increased sympathoadrenal activity due to preoperative anxiety (Derbyshire and Smith, 1984). On the other hand, the hypotension caused by certain anaesthetics may increase plasma catecholamines via baroreceptor-mediated activity (Joyce et al., 1983).

Halter et al. (1977) found that induction of anaesthesia by thiopentone, followed by inhalation of halothane decreased plasma adrenaline concentrations whereas noradrenaline remained unaltered. On the other hand, Philbin et al. (1979) found that plasma noradrenaline levels was increased by thiopentone induction and inhalation of halothane, whereas adrenaline, renin and vasopressin were unaltered. Using thiopentone and morphine for induction followed by halothane anaesthesia, Hoar et al. (1980) found that adrenaline levels decreased significantly, but noradrenaline levels were not altered. In the latter two studies however, the patients studied were treated with β-adrenergic blockers and other drugs till the day of surgery (Philbin et al., 1979; Hoar et al., 1980), which may have had some effect on
their catecholamine responses.

Philbin et al (1981) reported subsequently that induction of anaesthesia with halothane and nitrous oxide was associated with a significant decrease in plasma adrenaline and noradrenaline concentrations. On the other hand, Joyce et al (1982) have recorded that induction of anaesthesia with halothane and nitrous oxide causes a significant increase in noradrenaline values, which is continued upto the third stage of anaesthesia.

These conflicting data can be explained on the basis of direct and indirect effects of halothane anaesthesia on catecholamine secretion. Although halothane may inhibit the secretion of adrenaline and noradrenaline directly, it also causes a decrease in arterial blood pressure due to reduction of myocardial contractility (Smith, 1981) and inhibition of baroreceptor responses (Duke, Fownes and Wade, 1977). The resulting hypotension reflexly stimulates the sympatho-adrenal system and causes the release of catecholamines. Endotracheal intubation may be another factor causing the release of catecholamines during induction, as has been shown by several studies (Russell et al, 1981; Cummings et al, 1983; Derbyshire et al, 1983). Furthermore, there is some evidence to show that halothane anaesthesia may alter noradrenaline kinetics by increasing its fractional pulmonary extraction (Naito and Gillis, 1973).

Hamberger and Jarnberg (1983) have found that induction of anaesthesia with thiopentone and enflurane causes a decrease in adrenaline levels, but noradrenaline levels remain unaffected. Roizen et al (1981) found that incremental doses of halothane, enflurane and morphine were capable of progressively attenuating the adrenergic response (as measured by changes in noradrenaline levels, heart rate and blood pressure) to skin incision.
Recently, Joyce et al (1983) have reported that thiopentone anaesthesia without surgery causes a small, but significant decrease in plasma noradrenaline concentrations. On the other hand, Derbyshire et al (1984) found that no change in noradrenaline levels occurs after induction of anaesthesia with thiopentone (2-3 mg/kg). Thus, it may be concluded that thiopentone has minimal, if any effects on catecholamine release.

The effects of high-dose fentanyl anaesthesia on catecholamine release in patients undergoing cardiac surgery were first investigated by Lappas et al (1980), who found that plasma adrenaline or noradrenaline concentrations did not change during anaesthetic induction with fentanyl (75 µg/kg). On the other hand, Stanley et al (1980) found that same doses of fentanyl caused a significant decrease in adrenaline and noradrenaline during induction, whereas dopamine concentrations did not change. Recent studies have shown that anaesthetic induction with fentanyl 60 µg/kg (Sebel et al, 1981) or 100 µg/kg (Kono et al, 1981) do not cause any changes in plasma adrenaline or noradrenaline concentrations. Hicks et al (1981) found that graded doses of fentanyl (15 and 30 µg/Kg) increased plasma noradrenaline concentrations, whereas the continued injection of fentanyl upto 50 µg/kg was associated with a decrease in noradrenaline concentrations to the pre-induction values (Hicks et al, 1981). This study shows that the cardiovascular effects of fentanyl may cause an initial increase in catecholamine secretion, but higher doses of fentanyl inhibit this response probably by a direct suppression of catecholamine release from the adrenal medulla and extra-medullary chromaffin tissue (Costa et al, 1980).

In contrast, the use of high dose morphine anaesthesia (3 mg/kg) was found to be associated with substantial increases in plasma adrenaline and noradrenaline concentrations (Hoar et al, 1980). It is well-known that
morphine causes hypotension during induction due to a marked release of histamine (Philbin et al, 1981) and also by a direct effect causing systemic vasodilation (Lowenstein et al, 1972); thus, it may stimulate the release of catecholamines via baroreceptor-mediated sympathetic activity. Such effects were not observed with fentanyl anaesthesia due to the lack of histamine release during fentanyl anaesthesia (Rosow et al, 1981).

Spinal and epidural analgesia have also been shown to decrease plasma catecholamine concentrations due to a blockade of the spinal sympathetic ganglia (Pflug and Halter, 1981; Engquist et al, 1980; Kehlet et al, 1980).

In conclusion, although there are minor differences between the isolated effects of various anaesthetic techniques on the sympatho-adrenal system, these differences are small as compared to the changes produced surgical trauma. Thus, of greater importance are the effects of these anaesthetic techniques on the sympatho-adrenal activation produced by surgical trauma.

Effects of surgery:-- An increase in plasma noradrenaline concentrations was observed even with skin incision at the start of surgery (Roizen et al, 1981) and the sympathoadrenal response stimulated by surgical trauma is well-known. Initial studies using fluorometric assays, however, did not find any changes in the catecholamine levels of patients undergoing general surgical procedures (Nikki et al, 1972; Kehlet et al, 1974) or even cardiac surgery (Butler et al, 1977). However, subsequent studies have shown marked changes in plasma catecholamine concentrations in patients undergoing non-cardiac or cardiac surgery.

The adrenaline and noradrenaline response to abdominal surgery was first described by Halter et al (1977). They reported substantial increases in
the plasma concentrations of adrenaline and noradrenaline at the end of surgery, which were maintained at 2 hours after the procedure. These findings were confirmed by Nistrup Madsen et al (1978), who found that cyclic AMP and adrenaline concentrations increased during surgery and the changes in the two hormones were correlated with each other. Thus, they proposed that adrenaline release during surgery mediated its effects via an increase in the second messenger, cyclic AMP (Nistrup Madsen et al, 1978). Engquist et al (1980) found that the adrenaline responses was smaller in patients undergoing tympanoplasty as compared to patients undergoing hysterectomy. They also demonstrated that the responses to surgical stress could be blocked by epidural anaesthesia (Engquist et al, 1980). Pflug and Halter (1981) obtained similar effects with spinal anaesthesia.

These results were confirmed subsequently by several workers (Philbin et al, 1979; Brown et al, 1982; Brismar et al, 1982; Hamberger and Jandberg, 1983) and it was demonstrated that catecholamine responses to abdominal surgery can be inhibited by morphine (Taborsky et al, 1982) or enflurane (Hamberger and Jarnberg, 1983). In addition, recently reported studies have shown that the catecholamine responses to surgery can be blocked even by small doses of fentanyl, given as a single induction dose (Campbell et al, 1984) or as a continuous infusion (Pathak et al, 1985). These are the first reports which have shown that low doses of fentanyl cause an effective suppression of catecholamine responses in patients undergoing non-cardiac surgery, similar to the effects of high-dose fentanyl anaesthesia in patients undergoing cardiac surgery and cardiopulmonary bypass.

Although Replogle et al (1962) found substantial increases in plasma catecholamine concentrations during cardiopulmonary bypass, other early studies using fluorometric assays for measurement of catecholamines (Hine
et al, 1976; Tan et al, 1976) failed to detect any changes. Using a radioenzymatic assay, Hoar et al (1980) demonstrated that cardiac surgery and particularly, cardiopulmonary bypass (CPB) were associated with massive increases in plasma concentrations of adrenaline and noradrenaline. These findings were confirmed by Stanley et al (1980), who also found that the catecholamine response was blocked by high-dose fentanyl anaesthesia (75 μg/kg) up to the start of CPB, but not thereafter. These findings were confirmed by subsequent studies (Kono et al, 1981; Sebel et al, 1981; Zurick et al, 1982). During the postoperative period, Engelman et al (1983) have shown that plasma adrenaline remained elevated on the first and second postoperative day, whereas plasma noradrenaline concentrations were elevated for more than 3 days after surgery (Engelman et al, 1983).

Thus, it is concluded that non-cardiac or cardiac surgery are potent stimuli for the catecholamine secretion in adult patients. The responses to non-cardiac surgery may be obtunded or abolished by various anaesthetic techniques, but the responses stimulated by cardiopulmonary bypass are not abolished by currently used anaesthetic techniques. It is likely that these catecholamine responses would have major metabolic effects in the postoperative period.

1.3.3 Pituitary hormones :-

Apart from the secretion of catecholamines, the initiation of the stress response from the hypothalamus also involves alterations in the secretion of hormones from the anterior pituitary: the adrenocorticotropic hormone (ACTH), growth hormone (GH), prolactin, gonadotrophins and thyroid stimulating hormone (TSH); and from the posterior pituitary: arginine vasopressin (AVP). Although other peptides (such as β-melanotropin, which may be involved with aldosterone secretion (Matsuoka et al, 1981), or
pro-γ-melanotropin, which may increase the adrenal responses to ACTH (Al-Dujaili et al, 1981)) are also secreted from the pituitary gland, their role in the mediating or modulating the stress response to surgical trauma is not well-characterised.

**Adrenocorticotrophic hormone** : Newsome and Rose (1971) found that plasma ACTH concentrations were not altered during the induction of anaesthesia, but were raised markedly at 1 hour after the start of surgery; this intra-operative increase was abolished by spinal anaesthesia (Newsome and Rose, 1971). Oyama et al (1968) found that ACTH was released in response to anaesthetic agents such as diethyl ether or halothane (Oyama and Takiguchi, 1970). During the surgical operation, Oyama has proposed that pulses of ACTH may be released intermittently into the circulation (Oyama, 1973).

**Growth hormone** : In patients undergoing abdominal surgery, Ross et al (1966) found that plasma growth hormone concentrations increased during surgery and were raised in the early postoperative period. Charters et al (1969) found that plasma GH increased during surgery but had returned to preoperative concentrations by the end of surgery. Newsome and Rose (1971) also found substantial increases in plasma GH concentrations during surgery. These findings were confirmed in several studies and it was documented that the raised GH concentrations were maintained only upto 2 hours after surgery (Noel et al, 1972; Reier et al, 1973; Wright and Johnston, 1975; Brandt et al, 1976; Hall et al, 1978; Aarimaa et al, 1978; Cooper et al, 1979; Walsh et al, 1981; Lehtinen et al, 1981).

The intra-operative increase in plasma GH concentrations was found to be proportional to the extent of surgical trauma (Wright and Johnston, 1975; Aarimaa et al, 1978). The GH response was found to be inhibited by spinal
or epidural anaesthesia (Newsome and Rose, 1971; Noel et al, 1972; Brandt et al, 1976), by large doses of morphine (Reier et al, 1973), fentanyl (Hall et al, 1979) or sufentanil (Bovill et al, 1983) used for anaesthesia. Cooper et al (1979) found that epidural anaesthesia extending to a level of T10 did not alter the GH response to surgery; this was probably due to a low level of the block. Elliott and Alberti (1983) have suggested recently that patients undergoing open-heart surgery may belong either to a group of 'high responders' or to a group of patients who show hardly any growth hormone responses to surgical stress.

It is questionable whether the elevation of growth hormone during surgery has any significant influence on the metabolic stress response to surgery particularly in view of the short duration of these changes. It has been shown that hypophysectomised patients receiving steroid replacement therapy have an apparently normal metabolic response to surgery (Thoren, 1974).

**Prolactin**: It has been documented that plasma prolactin concentrations show the largest increases during surgery (Noel et al, 1972; Brandt et al, 1976; Moore et al, 1980; Lehtinen et al, 1981), but have returned to presurgical values by 24 hours after surgery (Noel et al, 1972; Brandt et al, 1976). It appears that these responses are not modulated by the severity of surgical trauma, since Lehtinen et al (1981) found that the prolactin responses to laparotomy and laparoscopy were of a similar magnitude. The prolactin responses of postmenopausal women were found to be significantly greater than those of men (Moore et al, 1980).

It has been documented that the prolactin responses can be blocked by epidural anaesthesia (Brandt et al, 1976). In addition, it has been demonstrated that the release of prolactin is stimulated by opiate agonists
such as morphine (Tolis et al, 1975) and β-endorphin (Foley et al, 1979),
and decreased by opiate antagonists such as naloxone (Lehtinen et al,
1981). However, prolactin does not appear to have any role in mediating the
metabolic stress response to surgical trauma.

**Gonadotrophins**: Charters et al (1969) found that the concentrations of
plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) were
unaltered during and after surgery. However, Carstensen et al (1972) found
that LH values were raised in the week following surgery and this finding
was confirmed by Oyama et al (1977). In addition, a transient increase in
plasma LH concentrations during surgery was found in several studies (Aono
et al, 1972; Oyama et al, 1977; Lehtinen et al, 1981), whereas plasma FSH
was found to be unchanged.

**Thyroid stimulating hormone**: In some studies, no significant changes in
plasma TSH concentrations in response to anaesthesia (Oyama, 1973) or
surgery (Burke, 1971; Chan et al, 1978) have been documented. However,
Adami et al (1978) reported a transient increase in plasma TSH during
surgery. Thereafter, plasma TSH values were found to be decreased during
the two days following surgery, and it was suggested that this was an
effect of the cortisol secretion during and after surgery (Adami et al,
1978). Elliot and Alberti (1983) have suggested that plasma TSH may increase
soon after skin incision, but this finding has not been confirmed.

**Arginine vasopressin**: The secretion of AVP in adult patients undergoing
surgery may be stimulated by various anaesthetic agents, e.g., halothane,
methoxyflurane or diethyl ether (Oyama, 1973), and substantially by the
surgical procedure (Moran et al, 1964). In patients undergoing non-cardiac
surgery, plasma AVP concentrations were found to increase rapidly after the
start of surgery (Cochrane et al, 1981) and were found to be proportional
to the severity of the surgical stress (Moran et al, 1964; Wu and Zbuzek,
1982; von Bormann et al, 1983). In patients undergoing cholecystectomy,
plasma AVP values remained elevated for upto 6 hours after surgery
(Cochrane et al, 1981); but following more extensive abdominal or thoracic
surgery the elevated values were documented for more than 5 days
postoperatively (von Bormann et al, 1983).

In patients undergoing cardiac surgery, marked increases in plasma AVP
concentrations were found before the start of cardiopulmonary bypass (CPB)
(Philbin and Coggins, 1978) and further increases were observed in response
to CPB (Philbin et al, 1979; Simpson and Forsling, 1977; Crone et al, 1982).

Although Cochrane et al (1981) found that the AVP response was not altered
by epidural anaesthesia, the patients receiving epidural anaesthesia in
their study had a substantial fall in the blood pressure during surgery,
which may have contributed to the increase in plasma AVP values. On the
other hand, it was found that the AVP responses during surgery can be
abolished effectively with the use of epidural anaesthesia (Bonnet et al,
1982; von Bormann et al, 1983), an effect which can be prolonged into the
postoperative period (von Bormann et al, 1983). In addition, the AVP
responses of patients undergoing cardiac surgery were abolished by morphine
(Philbin and Coggins, 1978; Crone et al, 1982) and fentanyl anaesthesia
(Stanley et al, 1979; Crone et al, 1982) before the start of CPB, and were
decreased in response to CPB (Crone et al, 1982).

The physiological role of AVP secretion during surgery is probably as a
vasopressor rather than for the regulation of plasma osmolality, since the
increase during surgery is five or ten times the concentration required for
osmoregulation (Crone et al, 1982). In addition, these changes are not accompanied by changes in plasma osmolality (Philbin et al, 1977; Stanley et al, 1979), although the urine produced after major surgery is always hyperosmolar and its volume depends on the solute load being excreted (Le Quesne et al, 1985). In addition, as discussed below, AVP may have important effects on the regulation of ketogenesis during surgery (Williamson, 1981).

1.3.4 Pancreatic hormones :-

Of the hormones secreted by the endocrine pancreas, insulin and glucagon are the most important with respect to the regulation of metabolism during surgery. The secretion of these hormones in the peri-operative period is primarily controlled by two opposing influences, those of blood glucose and plasma adrenaline concentrations, in addition to other non-specific factors which may regulate islet cell function (Halter et al, 1984).

Insulin : Ross et al (1966) found that plasma insulin concentrations were decreased during surgery and were elevated postoperatively in patients undergoing abdominal surgery. They also documented a significantly reduced tolerance to intravenous glucose, despite raised insulin concentrations in the postoperative period (Ross et al, 1966). Similar findings were reported by Allison et al (1968) from a study of burned patients and later, were confirmed with regard to patients undergoing surgery (Allison et al, 1969). These findings have been confirmed in several studies of patients subjected to non-cardiac surgery (Horrelt et al, 1969; Aarima et al, 1973; Wright et al, 1974; Giddings, 1974; Russell et al, 1975; Brandt et al, 1976; Cooper et al, 1980; Walsh et al, 1983), as well as to cardiac surgery with cardiopulmonary bypass (Allison, 1971; Mills et al, 1972; Kobayashi et al, 1980; Walsh et al, 1981; Kuntschen et al, 1985). It has been proposed that
the duration of insulin suppression corresponds with the 'ebb phase of injury' as defined by Cuthbertson in 1942, and has been documented for upto 24 hours after surgery (Walsh et al, 1981).

Studies both in vitro and in vivo have established that catecholamines inhibit insulin secretion by an α-adrenergic mechanism, but also stimulate insulin release by a β-adrenergic mechanism (Halter et al, 1984). Thus, the α-adrenergic stimulation caused by the marked adrenaline release in response to surgery may be responsible for the insulin suppression (Halter et al, 1984). Since the effect of adrenaline in low plasma concentrations is predominantly β-adrenergic, insulin suppression may not be observed in these circumstances (Young and Landsberg, 1977). Thus, it was found that α-adrenergic blockade could increase insulin secretion during surgery (Nakao and Miyata, 1977), whereas patients given β-blocking drugs like propanolol were found to have a lower plasma insulin concentrations during surgery (Cooper et al, 1980). Walsh et al (1983) have reported recently that insulin suppression during surgery was overcome by a strong glycaemic stimulus. However, this effect has not been confirmed. Kuntschen et al (1985) have demonstrated that in patients undergoing cardiac surgery and normothermic CPB, there is a reduction in insulin release as well as insulin action during surgery. The insulin resistance observed after surgery may be mediated mainly by the effects of adrenaline release during (Bessey et al, 1983).

Although insulin secretion is suppressed during surgery, it may still maintain a check on the catabolic effects of the counter-regulatory hormones, the lack of this effect is observed in insulin-deficient diabetic patients, in whom rapid and severe catabolic changes may result during stressful states (Alberti et al, 1980; Mills et al, 1973). The use of
insulin to treat the metabolic changes associated with stress is discussed below (Section 1.1.5).

Glucagon: In patients undergoing a variety of surgical procedures, Russell et al (1975) found that plasma glucagon concentrations increased significantly during surgery, but a further and substantial increase was observed on the first postoperative day which was maintained up to the fifth day after surgery. However, in patients subjected to abdominal hysterectomy Brandt et al (1976) found only slight and insignificant changes in plasma glucagon concentrations, the pattern of which was not altered in patients given epidural anaesthesia during the surgical procedure. These findings were contradicted by Foster et al (1979), who found that plasma glucagon concentrations were elevated in patients undergoing abdominal surgery at 2 days postoperatively, but had returned to preoperative concentrations at 4 days after surgery.

In patients undergoing cardiac surgery, Kobayashi et al (1980) found that plasma glucagon values were unchanged during cardiopulmonary bypass (CPB) and were found to be raised at 24 hours after surgery. Similar findings during cardiac surgery and CPB were reported by Teramoto et al (1980) and plasma glucagon concentrations were raised significantly at 6 hours after surgery, reached a peak at 24 hours after surgery and elevated values were maintained up to 5 days postoperatively. A similar pattern of changes in plasma glucagon has been found after severe trauma (Lindsey et al, 1974; Meguid et al, 1974) and burns (Wilmore et al, 1974; Batstone et al, 1976).

Experimental studies on animals (Eigler et al, 1980) and human volunteers (Shamoon et al, 1981; Bessey et al, 1984) have amply demonstrated the physiological role of glucagon in mediating the metabolic stress response.
Thus, infusion of glucagon was found to increase glucose production, which was potentiated by adrenaline infusion and sustained by cortisol (Eigler et al, 1980; Shamoon et al, 1981). In addition, it has been demonstrated that glucagon increases gluconeogenesis and urea production and utilization of the free amino acid pool (Wolfe et al, 1979; Boden et al, 1984).

1.3.5 Adrenocortical hormones :-

The effects of anaesthesia and surgery on the secretion of corticosteroid hormones have been studied extensively in previous (Brunt and Ganong, 1963) and recent (Elliott and Alberti, 1983) investigations. The secretion of glucocorticoids, mainly cortisol, plays a central role in mediating the metabolic response to surgical or traumatic stress (Alberti et al, 1980). On the other hand, the secretion of mineralocorticoids, mainly aldosterone, mediates the electrolyte fluxes following surgery (Le Quesne et al, 1985).

Cortisol : The changes in plasma concentrations of cortisol are probably initiated by the secretion of ACTH; however, the plasma ACTH concentrations after surgery are far greater than those required to produce a maximal cortisol response and the pituitary-adrenocortical feed-back mechanism is not functional during or after surgery since the plasma concentrations of both hormones are found to be elevated (Traynor and Hall, 1981).

Before the start of surgery, a decrease in plasma cortisol concentrations was observed during anaesthetic induction with several anaesthetic drugs such as halothane (Werder et al, 1970), enflurane, thiopentone or pentobarbital (Oyama, 1973); whereas anaesthetic agents like diethyl ether or ketamine caused an increase in plasma cortisol values (Oyama, 1973). Recently, it was demonstrated that etomidate causes a marked suppression of cortisol secretion by a direct inhibition of 11β-hydroxylation in the
adrenocortical cells (Fry and Griffiths, 1984; Wagner et al, 1984; DeJong et al, 1984) and prolonged etomidate infusion has been implicated in an increased mortality in critically ill patients (Watt and Ledingham, 1984).

Effects of surgery: It has been well-documented that plasma cortisol concentrations increase with the start of surgery and peak levels were reached within a few hours after surgery (Johnston, 1964; Alberti et al, 1980). These findings have been confirmed by several studies in adult patients undergoing general surgical procedures (Ross et al, 1966; Bromage et al, 1971; Lush et al, 1972; Gordon et al, 1973; Bowen and Richardson, 1974; Cosgrove and Jenkins, 1974; Clarke et al, 1974; Reier et al, 1974; Oyama et al, 1977; Namba et al, 1980; Moore et al, 1980; Cooper et al, 1981; Haxholdt et al, 1981; Engquist et al, 1981; Cowen et al, 1982; Cooper et al, 1982; Porter et al, 1983) as well as patients subjected to cardiac surgery with cardiopulmonary bypass (George et al, 1974; Yokota et al, 1977; Taylor et al, 1978; Walsh et al, 1981; Kono et al, 1983; Bovill et al, 1983). Furthermore, it was found that the increase in plasma cortisol concentrations was generally proportional to the severity of the trauma, whether surgical or accidental (Clarke, 1970; Kudoh et al, 1973; George et al, 1974; Nistrup Madsen et al, 1976; Batstone et al, 1976; Oyama et al, 1977; Stoner et al, 1977; Stoner et al, 1979; Foster et al, 1979; Alberti et al, 1980). The duration of the increased plasma cortisol concentrations was related either to the severity of surgical trauma or to the development of postoperative complications. Thus, elevated cortisol concentrations were found by Brandt et al (1978) on the first postoperative day, by Foster et al (1979) on the fourth postoperative day and by Oyama et al (1977) during the first postoperative week.

Several anaesthetic techniques have been used to decrease the magnitude and

Campbell et al (1984) have shown recently that the cortisol response to upper abdominal surgery was inhibited even with moderate doses of fentanyl. Extradural analgesia with small doses of morphine (Moore et al, 1984) or diamorphine (Cowen et al, 1982) was also found to inhibit the postoperative cortisol responses to major surgery. The effects of opiate drugs in large doses intravenously or in the extradural space were probably due to their interaction with opiate receptors in the hypothalamus or in the spinal cord respectively, thereby obtunding the effects of the surgical stimulus. In this respect, it is interesting that large doses of fentanyl had no effect on the established cortisol response to surgery (Bent et al, 1984).

The metabolic effects of increased cortisol secretion during and after surgery may be much greater than expected since the plasma cortisol binding capacity was found to be decreased during cardiac and non-cardiac surgery (Uozumi et al, 1972). Thus, the proportion of the non-protein bound and physiologically active hormone is increased to a greater extent than is evident from changes in plasma cortisol concentration. Cortisol is known to stimulate proteolysis and the release of gluconeogenic amino acids from
extrahepatic tissues, mainly skeletal muscle (Karl et al, 1976; Muhlbacher et al, 1984; Lund and Williamson, 1985), and causes a redirection of carbon flow from glutamine toward alanine formation. In the liver, glucocorticoids have mainly a 'permissive' effect on the stimulation of gluconeogenesis by glucagon (Newsholme and Leech, 1983).

Peri-operative changes in the other glucocorticoid hormones such as corticosterone, 11-deoxycortisol and cortisone have not been studied to a similar extent as cortisol. However, since the plasma concentrations of these hormones have been found to change during and after surgery in a similar pattern to that of plasma cortisol (Uozumi et al, 1972; Moore et al, 1985) and since they are known to have similar, though less potent, effects on intermediary metabolism, it may be assumed that their role in the surgical stress response of adult patients is similar and secondary to that of cortisol.

Aldosterone: In patients undergoing major abdominal surgery, plasma aldosterone concentrations were found to increase within minutes after the start of surgery and remained elevated for upto 24 hours after surgery (Enquist et al, 1978; Cochrane, 1978; Brandt et al, 1979). Similar changes have been documented in recent studies from patients undergoing abdominal surgery, although the duration of raised plasma aldosterone concentrations was short-lived (Wagner and White, 1984; Fragen et al, 1984; Moore et al, 1985). In patients undergoing cardiac surgery and cardiopulmonary bypass (CPB), plasma aldosterone concentrations increased before the start of CPB (Kono et al, 1981), and were found to increase markedly and progressively during cardiopulmonary bypass (Bailey et al, 1975).

These studies have also shown that the aldosterone response to surgery can
be inhibited by intravenous saline given during surgery (Engquist et al., 1978; Cochrane, 1978), epidural analgesia (Brandt et al., 1979), large doses of fentanyl (Kono et al., 1981) or etomidate anaesthesia (Wagner and White, 1984; Fragen et al., 1984; Moore et al., 1985). Saline administration during surgery probably inhibits the stimulation of aldosterone secretion caused by renin release, epidural or fentanyl anaesthesia may obtund the aldosterone response by decreasing ACTH release, whereas etomidate directly inhibits the formation of aldosterone in adrenal cortical cells.

1.3.6 Renin-angiotensin system :-

A three-fold increase in the plasma renin activity (PRA) of adult patients during surgery was documented by Robertson and Michelakis (1972). In patients undergoing cardiac surgery, Bailey et al. (1975) found that PRA increased markedly with the start of surgery and elevated levels were maintained during cardiopulmonary bypass; the changes in PRA were closely related to the changes in blood pressure during the procedure. Jakubowski and Taube (1975) found that PRA increased after induction of anaesthesia and remained elevated up to the first postoperative day. An increase in PRA in response to surgery, but not anaesthesia, has been confirmed by subsequent studies (Bevan et al., 1975; Brandt et al., 1979; Watkins et al., 1979; Philbin et al., 1981), however, the raised PRA had returned to normal levels within one hour after surgery (Brandt et al., 1979; Kanto et al., 1981).

Peri-operative changes in the PRA were found to be inhibited by epidural anaesthesia (Bevan et al., 1975; Brandt et al., 1979) and it was proposed that this effect was due to a blockade of afferent impulses from the surgical area as well as efferent impulses via the renal nerves during surgery. In contrast, high-dose fentanyl anaesthesia was found to have little or no effect on the changes of PRA in patients undergoing cardiac surgery.
surgery (Kono et al, 1981; Zurick et al, 1982).

1.3.7 Thyroid hormones :-

Studies on the changes in thyroid hormones in patients undergoing surgery have shown conflicting data with regard to plasma thyroxine concentrations. In patients undergoing various surgical procedures, Burr et al (1975) found decreased plasma thyroxine values on the first, second and fifth days after surgery. A similar decrease during and/or after surgery has been reported in subsequent studies (Adami et al, 1978; Elliott and Alberti, 1983) which was associated with a decrease in the thyroxine binding capacity of plasma (Adami et al, 1978). On the other hand, Brandt et al (1976) found an increase in plasma thyroxine during surgery with general anaesthesia, which was blocked by epidural anaesthesia. Similar findings during surgery were reported by Chan et al (1978) and Prescott et al (1979), who also found that plasma thyroxine was decreased significantly after surgery.

In contrast, similar peri-operative changes in plasma tri-iodothyronine concentration ($T_3$) have been reported in all these studies. Thus, a consistent fall in plasma $T_3$ and an increase in plasma reverse $T_3$ was documented, giving rise to a marked decrease in the $T_3/rT_3$ ratio during surgery (Burr et al, 1975; Brandt et al, 1976; Chan et al, 1978; Adami et al, 1978, Prescott et al, 1979). The decreased plasma $T_3$ concentrations persisted for up to 7 days after surgery (Burr et al, 1975; Chan et al, 1978; Adami et al, 1978). Prescott et al (1979) proposed that the altered peripheral metabolism of $T_3$ giving rise to these changes was due to cortisol release during surgery, but this is unlikely since Brandt et al (1976) have documented the decrease in $T_3/rT_3$ ratios even in patients whose cortisol responses were abolished by epidural anaesthesia. It has been speculated that these changes may represent an adaptation response to...
the hypermetabolism and increased oxygen consumption observed after major surgery (Elliott and Alberti, 1983).

1.3.8 Conclusion :-

Thus, the endocrine response of adults patients to surgical trauma is characterised mainly by an increase in the circulating concentrations of the catabolic hormones and a concomitant decrease in plasma concentrations of the global anabolic hormone, insulin. The magnitude and duration of this response, particularly with respect to changes in plasma cortisol, catecholamines, glucagon, growth hormone and vasopressin concentrations, may be roughly proportional to the extent of the surgical injury. In addition, changes in circulating concentrations of some of these hormones may be prolonged in patients with postoperative complications. These hormonal changes may have profound effects on the metabolic homeostasis of patients during and after surgery.

1.4 THE METABOLIC RESPONSE TO SURGERY :

1.4.1 Carbohydrate metabolism :-

The changes in carbohydrate metabolism are characterised primarily by a substantial hyperglycaemic response during and after surgery, which may be mediated both by an increase in glucose production and a decrease in peripheral glucose utilization.

Hyperglycaemia : An increase in blood glucose concentrations has been observed invariably in adult patients undergoing surgical, accidental or burn injury. From several studies on patients undergoing general surgical procedures (Aarimaa et al, 1978; Alberti et al, 1980; Allison et al, 1969; Bent et al, 1984; vonBormann et al, 1983; Brandt et al, 1976a; Bromage et
al, 1971; Campbell et al, 1984; Clarke, 1970; Clarke et al, 1974; Cooper et al, 1979; Cooper et al, 1981; Cowen et al, 1982; Engquist et al, 1977; Engquist et al, 1981; Foster et al, 1979; Hall et al, 1978; Halter et al, 1984; Haxholdt et al, 1981; Horrelt et al, 1969; Kehlet et al, 1979; Moore et al, 1981; Nistrup-Madsen et al, 1976 and 1978; Russell et al, 1975; Stjernstrom et al, 1981; Walsh et al, 1983; Wright et al, 1974), it was documented that blood glucose concentrations increase shortly after the start of surgery, this increase was continued during the procedure to reach peak levels towards the end of surgery. Furthermore, the hyperglycaemic responses to surgery were found to be related to the severity of the surgical trauma (Weddell and Gale, 1934; Clarke, 1970; Wright et al, 1974; Aarimaa et al, 1978; Traynor and Hall, 1981).

In patients undergoing cardiac surgery, the hyperglycaemic response was further accentuated particularly during and after cardiopulmonary bypass (Allison, 1971; Bevan and Rosales, 1979; Brandt et al, 1978a; Crone et al, 1982; Kobayashi et al, 1980; Kono et al, 1981; Mills et al, 1973; Philbin et al, 1981a; Sebel et al, 1981; Stanley et al, 1980; Teramoto et al, 1980; Walsh et al, 1981; Zurick et al, 1982; Kuntschen et al, 1985). In a recent report, McKnight et al (1985) have shown that the continuous monitoring of blood glucose in patients undergoing cardiac surgery and cardiopulmonary bypass revealed several changes which were not detected on intermittent measurements, the most prominent of which were a sharp fall in blood glucose concentrations with the start of CPB and a marked increase observed at the time of rewarming after deep hypothermia (McKnight et al, 1985).

The relative contributions of increased glucose production or decreased glucose utilization to the production of this hyperglycaemic response have been debated frequently. The evidence for each of these mechanisms has been
obtained mostly from labelled-substrate turnover studies or from arterio-
venous catheterisation studies across the splanchnic circulation or
skeletal muscle beds.

Glucose production: The primary pathways for endogenous glucose
production would be glycogenolysis or gluconeogenesis. Sunzel (1963) found
that the hepatic glycogen content of patients subjected to surgical trauma
was decreased substantially at the end of surgery. These changes in liver
glycogen content were not affected by the calories supplied to patients
preoperatively or by the varying amounts of dextrose given intravenously
during surgery. Using glucose-turnover studies in patients undergoing
elective surgery, Long et al (1971) found that glucose turnover and glucose
oxidation rates two days after surgery were similar to the preoperative
values. In critically ill patients with major injury or sepsis the glucose
turnover and glucose oxidation rates were more than double the values from
normal controls (Long et al, 1971). In similar patients, Gump et al (1974)
catheterised the splanchnic circulation and found that glucose production
was not inhibited by a large intravenous glucose load. In a subsequent
study (Gump et al, 1975), they found that the splanchnic glucose production
was mainly due to gluconeogenesis, as shown by a marked uptake of
gluconeogenic amino acids, mainly alanine, and the increased production of
glucose and urea. However, the uptake of other gluconeogenic precursors
such as lactate, pyruvate and glycerol was not measured. An exogenous
glucose load was found to suppress gluconeogenesis in normal controls but
not in septic, postoperative patients (Gump et al, 1975).

Further evidence for increased glucose production in patients undergoing
abdominal surgery was obtained from arteriovenous catheterisation studies
across the muscle bed of the leg reported by Stjernstrom et al (1981a).
They found that glucose uptake in the leg was not altered during or after surgery, whereas the blood glucose concentrations increased substantially during the corresponding period. The release of lactate increased markedly during surgery to levels which exceeded glucose uptake and was associated with an increased release of pyruvate, alanine and glycerol. In a combined study of splanchnic as well as peripheral uptake/release of circulating metabolites (Stjernstrom et al, 1981b), they found an increased splanchnic release of glucose which was associated with an increased uptake of the gluconeogenic precursors: lactate, pyruvate, alanine and glycerol. In this study, however, a decrease in the uptake of glucose by skeletal muscle was also documented during surgery (Stjernstrom et al, 1981b).

Thus, the available evidence indicates that increased glucose production from the liver and, to a lesser extent, from the kidneys may contribute substantially to the hyperglycaemic response. However, a decreased glucose utilization during surgery can not be ruled out on the basis of these data.

Glucose utilization: The evidence for a decreased utilization of glucose peri-operatively has been based mainly on intravenous glucose tolerance tests done before, during and after the surgical procedure. Ross et al., (1966) found that glucose tolerance was impaired at 24 and 48 hours after surgery, and a decreased glucose utilization coefficient was associated with a decreased insulin response to the glucose load. Allison et al (1969) performed glucose tolerance tests during surgery and also found a 'diabetic pattern' of changes in blood glucose concentration. Similar findings were obtained by Aarimaa et al (1973) during surgery, and it was found that the impaired glucose tolerance was maintained for two days after surgery.

Wright et al (1974) carried out glucose tolerance tests in three groups of
patients who were subjected to an increasing severity of surgical stress and found that the decrease in glucose utilization during surgery was related to the extent of surgical trauma. Furthermore, a decreased glucose utilization was documented up to the fifth postoperative day in patients undergoing minor surgery, up to the eighth postoperative day in patients undergoing moderate surgery and beyond the eighth day after surgery in patients undergoing major surgical stress (Wright et al, 1974). A decrease in glucose utilization peri-operatively was also proposed by Walsh et al (1983) who found that surgical hyperglycaemia was accentuated markedly in patients given dextrose infusion during surgery.

In adult patients undergoing cardiac surgery, a marked hyperglycaemia was observed in patients who received a glucose load in the pump priming fluid (Mills et al, 1973), thereby indicating a decreased glucose utilization in the postoperative period. Recent evidence from patients undergoing normothermic cardiopulmonary bypass (Kuntschen et al, 1985) also indicates that a decreased glucose utilization during and after surgery is responsible for the hyperglycaemic response observed in patients subjected to cardiac surgery. Furthermore, Kuntschen et al (1985) have confirmed that this decrease in glucose utilization is associated with a concomitant suppression of insulin secretion and resistance to its action.

It may be pointed out that the rates of glucose utilization in patients with extensive injuries, burns or sepsis (Allison et al, 1968; Wilmore, 1981; Long et al, 1971) can not be extrapolated to patients undergoing surgical trauma. This is because the extent of injured tissues in surgical patients would be quantitatively much less than patients with burns or sepsis. Im and Hoores (1970) have demonstrated markedly increased rates of glucose utilization and lactate production in injured tissues, since 70% of
the energy requirements in such tissues are obtained from glycolysis. Thus, normal or increased rates of glucose utilization may be documented in patients with burns or sepsis which would be contributed almost entirely by the injured area (Wilmore, 1981).

Concomitant with the surgical hyperglycaemia, an increase in blood lactate and blood pyruvate concentrations has been documented in several studies (Hall et al, 1978; Walsh et al, 1981; Walsh et al, 1983; Bent et al, 1984). As shown by Stjernstrom et al (1981a) the origin of these metabolites may be from the skeletal muscles due to an activation of the Cori cycle by adrenaline (Kusaka and Ui, 1977). In addition, it is likely that lactate production in injured tissues may contribute partially to the increased lactate concentrations after surgery (Im and Hoores, 1975).

Thus, it is concluded that the hyperglycaemic response to surgery may result from a combination of increased production and decreased utilization of glucose. The hormonal changes mediating the hyperglycaemic response have been described in the previous section. These hormonal changes may cause the stimulation of glycogenolysis and gluconeogenesis after surgery, with a decreased glucose utilization particularly during the surgical procedure. The relative proportion of these mechanisms may depend upon various factors in addition to severity of surgical trauma and anaesthetic management of the patient. The latter variable is of particular interest, since the hyperglycaemic response can be altered by specific anaesthetic techniques.

**Effects of anaesthesia** : The hyperglycaemic response to surgery can be decreased or abolished by those anaesthetic procedures which also inhibit the peri-operative changes in catecholamines and cortisol. Thus, a decreased hyperglycaemic response has been documented in patients given
epidural anaesthesia (Bromage et al, 1971; Engquist et al, 1977; Cooper et al, 1979; Kehlet et al, 1979) or fentanyl anaesthesia during non-cardiac surgery (Cooper et al, 1981; Cooper et al, 1982; Campbell et al, 1984) as well as during cardiac surgery up to the start of the cardiopulmonary bypass (Sebel et al, 1981; Walsh et al, 1981). Extradural anaesthesia given with diamorphine was also found to inhibit the hyperglycaemic response to major abdominal surgery (Cowen et al, 1982). Wiklund and Jorfeldt (1975) found that the splanchnic release of glucose was decreased significantly even by a small dose of fentanyl given after surgery. However, fentanyl given in high doses was found to have little effect on the established hyperglycaemic response to surgery (Bent et al, 1984). Thus, the changes in blood glucose concentration can be inhibited by those of anaesthetic techniques which were found to obtund the hormonal stress response.

1.4.2 **Protein metabolism** :-

The changes in protein metabolism after major surgery are characterised by a negative nitrogen balance which is the net result of increased protein breakdown and decreased protein synthesis in extrahepatic tissues, together with the utilization of amino acids for gluconeogenesis and synthesis of acute phase reactants in the liver and for the healing process in injured tissues. Although previous studies on peri-operative protein metabolism have concentrated almost entirely on the nitrogen balance after surgery (Cuthbertson, 1979), turnover studies using radiolabelled amino acids and arteriovenous catheterisation studies across organs have been used recently to define the various components of nitrogen loss after surgery.

**Negative nitrogen balance** : Urinary nitrogen excretion is increased in patients undergoing major abdominal surgery and remains elevated for up to five days after surgery. During this period, it has been calculated that
the patient may lose amounts of nitrogen equivalent to 500 grams of lean muscle tissue per day (Johnston, 1964). The duration and magnitude of postoperative nitrogen loss was related primarily to the severity of the surgical operation (Williamson et al., 1977; Foster et al., 1979; Fleck, 1980). The relationship of injury severity and nitrogen loss was also observed in patients with accidental trauma (Cuthbertson, 1979; Oppenheim et al., 1980) and burns (Blackburn, 1981).

Smith et al. (1975) found that patients who developed hyperketonaemia after accidental injury were found to have a decreased nitrogen excretion during the 24 hours following injury as compared to a group of similar patients who remained normoketonaemic after injury. This finding was confirmed in a later study of injured patients (Williamson et al., 1977) and was also observed in patients undergoing major surgery (Rich and Wright, 1979). The relationship of increase plasma ketone bodies to nitrogen excretion is discussed below. In patients who were given epidural anaesthesia during surgery and for 24 hours postoperatively, Brandt et al. (1978) found that the cumulative nitrogen loss over the five days following surgery was decreased significantly as compared to a control group of patients. This effect was associated with a complete inhibition of the changes in blood glucose and plasma cortisol concentrations (Brandt et al., 1978).

**Changes in plasma amino acids:** The total plasma amino acids were found to be decreased slightly in patients undergoing minor and moderate surgical trauma (Vinnars et al., 1975; Johnston et al., 1980). The decrease during surgery was found in the plasma concentrations of gluconeogenic amino acids, particularly alanine (Johnston et al., 1980; Elia et al., 1980a), whereas the branched-chain amino acids (BCAA) have been found to increase after major injury (Johnston et al., 1980; Wedge et al., 1976). These changes were found
to be associated with similar changes in the intramuscular concentrations of BCAAs (Vinnars et al, 1975) and the whole-body turnover of leucine was found to be increased after major injury (Elia et al, 1980b).

Thus, these changes in combination with the release of gluconeogenic amino acids found in catheterisation studies (Stjernstrom et al, 1981a) suggest that skeletal muscle may be catabolised postoperatively, resulting in the production of gluconeogenic amino acids mainly alanine and glutamine, (Karl et al, 1976; Muhlbacher et al, 1984) which are substrates for the hepatic or renal gluconeogenic pathways (Lund and Williamson, 1985). Muscle catabolism may also be associated with the release of a proportion of BCAAs that are not oxidised in the skeletal muscle (Elia et al, 1980).

3-Methylhistidine excretion: 3-Methylhistidine was proposed as a marker for myofibrillar protein breakdown since it is formed by post-translational methylation of histidine residues; after proteolysis, it is not metabolised further or used for de novo protein synthesis and is excreted in the urine quantitatively (Young and Munro, 1978). Williamson et al (1977) found that the excretion of 3-methylhistidine was related to the degree of trauma and the nitrogen excretion of patients with accidental or surgical injury and proposed that skeletal muscle protein breakdown makes a substantial contribution to the nitrogen loss after injury. Similar findings in patients undergoing surgery were reported by Gross et al (1978) and Foster et al (1979), whereas Elia et al (1981) extended its applicability to various other clinical situations associated with an increased rate of protein breakdown.

Using turnover studies of labelled histidine, Millward et al (1980) found that non-skeletal muscle sources made a substantial contribution to the
total amount of 3-methylhistidine excreted in urine. They proposed that the turnover rates of protein in the gastrointestinal tract, skin, vascular bed and lung were much faster than those of myofibrillar protein, thereby contributing significantly to the amount of 3-methylhistidine excreted in the urine (Rennie and Millward, 1983). Furthermore, they found that in patients undergoing moderate or severe degrees of surgical trauma, there was no change in the efflux of 3-methylhistidine from the leg whereas the whole-body production of 3-methylhistidine increased (Rennie and Harrison, 1984). However, it is not disputed that the increased excretion of 3-methylhistidine is associated with an increased whole-body nitrogen loss, from the breakdown of actin and myosin chains in a variety of tissues (Rennie and Millward, 1983). Thus, protein turnover studies using labelled amino acids and arteriovenous catheterisation studies are the only means presently available for studying the tissue-specific changes in protein synthesis and breakdown.

Protein turnover studies: Amino acid turnover studies in animal models have shown that the physiological modulation of skeletal muscle mass is due mainly to changes in the protein synthesis rates, whereas protein breakdown rates follow adaptively. On the other hand, in visceral tissues the facilitative process is protein breakdown with changes in synthesis rates being less important (Rennie and Harrison, 1984).

Whole-body protein turnover studies in patients undergoing minor or moderate degrees of surgery (O'Keefe et al., 1974; Crane et al., 1977; Kien et al., 1978), have found that the rates of protein synthesis were decreased significantly whereas the rates of protein breakdown were unaltered. On the other hand, in patients with severe injury (Birkhahn et al., 1980) and sepsis (Long et al., 1977), the rates of protein turnover were increased
markedly, associated with an 80% increase in the rate of protein breakdown together with a smaller increase in the rate of protein synthesis. In patients with sepsis and trauma, Clowes et al (1983) have identified a circulating plasma peptide which may be responsible for mediating the increased rates of muscle proteolysis observed in such patients.

Thus, it may be concluded that the protein catabolism following major surgery, trauma or sepsis is mediated by the complex interaction of a large number of regulating factors. Nevertheless, it is likely that therapeutic manipulation of the protein metabolism would provide the greatest benefits in terms of reduction of morbidity and mortality in this group of critically ill patients (Moyer et al, 1981).

1.4.3 Fat metabolism:

The catabolic hormonal milieu following surgery or other forms of injury stimulates the mobilisation of fatty acids from the adipose tissue which, in some cases, may be associated with the increased formation of ketone bodies. These changes, though less well-characterised than the concomitant changes in carbohydrate and protein metabolism, may be the most important for energy supply in the post-traumatic state.

Lipolysis: Increased plasma concentrations of non-esterified fatty acids (NEFA) were documented in patients with burns by Allison et al (1968) and were found to be associated with a decreased glucose tolerance. In patients undergoing surgery (Allison et al, 1969) an increase in plasma NEFA was found to be associated with the emotional stress of being brought to the operation theatre and a further increase was observed during surgery. Similar findings were reported by several studies in patients undergoing surgery (Horrelt et al; 1969; Cooperman, 1970). In contrast, no changes in
plasma NEFA were found during or soon after surgery by Foster et al (1979) or Kehlet et al (1979); the latter study found a decrease in plasma NEFA concentrations in patients given epidural anaesthesia during surgery. Following accidental trauma, Meguid et al (1974) found that the increase in plasma NEFA concentrations was related to the severity of trauma, whereas Oppenheim et al (1980) found no correlation between the plasma NEFA values soon after injury and the severity of trauma; this disparity may be related to different methods used for classifying the severity of trauma in the two studies. The glycerol released from lipolysis is taken up by liver cells and phosphorylated to enter the gluconeogenic pathway (Gump et al, 1975).

Using indirect calorimetry in patients undergoing elective surgery, Kinney et al (1970) found that 75 to 90% of the energy requirement was provided by fat metabolism whereas proteins provided the remainder. To some extent, the mobilized NEFA may undergo conversion to ketone bodies or triacylglycerols in the liver (Williamson, 1981).

**Production and utilization of ketone bodies:** Although the raised plasma NEFA concentrations and the suppression of insulin secretion after trauma may favour the increased production of ketone bodies; however, a number of studies have documented no change (Horrelt et al, 1969; Cooper et al, 1979), a slight increase (Foster et al, 1979; Brandt et al, 1978; Oppenheim et al, 1980) or a substantial increase (Kehlet et al, 1979) in the plasma concentrations of ketone bodies during surgery. As stated previously, the normoketonaemia observed in some patients after injury (Smith et al, 1975; Williamson et al, 1977) or major surgery (Rich and Wright, 1979) was associated with an increased nitrogen excretion in comparison to patients who were hyperketonaemic after injury. The lack of ketogenesis in some patients was proposed to be an effect of vasopressin release after injury.
(Williamson, 1981). Thus, the decreased availability of ketone bodies as a fuel may lead to an increased need for amino acid oxidation in skeletal muscles as a source of energy (James, 1981) which, in turn, may result in the increased nitrogen loss documented by Smith et al (1975). Furthermore, the plasma concentrations of ketone bodies were found to be normal or decreased in patients exposed to severe degrees of trauma (Williamson, 1977) which may also be related to the marked release of vasopressin in these patients.

1.4.4 Conclusion:

The adult patient exposed to surgical or accidental trauma undergoes a variable period of catabolism, the severity and duration of which may be related to the extent of the trauma and the presence of complications such as sepsis. The metabolic response to minor or moderate surgery can be altered by particular anaesthetic techniques, but manipulation of the metabolic response of the severely injured patient is difficult and is an area of research which may provide important clinical benefits in the management of this group of patients.

1.5 CLINICAL IMPLICATIONS:

The metabolic response to severe degrees of surgical or accidental trauma may be associated with a number of undesirable effects in the postoperative period. A markedly stimulated stress response may be associated with a hypermetabolic state, increased oxygen consumption, raised temperature, increased protein turnover and metabolic energy requirements, increased cardiac output, impaired hepatic and renal function, and an increased susceptibility to infection (Wilmore, 1980; McMenamy et al, 1981; Kehlet, 1979). Thus, adult patients undergoing severe stress are at a greater risk...
for complications such as cardiac insufficiency, myocardial infarction, pulmonary insufficiency, etcetra.

In such patients, thromboembolic complications, gastric stress ulcers, prolonged fatigue and convalescence may also be observed following major surgery (Rose and King, 1978, Kehlet, 1979). In some cases, hyperglycaemia during cardiac surgery may lead to a fatal hyperosmolar non-ketotic coma (Mills et al, 1973). The stress response may also be associated with a persistent metabolic acidosis in the postoperative period, which may have secondary detrimental effects on a compromised cardiopulmonary system (Bunker, 1962). However, it must be emphasized that these complications are mainly limited to the patients undergoing severe degrees of surgical trauma and would not be expected in the healthy patient undergoing moderate and elective surgery.

On the other hand, the special requirements of a wound may be fulfilled, to some extent, by the metabolic changes following trauma. Thus, the surgical hyperglycaemia provides for the increased glucose requirements of injured tissues (Im and Hoores, 1979); the proteolysis and mobilisation of amino acids in various body tissues may provide amino acid residues for repair (particularly methionine and cysteine) and for the production of acute phase reactants by the liver; the lipolysis and ketogenesis may provide an alternate source of fuel for various tissues such as the muscles and brain, whereas the gluconeogenesis following injury may provide a glucose supply for vital tissues (Wilmore, 1981; Elliott and Alberti, 1983).

However, there is some evidence to suggest that these processes can become life-threatening if catabolic activity is present in excess or if recovery does not take place within a reasonable period of time. In such cases, a
severe catabolic drive may continue even after the stressful stimulus which triggered it, is no longer present (Rodeman et al, 1983). From an analysis of several parameters of the stress response, Moyer et al (1981) were able to discriminate between patients with trauma and sepsis who survived and those who did not survive. Some of the variables which differentiated most between the two groups were: urea, lactate, the sum of non-essential amino acids, α-aminobutyrate, glucagon and glucose. In this context, it is tempting to also include the observation of Keeri-Szanto (1983) who found that patients given demand analgesia for postoperative pain were discharged from hospital 30% earlier than patients given conventional analgesic regimens. Is it possible that adequate analgesia decreased the duration of postoperative metabolic changes and thus, led to an earlier discharge? The observation of Brandt et al (1976), who found a reduction in postoperative nitrogen loss in patients kept pain-free with epidural analgesia after surgery, suggests that it might be possible.

Thus, attempts to manipulate the metabolic response of severely stressed patients may be a desirable therapeutic goal. Furthermore, it is likely that effects on the protein metabolism following stress may be the key changes required for obtaining the clinical benefits of such treatment.

1.6 MANIPULATION OF THE STRESS RESPONSE:

In addition to the anaesthetic techniques used for decreasing the stress response (the effects of which have been described above), several investigators have used hormonal or nutritional means to manipulate the metabolic stress response.

1.6.1 Hormonal therapy:
The use of high doses of insulin and glucose to decrease catabolism in patients with moderate to severe burns was first suggested by Hinton et al (1971), and was found to be associated with a decreased excretion of urea and potassium. Using patients as their own controls in three-day crossover studies, Woolfson et al (1979) found that insulin and glucose caused a marked decrease in the urea production rates of catabolic patients and the protein-sparing effect of insulin was proportional to the initial urea production rate. In patients who were not in a catabolic state at the time of study, these effects were not observed (Woolfson et al, 1979).

Foster et al (1980) compared the protein-sparing effects of several intravenous fluid regimens and found that infusion of insulin and glucose was associated with a decrease in total nitrogen excretion as compared to groups of patients who received saline infusion or glucose alone. Hall et al (1983) have shown recently that a low-dose infusion of insulin during and after surgery caused a decrease in the concentrations of blood glucose, non-esterified fatty acids and 3-hydroxybutyrate during and soon after surgery, whereas other variables were unaltered.

On the other hand, contrary evidence has been reported by Powell-Tuck et al (1984), who found that insulin given with total parenteral nutrition to patients undergoing major intestinal surgery had no effects on the nitrogen balance or the protein turnover rates (as measured by injection of a tracer dose of $^{15}$N-glycine) of these patients, as compared to a similar group of patients who received total parenteral nutrition without the addition of insulin. However, the results of this study need to be confirmed since the dose of insulin given and the number of patients studied were much smaller than in the previous reports.
Other forms of hormonal therapy for decreasing postoperative nitrogen loss have included the use of growth hormone or anabolic steroids. The former approach was used by Wilmore et al (1974) in patients with severe burns who documented an increased nitrogen retention and a marked increase in plasma insulin concentrations during the study (Wilmore et al, 1974). It is likely that the anabolic effects of growth hormone observed in this study were probably due to the release of insulin that was documented. The use of anabolic steroids was studied by Johnston and Chennour (1963) and Tweedle et al (1972). Both studies found that anabolic steroids decreased the nitrogen loss of patients on a low calorie diet, whereas the nitrogen excretion of patients on a high-calorie diet was not affected.

Thus, the use of insulin infusions peri-operatively presents the most likely hormonal therapy to decrease the nitrogen loss following surgery.

1.6.2 Nutritional therapy:

The effects of intravenous hyperalimentation on surgical wound healing was first investigated by Bozzetti et al (1975). In patients undergoing major abdominal surgery, they found that the rate of wound healing was enhanced in patients who received increased amounts of calories and amino acids during the five days after surgery.

Foster et al (1980) investigated the effects of various protein-sparing intravenous fluid regimens in patients undergoing abdominal surgery. They found that the nitrogen balance in patients given glucose infusions after surgery was not altered from that of control patients given only saline; but the addition of insulin was associated with a decrease in postoperative nitrogen loss. The infusion of amino acids was associated with an increased total nitrogen excretion postoperatively, but the net nitrogen loss after
surgery was decreased substantially (Foster et al, 1980). Recent interest has been stimulated in the use of parenteral nutrition solutions containing a large proportion of branched-chain amino acids (BCAA). Cerra et al (1982) found that in patients undergoing abdominal surgery, a positive nitrogen balance was achieved earlier with the use of BCAA-enriched parenteral nutrition as compared to patients given the routine parenteral nutrition solutions. Thus, they concluded that BCAA may specifically stimulate protein synthesis in stressed patients (Cerra et al, 1982). However, no change in 3-methylhistidine excretion was observed in their study. They have reported recently that infusion of BCAA-enriched solutions in patients undergoing severe surgical stress was associated with an improvement in their immune responses (Nuwer et al, 1983).

The reasons for these effects are unclear, particularly since McNurlan et al (1982) have found that leucine does not stimulate protein synthesis in the rat model. Could there be a synergistic action from the infusion of all BCAAs, rather than leucine alone? It is evident that further detailed studies will be required before an ideal nutritional regimen can be proposed for patients undergoing major surgery.

1.6.3 Effects of temperature :
Campbell and Cuthbertson (1966) first showed that raising the environmental temperature in which injured patients were nursed was associated with a decrease in the extent of nitrogen loss after surgery. This finding has been confirmed by several investigators and several units treating patients with severe burns now nurse their patients at a higher ambient temperature.

1.6.4 Conclusion :
The therapeutic manipulation of the hormonal and metabolic changes following severe stress may be necessary for an improvement in clinical outcome. However, the methods presently available are probably incomplete for dealing with this exceedingly complex metabolic phenomenon. Of the methods described above, insulin infusion and an increase of environmental temperature are the only ones in current clinical use. A simple solution to these metabolic problems seems unlikely.
CHAPTER II: SURGICAL STRESS AND THE NEWBORN INFANT
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2.3 AIMS OF THIS STUDY
2.1 THE RESPONSES OF NEONATES AND OLDER INFANTS UNDERGOING SURGERY

Despite the wealth of information available on the hormonal and metabolic responses of adult patients to surgical trauma, remarkably little is known about the responses of newborn infants undergoing surgery. This is probably due to the large quantities of blood that were required for measurement of hormonal or metabolic variables till recently, the relatively small numbers of neonates that were operated upon and their high morbidity and mortality till almost a decade ago.

In 1938, Herzfeld reported his series of a thousand paediatric herniotomies many of which were performed on newborn infants. Advances in peri-operative care and surgical technique, the availability of antibiotics and other supportive measures caused a decrease in the operative mortality of newborn infants during the 1940's. Thus, R M Moore in 1946, and T V Santulli in 1954 stated that the physiological changes due to surgery in neonates were not different from those in adults, except that the changes were more acute. This concept was refuted by P P Rickham in 1957, based on data obtained from studies of fluid, electrolyte and nitrogen balance in nine neonates undergoing surgery (Rickham, 1957a).

2.1.1 EARLY STUDIES ON ELECTROLYTE AND NITROGEN BALANCE:

Rickham's pioneering work focussed attention on the fact that newborn infants were not physiologically similar to adults, especially in the context of their response to surgery. On the basis of metabolic balance
studies of sodium, chloride, potassium and nitrogen, he found that neonates did not excrete excess potassium after surgery and the potassium/nitrogen ratio in urine was identical to that of lean muscle tissue (Rickham, 1957a). He concluded that this pattern of changes was due to starvation and that surgery per se did not contribute to the electrolyte changes observed.

From similar studies, Colle and Paulsen (1959a) found that neonates were not capable of renal conservation of sodium or chloride but their nitrogen loss after surgery was similar to that of adults. From electrolyte balance studies on neonates with congenital oesophageal atresia (Hughes et al, 1965) and duodenal obstruction, Wilkinson and co-workers (1965) concluded that starvation before or after surgery was the single most important factor determining the electrolyte changes in newborn infants. They stressed the importance of starting milk feeds as soon as possible after operation to achieve normal homeostasis and a positive nitrogen balance (Wilkinson et al, 1965). Similar results were obtained by other studies on the electrolyte fluxes of newborn infants undergoing surgery (Peonides et al, 1963; Suzuki et al, 1968).

However, contrary results were reported by Knutrud (1965). From a study of 35 neonates undergoing surgery, he found a severe post-operative nitrogen loss with markedly negative balances up to a week following surgery, whereas the urinary losses of potassium, magnesium and phosphate and the urinary potassium/nitrogen ratio were similar to that of adult surgical patients. Knutrud concluded that electrolyte changes following surgery in neonates and adults were fundamentally the same.

From nitrogen balance studies on 39 infants ( <2 years of age) and 56 older children ( >2 years of age) undergoing surgery, Sukarochana et al (1965)
demonstrated the protein-sparing effects of the intravenous infusion of dextrose or protein hydrolysate solutions. The minimum amount of calories required to demonstrate a protein-sparing effect was 25 calories/kg/day for children over 2 years of age and 60 calories/kg/day for infants under 2 years of age. Grewal et al. (1969) measured the blood levels of urea and electrolytes, and nitrogen balances in 18 infants less than 2 years of age and 82 children over 2 years of age. They found a negative nitrogen balance in all cases, the duration and severity of which were proportional to the extent of surgical trauma. The negative nitrogen balance was maintained for a longer period postoperatively in the older children as compared to infants less than 2 years of age (Grewal et al., 1969).

Bennett et al. (1970a and 1970b) measured the electrolyte excretion of 15 neonates undergoing surgery and proposed that the fluid and electrolyte requirements of neonates should be supplemented adequately after surgery.

Thus, electrolyte and nitrogen excretion were investigated in several early studies of neonates and older infants undergoing surgery. The uniform and most significant finding from these studies was the substantial loss of nitrogen and negative nitrogen balance following surgery in infants less than 2 years of age. However, the hormonal and metabolic changes associated with this finding were not investigated to a similar degree in this group of patients.

2.1.2 THE ENDOCRINE RESPONSE TO SURGERY:

2.1.2.1 Catecholamines:

The plasma concentrations of adrenaline and noradrenaline were measured before and after surgery in 19 infants (5 weeks to 18 months of age) by
Talbert et al (1967). There were no significant changes in the plasma catecholamines during surgery and it was concluded that the measurement of catecholamines was not sensitive to the relatively minor stress of inguinal herniorrhaphy.

Although there are no published data on the catecholamine responses of neonates undergoing surgery, it has been demonstrated that there is a marked release of catecholamines at birth (Lagercrantz and Bisoletti, 1977; Nakai and Yamada, 1978; Eliot et al, 1980) which may be further stimulated in neonates exposed to fetal distress or birth asphyxia (Nakai and Yamada, 1978; Lagercrantz and Bisoletti, 1977).

2.1.2.2 Pituitary hormones :-

Of the pituitary hormones, plasma vasopressin concentrations were measured in 2 neonates and 3 infants before and during major abdominal surgery by Hoppenstein et al (1968); a marked increase in response to surgical stress was documented in all cases.

There are no published data on the changes in anterior pituitary hormones in neonates undergoing surgery.

2.1.2.3 Adrenocortical hormones :-

The urinary concentrations of 17-hydroxycorticosteroids (17-OHCS) were measured by Colle et al (1960) in neonates undergoing surgery and were raised after surgery in neonates over a week of age, whereas they remained unchanged in neonates less than one week of age. These results were contradicted by Haugen et al (1967) who found that 17-OHCS concentrations in urine were increased also in neonates operated within a week after birth, a similar increase was observed plasma 17-OHCS concentrations which
were measured a single neonate undergoing surgery.

In neonates exposed to fetal distress, birth asphyxia, respiratory distress syndrome or various other neonatal problems, similar measurements of the adrenocortical hormones have demonstrated the neonatal responses to 'stressful' stimuli (Venning et al, 1949; Hillman, 1961; Cathro et al, 1969; Baden et al, 1973). Using a labelled-hormone infusion technique, Kenny et al (1963) have shown that term and preterm neonates have a normal cortisol production rate soon after birth.

Plasma cortisol concentrations were measured by Miura et al (1978) in 3 neonates undergoing surgery within one week after birth and a substantial increase was documented in all neonates. Obara et al (1984) have recently reported plasma cortisol measurements in 7 neonates and 14 infants undergoing surgery. They found no significant changes in plasma cortisol concentrations of neonates during or at the end of surgery. In the infants studied, plasma cortisol concentrations increased significantly during surgery. The cortisol responses of infants given anaesthesia with nitrous oxide were found to be significantly greater than the infants who received halothane and nitrous oxide anaesthesia during surgery (Obara et al, 1984). This recent finding shows that the anaesthetic management during surgery can modify the hormonal response of older infants undergoing surgery; it may be possible that the hormonal responses of newborn infants can be modified in a similar manner.

Thus, it has been documented that the adrenal cortex of newborn infants is capable of responding to the stressful stimuli present at birth. In the only study which reported plasma cortisol concentrations in newborn infants undergoing surgery, no significant changes were documented in response to
2.1.2.4 Pancreatic hormones :-

Baum et al (1968a) reported peri-operative changes in plasma concentrations of insulin in one neonate and 9 older infants undergoing cardiac surgery and hypothermic cardiopulmonary bypass. Plasma insulin concentrations decreased during cooling in all cases, particularly when body temperatures fell below 30°C. Despite substantial hyperglycaemia, insulin values remained low during surgery in all cases and increased at the time of rewarming in all infants but not in the neonate who was studied. They concluded that insulin secretion was suppressed during hypothermia in infants undergoing cardiac surgery.

There are no published data on the changes in plasma glucagon, or other pancreatic hormones in neonates or older infants undergoing surgery.

2.1.2.5 Conclusion :-

Thus, the only information available from the published data on hormonal responses of neonates undergoing surgery is the tendency towards an increased secretion of corticosteroid hormones during surgery. It is therefore, evident that the endocrine response of newborn infants to surgical stress has not been investigated adequately.

2.1.3 THE METABOLIC RESPONSE TO SURGERY :

2.1.3.1 Carbohydrate metabolism :-

Hyperglycaemia and hyperlactataemia in response to surgery have been documented in several studies of neonates and older infants undergoing cardiac and non-cardiac surgical procedures.
**Non-cardiac surgery** : Bunker et al (1952) studied the effects of ether anaesthesia in infants undergoing general surgical procedures and found that a metabolic acidosis developed during surgery which was associated with a substantial increase in blood lactate concentrations.

Elphick and Wilkinson (1981) studied 14 neonates undergoing surgery and also observed an increase in blood glucose values which was maintained, in some cases, for up to 8 hours after surgery. Intravenous glucose tolerance tests were performed before and after surgery in another 4 neonates and it was found that the glucose clearance rate was decreased postoperatively in 3 neonates but was increased in one neonate (Elphick and Wilkinson, 1968). In contrast to other studies on neonates undergoing surgery, blood lactate concentrations were found to decrease during surgery (Elphick, 1972).

In neonates undergoing various surgical procedures, Pinter (1972 and 1973) found that blood glucose and blood lactate concentrations were increased substantially at the end of surgery but had returned to preoperative values by 6 hours after surgery. There were no significant differences between the responses of neonates with 'alimentary' or 'non-alimentary' congenital anomalies. From these studies (Pinter, 1973; Elphick and Wilkinson, 1981), it was concluded that anaesthesia and surgery caused a temporary metabolic disturbance in newborn infants, the causes for which were not clear.

**Cardiac surgery** : In addition to the changes in plasma insulin, Baum et al (1968) reported a massive increase in blood glucose values during cardiac surgery and hypothermic cardiopulmonary bypass. It is interesting to note that the peak glucose concentration in the only neonate studied was twice the peak glucose values documented in the older infants undergoing
cardiac surgery. Furthermore, an insulin response to this hyperglycaemia was observed in all the older infants, whereas it was absent in the neonate. Blood lactate concentrations were also found to increase markedly during surgery in all cases (Baum et al, 1968b).

A similar study was reported by Johnston et al (1974) on one neonate and 10 older infants (3 months to 2 years of age) undergoing cardiac surgery and hypothermic cardiopulmonary bypass; blood glucose values were found to increase markedly during the surgical procedure in all cases. In children (3 months to 7 years of age) undergoing cardiac surgery and cardiopulmonary bypass, Bevan and Rosales (1979) found that glucose utilisation was decreased markedly during the procedure and was associated with low plasma insulin concentrations. From these studies, it was concluded that the changes in glucose homeostasis in small children were similar to those in adult patients undergoing cardiac surgery (Baum et al, 1968a; Bevan and Rosales, 1979; Johnston et al, 1974).

2.1.3.2 Fat metabolism :-
Changes in fat metabolism during surgery were first investigated by Talbert et al (1967) in infants undergoing inguinal herniorrhaphy. They found a significant increase in plasma concentrations of non-esterified fatty acids (NEFA) at the end of surgery and concluded that this was indicative of lipolysis in response to surgery.

In infants undergoing cardiac surgery, Baum et al (1968b) found that concentrations of blood glycerol increased markedly during surgery, but plasma NEFA were unaltered during the procedure and decreased at the time of rewarming from deep hypothermia. The disparity of changes in plasma NEFA and glycerol was ascribed to the increased utilization of fatty acids
during surgery and the decreased metabolism of glycerol in liver cells, which was proposed to be a result of the deep hypothermia during cardiac surgery (Baum et al, 1968).

In neonates undergoing non-cardiac surgery, Pinter (1973) found an increase in plasma NEFA concentrations during surgery and a further increase postoperatively, whereas Elphick and Wilkinson (1981) found no significant changes. In the latter study, a decrease in plasma triglycerides was documented at 18 hours after surgery but the plasma concentrations of lipoproteins, phospholipids and cholesterol were unchanged during and after surgery (Elphick and Wilkinson, 1981). Although these changes were not commented upon, it is possible that they were contributed by the effects of starvation since neonates in both studies did not receive any calories from a variable duration preoperatively up to the end of the study period.

From these variable data, it is not possible to draw firm conclusions regarding the changes in fat metabolism in the neonate or older infant undergoing surgery.

2.1.3.3 Protein metabolism :-

As described above, early studies have documented a negative nitrogen balance in neonates and older infants subjected to surgical trauma, the duration and severity of which, in one study, was found to be related to the extent of surgical trauma (Grewal et al, 1969). In a recent report, Greenall et al (1983) have also shown that the amount of nitrogen loss in neonates undergoing surgery was related to the extent of surgical trauma.

Since nitrogen balance is only a crude reflection of changes in the rates of protein synthesis and protein breakdown occurring in the various body
tissues, other measures such as 3-methylhistidine excretion and protein turnover studies have been used for studying protein metabolism in preterm and term neonates. These methods have not been applied to the study of neonates undergoing surgery, but preliminary data are available particularly with regard to clinically 'stressed' preterm neonates.

**Protein turnover studies**: Protein turnover studies in one term and six preterm neonates were first reported by Pencharz et al (1977), who found that the rate of protein flux was approximately eight times higher in these cases as compared to adult subjects, and was even higher in neonates small-for-gestational-age (Pencharz et al, 1981). Nissim et al (1983) found that the rate of protein turnover in preterm neonates was higher than in term neonates which, in turn, had greater turnover rates than older infants and children. However, all these studies were performed in healthy neonates and the effects of stress due to clinical illness were not investigated.

**Excretion of 3-methylhistidine**: An increased rate of myofibrillar protein breakdown, as assessed by the 3-methylhistidine/creatinine ratio (3-MH/Cr), was found in preterm neonates who were clinically ill and losing weight at the time of study (Seashore et al, 1980; Ballard et al, 1979). The urinary 3-MH/Cr ratios were related closely with the amount of nitrogen loss in these neonates (Ballard et al, 1979; Seashore et al, 1980).

From these studies, it has been proposed that the regulation of protein metabolism in newborn infants is different to that of adults. The high rates of whole-body protein turnover are probably a result of rapid growth in this period. Although it has been demonstrated that the modulation of skeletal muscle mass in adult humans or animals is achieved mainly by alterations in the rate of protein synthesis with little or no change in
protein breakdown (Rennie and Harrison, 1984), however, the reverse situation probably prevails in the newborn infant or animal (Pencharz et al, 1977; Nissim et al, 1983; Pencharz et al, 1981). Thus, in neonates the changes in skeletal muscle mass are probably the result of changes in protein catabolism whereas the protein synthesis follows adaptively. Preliminary evidence for this hypothesis was presented by Ogata and Holliday (1976), who found marked changes in the protein breakdown rates of newborn guinea pigs exposed to starvation whereas protein synthesis rates changed little or not at all. In addition, the protein-sparing effects of glucose infusion were mediated through a decrease in protein breakdown rates whereas the rate of protein synthesis was not affected (Ogata and Holliday, 1976).

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**Speculation:** These findings, together with the increased rate of protein turnover may imply that the protein catabolism following surgical stress would cause a greater loss of body tissues in newborn infants as compared to adult patients. It is also possible that due to these characteristics, the postoperative protein catabolism in newborn infants could be manipulated more easily than that of adult patients.

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**2.1.3.4 Conclusion:**

The metabolic response to surgery has been investigated sparsely in the neonatal age group. Apart from the demonstration of hyperglycaemia during surgery and a negative nitrogen balance in the postoperative period, the available data are conflicting and variable. Furthermore, the effects of prematurity, different degrees of surgical stress, anaesthetic management or other peri-operative factors have not been investigated. This is despite the fact that alterations in peri-operative metabolic homeostasis may have far-reaching clinical implications for the neonate undergoing surgery.
2.1.4 CLINICAL IMPLICATIONS:

The concept of an endocrine and metabolic stress reaction to surgery assumes greater significance in the context of newborn infants. Even the normal neonate exists in a precarious metabolic state as it adapts to the postnatal environment and to post-natal nutrition (Bougeneres et al, 1982; Aynsley-Green, 1982). During this transition period a number of hormonal and metabolic changes are known to occur, disruption or derangement of which may lead to detrimental consequences such as hypoglycaemia, acidosis, hyperglycaemia or electrolyte imbalance.

In contrast to the adult, a neonate has limited body reserves of fat, protein and carbohydrate. In addition, it has to meet the metabolic cost of rapid growth and organ maturation in the neonatal period (Wilkinson 1976; Davies, 1981).

In this setting, any injury would necessitate a metabolic expenditure for healing and repair. This is often combined with the effects of partial or total starvation before and/or after surgery (Wilkinson 1965). In addition, hypothermia may develop during anaesthesia and surgery (Dilworth 1973, Tsingoglou and Wilkinson 1971). The combined effect of these factors could be particularly disadvantageous, if not life-threatening, for the seriously ill or premature infant.

A high postoperative mortality in newborn infants has been reported by several authors (Rickham, 1957b; Knutrud, 1965; Calverley and Johnston, 1972; Ryan, 1973; Wong et al, 1974; Haselby et al, 1982; Kiely, 1984; Anand and Aynsley-Green, 1985). The incidence of peri-operative cardiac arrest was found to be relatively higher in newborn infants than in any other age
group (Snyder, 1953; Rackow et al, 1961). Deaths during surgery or within 48 hours after anaesthesia also occur more frequently in infants as compared to children (Graff et al, 1964) or adults (Turnbull et al 1980). In infants with severe illness due to a variety of causes, a high incidence of gastric stress ulcers has been documented (Bell et al, 1981). In contrast to adult patients, stress ulcers in the infant have a greater frequency of perforation and a high mortality was found to be associated with them (Bell et al, 1981).

In addition, the hyperglycaemia and metabolic acidosis following surgical stress may have special consequences for the newborn infant undergoing surgery. A marked hyperglycaemic response may cause significant alterations in the plasma osmolality during surgery (Gennari, 1984) and can have detrimental effects on the renal cortex or cerebral substance (Finberg, 1967); and may even lead to intraventricular haemorrhage (Arant and Gooch, 1978). The detrimental effects of a hyperosmolar state are of even greater potential significance in preterm neonates, particularly when associated with the development of a metabolic acidosis (Levene and De Vries, 1984).

A metabolic acidosis in neonates undergoing surgery has been documented by several workers (Knutrud, 1965; Pinter, 1972 and 1973; Scott and Inkster, 1973; Johnston et al, 1974; Baum et al, 1968b). In published reports on the outcome of neonates undergoing surgery, a persistent metabolic acidosis in the postoperative period was associated with a poor clinical outcome (Ward et al, 1970; Lipman et al, 1976).

Thus, it is evident that metabolic homeostasis in the newborn infant undergoing surgery may play an important role in their clinical outcome following surgery. Details of anaesthetic and peri-operative management may
affect profoundly the maintenance of such homeostasis. However, there is little agreement with regard to the anaesthetic or peri-operative clinical management of neonates undergoing surgery, which may be based on empirical principles or personal preference.
2.2 ANAESTHETIC MANAGEMENT OF NEWBORN INFANTS

2.2.1 INTRODUCTION :-

From recent published reviews of neonatal anaesthetic techniques it is evident that there exists little agreement amongst paediatric anaesthetists regarding the need for anaesthesia and the optimum choice of anaesthetic agents for newborn infants undergoing surgery. This is largely due to the lack of recent studies on the physiological effects of various anaesthetic agents in the neonatal age group. Thus, the techniques used presently were found to be based on the principles evolved by Jackson Rees (1950).

The question whether anaesthesia is necessary at all in newborn infants undergoing surgery has been raised in previous (Rackow et al, 1961; Bush and Stead, 1962; Calverley and Johnston, 1972; Downes and Raphaely, 1973) and current reviews of neonatal anaesthetic techniques (Shaw, 1982; Lipman et al, 1976; Vivori and Bush, 1977; Inkster, 1977; Brown and Fisk, 1979; Betts and Downes, 1984). In some reviews, the use of nitrous oxide and/or halothane has been advocated on the basis of personal preference (Ward et al, 1970; Yamamoto et al, 1972; Ryan, 1973; Steward et al, 1974; Goudsouzian and Ryan, 1976; Salanitre and Rackow, 1977; Salem and Bennett 1980, Dierdorf and Krishna 1981). In addition, recent interest has been shown in the administration of fentanyl intravenously to neonates undergoing cardiac or non-cardiac surgical procedures (Robinson and Gregory, 1981; Krishna et al, 1981; Hickey and Hansen, 1984; Haselby et al, 1982; Vacanti et al, 1984).

2.2.2 PREMEDICATION:--

Sedative drugs such as barbiturates or narcotics before surgery are not advocated currently (Shaw, 1982; Krishna et al, 1981; Salem and Bennett,
but the use of atropine pre-operatively has been advised in some reviews (Salem and Bennett, 1980; Smith, 1978) and refuted in others (Dierdorf and Krishna, 1981; Newman and Hansen, 1980).

2.2.3 MUSCLE RELAXANTS

The use of muscle relaxants in neonatal anaesthesia became popular in the 1950s because of three main advantages: (a) they were presumed to be less toxic than inhalation anaesthetic agents, (b) they provided better respiratory control and operating conditions, and (c) they allowed the use of diathermy (Bush and Stead 1962).

D-tubocurarine: In 1955, Stead had found that neonates were more sensitive to d-tubocurarine than adults and this finding has been confirmed in subsequent studies (Bush and Stead, 1962; Walts and Dillon, 1969; Cook, 1981, Fisher et al, 1982). However, the elimination half-life of d-tubocurarine was found to be longer in neonates than in adults (Fisher et al, 1982) but due to its distribution in a larger extra-cellular volume, lower plasma concentrations were obtained from equivalent doses (Fisher et al 1982, Cook 1981). Therefore, the dose requirements proposed for neonates are not different from those of adults although second and subsequent doses for maintenance of relaxation in neonates may not be required.

Succinylcholine: Neonates were found to be more resistant to succinylcholine than adults for doses administered on the basis of body weight (Bush and Stead 1962); but equivalent to them when dosage was based on body surface area (Walts and Dillon, 1969, Cook 1981). However, serious adverse effects may follow succinylcholine administration in neonates such as profound bradycardia, hyperkalemia and myoglobinemia (Cook, 1981). An acute fulminant pulmonary oedema has also been documented in some neonates.
after injection of succinylcholine (Cook and Westman, 1981).

2.2.4 **HALOTHANE ANAESTHESIA IN NEONATES** :-

Halothane, as the sole anaesthetic agent or in combination with nitrous oxide is used widely in the current anaesthetic practice for newborn infants. Its advantages are the pleasant, non-irritating odour; less danger of producing secretions, bronchospasm or laryngospasm; the rapidity of action and its potent anaesthetic and amnesic properties.

**Anaesthetic requirement**: The estimation of anaesthetic requirement is based on the measurement of MAC (Minimal Alveolar Concentration), which is the alveolar concentration of an anaesthetic gas at which 50% of patients move in response to a single stimulus (skin incision). The MAC of halothane for different age groups was investigated by Gregory et al (1969) who found that it was highest in infants less than 6 months of age. A similar study by Nicodemus et al (1969) arrived at the same conclusion. Since then was accepted that neonates required a significantly higher concentration of halothane than older children or adults for effective anaesthesia.

However, in the age group of 'less than 6 months', only two neonates (and 10 older infants) were included in the former study and an unspecified number of the 6 infants included in the latter study were neonates. Lerman et al (1983) have shown recently that the anaesthetic requirement of neonates is much less than that of infants between 1 and 6 months of age, who were found to have the highest anaesthetic requirement of all age groups. Thus, MAC of halothane for neonates is achieved at lower concentrations than have been used since 1969, and studies investigating the side-effects of halothane were performed at concentrations which represented an overdosage of halothane for neonates (Lerman et al, 1983).
Cardiovascular effects: The uptake of halothane is faster in neonates than in adults (Salanitre and Rackow, 1969; Eger et al, 1971), due to: (1) a greater rate of alveolar ventilation relative to the functional residual capacity of the neonate, (2) greater perfusion and ventilation on a weight basis, (3) diversion of a greater fraction of cardiac output to highly perfused tissues, and (4) a low blood-gas partition coefficient for halothane in neonates (Lerman et al, 1984). Thus, at the time of induction of anaesthesia the uptake of halothane in the heart and brain of neonates and older infants would be faster than in other age groups (Brandom et al, 1983). These factors may explain the increased incidence of hypotension (Diaz and Lockhart, 1979; Gregory, 1982), bradycardia (Diaz and Lockhart, 1979) and cardiac arrest (Rackow et al, 1961) that have been documented during halothane anaesthesia in newborn infants. However, some of these effects may have been due to halothane concentrations in excess of those required by newborn infants (Lerman et al, 1983).

Effects on temperature regulation: It has been documented that halothane anaesthesia causes a greater temperature loss in infants and young children than other anaesthetic agents (Engelman and Lockhart, 1972). This effect has ascribed to the peripheral vasodilation, inhibition of non-shivering thermogenesis and of central homeothermic regulation caused by halothane anaesthesia (Engelman and Lockhart, 1972; Dilworth, 1973).

Hepatotoxicity: There is substantial evidence that the use of halothane in paediatric patients does not cause the acute liver failure that has been found in adult patients. From a review of 82,000 cases, Wark (1983) found a two cases of postoperative hepatitis both of which were mild and of short duration. Warner et al (1984) found a single case of self-limiting jaundice
from a review of 200,311 paediatric cases who had received halothane, many of them for several successive surgical procedures.

**Metabolic effects**: The metabolic effects of halothane mainly stem from the changes in liver metabolism and hormonal secretion observed during halothane anaesthesia.

In the perfused rat liver, Biebuyck et al (1972a) found that addition of halothane to the perfusate caused a marked inhibition of gluconeogenesis, glycolysis, ureagenesis and a decrease in oxygen consumption. These changes were associated with a 16-fold increase in lactate production which returned to normal within 15 min after withdrawal of the anaesthetic. The addition of a fatty acid (oleate) to the perfusion medium was found to inhibit these changes (Biebuyck et al, 1972b).

In addition, halothane anaesthesia has been shown to suppress catecholamine secretion in several studies in animals (Perry et al, 1974; Roizen et al, 1974) and adult patients undergoing surgery (Roizen et al, 1981). The suppression of insulin secretion by halothane anaesthesia was also observed in the perfused rat pancreas (Aynsley-Green et al, 1973). It is possible that these hormonal changes may affect the metabolic alterations during halothane anaesthesia.

Thus, although halothane anaesthesia is associated with effects on various physiological systems, its safety and efficacy for use in paediatric anaesthetic practice have been proved over the past three decades.

**2.2.5 FENTANYL ANAESTHESIA IN NEONATES**

The use of intravenous fentanyl anaesthesia in neonates has been advocated
in recent years due to the greater cardiovascular stability seen in poor-risk patients (Robinson and Gregory, 1981; Hickey and Hansen, 1984), the availability of 100% oxygen if required by the neonate and since it may provide analgesia and a smooth transition to ventilatory management in the postoperative period (Vacanti et al, 1984).

Respiratory effects: Fentanyl is a potent opiate drug and particularly in view of the increased sensitivity of newborn infants to the respiratory depression caused by opiate drugs (Evans et al, 1976, Way et al, 1965), the use of fentanyl has been advocated for neonates who are likely to be ventilated in the postoperative period (Haselby et al, 1982; Bikhazi, 1981; Allen, 1980).

Cardiovascular effects: Minimal cardiovascular effects have been found with the use of fentanyl anaesthesia in neonates. After the injection of 50 μg/kg or 75 μg/kg of fentanyl to neonates or infants undergoing cardiac surgery, Hickey and Hansen (1984) found slight, but significant decreases in the heart rate, mean arterial pressure and the diastolic blood pressure. These changes were not considered to be clinically important (Hickey and Hansen, 1984) and were not observed when a smaller dose of fentanyl (25 μg/kg) was given to a similar group of infants (Hickey et al, 1985).

Vacanti et al (1984) have found that the use of fentanyl anaesthesia in neonates undergoing surgery for congenital diaphragmatic hernia provided a remarkable degree of cardiovascular stability which was associated a decreased hyperreactivity of the pulmonary vasculature. Robinson and Gregory (1981) also found that fentanyl anaesthesia in preterm neonates undergoing ligation of a patent ductus arteriosus provided cardiovascular stability.
Hormonal and metabolic effects of fentanyl anaesthesia have been discussed in the previous chapter.

Thus, apart from the respiratory depression caused by fentanyl anaesthesia, the advantages of its use in paediatric patients have been recognised recently. In adult patients, it is popularly used not only for the effects described above but also due to an inhibition of the hormonal and metabolic stress response.
2.3 **AIMS OF THIS STUDY** :-

1. To define the endocrine and metabolic response of newborn infants to surgical trauma.

2. To identify differences between the response of preterm and term neonates undergoing surgery.

3. To examine alterations in the response to surgical stress by currently used neonatal anaesthetic techniques.

4. To consider methods for improving the anaesthetic and postoperative management of neonates, based on a clearer understanding of the effects of surgery and anaesthesia on neonatal physiology.

5. To compare data obtained from newborn infants with published data from adult patients undergoing surgery.
CHAPTER III: LABORATORY METHODS
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3.1 BLOOD SAMPLING:

Blood samples were drawn by peripheral venepuncture in the Preliminary Study and the Halothane Trial (Chapters 4 and 6 respectively) and from an indwelling arterial catheter in the Fentanyl Trial and Cardiac Study (Chapters 7 and 8 respectively). During the withdrawal of blood by peripheral venepuncture the duration and degree of venestasis was reduced to a minimum. The volume of each blood sample depended on the weight of the newborn infant, such that not more than 1% of blood volume was obtained at each sampling point and not more than 5% of blood volume was sampled during the entire study period. The blood specimens obtained were handled in the following manner:

(a) 0.4 ml was placed into a tube containing 5 ml of ice-cold 5% perchloric acid (PCA) (prepared by mixing 40 ml of perchloric acid (assay 60-62%, sp.gr. 1.54; Analar, BDH) with 440 ml of distilled water) and thoroughly mixed. The tube was weighed on a top pan balance before and after the addition of PCA, and was weighed again after the addition of blood. The PCA served to denature and precipitate proteins and destroy cells, thereby stopping further metabolic changes in the blood. PCA tubes containing blood were stored at 4°C for up to 96 hours before further laboratory processing (Bergmeyer et al, 1974).

After extraction and neutralization, this sample was used subsequently to measure blood glucose, lactate, pyruvate, acetoacetate, 3-hydroxybutyrate, alanine and glycerol by specific enzymatic methods.

The remaining blood sample was aliquoted into heparinised tubes with and without trasylol. After storing in crushed ice for less than 30 min, these aliquots were centrifuged at 3000 rpm for 15 min at 4°C, the plasma was
pipetted off and stored in 2 ml plastic tubes.

(b) 0.25 ml was collected into a small lithium heparin tube containing 500 KIU of Trasylol (Bayer).

The plasma sample containing Trasylol was used for the measurement of pancreatic glucagon by radioimmunoassay. Trasylol was required to prevent the proteolytic degradation of glucagon during collection and storage.

(c) The remaining blood sample (0.5-2.0 ml) was collected into another 2 ml lithium heparin tube.

The plasma sample without Trasylol was divided into three aliquots for the measurement of insulin by radioimmunoassay, measurement of adrenaline and noradrenaline by radioenzymatic assay and the measurement of aldosterone, corticosterone, deoxycorticosterone, progesterone, 17-hydroxyprogesterone, 11-deoxycorticisol, cortisol and cortisone by liquid chromatographic separation and radioimmunoassay. The plasma aliquot for measurement of catecholamines was stored at -70°C whereas other plasma aliquots were stored at -20°C.

(d) In the Halothane Trial, 0.25 ml of blood was also collected from some babies into a small potassium EDTA tube, centrifuged in a similar manner, the plasma being separated and stored at -20°C. This plasma was used for the measurement of plasma free fatty acids and triglycerides by specific enzymatic methods. Plasma obtained from a lithium heparin tube was not used for these assays since the activation of lipoprotein lipase by heparin would give rise to aberrant values.
TABLE 3.1

Each blood sample ~ 0.85 ml/Kg body weight

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<tr>
<th>Amount</th>
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<td>4 ml</td>
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<td>0.25 ml</td>
<td>K⁺ EDTA</td>
<td>Heparin with Trasylol</td>
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<td>0.25 ml</td>
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<td>0.5-2.0 ml</td>
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<td></td>
<td>Plasma separation</td>
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<td>Storage at -20 °C</td>
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<td>Cortisone</td>
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Table 3.1: Scheme to show the division of each blood sample into aliquots; collection, extraction and storage requirements; lists of metabolic or hormonal variables measured in each aliquot.
3.2 MEASUREMENT OF METABOLIC VARIABLES:

3.2.1 Preparation of samples:

The extraction and preparation of samples was performed at 4°C. After initial storage, the PCA tubes containing blood were centrifuged at 3000 rpm for 10 min. The supernatant was decanted into pre-weighed labelled 10 ml plastic tubes which were then reweighed. One drop of universal indicator (BDH) was added to the supernatant followed by a 20% solution of Potassium hydroxide (KOH) (Analar, BDH), which was added drop-wise until the pH lay between 7 and 8.

The tubes were reweighed and centrifuged for 10 min at 3000 rpm, and the supernatant ‘neutral extract’ was used for metabolite analysis. Pyruvate and acetoacetate were measured immediately after neutralisation and the other metabolites were measured soon thereafter or after storage at -20°C for up to 2 weeks.

3.2.2 Principles of enzymatic analysis:

The enzymatic assay of metabolic substrates is based on the principle that a specific enzymatic reaction in which the substrate participates is coupled with the reduction of NAD/NADP or oxidation of NADH/NADPH. The pyridine nucleotides (NAD, NADP) absorb light at 260 nm, and in the reduced state (NADH, NADPH) they have an additional absorption band with a maximum at 340 nm. By measurement of the optical density at 340 nm, the enzymatic conversion of the substrate can be followed directly in the spectrophotometer cuvette. Regardless of whether NAD accepts H⁺ or whether NADH donates H⁺, at this wavelength optical density increases or decreases by 6.22 units (light path 1 cm) with the production or
consumption of 1 umole of NADH/NADPH. Since in a specific enzymatic reaction, 1 umole of substrate usually co-reacts with 1 umole of NAD/NADP (or NADH/NADPH), the change in optical density will reflect accurately the amount of substrate consumed by the reaction. When the assay conditions are optimum, conversion of the substrate is practically complete and the optical density difference (ODD) can be used to calculate the concentration of substrate in the blood sample by multiplying with an appropriate dilution factor.

Since many enzymatic reactions are equilibrium reactions, in order to make an end-point measurement the equilibrium of the reaction has to be displaced such that it favours the complete consumption of the substrate. The reaction equilibrium can be influenced by several factors such as increase in substrate or cofactor concentration, variation of pH, presence of trapping agents, or the use of regenerating reactions in which one of the co-substrates may be regenerated by a secondary reaction. If none of the reactants or products of an enzymatic reaction lends itself to spectrophotometric measurement, it is often possible to transform one of the products by another enzymatic reaction which can be easily measured (eg, measurement of glucose, glycerol, free fatty acids and triglycerides). The former reaction, in which the substrate to be determined is transformed is known as the auxiliary reaction, whereas the reaction used for actual measurement is known as the indicator reaction. Both reactions can usually be carried out in the same assay mixture (Bergmeyer, 1974).

Specificity of an enzymatic assay depends on the purity of the enzyme preparation whereas precision depends on the provision of optimum assay conditions. The sensitivity of enzymatic assays is limited by the fact that sufficient conversion of NAD/NADP (or NADH/NADPH) must take place so as to
duce a measurable change in the optical density.

1.3 Materials:
In assays were performed using a Pye Unicam SP6-550 UV/Visible spectrophotometer at 340 nm and 1 cm light path plastic cuvettes (Hughes and Hughes, Romford, Essex). The spectrophotometer showed a linear response to increments in NADH or NADPH, within the assay ranges. Enzymes and cofactors were obtained from the Boehringer Corporation, Lewes, Sussex; reagents from BDH Chemicals, Poole, Dorset.

1.4 Quality control:
Six or more cuvettes containing substrate from a standard solution of 1 ml/L were incorporated into each assay. Standards for each metabolite were prepared from concentrated solutions and stored at -20°C. All assays contained two or more 'Control' cuvettes which were included to measure the metabolite-specific optical density changes before and after addition of enzyme. A blank cuvette, to which enzyme was not added, was included at the beginning of each assay. 'Blank' cuvettes were used to standardise the spectrophotometer settings and to correct for spontaneous decrease in the optical density. These quality control measures were employed for all metabolite assays.

1.5 Precision and accuracy:
Precision and accuracy of these methods were measured by the intra-assay and inter-assay coefficients of variation, as well as from the recovery of added known standard solutions to unknown samples (Table 3.2).

1.6 Measurement of D-Glucose: Blood glucose levels were measured according to the method described by Bergmeyer, Bernt, Schmidt and Stork
Reaction sequence:

(a) Auxiliary reaction

\[ \text{Mg}^{++} \]

\[
\text{GLUCOSE} \rightarrow \text{GLUCOSE-6-PHOSPHATE} \]

\[ \text{ATP} \rightarrow \text{ADP} \]

(b) Indicator reaction:

\[ \text{glucose-6-phosphate dehydrogenase} \]

\[ \text{Mg}^{++} \]

\[
\text{GLUCOSE-6-PHOSPHATE} \rightarrow \text{6-PHOSPHOGLUCONATE} \]

\[ \text{NADP} \rightarrow \text{NADPH} + \text{H}^+ \]

At pH 7.5, equilibrium for the indicator reaction is far to the right which ensures the completion of both reactions (since glucose-6-phosphate formed in the former is rapidly used up in the latter reaction). Although hexokinase catalyses the phosphorylation of several other monosaccharides, specificity is provided by glucose-6-phosphate dehydrogenase (G6PD) with which hexose or pentose esters other than glucose-6-phosphate do not react.

Buffer solution for assay:

20 ml 0.1M tris buffer pH 8.0;

2 ml 0.1M magnesium chloride;

2 ml 0.01M ATP;

2 ml 1% NADP

0.13 ml of G6PD (1 mg/ml).

This was prepared freshly for each assay.

Total volume in each cuvette was 2.0 ml. In the sample cuvettes, this consisted of 0.1 ml of neutralised PCA extract, 0.9 ml of distilled water and 1 ml of assay buffer; in the standard cuvettes 0.1 ml of 1mM glucose, 0.9 ml of water and 1 ml of assay buffer was added.

The cuvettes were read at 340 nm before and 15 minutes after the addition
of 0.005 ml of hexokinase.

3.2.7 Measurement of L-(+)-Lactate: Levels of blood lactate were measured according to the method described by Gutmann and Wahlefeld (pp.1464-1468, Bergmeyer, 1974).

Reaction sequence

\[
\text{lactate dehydrogenase} \quad \text{LACTATE} \quad \rightarrow \quad \text{PYRUVATE}
\]

\[
\text{NAD}^+ \quad \rightarrow \quad \text{NADH} + \text{H}^+
\]

The equilibrium of this reaction lies well on the side of lactate and NAD. Therefore, in order to ensure the complete conversion of lactate, the reaction products have to be removed from the equilibrium. Protons are trapped by an alkaline reaction medium; the pyruvate reacts with hydrazine hydrate in the buffer solution to form pyruvate hydrazone and, in addition, a large excess of NAD and enzyme is used to obtain a sufficiently rapid end-point. Lactate dehydrogenase reacts only with L-(+)-lactate and thus provides specificity for the assay.

Buffer solution for assay:

- 40 ml 0.2 M tris;
- 5 ml hydrazine hydrate 100%;
- 25 mg EDTA;

Made up to 100 ml with distilled water.

The pH of the buffer solution was adjusted to pH 9.5 with 1 M hydrochloric acid and it was stored for upto two weeks at 4°C. Before use, 1 ml of 1% (w/v) NAD was added to every 9 ml of the buffer used for each assay.

The total volume in each cuvette was 2.0 ml. In the sample cuvettes, this consisted of 0.2 ml of neutralised PCA extract, 0.8 ml of water and 1 ml of assay buffer; the standard cuvettes contained 0.1 ml of 1mM Na-L-lactate, 0.9 ml of water and 1 ml of assay buffer.
All cuvettes were read at 340 nm before and 35 minutes after the addition of 0.02 ml of lactate dehydrogenase.

3.2.8 Measurement of D-(-)-3-hydroxybutyrate: Blood levels of 3-hydroxybutyrate were measured according to the method described by Williamson and Mellanby (pp.1836-1839, Bergmeyer, 1974).

Reaction sequence:

\[
\begin{align*}
& 3\text{-OH-butyrate dehydrogenase} \\
& 3\text{-OH-BUTYRATE} \rightarrow \text{ACETOACETATE} \\
& \text{NAD}^+ \quad \text{NADH} + \text{H}^+
\end{align*}
\]

At pH 8.5 equilibrium is reached when approximately 40% of the 3-hydroxybutyrate is oxidised to acetoacetate. However, the presence of hydrazine in the buffer solution traps the acetoacetate formed as a hydrazone and the reaction proceeds quantitatively from the left to right. Due to low activity of the 3-hydroxybutyrate dehydrogenase preparations it is necessary to use excess quantities of the enzyme. 3-Hydroxybutyrate dehydrogenase is not completely specific for 3-hydroxybutyrate and the higher analogues, eg, 3-hydroxypentanoic and 3-hydroxyhexanoic acids also react but at much slower rates (Bergmeyer, 1974).

Buffer solution for assay:

- 70 ml 0.1 M tris buffer pH 8.5
- 0.25 ml hydrazine hydrate 100%
- 25 mg EDTA

Made up to 100 ml with distilled water.

The pH of the assay buffer solution was adjusted to pH 8.5 with 1 M hydrochloric acid and it was stored for up to two weeks at 4°C. Before use, 1 ml of 1% (w/v) NAD was added to 10 ml of assay buffer for each assay.

The total volume in each cuvette was 2 ml. In the sample cuvettes, this
consisted of 1 ml of neutralised PCA extract and 1 ml of cocktail; the standard cuvettes contained 0.1 ml of 1mM D,L-3-hydroxybutyrate, 0.9 ml water and 1 ml buffer. The 3-hydroxybutyrate standard was stored at -20°C as 0.1 M (0.252 gm in 10 ml H₂O) and diluted just before use. The cuvettes were read at 340 nm before and 90 minutes after addition of 0.01 ml of 3-hydroxybutyrate dehydrogenase.

3.2.9 Measurement of L-Alanine: Blood alanine concentrations were measured according to the method described by Williamson (pp. 1679-1682, Bergmeyer, 1974)

\[
\begin{align*}
\text{Reaction sequence:} \\
\text{alanine dehydrogenase} \\
\text{ALANINE} \rightarrow \text{PYRUVATE} \\
\text{H}_2\text{O} + \text{NAD}^+ \rightarrow \text{NAD}^+ + \text{NADH} + \text{NH}_4^+
\end{align*}
\]

This reaction proceeds quantitatively from left to right in the presence of low H⁺ ion concentrations (pH 9). Hydrazine hydrate is included in the buffer solution in order to trap the pyruvate formed by conversion to pyruvate hydrazone.

\[
\text{Buffer solution for assay:}
\]

\[
\begin{align*}
40 \text{ ml 0.2 M tris buffer} \\
4 \text{ ml hyrazine hydrate 100\%} \\
25 \text{ mg EDTA;}
\end{align*}
\]

Made up to 80 ml with distilled water.

The pH of the buffer solution was adjusted to pH 9 with approximately 6 ml of 1 M hydrochloric acid and it was stored for up to 14 days at 4°C. Before use, 1 ml of 1% (w/v) NAD was added to 10 ml of assay buffer for each assay.

The total volume in each cuvette was 2 ml. In the sample cuvettes, this consisted of 0.5 ml of neutralised PCA extract, 0.5 ml of water and 1 ml of
assay buffer; the standard cuvettes contained 0.1 ml of 1mM alanine, 0.9 ml of water and 1 ml of assay buffer.

All cuvettes were read at 340 nm before and 60 minutes after addition of 10 ul of alanine dehydrogenase. They were read again at 5 minute intervals until two consecutive readings of the optical density were identical.

3.2.10 Measurement of Pyruvate and Acetoacetate: Since their assay conditions are very similar, pyruvate and acetoacetate were measured sequentially in the same sample according to a combination of the methods respectively described by Czok and Lamprecht (pp. 1446-1451, Bergmeyer, 1974) and Mellanby and Williamson (pp. 1840-1843, Bergmeyer, 1974).

**Reaction sequence:**

\[
\text{lactate dehydrogenase} \\
\text{PYRUVATE} \rightarrow \text{LACTATE} \\
\text{NADH} + H^+ \rightarrow \text{NAD}^+ \\
3\text{-hydroxybutyrate dehydrogenase} \\
\text{ACETOACETATE} \rightarrow \text{3-HYDROXYBUTYRATE} \\
\text{NADH} + H^+ \rightarrow \text{NAD}^+ \\
\]

At pH 7.0 the equilibrium of the former reaction is sufficiently far to the left to ensure a quantitative measurement of pyruvate levels provided that the NADH concentration is not less than 0.01 mM. Lactate dehydrogenase reacts with hydroxypyruvate and glyoxylate in addition to pyruvate, but these substrates are in negligible concentrations in normal blood.

At the same pH and with a suitable excess of NADH, at least 98% of the acetoacetate is reduced to 3-hydroxybutyrate. However, due to the low activity of 3-hydroxybutyrate dehydrogenase preparations the latter reaction proceeds at a much slower rate than the former. Also, 3-hydroxybutyrate dehydrogenase is not absolutely specific for
acetoacetate; the higher homologues eg, 3-oxopentanoic and 3-oxohexanoic acids also react but at considerably slower rates (Bergmeyer, 1974).

**Buffer solution for assay:**

10 ml 0.1 M Potassium phosphate buffer pH 7.0

0.6 ml 0.5% (w/v) NADH

A fresh solution was prepared for each assay. The total volume in each cuvette was 2.0 ml. In the sample cuvettes, this consisted of 1 ml of neutralised PCA extract and 1 ml of assay buffer. Separate cuvettes were used for pyruvate and acetoacetate standards and contained 0.1 ml of 1 mM pyruvate or acetoacetate respectively, together with 0.9 ml of water and 1 ml of assay buffer.

The cuvettes were read at 340 nm before and 5 minutes after the addition of 0.005 ml of lactate dehydrogenase. Thereafter, 0.01 ml of 3-hydroxybutyrate dehydrogenase was added to each cuvette and they were read again at 35 minutes and at 5 minute intervals thereafter, until there was no further change in the optical density.

3.2.11 Measurement of Triglycerides:

Triglyceride levels were measured on plasma samples according to the method described by Eggstein and Kuhlmann (pp. 1825-1835, Bergmeyer, 1974). Plasma triglycerides were initially hydrolysed to produce glycerol and free fatty acids and the glycerol liberated by hydrolysis was measured enzymatically as described in the next section. The concentration of free glycerol, measured in neutralised PCA extract was subtracted from the total glycerol measured after alkaline hydrolysis of the plasma sample to obtain the glyceride-glycerol concentration, which was known to be numerically equal to the triglyceride content of the plasma.

**Reaction sequence:**

(a) Hydrolysis:
70 °C

\[
\text{TRIGLYCERIDE} + 3\text{H}_2\text{O} \rightarrow \text{GLYCEROL} + 3 \text{FATTY ACIDS}
\]

Alcohol KOH

(b) Indicator reaction:

- glycerokinase
- pyruvate kinase
- lactate dehydrogenase

\[
\text{GLYCEROL} + \text{PHOSPHOENOLPYRUVATE} \rightarrow \text{GLYCEROL-3-PO}_4 + \text{LACTATE}
\]

\[
\text{ATP} \quad \text{ADP} \quad \text{ATP} \quad \text{NADH} \quad \text{NAD}^+
\]

Hydrolysis: 0.2 ml of plasma was added to 0.5 ml of alcoholic KOH (0.5 N KOH in 98% ethanol). The mixture was incubated at 70°C for 30 minutes and then cooled. Thereafter, 1.5 ml of 0.1 M MgSO\(_4\) was added to precipitate protein, and the samples were centrifuged at 3000 rpm for 10 min. The triglycerides present in plasma produced equimolar quantities of glycerol. This glycerol produced by hydrolysis was contained in the supernatant solution, 0.5 ml of which was used for the enzymatic measurement of total glycerol. Known standard solutions of glycerol tripalmitate (1mM, 2mM, and 3mM) were also treated in the same way as plasma samples and subjected to the complete assay procedure. Glycerol tripalmitate was stored for up to 3 weeks as a 10mM solution in chloroform.

3.2.12 Measurement of Glycerol:

The levels of blood glycerol were assayed according to the method described by Eggstein and Kuhlmann (pp. 1825-1831, Bergmeyer, 1974).

Reaction sequence:

- glycerokinase

\[
\text{GLYCEROL} + \text{ATP} \rightarrow \text{GLYCEROL-3-PO}_4 + \text{ADP}
\]
pyruvate kinase

\[ \text{ADP} + \text{PHOSPHOENOLPYRUVATE} \rightarrow \text{ATP} + \text{PYRUVATE} \]

lactate dehydrogenase

\[ \text{PYRUVATE} + \text{NADH} + \text{H}^+ \rightarrow \text{LACTATE} + \text{NAD}^+ \]

At a pH of 7.4 and in the presence of Mg\(^{++}\) ions, the equilibria for all three reactions are sufficiently far to the right to ensure that there is a quantitative consumption of glycerol in the first reaction and that the indicator reactions rapidly proceed to completion.

**Buffer solution for assay:**

30 ml 0.1 M tris buffer pH 7.4
3 ml 0.1 M magnesium chloride
35 mg phosphoenolpyruvate
50 mg ATP
12.5 mg NADH
0.2 ml lactate dehydrogenase
0.2 ml pyruvate kinase

This was made up immediately before use and the pH adjusted to pH 7.4 with 0.2 M tris buffer.

**Triglycerides:** The total volume in each cuvette was 2 ml. In the sample cuvettes, 0.5 ml of the hydrolysed plasma sample was added to 1 ml of assay buffer and 0.5 ml of distilled water.

**Glycerol:** The sample cuvettes contained 1ml of assay buffer and 1 ml of neutralised PCA extract.

'Blank' cuvettes contained 0.5 ml of buffer mixture and 1.5 ml of distilled water whereas 'Control' cuvettes contained 1 ml of assay buffer and 1 ml of distilled water. 'Standard' cuvettes contained 0.1 ml of 1 mM glycerol, 0.9ml of water, and 1 ml of assay buffer. Glycerol standard was stored as a
0.1 M solution at -20°C and 0.05 ml was diluted to 5 ml for each assay. The cuvettes were read at 340 nm before and 40 minutes after addition of 0.01 ml of glycerokinase. Thereafter, they were re-read at 10 minute intervals until there was no further change in the optical density.

3.2.13 Measurement of Non-esterified fatty acids:
Non-esterified fatty acids were measured in duplicates of plasma samples which were collected and stored as described in 3.1. The method of Shimizu et al (1979) was used for the measurement of non-esterified fatty acids (NEFA), a method based on the activation of NEFA by Acyl-Co A synthetase.

Reaction sequence:

\[
\text{Acyl Co A synthetase}
\]

\[
\text{NEFA + COENZYME A + ATP} \rightarrow \text{ACYL COENZYME A + AMP + PPi}
\]

\[
\text{Myokinase}
\]

\[
\text{AMP + ATP} \rightarrow 2 \text{ADP}
\]

\[
\text{Pyruvate kinase}
\]

\[
2 \text{ADP + PHOSPHOENOLPYRUVATE} \rightarrow 2 \text{ATP + 2 PYRUVATE}
\]

\[
\text{Lactate dehydrogenase}
\]

\[
2 \text{PYRUVATE + 2 NADH} \rightarrow 2 \text{LACTATE + 2 NAD}^{+}
\]

At pH 8.0 and in the presence of sufficient quantities of ATP and coenzyme A, the reaction catalysed by Acyl CoA synthetase favours the production of acyl coenzyme A. Thus, the production of AMP is used as a measure of NEFA activation through an indicator system provided by successive reactions catalysed by myokinase, pyruvate kinase and lactate dehydrogenase. The equilibria of all three reactions in the indicator system are sufficiently far to the right to ensure that the overall rate of the reaction is limited only by the concentration of NEFA. Acyl Co A synthetase only activates
monocarboxylic acids with 6 to 18 carbon atoms and does not affect other

carboxylic acids and lipids in human plasma thereby providing a high

specificity for the method (Shimizu et al, 1979).

**Buffer solution for assay :**

200 ml 0.1 M tris pH 8.0

35.1 mg EDTA (0.6 mM)

406.6 mg magnesium chloride (10 mM)

2 ml triton X-100

This was stored at 4°C. Immediately before each assay, the following

reagents were added to 36 ml of buffer solution :

1.6 ml NADH 0.5% (w/v)

1.0 ml ATP 0.1 M

1.0 ml phosphoenolpyruvate 0.2 M

0.2 ml myokinase 2 mg/ml

0.4 ml pyruvate kinase 1 mg/ml

0.2 ml lactate dehydrogenase 10 mg/ml

The total volume in each cuvette was 2.005 ml. In the sample cuvettes, this

consisted of 0.04 ml of plasma, 1.95 ml of buffer mixture and 0.015 ml of

Acyl Co A synthetase. The plasma volume was replaced by 0.04 ml of water in

the single 'blank' and quadruplicate 'control' cuvettes, and by 0.04 ml of

1 mM NEFA standard solution in the quadruplicate 'standard' cuvettes. The

NEFA standard consisted of 1:1:1:1 mixture of myristic, palmitic, stearic

and oleic acids in 25% Triton X-100 and was stored at -20°C.

The cuvettes were read at 340 nm before and 20 minutes after the addition

of Coenzyme A to all cuvettes except the blank. Further readings were taken

at 5 minute intervals until the readings of optical density were constant.

3.2.14 **CALCULATIONS FOR METABOLITE ASSAYS :**
Calculations for glucose, lactate, 3-hydroxybutyrate, alanine, pyruvate, acetoacetate and glycerol:

All metabolite calculations are based on the change in optical density measured at 340 nm in the sample cuvettes following addition of enzyme, and after subtraction of the non-specific change occurring in the 'control' cuvettes. Thus:

Optical density = Change in absorbance for sample cuvette minus change in absorbance for control cuvette.

Since the molar extinction coefficient of NADH is 6.22 cm²/μmol, the amount of substrate in the cuvette = \( \frac{\text{ODD} \times \text{total volume in cuvette}}{6.22} \)

This is multiplied by the dilution factor for each sample to give the concentration of substrate (μmol/ml = mmol/L) in the blood sample:

\[
\frac{\text{wt. blood} + \text{PCA}}{\text{wt. neutral extract}} \times \frac{\text{total vol in cuvette}}{\text{wt. blood}} \times \frac{\text{ODD}}{\text{wt. acid extract}} \times \frac{\text{vol. neutral extract}}{6.22}
\]

Calculation for metabolite standards:

This is based on the same principle as the calculation for the metabolite samples.

\[
\frac{\text{total vol in cuvette}}{\text{vol of std in cuvette}} \times \frac{\text{ODD}}{6.22} = \frac{2 \text{ ml} \times \text{ODD}}{0.1 \text{ ml} 	imes 6.22} = 3.215
\]

Calculation for triglycerides:

The calculation of the dilution factor for triglycerides is different to that of the metabolites measured in neutralised PCA extract. The dilution of triglycerides during hydrolysis is also corrected for by the following calculation:

\[
\frac{\text{total vol of hydrolysate}}{\text{vol of hydrolysate used}} \times \frac{\text{total vol in cuvette}}{\text{vol of plasma used}} \times \frac{\text{ODD}}{6.22} = \frac{2.2 \times 2.0 \times \text{ODD}}{0.5 \times 0.2 \times 6.22} = 7.074 = \text{Total glycerol in plasma (μmol/ml = mmol/L)}
\]

Triglyceride concentration (mmol/L) = Total glycerol - Free glycerol

Calculation for non-esterified fatty acids:

Two molecules of NADH are oxidised for each molecule of NEFA activated by Acyl CoA synthetase and since the plasma sample undergoes dilution only
in the cuvette, the dilution factor for NEFA can be calculated as such:

\[
\frac{\text{total vol in cuvette}}{\text{vol of plasma used}} = \text{ODD} \times 4.02 = \text{NEFA (\mu mol/ml = mmol/l).}
\]

\[
\text{vol of plasma used} \times 6.22 \times 2
\]
**Table 1:** METHODS: Precision and accuracy for the measurement of blood metabolite concentrations.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>No. of Samples</th>
<th>Mean ± SD (mmol/L)</th>
<th>Coeff.</th>
<th>No. of Assays</th>
<th>Mean ± SD (mmol/L)</th>
<th>Coeff.</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>20</td>
<td>0.99 ± 0.01</td>
<td>1.5%</td>
<td>29</td>
<td>1.01 ± 0.04</td>
<td>1.6%</td>
<td>101.0%</td>
</tr>
<tr>
<td>Lactate</td>
<td>20</td>
<td>0.99 ± 0.02</td>
<td>2.3%</td>
<td>31</td>
<td>0.99 ± 0.03</td>
<td>2.1%</td>
<td>100.9%</td>
</tr>
<tr>
<td>Hydroxybutyrate</td>
<td>20</td>
<td>0.94 ± 0.01</td>
<td>1.1%</td>
<td>26</td>
<td>1.00 ± 0.03</td>
<td>2.8%</td>
<td>97.1%</td>
</tr>
<tr>
<td>Alanine</td>
<td>20</td>
<td>1.00 ± 0.01</td>
<td>1.2%</td>
<td>27</td>
<td>1.00 ± 0.03</td>
<td>1.4%</td>
<td>98.1%</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>20</td>
<td>0.91 ± 0.02</td>
<td>1.7%</td>
<td>55</td>
<td>0.94 ± 0.06</td>
<td>6.4%</td>
<td>97.0%</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>20</td>
<td>0.80 ± 0.01</td>
<td>1.6%</td>
<td>55</td>
<td>0.84 ± 0.04</td>
<td>4.7%</td>
<td>100.4%</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>20</td>
<td>1.07 ± 0.03</td>
<td>2.9%</td>
<td>6</td>
<td>1.04 ± 0.02</td>
<td>2.0%</td>
<td>-</td>
</tr>
<tr>
<td>Glycerol</td>
<td>20</td>
<td>1.08 ± 0.01</td>
<td>1.0%</td>
<td>23</td>
<td>1.02 ± 0.03</td>
<td>2.8%</td>
<td>100.7%</td>
</tr>
<tr>
<td>Non-esterified</td>
<td>20</td>
<td>0.93 ± 0.02</td>
<td>2.2%</td>
<td>8</td>
<td>0.96 ± 0.01</td>
<td>1.4%</td>
<td>98.7%</td>
</tr>
</tbody>
</table>

All values were measured on standard samples containing 1 mmol/L of substrate. The recovery of each metabolite was estimated by the addition of known standard solutions to unknown samples.

(Coeff. = Coefficient of variation).
3.3 MEASUREMENT OF PLASMA INSULIN:

The first radioimmunoassay, described by Berson and Yalow in 1958, was developed for the measurement of plasma insulin. In this study, plasma insulin was measured by a radioimmunoassay method described by Albano et al. (1972) using activated charcoal for separation of the free and bound hormone. A disequilibrium assay was set up with a 5-day incubation period and all plasma samples were assayed in duplicate with 50 μl of plasma in each tube.

3.3.1 Principle:

The essential principle of the insulin radioimmunoassay, as for any other radioimmunoassay technique, is the reaction of a fixed amount of specific insulin antibody with a mixture of the plasma sample to be assayed and a constant amount of radioactively labelled pure insulin. After the reaction has been initiated by incubating the plasma sample with insulin antibody for approximately 48 hours, the radioactively labelled insulin is added to compete for the remaining binding sites on the antibody complex. The reaction is allowed to approach completion over the subsequent 72 hours, and the antibody-bound insulin is separated from the free hormone by differential adsorption on to albumin-coated charcoal. The distribution of radioactivity between the free and bound forms of insulin is then determined. Due to competition for the limited number of binding sites available, it is expected that the amount of radioactively labelled insulin bound to antibody will decrease as the concentration of the unlabelled insulin in the plasma sample increases. Thus, results obtained with samples
of unknown insulin concentration can be compared with standard curves obtained by the measurement of samples to which known amounts of pure insulin standard have been added.

3.3.2 Optimum conditions for assay:
The buffer used for this assay consists of 0.19 M sodium phosphate solution pH 7.4 and also contains Thiomersal as a bacteriostatic agent. Before use, bovine serum albumin is added to the buffer solution since it prevents the binding of free hormone to surfaces and favours the formation of antigen-antibody complexes. The insulin standards used for constructing the standard curve, the radioactively labelled insulin tracer and the insulin antibody are all diluted in the working solution of the buffer. During incubation, the assay tubes are stored at 4°C in order to minimize proteolytic degradation and evaporation and also because the avidity of antibodies increases at lower temperatures.

3.3.3 Preparation of materials:

3.3.3.1 Concentrated buffer solution:

7.80 gm NaH₂PO₄.2H₂O
101.15 gm Na₂HPO₄
0.25 gm Thiomersal
1000 ml Warm distilled water

The sodium phosphate salts and Thiomersal were dissolved in 800 ml of warm distilled water and made up to 1 litre. The pH of the solution was checked and if necessary, was adjusted to pH 7.4. The buffer solution was stored at room temperature out of direct sunlight.

3.3.3.2 Working buffer solution:

For use in the radioimmunoassay the concentrated buffer solution was diluted in multiples of the following proportion and mixed for 30 min.
20 ml Concentrated buffer solution  
80 ml Distilled water  
0.3 gm Bovine serum albumin

3.3.3.3 Standard Insulin solutions:
Human monocomponent insulin was obtained from Novo Laboratories, Basingstoke. Each vial containing 0.1 mg of freeze-dried pure insulin was reconstituted with 1 ml of distilled water and divided into 4 aliquots of 0.25 ml each, which were stored at -20 °C. From the above aliquot 0.05 ml was diluted in sodium phosphate buffer containing 1% human albumin to obtain a standard insulin solution of 72 nmol/L (10 Units/L). This was further divided into 0.2 ml aliquots which were also stored at -20 °C. For use in the assay, 0.025 ml of the 72 nmol/L insulin standard was diluted in 20 ml of working buffer solution to give 0.09 pmol/ml of insulin standard solution. This was used for constructing the 0-576 pmol/L standard curve required in this assay.

3.3.3.4 Insulin free plasma:
Heparinised blood was obtained from the umbilical cord of normal neonates, centrifuged at 3000 rpm for 10 min at 4 °C and the plasma separated. Non-haemolysed plasma was obtained from the cord blood of several neonates at birth was pooled and 1 gm of Norit OL charcoal (Hopkins and Williams) was added for every 10 ml of plasma. The charcoal suspension was thoroughly stirred for 20 minutes and then centrifuged at 3000 rpm for 10 min. The supernatant plasma was separated from charcoal and allowed to sediment at 4 °C overnight. This was then re-centrifuged at 3000 rpm for 30 minutes to remove the remaining traces of charcoal, divided into 1.5 ml aliquots and stored at -20 °C.

3.3.3.5 Insulin antibody:
Anti-Insulin Antibody (RD 10) was obtained from Wellcome Research Laboratories, Beckenham. Each vial contained the freeze-dried residue of
0.5 ml of a 1:1000 dilution of guinea-pig antiserum in a buffer containing 0.04 M sodium phosphate, 0.5% bovine albumin and 0.1% sodium azide, pH 7.4. For use in the assay, the antibody residue was dissolved in 0.5 ml distilled water, left for 30 minutes and divided into aliquots of 0.25 ml. From one aliquot, 0.2 ml was diluted accurately in 20 ml of working buffer solution to give a titre of 1:200,000 and this was used for the assay. The other aliquot was stored at -20 °C for up to 2 weeks.

3.3.3.6 Plasma protein fraction:

This was obtained as a 5% human albumin fraction from Blood Products Laboratory, Elstree, Herts (UK) and was divided into 10 ml aliquots which were stored at -20 °C.

3.3.3.7 Radioactive Insulin Tracer:

Insulin labelled with $^{125}$I was obtained from The Radiochemical Centre, Amersham, Bucks. It was diluted with the working solution of buffer to a final concentration of 100,000 cpm/ml (0.35–0.5 ml of tracer solution in 20 ml of buffer, depending on specific activity) which was used in the assay.

3.3.4 ASSAY PROTOCOL:

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Insulin content</th>
<th>Buffer</th>
<th>Std.</th>
<th>IFP</th>
<th>Plasma samples</th>
<th>Ab</th>
<th>Tracer</th>
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<td>50</td>
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<td>100</td>
</tr>
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<td>100</td>
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<td>50</td>
<td>0</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>2 x tracer</td>
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<td>0</td>
<td>50</td>
<td>0</td>
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<td>200</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>650</td>
<td>100</td>
</tr>
</tbody>
</table>

Insulin content = pmol/L, corresponding to a standard curve of 0–80 mU/l
with a plasma sample volume of 50 μl. All volumes are given in μl.

**Blank** tubes contained all the assay reagents except the antibody, in order to evaluate the non-specific binding of labelled hormone to plasma proteins, etc.

**Standard** tubes contained increasing concentrations of pure insulin hormone in order to construct a nine-point standard curve, against which the binding of the unknown plasma samples was compared.

**Zero** hormone tubes were run at frequent intervals throughout each assay in order to detect and assess the degree of any drift in the binding of labelled hormone to antibody.

**1/2 tracer and 2 x tracer** tubes contained half and twice the concentration of the labelled hormone that was present in the standard or sample tubes respectively. The half-tracer tubes were useful in detecting the presence of a 'hump' in antibody binding and the double-tracer tubes were used for calculating the specific activity of the labelled hormone.

**Excess** antibody tubes contained only the antibody and tracer and were included in order to measure the immunological integrity of the labelled hormone.

**Quality control samples**: In each assay, 4 or more samples with known concentration of insulin were included in order to measure the inter-assay coefficient of variation.

**3.3.5 Charcoal separation**:  
10 mg of charcoal was added to each tube for adsorption of the unbound hormone. The charcoal suspension was made up on the night before it was used, in multiples of the following proportion:

- 1 gm Norit OL charcoal (Hopkins and Williams)
- 8 ml working solution of buffer
- 2 ml 5% Human albumin fraction
Mix by stirring for at least 30 minutes.

Before use, the charcoal suspension was stirred for 15 min and 0.1 ml of charcoal suspension was added to each assay tube. The tubes were briefly mixed on a Rotamixer and within 20 min were centrifuged at 3000 rpm for 30 min at 4 °C.

Immediately thereafter, the supernatant was separated from the charcoal using a Pasteur pipette.

3.3.6 Counting procedure and calculations:

The radioimmunoassay tubes were counted in a LKB-Wallac 1270 Rackgamma counter for 500 seconds or up to a maximum of 5000 counts. Radioactivity was counted only in the supernatant portion of each sample and the percentage of bound hormone was calculated by assuming that the radioactivity in the supernatant of zero tubes represented 100% bound hormone. Thus:

\[
\text{Percentage of bound hormone} = \frac{\text{cpm (standards or unknowns)}}{\text{cpm (zero tubes)}} \times 100
\]

All calculations were performed directly by the on-line 'Silent 700' Electronic Data Terminal (Texas Instruments Inc.) linked to the gamma counter. An optimised standard curve was calculated by the computer using a modified Gaussian regression of the third order for reciprocal standard values. Unknown sample concentrations were then obtained by measuring from the calculated standard curve.

3.3.7 Precision and accuracy:

The intra-assay coefficient of variation was 7.5% at a low insulin concentrations and 5.2% at higher insulin concentrations. The inter-assay coefficient of variation was determined only from the pooled plasma with low insulin concentrations and was found to be 10.7% (Table 3.3).
3.4 MEASUREMENT OF PLASMA GLUCAGON :

A radioimmunoassay method for the measurement of glucagon was first described by Unger and co-workers (1959). In this study, a radioimmunoassay method described by Ghatei et al (1983) was used which was specific for the measurement of pancreatic glucagon. An equilibrium assay was set up with a 5-day incubation period using differential adsorption on to dextran-coated charcoal for separation of the bound and unbound tracer. All plasma samples were assayed in duplicate with 50 ul of plasma in each tube; all samples belonging to the same study were measured in a single radioimmunoassay procedure.

3.4.1 Principle :
The basic principles of radioimmunoassay, as described for insulin in 3.4.1 also apply to the measurement of glucagon. However, the presence of glucagon in very low concentrations in human plasma imposes stringent conditions on the method used for its measurement. The measurement of low concentrations requires an antibody of high avidity; the presence of several molecular forms of glucagon-immunoreactivity or interfering substances in human plasma, eg, the 'big plasma glucagon' associated with the gamma globulin fraction, or the extended forms of glucagon secreted by cells of the intestinal mucosa; which may be present in higher concentrations than pancreatic glucagon, requires an antibody of high specificity.

Using synthetic glucagon fragments, Assan and Slusher (1972) have shown that antibodies specific to pancreatic glucagon were directed towards the C-terminal region of the glucagon molecule, whereas nonspecific antibodies which cross-reacted markedly with other intestinal peptides were directed
towards the N-terminal and central regions of the molecule. Furthermore, isolation and characterisation of gut glucagons has shown that these molecules are extended at the C-terminus and so do not cross-react with C-terminally directed antisera (Ghatei et al., 1983), whereas the pancreatic hormone cross-reacts completely with C-terminally directed antisera. Thus, for the specific measurement of pancreatic glucagon, the antiserum used must contain C-terminally directed glucagon antibodies of high avidity, and with a low susceptibility to interference by 'big plasma glucagon'.

A microstandard curve has to be constructed for comparing the unknown samples, to enable the accurate measurement of very low circulating concentrations of pancreatic glucagon. In addition, the assay error has to be minimized as much as possible.

3.4.2 Optimum conditions for assay:
The buffer used for this assay is 0.05 M barbitone sodium buffer at pH 8.0-8.2 which contains sodium azide as a bacteriostatic agent. Before use, a relatively large quantity of bovine serum albumin is added to the buffer solution to block the surface adsorption of glucagon molecules and thereby also to moderate the effect of charcoal, which by its strong adsorption would otherwise strip peptide molecules from the antibody complex. In addition, Trasylol (Bayer) is added to the buffer solution in order to decrease the extent of proteolytic degradation by trypsin-like enzymes during incubation. The assay tubes are stored at 4°C during incubation in order to further minimize proteolytic degradation, to decrease evaporation during storage and to increase the avidity of the glucagon antibody.

3.4.3 Preparation of materials:

3.4.3.1 Buffer solution:
The buffer used for radioimmunoassay of glucagon was 0.05 M barbitone sodium (Veronal) buffer.

51.55 gm  barbitone sodium  
20.00 ml  5 M hydrochloric acid  
2.50 gm  sodium azide (NaN₃)  
5000 ml  distilled water.

5 litres of distilled water were pre-boiled and cooled; barbitone sodium was added and stirred for 20 mins till dissolved completely. Hydrochloric acid was then added and allowed to disperse well before adding sodium azide. The pH of the solution was then checked and if between pH 8.0-8.2, it was considered appropriate.

3.4.3.2 Working solution of buffer:

For use in the radioimmunoassay 100 ml of buffer was required for every 100 tubes. Bovine serum albumin and Trasylol were added to a final concentration of 1.5% and 0.5% respectively.

100 ml Veronal buffer  
5 ml 30 % BSA solution  
0.5 ml Trasylol injection (Bayer).

3.4.3.3 Standard Glucagon solutions:

Glucagon sub-standards were prepared from pure porcine glucagon (Novo) and calibrated by radioimmunoassay against a glucagon standard supplied by the WHO International Laboratory for Biological Standards and Control, Holly Hill, Hampstead, London. Each vial of glucagon sub-standard contained 1.8 pmol of pure porcine glucagon which was reconstituted in 1.8 ml of buffer solution to obtain a standard solution of 1 pmol/ml which was used for the 0-100 pmol/L standard curve.

3.4.3.4 Glucagon free plasma:

Glucagon free plasma was obtained by the charcoal stripping of time-expired plasma (which had remained at room temperature for >48 hours). Activated
charcoal (Norit GSX = 1 gm) was added to 20 ml plasma and stirred for 15 min at 4 °C. The suspension was then separated in a refrigerated centrifuge for one hour at 3000 rpm. Final traces of charcoal were removed by recentrifugation overnight. The supernatant was divided into 10 ml aliquots and stored at -20 °C.

3.4.3.5 Glucagon antibody:
The RCS-5 antibody, which is specific for pancreatic glucagon was used for this assay. It was stored at -20 °C at a dilution of 1:10. For a 5-day incubation period it was used in a dilution of 1:50,000, obtained by diluting 1 ul in 3 ml of buffer solution for each 100 tubes in the assay.

3.4.3.6 Radioactive Glucagon tracer:
The $^{125}$-iodinated glucagon was obtained by trace-iodination followed by purification of the monoiiodinated glucagon using high resolution ion exchange chromatography. This method, first described by Jorgensen and Larsen (1972) is the most successful since it prevents gross oxidative damage to the glucagon molecule and the damaged and unlabelled peptides are then completely removed during purification. The labelled glucagon was stable for upto 3 months in 1 ml aliquots stored at -20 °C. The aliquot used was diluted in order to get approximately 30 counts/10 sec in 100 ul of the diluted tracer. For this, 100 ul of tracer was diluted in 10 ml of the glucagon buffer and adjustments were made by adding further amounts of tracer or buffer as required. (The iodination procedure was performed by N.D. Christofides.)
### ASSAY PROTOCOL:

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Glucagon content</th>
<th>Buffer</th>
<th>Std. X</th>
<th>GFP</th>
<th>Samples</th>
<th>Ab</th>
<th>Tracer</th>
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<tr>
<td>2 x</td>
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<td>50</td>
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<td>0</td>
<td>50</td>
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<td>0</td>
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<tr>
<td>Std.</td>
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<td>100</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>600</td>
<td>50</td>
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</table>

Glucagon content = fmol/tube
All volumes are given in μl.

Blank tubes contained all the assay reagents except the antibody, in order to evaluate the non-specific binding of labelled hormone to plasma proteins, etc.

Standard tubes contained increasing concentrations of pure glucagon hormone in order to construct a ten-point standard curve, against which the binding of the unknown plasma samples was compared.

Zero hormone tubes were run at frequent intervals throughout each assay so as to detect and assess the degree of any drift in the binding labelled hormone to antibody.

1/2 x and 2 x tracer tubes contained half and twice the concentration of the labelled hormone that was present in the sample tubes respectively. The half-tracer tubes were useful in detecting the presence of a 'hump' in antibody binding or whether the addition of lesser amount of label could result in increased sensitivity. The double-tracer tubes were used for calculating the specific activity of the labelled hormone.

Excess antibody tubes contained only the antibody and tracer and were
included in order to measure the immunological integrity of the labelled hormone.

Quality control samples: In each assay, four or more samples with known concentration of glucagon were included in order to measure the inter-assay coefficient of variation.

3.4.5 Charcoal separation:
5 mg of charcoal was added to each tube for adsorption of the unbound hormone. The charcoal suspension was made up in multiples of the following proportion:

- 2.0 gm Norit OL charcoal
- 0.2 gm Dextran T70 (Pharmacia)
- 100 ml Working solution of buffer

Mix by stirring for at least 30 minutes. Into each assay tube, 0.25 ml of charcoal suspension was dispensed and the tubes were centrifuged at 3000 rpm for 20 min at 4 °C. Immediately thereafter, the supernatant was separated from the charcoal pellet with a Pasteur pipette. To prevent contamination of the counter well with radioactive iodine, all assay tubes were sealed with low melting point wax before counting.

3.4.6 Counting procedure and calculations:
Both the free and antibody-bound tracer fractions were counted in order to reduce error in calculating the percentage of antibody-bound hormone. Thus,

\[
\text{Percentage of bound hormone} = \frac{\text{cpm (supernatant)}}{\text{cpm (supernatant)} + \text{cpm (charcoal)}} \times 100
\]

Radioactivity was counted in four Nuclear Enterprises 1600 Gamma counters linked in series to a microprocessor unit; each counter was capable of counting 16 samples simultaneously. Results were calculated by a Sirius microcomputer using point to point straight lines for constructing the
standard curve. Unknown sample concentrations were obtained by measurement from this standard curve.

\textbf{3.4.7 Precision and accuracy :-}

The intra-assay coefficient of variation was measured near the middle of the standard curve and was found to be 7.9\%, whereas the inter-assay coefficient of variation from the small number of assays was found to be 9.4\% (Table 3.3).
3.5 MEASUREMENT OF PLASMA ADRENALINE AND NORADRENALINE:

The measurement of catecholamines may be performed accurately by several alternative methods using variations of the radioenzymatic technique or the use of high pressure liquid chromatography (HPLC). The fluorimetric and bio-assay techniques are regarded as relatively inaccurate in comparison to these methods, particularly with respect to the measurement of adrenaline concentrations. The radioenzymatic assays are based on the conversion of the catecholamine being measured into a labelled derivative, this reaction occurring in the presence of a specific enzyme and a labelled co-substrate. There are two basic varieties, depending on the enzyme preparation being used to catalyse the reaction: catechol-o-methyl transferase (COMT-REA) or phenylethanolamine-N-methyltransferase (PNMT-REA); the latter is less accurate and can be used to measure only noradrenaline concentrations. The HPLC methods may involve the use of either fluorimetry or electrochemical detection in the final step of the assay. On the other hand, the REA techniques are based on labelling of the catecholamines with one or two (\(^{14}\)C and \(^{3}\)H) isotopes in the initial stages of the assay. In this study, a double-isotope COMT-REA, described by Brown and Jenner (1981) was used for measurement of plasma adrenaline and noradrenaline concentrations.

A double-isotope REA technique for measurement of 'total' catecholamines in blood was first described by Engelman, Portnoy and Lovenberg in 1968; they later modified this method to include a thin-layer chromatography step in order to measure the plasma concentrations of adrenaline and noradrenaline separately (Engelman and Portnoy, 1970). However, the method used in the present study was a modification of two single-isotope methods (Peuler and Johnson, 1977; Da Prada and Zurcher, 1976) in which the efficiency of methylation with \(^{14}\)C and \(^{3}\)H-methyl donors was used to correct for the
inter-plasma variation in inhibition of catechol-0-methyl transferase (COMT) activity as well as for the loss of recovery during multiple extraction stages of the assay; thus permitting a much greater precision and sensitivity than the previous methods described for the measurement of catecholamines.

3.5.1 Principle:
Radioenzymatic assays (REA) are based on the conversion of the substance being measured to a labelled derivative in the presence of a specific enzyme and a labelled co-substrate. In REA, the amount of label must be saturating in order to ensure that the rate of reaction depends solely on the concentration of unknown hormone in the plasma sample. Subsequently, the labelled hormone has to be separated from a large excess of the unreacted label and this involves several steps of extraction, from which recovery is relatively low and variable.

In this assay, adrenaline and noradrenaline are methylated with $^{14}$C and $^3$H-labelled methyl groups from S-adenosyl-L-methionine (SAM) in the presence of COMT. High sensitivity is achieved by use of high specific activity $^3$H-SAM (60-85 Ci/mmol); low background counts are maintained by the prior removal of potential contaminating catecholamines from the enzyme preparation. Specificity is ensured by a thin-layer chromatography step in the extraction procedure and, to some extent, by the COMT enzyme. Precision is achieved in two ways. First, by simultaneous methylation of the plasma sample with $^{14}$C AND $^3$H methyl groups (which corrects for the variable inhibition of COMT by the plasma sample) and second, by the addition of the formed $^{14}$C-metanephrines to the $^3$H-metanephrines after the termination of methylation, which then corrects for the loss of labelled hormone during the multiple procedures used for extraction.
3.5.2 Principle of differential extraction:
Before the thin-layer chromatography (TLC), several 'solvent extractions' are employed to eliminate as much background $^3$H-SAM as possible (see Fig 3.2). These are based on the property that catecholamines and their methylated derivatives are weak alkalis (pK 9-10), which, at a physiological pH, are fully ionised and water soluble. With ion-pair reagents such as tetraphenyl boron (Tpb), metanephrines form a non-polar complex or 'ion-pair' which is soluble in non-aqueous solvents and unstable at an acid pH. Thus, in the presence of Tpb the labelled metanephrines are extracted into ether at pH 8 and 'back-extracted' into a much smaller volume of acid; thus causing an elimination of excess $^3$H-SAME and concentration of the aqueous phase to a small volume that can be readily applied to the TLC plate.

3.5.3 Optimum conditions for assay:
The plasma samples are incubated with the enzyme COMT and (in separate tubes) with $^3$H- and $^{14}$C-SAM. The methylation of catecholamines by COMT is optimum at pH 8.4, and this is maintained by a tris buffer used for the reaction. The buffer also contains Mg$^{++}$ ions which are an absolute requirement for COMT activity and EGTA (Ethylene glycol-bis-(beta-aminooethyl ether)-tetra acetic acid) which chelates Ca$^{++}$ ions but not the Mg$^{++}$ ions. Benzylhydroxylamine is also added to the incubation mix since it inhibits the enzyme DOPA decarboxylase, which is a contaminant of the COMT enzyme preparation. DOPA is present in the plasma in much higher concentrations than catecholamines; if decarboxylated to dopamine, the latter is o-methylated by COMT and can interfere with the measurement of adrenaline and noradrenaline. The incubation is carried out at a temperature of 35°C at which enzyme activity is highest. The presence of
haemolysis in the sample gives inaccurate results for catecholamine estimation, possibly due to inhibition of the enzyme and due to consumption of the label by non-catechol substrates (Causon, Murphy and Brown, 1982).

3.5.4 Preparation of materials:

3.5.4.1 COMT buffer solution:

9.69 gm trizma base (Sigma T-1503)
3.04 gm MgCl₂ (BDH)
4.88 gm EGTA (Sigma E-4378)
100 ml distilled water

The buffer mixture was stirred for 20 min and the pH was checked and adjusted to pH 8.4 with hydrochloric acid if necessary.

3.6.4.2 Standard adrenaline and noradrenaline solutions:

Catecholamine standards were obtained from Sigma Laboratories. 25 mg of adrenaline (L-Epinephrine, Sigma E-4250) or noradrenaline (Arterenol free base, Sigma A-7257) were weighed and dissolved in 50 ml of 0.1 M hydrochloric acid (HCl) to give a concentration of 0.5 mg/ml. 1 ml of this standard solution was diluted further in 50 ml of 0.1 M HCl to give a standard concentration of 10 ug/ml which was used in the assay.

3.5.4.3 'Cold carrier' solution:

The 'cold carrier' solution containing unlabelled metanephrines was required for stopping the enzymatic reaction in the ³H tubes at the end of the incubation period, and was also used for 'back-extraction' of the labelled catecholamines into an acidic medium.

500 mg 3-methoxytyramine
500 mg metanephrine
500 mg normetanephrine
50 ml 0.1 M hydrochloric acid

The metanephrines were dissolved in 0.1 M HCl and this solution was diluted
1:10 with 0.1 M HCl for use in the assay.

3.5.4.4 Benzylhydroxylamine solution:
A solution of 5 mg/ml of O-benzylhydroxylamine (Sigma B-8130) was made by dissolving 250 mg of the salt in 50 ml of distilled water.

3.5.4.5 Radioactive S-adenosyl-L-methionine:
Labelled S-adenosyl-L-(Methyl-\(^{3}\)H) methionine containing 65-85 Ci/mmol (TRK 581) was obtained from The Radiochemical Centre, Amersham, UK; and adenosyl-L-methionine, S-(Methyl-\(^{14}\)C) was obtained from New England Nuclear Corporation Inc., Cambridge, USA. They were divided into aliquots of 360 ul and 100 ul respectively and stored under liquid nitrogen.

3.5.4.6 COMT enzyme preparation:
The enzyme used in this assay was prepared by the method of Axelrod and Tomchick (1958) from rat livers, after pretreatment of the rats with oestradiol (1 mg intra-muscularly, twice a day) and 6-hydroxydopamine (100 mg/kg intra-peritoneally once daily) for two days before the procedure. (The method was performed by R.C. Causon.)

3.5.5 ASSAY PROCEDURE:
The assay procedure was carried in 3 sequential stages: incubation, during which the catecholamines were methylated with labelled SAM; extraction, during which the large excess of labelled SAM was removed and separation, in which metanephrine and normetanephrine were separated by thin layer chromatography, oxidised and prepared for scintillation counting.

3.5.5.1 Incubation:
Each assay contained 4 or more racks of tubes, which included one blank and at least one duplicate of standards and pooled neonatal plasma in each rack. Into triplicate sets of plastic tubes (Sarstedt no. 55.526), 0.05 ml
of plasma from each sample was pipetted; the standard tubes contained a
typical non-haemolysed neonatal plasma and 0.005 ml of 100 ng/ml adrenaline
and 100 ng/ml noradrenaline solutions; the pooled plasma tubes contained
0.05 ml plasma derived from the cord blood of unstressed neonates; whereas
the blanks contained 0.05 ml of distilled water. The incubation mixture was
prepared in the following proportion for 4 racks:

6 mg glutathione reduced form (Sigma G-4251)
2.8 ml tris/Mg/EGTA buffer, pH 8.4
12 mg COMT enzyme (variable, depending on its activity)
0.02 ml benzylhydroxylamine, 5 mg/ml

This buffer mixture was divided into two parts:

(A) 1.55 ml, to which was added:
0.72 ml $^3$H-SAM (65-85 Ci/mmol)

and, (B) 1.25 ml, to which was added:
0.02 ml $^{14}$C-SAM (57.6 mCi/mmol)
0.05 ml adrenaline standard, 10 ug/ml
0.05 ml noradrenaline standard, 10 ug/ml

0.025 ml of solution (A) was added to the first two rows ($^3$H tubes) of
each rack and 0.025 ml of solution (B) was added to the third row ($^{14}$C
tubes) of each rack. All racks were incubated for 1 hour in a water bath at
35 °C.

After incubation, 0.02 ml of cold SAM solution 2 mg/ml in 1 M Borate pH 8,
was added to the third row of each rack ($^{14}$C tubes only) in order to stop
the reaction and these tubes were then gently vortexed. From the $^{14}$C
tubes, 0.04 ml was carefully pipetted into each of the corresponding $^3$H
tubes, the $^{14}$C tubes were then discarded. The $^3$H tubes were mixed by
gentle vortexing. A mixture to stop the reaction from proceeding further in
the $^3$H tubes was prepared in the following proportion for 4 racks:
4 ml 1 M borate solution, pH 8
20 mg tetraphenylboron (Aldrich T-2540-2)
0.04 ml cold carrier solution

0.05 ml of this mixture was added to all the $^3$H tubes.

3.5.5.2 Extraction:
Immediately after adding the stopping mixture, 2 ml of diethyl ether (AR BDH) was dispensed into each tube. (The stopping mixture was added to all tubes in one rack and then ether was dispensed into the tubes of that rack, before proceeding to the next rack.) All tubes were vortexed in a multi-vortex mixer for 2 minutes and then centrifuged for 2 minutes at 1000g. The lower part of each tube was immersed briefly in a dry ice/acetone mixture in order to freeze the lower aqueous layer. The upper diethyl ether layer was immediately decanted into the glass tubes containing 0.035 ml of cold carrier solution (1 mg/ml in 0.1 M HCl). The glass tubes were vortexed for two minutes in a multi-vortex mixer and then centrifuged for 2 minutes at 1000g. The lower parts of each tube were again immersed in dry ice/acetone mixture to freeze the lower aqueous layer, the upper diethyl ether layer was discarded. The residual ether in each tube was blown off under vacuum in a Buchler Vortex Evaporator (Searle Ltd) for 45 seconds at room temperature.

3.5.5.3 Separation:
The cold carrier solution containing labelled metanephrines was spotted onto 19-channel TLC plates (Whatman LKD 5F) using glass capillaries and applicators (A R Howell Ltd). Thus, all the tubes from one rack could be spotted onto each TLC plate which were then, after an interval of 10 minutes, blown dry in cold air for 25 minutes. The chromatography tanks were equilibrated with an eluent system prepared from chloroform, methanol
and 70% ethylamine (AR MedB) in a proportion of 32 : 6 : 4 and the TLC plates were run in this for approximately 1 hour.

After chromatography, the plates were dried briefly in a fume cupboard, visualised under ultra-violet light and the spots containing metanephrine and normetanephrine were defined with a scalpel. Each spot was moistened with a drop of distilled water and scraped into small scintillation vials (Beckmann) containing 0.5 ml of 0.05 M ammonium hydroxide at 4 °C. The vials were vortexed in a multi-vortex mixer for 5 minutes and then 0.025 ml of freshly prepared 4% sodium-m-periodate solution (Sigma S-1878) for oxidation was added to each vial. The vials were briefly vortexed again and, after an interval of 10 minutes at room temperature, 0.05 ml of 1:1 mixture of glacial acetic acid (BDH) and 20% glycerol (BDH) was added to each vial followed by 5 ml of Beckman NA scintillation cocktail. All vials were capped and mixed by inversion for 2 minutes and allowed to stand overnight before counting.

3.5.6 Counting procedure and calculations:

Each vial was counted in a Beckman LS 2800 scintillation counter for 10 minutes and the $^{3}H/^{14}C$ dpm ratio was measured, thereby correcting for any loss of labelled hormones during extraction procedures. The $^{3}H/^{14}C$ dpm ratio for each sample was compared to that for samples to which a known amount of catecholamine standard was added. To 0.05 ml of a typical vehicle plasma 0.005 ml of adrenaline or noradrenaline 100 ng/ml standard was added thus giving a dilution factor of approximately = 10. Therefore, the unknown catecholamine concentration (ng/ml) could be calculated by:

$$\frac{^{3}H/^{14}C \text{ dpm (SAMPLE)}}{^{3}H/^{14}C \text{ dpm (BLANK)}} \times 10$$

$$^{3}H/^{14}C \text{ dpm (STANDARD+VEHICLE)} - ^{3}H/^{14}C \text{ dpm (VEHICLE)}$$
For every assay, the mean of the $^3\text{H}/^1\text{C}$ dpm for blanks and standards from each rack was taken.

3.5.7 Precision and accuracy :-

The intra-assay coefficient of variation measured in pooled neonatal plasma obtained from the cord blood of unstressed neonates at birth was found to be 10.3% for adrenaline and 2.4% for noradrenaline; the inter-assay coefficient of variation measured from the same material was 13.2% for adrenaline and 13.8% for noradrenaline. The recovery of these hormones was measured by the addition of 10 µg/ml of adrenaline and noradrenaline standards to the plasma pool and was found to be 98% and 91% respectively.
Table 1: METHODS: Precision and accuracy for the measurement of plasma insulin, glucagon, adrenaline and noradrenaline concentrations.

<table>
<thead>
<tr>
<th></th>
<th>INTRA-ASSAY VARIATION</th>
<th>INTER-ASSAY VARIATION</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples</td>
<td>Mean ± SD</td>
<td>Coeff.</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>Standard (1)</td>
<td>10</td>
<td>76 ± 6</td>
</tr>
<tr>
<td></td>
<td>Standard (2)</td>
<td>15</td>
<td>189 ± 20</td>
</tr>
<tr>
<td>Glucagon (pmol/L)</td>
<td>10</td>
<td>19.6 ± 1.5</td>
<td>7.9%</td>
</tr>
<tr>
<td></td>
<td>Adrenaline (nmol/L)</td>
<td>10</td>
<td>0.16 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>Noradrenaline (nmol/L)</td>
<td>10</td>
<td>4.09 ± 0.10</td>
</tr>
</tbody>
</table>

All values were measured in pooled plasma samples. The recovery of catecholamines was obtained by addition of 10 μg/ml of Adrenaline and Noradrenaline standards to unknown plasma samples. (Coeff. = Coefficient of variation)
3.6 MEASUREMENT OF PLASMA STEROID HORMONE CONCENTRATIONS:

A method for the simultaneous determination of cortisone and cortisol using chromatography followed by radioimmunoassay was first described by Bro-Rasmussen, Buus and Trolle in 1962. In this study, the method developed by Sippell et al (1978a and 1978b) was used for the simultaneous measurement of 8 steroid hormones in each plasma sample. Separation of steroid hormones was performed by two chromatography steps followed by radioimmunoassay measurement of each hormone in the resulting concentrated eluate fraction.

3.6.1 Principle:

For chromatography, 10 parallel Sephadex LH-20 columns with gel dimensions of 750mm x 9mm are used and the solvent system consists of methylene chloride and methanol (98:2, v/v). The central component of the chromatographic method is a ten channel pulse damped, twin-piston precision pump which pumps exactly 40 ml of solvent/hour through the ten columns in reversed flow, ie, from bottom to top, thus facilitating the elimination of air bubbles and inhomogeneities within the packed gel. With this column 17-hydroxyprogesterone (17-OHP), corticosterone (B), 11-deoxycortisol (S), aldosterone (A), cortisone (E) and cortisol (F) are isolated (Sippell et al, 1978a); and the combined progesterone (P) and 11-deoxycorticosterone (DOC) fraction is separated by using another solvent system, consisting of water-saturated n-heptane : chloroform : ethanol (50:50:0.25) (Sippell et al, 1978b). This additional chromatographic run can be rapidly performed on ten parallel, manually operated 400 mm x 11 mm LH-20 columns whilst the more polar steroids B, E and F are being eluted from the automatically operated 750-mm columns.

The combination of chromatographic purification and sufficiently specific
antisera in the different radioimmunoassays gives a high degree of overall specificity for the steroid estimations. The measurement of any cross-contaminating steroids in the isolated chromatographic fractions is eliminated by the use of antisera in the respective radioimmunoassays which exhibit only negligible cross-reaction with the contaminating steroids. The basic principles of radioimmunoassay (RIA), as described for insulin and glucagon (3.4.1 and 3.5.1, respectively) are also applicable to the measurement of all steroid hormones.

3.6.2 Materials used:

3.6.2.1 Radioactive steroids:

- \([1,2,6,7-^3H] \)-D-aldosterone (NET-419), \([1,2,6,7-^3H] \)-corticosterone (TRK-406), \([1,2-^3H] \)-11-deoxycorticosterone (TRK-420),
- \([1,2,6,7,16,17-^3H] \)-progesterone (TRK-641), \([1,2,6,7-^3H] \)-17-hydroxyprogesterone (TRK-611) \([1,2-^3H] \)-11-deoxy cortisol (NET-295),
- \([1,2,6,7-^3H] \)-cortisol (TRK-407) and \([1,2-^3H] \)-cortisone (TRK-2376) were purchased either from The Radiochemical Centre, Amersham (UK) or from New England Nuclear Corp., Boston, MA (USA). All steroids had a specific radioactivity of 30-100 Ci/mmol and the radiochemical purity was 97-98%.

About 3 x 10^6 cpm of each steroid was repurified by chromatography on a 40 cm Sephadex LH-20 column using methylene chloride-methanol (98:2, v/v) as solvent. Purified radioactive steroids were stored in benzene-ethanol (9:1, v/v) at 4 °C for up to 3 months.

3.6.2.2 Chemicals:

Methylene chloride (Merck no. 6050), methanol (Merck no. 6009), ethanol (Merck no. 983), benzene (Merck no. 1779), chloroform (Merck no. 2445), n-heptane (Merck no. 3/9177) were used without further purification. Non-labelled steroids were obtained from either Merck, Darmstadt or Ikapharm, Ramat-Gan, Israel. Dextran T70 was supplied by Pharmacia,
Uppsala, Sweden; human gamma globulin by Behring, ORHE 04/05, Marburg, FRG; charcoal Norit A by Serva, Heidelberg, FRG. Scintillation cocktail Quickstint 402 (for aqueous solutions) and Quickstint 501 (for organic solutions) were purchased from Zinsser, Frankfurt, FRG.

3.6.2.3 **Instruments** :
All glassware used was made steroid-free by previous heating to 500 °C for up to 5 hours. Disposable 12 x 55 mm polystyrene tubes were used for the radioimmunoassays.

3.6.2.4 **Equipment** :
The pump used for automated chromatography was made of solvent resistant materials and was obtained from Ismatec Corp., Zurich, Switzerland. The RIA incubations were carried out in a gentle-shaking (20/min) water bath obtained from Julabo GmbH, Seelbach, FRG and for centrifugations a refrigerated 6/4 Lab centrifuge (Heraeus Christ, Osterode, FRG) with a manifold RIA tube swing out head was used. Radioactivity was counted in a Nuclear Chicago Isocap 300 Liquid Scintillation Multi-spectrometer (Zinsser, Frankfurt, FRG) with an efficiency of 60% for tritium.

3.6.3 **Preparation of solvents and buffers** :

3.6.3.1 **Solvent system 1** : The solvent used for mechanized multi-column chromatography, in which the primary separation of all steroids was carried out, was made up of:

- 2.5 litre methylene Chloride
- 50 ml methanol

3.6.3.2 **Solvent system 2** : This solvent was used for the separation of progesterone and deoxycorticosterone in the subsequent chromatographic step and was made up of:

- 500 ml chloroform
- 500 ml n-heptane
2.5 ml ethanol
7.0 ml distilled water

These ingredients were thoroughly mixed in a 2 litre separatory funnel and left standing for 20 minutes till there was complete separation of the aqueous and non-aqueous phases; the heavier non-aqueous phase was removed at the bottom and used for chromatography.

3.6.3.3 Gamma-globulin buffer: The buffer used in all radioimmunoassays and for diluting all antisera and internal standards was prepared in the following proportions and stored at 4°C for 3 months.

- 6.18 gm H$_3$BO$_3$ p.a. (Merck no. 165)
- 7.46 gm KCl p.a. (Merck no. 4933)
- 0.65 gm NaN$_3$ (Merck no. 6688)
- 1.20 gm bovine gamma globulin (Behringwerke)
- 78.0 ml 0.1 N NaOH (Merck no. 233)

2000 ml total volume with distilled water

Mix by stirring for 30 mins and check pH 7.2-7.4.

3.6.3.4 Charcoal suspension: The same composition of charcoal suspension was used for all radioimmunoassays; each tube was treated with 2.5 mg of charcoal for adsorption of the unbound hormone.

- 2.5 gm Norit A charcoal (Serva)
- 0.25 gm Dextran T70 (Pharmacia)
- 100 ml gamma globulin buffer

The charcoal suspension was mixed by vigorous stirring for at least 30 minutes before use and 100 μl was added to each tube.
3.6.4 ASSAY PROCEDURE:

The separation and measurement of steroid hormones by this method was performed in three distinct stages: extraction, chromatography and radioimmunoassay.

3.6.4.1 Extraction of plasma samples:

To 0.4 ml of plasma, 1500 cpm of each of the following steroids dissolved in 0.1 ml of gamma globulin buffer were added as internal standards: [1,2,6,7-3H]-D-aldosterone, [1,2,6,7-3H]-corticosterone, [1,2-3H]-deoxycorticosterone, [1,2,6,7,16,17-3H]-progesterone, [1,2,6,7-3H-17]-hydroxyprogesterone, [1,2-3H]-11-deoxycortisol, [1,2,6,7-3H]-cortisol and [1,2-3H]-cortisone. Plasma samples were made up to 1 ml with distilled water. Similarly, quality control samples were analysed in a volume of 1 ml. After thorough mixing and an equilibration period of at least 90 minutes at 4 °C, the plasma samples were manually extracted twice with 5 ml of cold (4°C) methylene chloride and the extract washed with 3 ml of distilled water. For better separation, the samples were centrifuged at 1000g for 10 min in a refrigerated centrifuge and the lower methylene chloride layer was separated from the upper aqueous layer by vacuum suction. The final extracts were evaporated under a gentle stream of dry nitrogen at 37 °C.

3.6.4.2 Multi-column chromatography:

Plasma extracts were redissolved in 0.2 ml of the solvent system consisting of methylene chloride and methanol (98:2, v/v) and then injected via a teflon septum into the base of 10 parallel 750 mm x 9 mm Sephadex LH-20 columns through which solvent was pumped at a constant rate of 40 ml/hour. The linear fraction collector was programmed to collect fractions.
corresponding to the various peaks of the steroids being eluted in sequence. Thus, a complete separation of 17-OHP (41-45, 5 ml), B (52-56, 5 ml), S (66-73, 8 ml), A (76-82, 7 ml), E (88-96, 9 ml), and F (155-178, 24 ml) could be obtained. The first fraction consisted of P and DOC (25-36, 11 ml) which was submitted to further chromatographic separation after drying the extract under nitrogen at 37 °C; dissolving in 1 ml of solvent system 2. Chromatography was performed manually on Sephadex LH-20 columns measuring 40 mm x 11 mm; P was collected as a 6 ml fraction between 13 and 18 ml whereas DOC was collected as a 9 ml fraction between 21 and 29 ml.

The fractions, each containing one of the 8 isolated steroids, were evaporated to dryness, redissolved in 2.0 ml of ethanol and divided into two aliquots (Table 3.4) for measurement of internal tracer recovery and for radioimmunoassay. The aliquot used for recovery was transferred to a scintillation vial; dried in vacuum at 50 °C and after re-dissolving with 0.1 ml gamma globulin buffer and 9 ml of scintillation cocktail, was allowed to stand for two hours before counting the radioactivity.

<table>
<thead>
<tr>
<th>HORMONE</th>
<th>RADIOIMMUNOASSAY</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>2 x 0.75 ml</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>Deoxy cortisolone</td>
<td>1 x 1.5 ml</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>17-hydroxyprogesterone</td>
<td>2 x 0.75 ml</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>2 x 0.75 ml</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>11-deoxycortisol</td>
<td>2 x 0.75 ml</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>2 x 0.75 ml</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>Cortisol</td>
<td>2 x 0.05 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Cortisone</td>
<td>2 x 0.10 ml</td>
<td>1.5 ml</td>
</tr>
</tbody>
</table>

Table 3.4 : Volume of ethanolic extracts used for recovery of internal standards and for radioimmunoassay of the steroid hormones.

3.6.4.3 Steroid radioimmunoassays:

The amount of ethanolic extract used for radioimmunoassay of each hormone is shown in table 3.4; all assays were performed in duplicate except for...
deoxycorticosterone, in which the expected low levels required measurement in a single large sample. Blanks were prepared in quadruplicate and standards were prepared in duplicate by evaporating 10, 25, 50, 100, 250, 500, 1000 and 3000 pg of the standard hormone in 0.1 ml of the respective ethanolic solutions at the same time as the plasma ethanolic extracts. All tubes in each radioimmunoassay were evaporated in vacuum at 50 °C. To compensate for contingent non-specific blanks that might be introduced into the RIA-tubes by the column eluate containing the unknown steroid, 200 ml of eluate collected during several pre-rinsing runs of the chromatographic columns was dried under nitrogen and redissolved in 200 ml of absolute ethanol. 0.6 ml of this solution was evaporated in each standard tube and in the blanks.

To the unknown tubes, standards and blanks in all the radioimmunoassays, 0.1 ml of gamma globulin buffer containing 6000 cpm of the respective tritiated steroid was added followed by 0.5 ml of the respective antiserum to each tube. The titres and origin of the antisera are shown in table 3.5.
Table 3.5: The origin and titre of antisera used for radioimmunoassay of the steroid hormones.

<table>
<thead>
<tr>
<th>HORMONE</th>
<th>ANTISERA TITRE</th>
<th>ORIGIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>1:5000</td>
<td>rabbit anti 11a-hydroxyprogesterone-11a-hemisuccinate-BSA antibody</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>1:40000</td>
<td>rabbit anti 11-deoxycorticosterone-3-CMO-BSA antibody</td>
</tr>
<tr>
<td>17-hydroxyprogesterone</td>
<td>1:55000</td>
<td>rabbit anti 17-hydroxyprogesterone-3-CMO-BSA antibody</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>1:12000</td>
<td>rabbit anti corticosterone-21-hemisuccinate-BSA antibody</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>1:15000</td>
<td>rabbit anti aldosterone-3-CMO-BSA antibody</td>
</tr>
<tr>
<td>11-deoxycortisol</td>
<td>1:15000</td>
<td>rabbit anti 11-deoxycortisol-3-CMO-BSA antibody</td>
</tr>
<tr>
<td>Cortisol</td>
<td>1:18000,</td>
<td>rabbit cortisone-21-hemisuccinate-BSA antibody</td>
</tr>
<tr>
<td>Cortisone</td>
<td>1:55000</td>
<td></td>
</tr>
</tbody>
</table>

CMO = (O-carboxymethyl) oxime, BSA = Bovine serum albumin.

After thorough mixing using a Rotamixer, the RIA tubes were incubated for 20 minutes at 37 °C with gentle shaking; then allowed to stand in an ice bath for 2 hours. Thereafter, 0.1 ml of vigorously stirred charcoal suspension was rapidly added to each tube and after an interval of 15 minutes, the bound and free fractions of the hormones were separated by centrifugation for 20 min at 4 °C. The supernatant containing the bound fraction was decanted directly into plastic vials containing 9 ml of the scintillation cocktail.

3.6.5 Counting procedure and calculations:

Radioactivity was counted in a Nuclear Isocap Chicago 300 Scintillation Multi-spectrometer with a statistical error of 2% or less. Standard curves were constructed by computer, using a spline algorithm prepared by Marschner et al, (1974). Unknown sample concentrations were then obtained
by using the calculated standard curve.

3.6.6 Precision and accuracy:

Precision and accuracy of the steroid assays were determined by the inter-assay coefficients of variation, calculated from the measurement of quality control plasma samples with known steroid content in 8 successive assays (Table 3.6). The sensitivity of this method was 0.03 ng/ml for progesterone, 11-deoxy corticosterone and 17-hydroxy progesterone; 0.13 ng/ml for corticosterone, 0.04 ng/ml for 11-deoxy cortisol, 0.02 ng/ml for aldosterone, 0.4 ng/ml for cortisone and 0.31 ng/ml for cortisol.
3.7 MEASUREMENT OF URINARY TOTAL NITROGEN:

The total nitrogen content of urine was measured by a micro-modification of the Kjeldahl method, which was described by Johan Kjeldahl in 1883 and is currently used as the standard method for measurement of nitrogen content in biological materials (Rosdahl and Mossberg, 1980).

3.7.1 Principle:

The Kjeldahl method is based on the principle that the nitrogen content of organic nitrogenous compounds is converted to ammonium sulphate when digested with concentrated sulphuric acid. The complete conversion of organic nitrogen to ammonium sulphate required an extremely prolonged reaction time in the original method (Kjeldahl, 1883), but can be shortened by the addition of catalysts such as, mercury, copper, titanium or selenium; and may be decreased further by the addition of oxidising agents such as hydrogen peroxide, potassium permanganate or perchloric acid. The addition of salts to raise the boiling point of the mixture also ensures that the digestion of nitrogenous compounds proceeds to completion.

Thereafter, the ammonium salts are converted to ammonia in the presence of strong alkali (eg, sodium hydroxide 35-40%), which is then steam distilled into a flask containing boric acid (4%). Finally, the ammonium borate formed is titrated with a standard solution of hydrochloric acid and the nitrogen content of the original sample can be calculated from the volume of acid required for complete titration.

3.7.2 Materials used:

All chemicals were obtained from BDH Chemicals, Poole (UK) except the catalyst tablets. The following materials were used without further
preparation:

Concentrated sulphuric acid 98% (sp.gr. 1.84) (Analar, BDH : Prod 10276)

Hydrogen peroxide 20 volumes (6% w/v) (Analar, BDH : Prod 10127)

Kjeltabs CQ : catalyst tablets containing 1.5 gm potassium sulphate and 0.15 gm copper sulphate (Thomson and Capper Ltd., Runcorn, Cheshire)

BDH 4.5 Indicator (BDH : Prod 21041) was used for titration.

3.7.2.1 Boric acid solution :-

Boric acid 98% (Analar, BDH : Prod 10058) 400 gm was added to 9 litres of distilled water, the mixture was heated and stirred till dissolved completely. This ~4% solution was used for dissolving the ammonia generated by steam distillation.

3.7.2.2 Sodium hydroxide solution :-

Sodium hydroxide pellets 98% (Analar, BDH : Prod 10252) 2000 gm were dissolved in 5 litres of distilled water to give a solution of 35-40%, which was used during the distillation procedure.

3.7.2.3 Hydrochloric acid solution :-

A vial containing 2 N hydrochloric acid (Convol, BDH : Prod 18037) was diluted volumetrically into 1 litre of distilled water to obtain a 0.1 N solution, 100 ml of this solution was further diluted with 900 ml distilled water to give a 0.01 N solution of hydrochloric acid which was used for titration.

3.7.3 Equipment used :-

For digestion of the urine samples the Digestion System 12 (1009 Digestor) was used and for steam distillation the Kjeltec System (1002 Distilling Unit) was used; both instruments were purchased from Tecator Ltd., Thornbury, Bristol. The test tubes used for digestion were also obtained from the same firm.
3.7.4 PROCEDURE:

The micro-Kjeldahl method for measurement of total nitrogen content of urine samples was carried out in three stages: digestion, distillation and titration. All urine samples were assayed in duplicate, two distilled water blanks were measured before the assay of urine samples and a blank was inserted between each urine sample assayed.

Into the digestion tubes were added, in the following order:

- 200 ul of urine sample
- 1 copper sulphate Kjeltab
- 2 ml of concentrated sulphuric acid
- and 1 ml of hydrogen peroxide.

The digestion block was pre-heated to 420 °C and the tubes were placed in it for a period of 20 minutes; thereafter, the tubes were allowed to cool and the digested samples were then diluted with 20 ml distilled water. The tubes with digested samples were placed in the distilling unit and ~10 ml of 35-40% sodium hydroxide solution was dispensed into them prior to distillation. Distillation was carried out for 5 min and the ammonia formed was dissolved in 4% boric acid solution, to which 1 drop of BDH 4.5 Indicator had been added. This solution was then titrated with 0.01 N hydrochloric acid till the indicator showed a distinctive light grey colour at pH 4.5.

3.7.5 Calculations:

The total nitrogen content of the urine was calculated by the following formula:

\[
\%N = 14.01 \times \frac{(\text{titrant vol. sample} - \text{titrant vol. blank}) \times \text{molarity of HCl}}{\text{sample (ml)}}
\]

3.7.6 Precision and accuracy:
The intra-assay coefficient of variation from 20 measurements of the same urine sample was 2.6% and the recovery of added Kjeldahl standard solutions to urine samples was 96%.
Table 3.6 METHODS: — Precision and accuracy for the measurement of plasma steroid hormone concentrations.

<table>
<thead>
<tr>
<th>STEROID</th>
<th>INTER-ASSAY VARIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASSAYS</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>8</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>8</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>8</td>
</tr>
<tr>
<td>Progesterone</td>
<td>8</td>
</tr>
<tr>
<td>17-Hydroxyprogesterone</td>
<td>8</td>
</tr>
<tr>
<td>11-Deoxycortisol</td>
<td>8</td>
</tr>
<tr>
<td>Cortisol</td>
<td>8</td>
</tr>
<tr>
<td>Cortisone</td>
<td>8</td>
</tr>
</tbody>
</table>

All values were measured in quality control plasma samples with known hormone concentration. (Coeff. = Coefficient of variation).
ACKNOWLEDGEMENT NOTE

1. The analysis of urine samples for measurement of creatinine and 3-methylhistidine concentrations was performed by Margaret Russell. The analysis for creatinine was performed by a Beckman ASTRA Automated Stat-Routine Analyser System (from Beckman-RIIC Ltd., High Wycombe) using the Jaffe rate colorimetry method for measurement of creatinine. The urinary analysis for 3-methylhistidine was carried out by an automated method using a modification of the reaction of fluorescamine with amines as described by Murray AJ, Ballard FJ, Tomas FM: Analytical Biochemistry (1981) 116: 537-544.

2. The measurement of plasma amino acids in a small number of patients (Chapter IV) was performed by Stephen Lloyd using a Chromaspek Ion-Exchange Chromatograph J180 (from Rank Hilger, Kent).
4.1 STUDY PROTOCOL
   4.1.1 Ethical considerations
   4.1.2 Entry of patients
   4.1.3 Blood sampling
   4.1.4 Collection of urine samples
   4.1.5 Preoperative management
   4.1.6 Anaesthetic management
   4.1.7 Postoperative management
   4.1.8 Statistical analysis
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4.1 STUDY PROTOCOL:

4.1.1 Ethical considerations: 
Approval of the Central Oxford Research Ethics Committee was obtained before the commencement of this study. The parents of each patient were given a detailed explanation of the purpose of this project before allowing the inclusion of their newborn infant in this study. Written consent for blood and urine sampling, as well as other observation procedures, was obtained from the parents on consent forms which incorporated a summary of the above verbal explanation.

In all infants studied, not more than 5% of the blood volume was sampled during the entire course of the study, a volume which can be tolerated without the need for replacement by transfusion. Blood samples were only collected at a time when venepuncture was performed for routine clinical investigations, thus avoiding any extra pain or discomfort for the sake of research. Apart from routine drug therapy and routine clinical monitoring, no special procedures were carried out as part of this study.

After detailed consultations, consent was obtained from all Paediatric, Surgical and Anaesthetic Consultants and Senior Registrars who were responsible for the care of surgical neonates. The approval of nursing staff in the Paediatric Wards, Paediatric Intensive Care Unit, Special Care Baby Unit and Operation Theatres was also obtained before commencement of this study.

4.1.2 Entry of patients: 
Newborn infants undergoing elective or emergency surgery within 44 weeks of post-conceptional age were considered eligible for inclusion in this study.
However, the following exclusions were made:

1. Infants born small-for-gestational age (birth weight less than 10th percentile for gestational age).
2. Preterm neonates weighing less than 750 grams at birth.
3. Neonates suspected to have congenital metabolic or hormonal disorders on the basis of clinical findings.
4. Neonates previously exposed to acute stress in the form of severe birth asphyxia, hypothermia, severe infection, trauma or haemorrhage within the 72 hours before surgery.

The gestational and post-natal age, pre-natal problems and record at birth, neonatal problems before operation, feeding history and pre-operative management were recorded on neonatal data sheets. Details of the anaesthetic management of each patient and clinical assessments of the anaesthetist were documented on anaesthesia record sheets whereas the post-operative management and clinical complications were recorded on post-operative data sheets. Examples of all data sheets are included in Appendix II.

4.1.3 Blood sampling:

Data from adults subjects have suggested that the major endocrine and metabolic changes after surgery are seen during the first 24 hours postoperatively (Elliot and Alberti, 1983). It was considered essential therefore, to examine this time period in the neonate, particularly in view of the limited blood volume that could be sampled from sick or preterm neonates during the entire period of observation. Thus, blood samples for the measurement of hormonal and metabolic variables were drawn before the induction of anaesthesia, at the end of surgery and at 6, 12 and 24 hours following surgery.
The association of other stressful stimuli, (eg, intubation, extubation, incidental hypothermia, etc.) occurring between the preoperative and end-operative blood samples were assumed to be part of the 'total stress' experienced by the neonate as a result of the surgical operation, the effects of which were measured. The isolated effects of anaesthetic induction could have been measured by taking another blood sample after induction of anaesthesia and before the start of surgery, but restrictions on the volume of blood sampling precluded such a measurement.

Postoperative hormonal and metabolic changes in adult patients undergoing surgery have been studied usually at time intervals measured from the start of surgery (eg, Kehlet et al, 1979) or from the induction of anaesthesia (eg, Clarke et al, 1974). In this study however, it was decided to obtain postoperative blood samples at time intervals measured from the end of surgery due to the variable duration of neonatal surgical operations. Thus, blood samples taken at 6, 12 and 24 hours postoperatively denote time intervals from the end of surgery, rather than from anaesthetic induction or skin incision; a difference which may be important when comparing the stress response of neonatal and adult patients.

Thus, in the preliminary study blood samples were obtained by peripheral venepuncture or from indwelling venous cannulae just before anaesthetic induction, at the end of surgery, and at 6, 12 and 24 hours after surgery.

4.1.4 Collection of urine samples:
An important effect of a postoperative catabolic state is the associated negative nitrogen balance, since this may have a bearing not only on the postoperative complications and clinical condition of the neonates
undergoing surgery but may also affect subsequent growth and maturation. It was, therefore, considered important to measure the changes in nitrogen excretion and the urinary 3-methylhistidine/creatinine ratio of neonates for up to 3 days postoperatively.

Thus, urine was collected from each infant during the three days following surgery in pooled samples. The method of collection was by the application of neonatal urine bags, since this was considered most convenient by the nursing staff and also because it obviated the need for lengthy extraction procedures which may be necessary when other methods of collection are adopted (Seashore and Seashore, 1976). Total nitrogen excretion was measured in neonates who were not fed during the three days following surgery and from whom there was no loss of urine during collection; urine collected from all other neonates entered in this study was used for the measurement of 3-methylhistidine/creatinine ratios.

Since the operated neonates included for the measurement of total nitrogen excretion did not receive enteral feeds during the 3 days after surgery, it was assumed that nitrogen balance could be estimated from the measurement of nitrogen intake and output, and that caloric intake would not need to be standardised. Thus, the parenteral nitrogen input was recorded on nitrogen balance charts and samples of parenteral nutrition solutions and blood or blood products were obtained for measurement of nitrogen content.

4.1.5 Pre-operative management:

Since the metabolic response of neonates during surgery could be influenced substantially by the degree of substrate supply and mobilisation before surgery, it was considered necessary to standardise the preoperative management of neonates included in this study. It was recommended that the
duration of preoperative starvation be limited to 4 hours before induction of anaesthesia. In all neonates, an intravenous infusion was started approximately 2 hours before the operation and adjusted whenever possible, to deliver dextrose at 4-6 mg/Kg/minute, which is the physiological glucose production rate of the newborn infant (Kalhan et al, 1980).

Excessive handling and stressful procedures were limited as much as possible in the immediate preoperative period. In most cases, transport to the operation theatre was carried out without transferring the neonate to another incubator or overhead heater and appropriate precautions were taken to prevent heat loss during transport. On the basis of an initial assessment of preoperative clinical condition, neonates were classified into 6 general categories:

1= Normal healthy infant undergoing elective surgery.
2= Patient not well, receiving intravenous fluids or symptomatic treatment for the surgical problem.
3= Patient not well, receiving intravenous fluids or antibiotics and/or curative treatment for surgical and associated problems.
4= Sick patient, requiring ventilation or continuous positive airway pressure and/or total parenteral nutrition and/or antibiotics; undergoing emergency surgery.
5= Very sick patient, ventilation and life support necessary, emergency surgery associated with risk of operative mortality.
9= Patient not assessed, not enough evidence documented.

4.1.6 Anaesthetic management:

After several discussions with members of the Anaesthetic Department, it was decided that the anaesthetic management of patients included in the preliminary study would depend on the judgement of the clinical
anaesthetist incharge for each case. It was considered justifiable and prudent to allow anaesthetic management to proceed without any attempt at standardisation since, at the start of this project, there was widespread resistance to the restrictions imposed by any experimental design, and also, since a primary objective of this project was to examine the effects of current anaesthetic practice. For patients included in the subsequent clinical trials it was decided that anaesthetic management would be based on the results obtained in the preliminary study.

Intravenous fluid therapy was continued at 4-6 mg/Kg/minute of dextrose during surgery and strict measures were taken during anaesthetic induction and the surgical procedure to ensure that there was minimal loss of heat.

4.1.7 Post-operative management:
To facilitate the metabolic interpretation of this study it was considered necessary to standardise postoperative clinical management, particularly with respect to intravenous fluid therapy and postoperative analgesia. The administration of intravenous fluids was regulated to maintain the same rate of dextrose infusion.

When this study was planned, it was observed that postoperative analgesic therapy for neonates was provided by widely variable regimens of morphine, diamorphine or papaveretum (Omnopon, Roche). Since narcotic drugs have well-documented effects on the hormonal and metabolic changes that characterise the stress response, it was considered necessary to standardise postoperative analgesia as much as possible within clinical limitations. It was proposed therefore, that only diamorphine would be used for neonates included in this study and that it would be given in a specific dose range (0.1-0.2 mg/Kg) after surgery. The timing of analgesia
was adjusted such that diamorphine was not injected during the two hours preceding a postoperative blood sample. However, it was emphasized that the clinical comfort of neonates would be the primary concern for regulating administration of analgesia and alterations to this regimen were allowed for specific cases.

4.1.8 Statistical analysis:
Non-parametric tests for statistical analysis were used in order to avoid any assumptions of the symmetry or distribution of the population studied. Another consideration in using these tests was to widen the applicability of the findings from this study, even if a small number of cases were studied in particular sub-groups of the patient population.

Wilcoxon's matched-pairs signed ranks test was used to analyse the changes in the measured variables from the preoperative levels. This test was considered appropriate since it was applied to related measurements in the same patient where each patient was used as his own control. Although wasteful of information obtained in the ratio scale of measurement, this test has a power-efficiency of 95.5% compared with the (parametric) paired T test (Siegel, 1956).

The Mann-Whitney U test was used to test for the significance of differences between two independent groups drawn from the same patient population. The advantages of using this test are that it can be applied to small numbers of patients and there is very little wastage of information, which makes it one of the most powerful alternatives to the unpaired T test (Siegel, 1956).

The Spearman rank correlation coefficient was used as a measure of association
between the measured variables. This coefficient has a power-efficiency of 91% when compared to the most powerful parametric measure of correlation, the Pearson r (Siegel, 1956), and avoids the assumptions of linearity or continuity of the correlations being examined. Spearman rank correlation coefficients were calculated only between values which had been obtained from the same blood or urine sample, and only between those variables where a physiological relationship was expected or known to occur.

4.2 SCORING METHOD FOR THE ASSESSMENT OF SURGICAL STRESS:

4.2.1 Background:
In adult patients undergoing surgery, studies of the stress response have been performed generally on patients subjected to a specific surgical procedure, so as to standardise the amount of surgical stress experienced by each patient. Thus, the hormonal and metabolic phenomena documented by these studies are related primarily to the circumstances of the surgical procedure and there exists very little basis on which the responses of patients undergoing different surgical procedures have been compared. A few studies, however, have attempted comparisons between patients undergoing different degrees of surgical stress based on the site of surgery, or other empirical grounds.

Thus, in 1934 Weddell and Gale observed that hyperglycaemia precipitated by intraperitoneal operations was greater than that after extraperitoneal operations. Green et al (1949) found that the degree of hyperglycaemia and nitrogen loss following battle injuries was proportional to the severity of the trauma. Clarke, Johnston and Sheridan (1970) demonstrated that increases in the level of cortisol, blood sugar and free fatty acids were markedly greater in patients undergoing intra-abdominal surgery than in
patients undergoing body surface surgery. Clarke (1970) also found that the stress of intra-abdominal operations was greater than that of thoracic or body surface surgery on the basis of surgical hyperglycaemia. Nikki et al (1972) measured plasma catecholamines in patients undergoing minor (ophthalmic) and major (abdominal) surgery and, probably due to the low sensitivity of their fluorometric assay, found no differences between the two groups during the operation, although patients subjected to abdominal surgery had higher catecholamine concentrations postoperatively.

Wright and Johnston (1975) divided their patients into groups undergoing minor (inguinal herniorrhaphy), moderate (vagotomy and pyloroplasty) and major (aortofemoral bypass graft) degrees of surgical stress and found that the duration and severity of glucose intolerance and the increase in growth hormone was much greater in the major surgical group as compared to the moderate surgical group, which, in turn, showed greater changes than the minor surgical group. With the measurement of plasma catecholamines, Butler et al (1977) were able to show that the stress of cardiac surgery is significantly greater than that of intra-abdominal surgery.

Aarimaa et al (1978) defined oesophageal resection as major surgical stress and exploratory laparotomy as moderate surgical stress and studied the changes in plasma insulin, growth hormone, non-esterified fatty acids, blood glucose and the urinary excretion of catecholamines in patients undergoing these surgical procedures. Patients subjected to major surgical stress had greater increases in blood glucose and growth hormone during surgery. Postoperatively, these patients had higher concentrations of glucose, growth hormone and insulin as compared to the moderate surgery group. The concentrations of free fatty acids and the urinary excretion of catecholamines were similar in both groups during and after surgery.
Bormann et al (1983) compared plasma vasopressin concentrations of patients undergoing localised abdominal surgery, extensive abdominal surgery and intra-thoracic surgery and found that the stress-induced elevation of plasma vasopressin was greatest after thoracic surgery, whereas extensive or localised abdominal surgery caused successively smaller increases in plasma vasopressin.

Thus, although an objective method for the assessment of different grades of surgical stress does not exist, differing endocrine and metabolic responses have been observed in adult patients undergoing different surgical procedures.

On the other hand, a number of scoring methods and predictive indices have been proposed for grading the severity of accidental trauma (Baker et al, 1974; Cowley et al, 1974), burn injury (Moores et al, 1975) as well as sepsis in the traumatised patient (Elebute and Stoner, 1983). As in the case of accidental trauma, these scoring methods have been related not only to the morbidity and mortality following multiple trauma (Baker et al, 1974) but also to hormonal and metabolic parameters of the stress response (Stoner et al, 1979; Oppenheim et al, 1980) (see Chapter IX).

4.2.2 Development of the 'Surgical Stress Score':

For the purposes of this study it was considered necessary to develop an objective method for the assessment of neonates undergoing different surgical procedures. This was required not only because of the lack of sufficient numbers of neonates undergoing a single surgical procedure; but also due to the presence of several non-surgical stress factors, such as prematurity, hypothermia or infection, which could be associated with an
operative procedure in neonates and could possibly influence the response to surgical trauma.

The proposed scoring method was based on 5 factors which contribute to the stress of surgical trauma, ie, the amount of blood loss, the site of surgery, the degree of superficial trauma, the extent of visceral trauma and the duration of the surgical procedure. Added to this basic framework were additional stress factors associated with the neonatal age group, such as hypothermia during surgery, localised or generalised infection and prematurity. To enable the applicability of this scoring method to neonates undergoing cardiac surgery, additional factors such as cardiopulmonary bypass, deep hypothermia and circulatory arrest were also included. The relative values of the various factors were decided on the basis of evidence in the adult literature or from specific studies of a particular factor (eg, Davenport et al, 1966). The scoring method as well as the considerations used for assigning values to the various parameters of surgical stress are presented in Chapter IX.
4.3 PRELIMINARY STUDY:

4.3.1 Description of patients and preoperative clinical management:
After informed discussion and written parental consent, 29 neonates (21
term and 8 preterm) undergoing surgery at a mean post-natal age of 19±3
days (mean ±SEM) were included in this study. The mean gestational age of
the patients was 36.2 ±0.9 weeks and birth weight was 2.5 ±0.2 Kg.

According to the assessment of preoperative condition, there were 6
patients in category 1, 7 patients in category 2, 8 patients in category 3
and 8 patients in category 4, no patients were critically ill (category 5)
at the time of surgery. 24 patients received intravenous dextrose
preoperatively at a mean rate of 4.6 ±0.4 mg/Kg/min and the mean duration
of preoperative starvation was 5.5 ±0.2 hours. 4 patients received TPN
preoperatively which was given for a mean duration of 6 ±3 days and was
stopped at 4 hours before surgery.

4.3.2 Anaesthetic and clinical management during surgery:
The rate of intravenous dextrose infusion during surgery was 5.3 ±0.4
mg/Kg/min (mean ±SEM, range 1.8-9.8); 5 patients received a blood
transfusion and the mean volume of blood transfused was 27 ±8 ml.

Several anaesthetic agents and muscle relaxants were given to neonates
during the surgical procedures. Nitrous oxide was given to 27 patients in a
concentration of 20%-70%; in addition, 11 patients received halothane
(0.5-2.0%), 8 patients received thiopentone sodium (0.5-6.3 mg/Kg) and 3
patients received fentanyl (2-4 µg/Kg) for anaesthesia. The muscle
relaxants used were suxamethonium (0.5-3.4 mg/Kg) for tracheal intubation
in 6 patients; during surgery, 19 patients received d-tubocurarine (0.3-1.3 mg/Kg), 3 patients received pancuronium (0.1 mg/Kg) and 1 patient received atrocurium (0.3 mg/Kg).

During anaesthesia, spontaneous respiration was allowed in 4 neonates whereas all other neonates were hand-ventilated at a rate of 60-140 breaths/min with an oxygen concentration ranging from 30-80%. At the end of surgery, reversal of relaxation was obtained with neostigmine (0.15 mg/Kg) and atropine (0.06 mg/Kg) in 16 of the 25 neonates receiving muscle relaxants, in the 9 other neonates ventilation was continued into the post-operative period.

4.3.3 Surgical procedures

According to the 'surgical stress score', 9 neonates were subjected to Grade I surgical stress (score 0-5), 18 patients to Grade II stress (score 6-10) and 2 patients to Grade III stress; no patients were exposed to Grade IV stress and the mean score obtained by all patients was 7.3± 0.5 (range 3-13). The mean duration of surgery was 48±5 min and the mean temperature loss during surgery was 0.9 ± 0.1 °C (range 0-3 °C).

4.3.4 Postoperative clinical management

The mean rate of dextrose infusion during the 3 days following surgery was 4.1 ± 0.4 mg/Kg/min. On the first postoperative day, 8 patients received analgesia with diamorphine (dose 0.13 ± 0.04 mg/Kg/day, mean ± SEM) and two patients received morphine (dose 0.1 mg/Kg/day); the first analgesic dose was given 5± 1 hours after surgery (range 1-10 hours).

4.3.5 Urinary collection

Pooled urine samples for measurement of the 3-methylhistidine/creatinine
ratio were obtained from 20 neonates in this study. Of these, 13 neonates were not fed during the 3 days following surgery and urine was collected in 24-hour pooled samples for measurement of total urinary nitrogen. In 5 neonates the urine collection was considered to be inaccurate due to excessive losses and these were discarded.

4.4 RESULTS OF THE PRELIMINARY STUDY:

4.4.1 Hormonal changes:

The overall results from the measurement of plasma insulin, adrenaline, noradrenaline and glucagon are listed in Table 4.1. Plasma concentrations of adrenaline, noradrenaline and glucagon were measured in the blood samples of only a limited number of patients in this study since laboratory techniques for the measurement of these variables were not available during the initial part of the study. No form of selection was exercised for the measurement of these variables in any specific group of patients.

The concentrations of plasma adrenaline (p<0.025) and plasma noradrenaline (p<0.05) increased significantly during surgery, but by 6 hours after surgery the concentrations of both hormones were not significantly different from their preoperative values. Plasma insulin concentrations did not change during surgery, but were found to be significantly raised at 6 hours (p<0.05) and 24 hours (p<0.05) postoperatively. Plasma glucagon concentrations were not altered during or after surgery, but by 24 hours postoperatively, plasma glucagon was significantly below the preoperative concentration (p<0.05).

Values for the insulin/glucagon ratio (Table 4.3), calculated from
measurements made on the same blood sample, did not change significantly
during surgery or in the postoperative period.

4.4.2 Metabolic changes :-
The overall results of metabolite analysis are listed in Tables 4.2 and 4.3.
Blood glucose concentrations were found to be increased significantly
(p<0.0001) at the end of surgery and at 12 hours (p<0.005) after surgery.
There was a significant increase in the concentrations of blood lactate
(p<0.0001), blood pyruvate (p<0.0001) and blood glycerol (p<0.001) at the
end of surgery; blood lactate concentrations were significantly elevated at
12 hours (p<0.025) after surgery, whereas blood pyruvate and glycerol had
reverted to their respective preoperative values by 6 hours after surgery.

The blood concentrations of acetoacetate did not change significantly
during or after surgery whereas blood 3-hydroxybutyrate was significantly
increased at the end of surgery (p<0.005) and at 12 hours postoperatively
(p<0.05). The increase in total ketone bodies during surgery (Table 4.3)
was highly significant (p<0.005), but at 6 hours postoperatively total
ketone bodies had returned to their preoperative levels. Non-esterified
fatty acids were measured in a small number of neonates (N=7) and were
found to be increased significantly at the end of surgery (p<0.025), but
had reverted to preoperative concentrations at 6, 12 and 24 hours after
surgery. Blood alanine concentrations increased significantly during
surgery (p<0.025) but had reverted to the preoperative values at 6, 12 and
24 hours after surgery.

Values for the molar lactate/pyruvate ratio, alanine/pyruvate ratio,
hydroxybutyrate/acetoacetate ratio and total gluconeogenic substrates (sum
of blood lactate, pyruvate, alanine and glycerol values) were calculated
from the above measured values in each blood sample. A significant decrease was found in the alanine/pyruvate ratio (p<0.005) at the end of surgery and at 6 hours postoperatively (p<0.05). The total gluconeogenic substrates in blood were increased markedly at the end of surgery (p<0.0001) and at 12 hours after surgery (p<0.025). The hydroxybutyrate/acetoacetate ratio was increased significantly at the end of surgery (p<0.05) and at 12 hours after surgery (p<0.05). There were no significant changes in the lactate/pyruvate ratio during or after surgery.

The insulin/glucose ratio was also calculated from each blood sample; it was found to be decreased significantly at the end of surgery (p=0.005) and significantly elevated at 24 hours postoperatively (p<0.05).

4.4.3 Hormonal-metabolic correlations :-
In several previous studies, the correlation between hormonal and metabolic variables at different sampling points has been assumed to imply a causal relationship, although in the absence of metabolite turnover studies and studies of end-organ responsiveness to changes in hormonal concentration; this assumption is not entirely valid. In view of the extremely difficult application of these techniques to the clinical situation of neonates undergoing surgery, it was considered reasonable to make these assumptions in the present study in order to consider the mechanisms for the metabolic changes documented.

The matrix of correlation coefficients for blood glucose values is presented in Table 4.4. At the end of surgery, concentrations of blood glucose were correlated strongly with plasma adrenaline (p<0.01), plasma glucagon (p<0.05) and the insulin/glucagon ratio (p<0.01), and had weaker associations with plasma insulin (p<0.01) and total gluconeogenic
substrates (p<0.01). At 6 hours postoperatively, significant correlations were found with plasma glucagon (p<0.05), plasma insulin (p<0.01), and the insulin/glucagon ratio (p<0.025).

The matrix of correlation coefficients for blood lactate values is presented in Table 4.5 and for blood pyruvate values in Table 4.6. Blood lactate concentrations at the end of surgery were correlated strongly with plasma adrenaline values (p=0.001) and were correlated weakly with plasma insulin values (p<0.05). At 6 hours after surgery, blood lactate values were correlated significantly with plasma adrenaline (p=0.05), plasma insulin (p=0.001) and the insulin/glucagon ratio (p<0.005). Similar correlations were also found for blood pyruvate values at the end of surgery and in the postoperative period. Thus, blood pyruvate concentrations were found to be correlated with plasma adrenaline at the end of surgery (p<0.05) and at 6 hours after surgery (p=0.05). Blood pyruvate was correlated with plasma glucagon levels at the end of surgery (p<0.05); with plasma insulin at the end of surgery (p<0.025), 6 hours (p=0.000), 12 hours (p<0.01) and 24 hours postoperatively (p<0.01). In addition, a significant correlation was found with the insulin/glucagon ratio at 6 hours after surgery (p<0.01).

Significant correlations were found between the lactate/pyruvate ratio and plasma adrenaline at the end of the operation ($r_s=0.78$, $N=9$, $p<0.01$) and at 6 hours postoperatively ($r_s=0.66$, $N=8$, $p<0.05$).

The blood concentrations of total ketone bodies were found to be strongly correlated with plasma glucagon concentrations preoperatively ($r_s=0.88$, $N=6$, $p<0.01$), but no significant correlations were found with the
catecholamines or glucagon values at the end of surgery or in the postoperative period.

Blood glycerol was found to be correlated strongly with plasma adrenaline ($r_s=0.66$, $N=9$, $p=0.025$) and plasma glucagon ($r_s=0.77$, $N=7$, $p=0.02$) at the end of surgery, and weakly to plasma insulin concentrations preoperatively ($r_s=0.32$, $N=29$, $p<0.05$) and at the end of surgery ($r_s=0.35$, $N=28$, $p<0.05$).

The matrix of correlation coefficients for blood alanine and total gluconeogenic substrates is presented in Table 4.7. Similar to the associations of blood pyruvate, blood alanine concentration also correlated significantly with plasma glucagon at the end of surgery ($p=0.02$) and with plasma insulin at 6 hours ($p=0.001$) and 12 hours postoperatively ($p=0.001$). In addition, a significant negative correlation was present between the alanine/pyruvate ratio and plasma adrenaline values at the end of surgery ($p<0.05$). Total gluconeogenic substrates in blood were found to be correlated strongly with plasma adrenaline values at the end of surgery ($p=0.005$), and with plasma insulin ($p=0.001$) and plasma glucagon ($p<0.005$) at 6 hours after surgery.

Since the levels of plasma catecholamines and plasma glucagon were measured in only a limited number of patients, this raises the question whether the hormonal-metabolic correlations derived from a small number of observations can be applied to the entire group of patients under investigation. It was considered justifiable to accept these correlations since there had been no selective bias for the measurement of these variables in any particular group of patients. Second, patients in whom the catecholamine and glucagon levels were measured were considered to be a representative sample of the
entire patient population since the mean and standard deviation of the metabolic data derived from these patients were almost identical to the corresponding values from the entire population. Furthermore, it has been proposed that the validity of any data correlations depends not only on the degree of correlation (i.e., the value of $r_s$) and its statistical significance, but also on the validity of the physiological relationship they propose (Gore, 1981). It was considered justified therefore, to accept those hormonal-metabolic relationships which showed a high degree of correlation ($r_s > 0.5$) and were representative of expected physiological relationships, even if they had been obtained from only a small number of patients. In addition, non-parametric correlation coefficients were used because they do not require the assumptions of a normal distribution of each variable and linear association between variables that are implied by parametric correlation (Altman, 1980) and thus provide a greater safeguard against the identification of spurious relationships (Seigel, 1956). It is, proposed however, that these relationships cannot be extrapolated beyond this particular data set and that they would require further verification in subsequent studies.

4.4.4 Urinary nitrogenous constituents :-

The results of urinary analysis are listed in Table 4.8. The urinary 3-methylhistidine/creatinine ratio was raised significantly on the second ($p<0.05$) and third ($p<0.05$) postoperative days. Total nitrogen excretion, measured on the urine samples from 8 patients was increased significantly on the second day after surgery ($p<0.025$) but values on the third postoperative day were not significantly different from values measured on the first day after surgery.

4.4.5 Clinical observations :-
In all neonates undergoing surgery, routine clinical monitoring of the heart rate, ECG and rectal temperature was carried out and recorded. The base-line heart rate, obtained before any other procedures were performed, was $139 \pm 5$ beats/min (mean ± SEM) and increased to a maximum of $182 \pm 22$ beats/min during the operation. The mean temperature loss during surgery was $0.9 \pm 0.1$ °C. The mean weight of patients decreased from a preoperative value of $2.6 \pm 0.2$ Kg to $2.5 \pm 0.2$ Kg after the first postoperative day and to $2.4 \pm 0.2$ Kg after the third postoperative day.

The neonates were evaluated regularly in the early postoperative period (0-24 hours) and upto the time of discharge from hospital. Several postoperative complications were observed particularly in preterm neonates and in the group of neonates undergoing moderate or severe surgical stress. The common postoperative complications are listed below.

1. Excessive blood loss (low packed cell volume postoperatively).
2. Extrasystoles and persistent tachycardia.
3. Repeated episodes of apnoea and bradycardia.
4. Gastric bleeding.
5. Postoperative oliguria (urine output <1ml/hr for >6 hours).
6. Excessive irritability.
7. Excessive weight loss (>10% body weight during 3 postoperative days).
8. Temperature variability.
9. Respiratory instability requiring increased oxygen or postoperative ventilation.

4.5 DISCUSSION:

The hormonal regulation of intermediary metabolism in newborn infants
undergoing anaesthesia and surgery has not been studied previously. In view of its preliminary nature, the present study was designed in order to obtain as much information as possible about the general features of the neonatal stress response while maintaining a simple format.

The preliminary analysis was performed using the data collected from all neonates included in this study although differences in the gestation, post-natal age, type of anaesthetic management, degree of surgical stress and other characteristics made this a heterogenous study population. It is proposed, however, that this set of patients is representative of the usual neonatal surgical population of any paediatric surgical unit.

The preoperative condition of the patients varied from the healthy term neonate admitted for elective surgery, to the sick preterm neonate requiring respiratory, cardiovascular and nutritional support in the period before surgery. Despite these differences, it is argued that the hormonal and metabolic variables measured in the blood sample taken just before induction of anaesthesia would be representative of the patient’s condition at that time and the changes documented at the end of surgery and thereafter would reflect the response to surgical stress. Moreover, the selection criteria eliminated those patients who had been exposed to acute stress in the 72 hours preceding surgery.

The patients were classified into 4 categories depending on their clinical condition before surgery and analysis of hormonal and metabolic data of the 6, 7, 8 and 8 patients in preoperative categories 1 to 4 respectively was carried out by the Kruskal-Wallis analysis of variance. There was no significant difference in the gestation, birth weight, peri-operative management; degree of surgical stress or anaesthetic management of the
neonates in these groups. There was no significant difference in any of the hormonal or metabolic variables measured at the end of surgery or in the postoperative period. Thus, the contention that hormonal or metabolic changes measured immediately after a surgical operation are probably not affected by the preoperative condition of patients may hold true, provided the subjects have not been exposed to acute stress.

Although the recommendations for standardisation of preoperative management were followed in general, a wide variation in clinical practice was obtained and the ranges for dextrose infusion rate and preoperative starvation were wider than expected (3-9 mg/Kg/min and 4-7 hours respectively). This may help to explain some of the variation in the hormonal and metabolic parameters measured before and after surgery. In the studies reported by Pinter (1973a) and Elphick and Wilkinson (1981), the duration of preoperative starvation was variable and neonates were not infused any glucose containing solutions before or during surgery. This policy, however, was not considered acceptable for the present study.

4.5.1 Hormonal changes :-

CATECHOLAMINES

In all neonates, anaesthesia and surgery caused a significant increase in the plasma concentrations of adrenaline and noradrenaline. The pattern of change in plasma noradrenaline was similar to that of adult patients (Halter et al, 1977; Nistrup Madsen et al, 1978; Hamberger and Jarnberg, 1983) but the increase in plasma adrenaline during surgery contrasts with some data available from adult subjects which have shown that adrenaline concentrations may fall (Hamberger and Jarnberg, 1983) or remain unchanged (Kono et al, 1981; Elliot and Alberti, 1983) during surgery and rise only during the postoperative period. However, earlier studies in adult patients
have documented a small rise in plasma adrenaline concentrations at the end of surgery (Halter et al, 1977; Nistrup Madsen et al, 1978) and the neonatal response was similar to these data. Furthermore, it is evident that the changes in plasma noradrenaline and adrenaline found in this study are greater in magnitude but much shorter in duration in comparison to the adult catecholamine response.

There are no published data on the catecholamine responses of newborn infants undergoing surgery. However, studies in term neonates have documented a marked release of catecholamines at the time of birth (Lagercrantz and Bisoletti, 1977; Nakai and Yamada, 1978; Eliot et al, 1980) which may be augmented in infants undergoing fetal distress (Nakai and Yamada, 1978), birth asphyxia (Lagercrantz and Bisoletti, 1977) or breech deliveries, and in infants of diabetic mothers (Artal et al, 1982). Cheek et al (1963), using a fluorometric technique, measured adrenaline and noradrenaline in normal term and preterm neonates soon after birth and found that preterm neonates had higher adrenaline values than the term neonates. Neonates with postmaturity and placental insufficiency had 8 times the adrenaline levels of normal term neonates, and preterm neonates with respiratory distress had a four-fold increase in adrenaline levels compared to normal preterm neonates (Cheek et al, 1963). On the other hand, Lagercrantz and Bisoletti (1977) have shown that preterm neonates have a smaller catecholamine response than term neonates to the process of birth as well as to birth asphyxia. These discrepancies may be related to the less accurate fluorimetric techniques used for measurement of adrenaline and noradrenaline in these studies. Thus, in summary, it has been documented previously that the adrenal medulla of preterm and term neonates was capable of responding to "stressful" stimuli present at birth.
In older infants (mean age 7 months) undergoing surgery for repair of bilateral inguinal hernia, Talbert et al (1967) found no significant changes in adrenaline or noradrenaline concentrations measured by a fluorometric technique before and after surgery, although plasma non-esterified fatty acids were increased significantly. They concluded that the stress of hernia repair was not sufficient to stimulate catecholamine release in these infants.

From the present study it may be concluded that the newborn infant is capable of mounting a catecholamine response to surgery, the characteristic features of which, in contrast to the adult response, are its short duration and an increase in plasma adrenaline concentration during surgery.

INSULIN

Plasma insulin concentrations did not change significantly by the end of surgery, but were raised significantly at 6 and 24 hours after surgery, a pattern of change somewhat similar to that of adult patients (Allison et al, 1968 and 1969; Wright et al, 1974; Walsh et al, 1981). The mechanism and significance of these changes will be discussed in relation to the concomitant metabolic alterations.

Plasma insulin concentrations have not been measured in newborn infants undergoing surgery except for a single neonate studied by Baum et al (1968). In older infants undergoing cardiac surgery, Bevan and Rosales (1979) (mean age of patients: 28 months) have reported no change during surgery and a postoperative increase in plasma insulin; whereas Baum et al (1968) (mean age of patients 6 months) have found a decrease in plasma insulin during deep hypothermia and a rise to values higher than preoperative concentrations during rewarming. However, the findings of the latter study
may not be reliable since the composition of cardiopulmonary bypass prime and intravenous fluid therapy were widely different in dextrose content. Moreover, the endocrine and metabolic changes were not subjected to statistical analysis.

GLUCAGON

Plasma glucagon concentrations, which were measured only in term neonates, had decreased significantly from preoperative values by 24 hours after surgery. This is in striking contrast to the adult stress response, where a marked increase in plasma glucagon has been documented between 12 and 24 hours postoperatively (Russell, Walker and Bloom, 1975). However, since plasma glucagon was measured in only a small number of patients (N=7), it was felt that this finding would need to be confirmed in subsequent clinical trials (Chapters 6, 7 and 8).

There are no published data on plasma glucagon changes in neonates or older infants undergoing surgery. In neonates exposed to fetal distress, it has been documented that plasma glucagon may be markedly raised after birth (Johnston and Bloom, 1973; Lucas et al, 1979). Fekete et al (1972) found that plasma glucagon concentrations were not altered in preterm and term neonates exposed to cold stress. Milner et al (1972a) found raised plasma glucagon concentrations in preterm and term neonates undergoing exchange transfusion for hyperbilirubinaemia. In a subsequent study, these levels were not decreased in response to dextrose infusion (Milner et al, 1972b). However, the latter 3 studies can be criticized on the grounds that a proteolytic enzyme inhibitor (eg., aprotinin) was not added to the blood samples. Moreover, Fekete et al (1972) studied infants 4-6 hours after a meal when plasma enteroglucagon may have been raised, whereas Milner et al (1972a and 1972b) gave no information about the effects of raised bilirubin...
levels on their radioimmunoassay. Thus, apart from the limited data obtained in this study and the observation of raised cord-plasma glucagon concentrations in babies exposed to fetal distress, there is no information on the effects of stressful stimuli on plasma glucagon secretion in newborn infants.

4.5.2 Metabolic changes :-

GLUCOSE

The most prominent metabolic effect of surgical stress in neonates was the highly significant hyperglycaemia which developed by the end of surgery in all neonates studied. During the postoperative period, blood glucose concentrations were significantly elevated at 12 hours following surgery. The magnitude of increase in blood glucose concentrations was much greater than the response of adult patients undergoing non-cardiac surgery of comparable severity (Clarke, 1968; Clarke, 1970; Walsh et al, 1983).

Pinter (1973a) found that blood glucose concentrations in neonates subjected to surgery were raised to a peak level of 6.8 mmol/l at the end of surgery, but were not increased significantly at 6, 12 or 24 hours after surgery. Elphick and Wilkinson (1981) reported that blood glucose values were raised in capillary blood samples obtained between 0-4 hours after surgery (mean values not given, approximately 7.4 mmol/l from graph) and had returned to preoperative values in blood samples obtained between 4-8 hours postoperatively. These results were not subjected to statistical analysis and it is apparent that blood glucose was elevated in only some of the neonates investigated (Elphick and Wilkinson, 1981). In addition, Elphick and Wilkinson (1968) performed intravenous glucose tolerance tests on 4 neonates before and after surgery and found that the glucose clearance rate was decreased after surgery in 3 neonates and increased in one neonate, and
that the rate of fall of glucose was independent of the absolute glucose concentrations in blood (Elphick and Wilkinson, 1968).

The mean end-operative blood glucose concentrations in both these studies were distinctly lower than the values documented in the present study and the hyperglycaemic responses were maintained for a much shorter duration postoperatively. These differences could be due to a greater degree of surgical stress experienced by the neonates in this study, or may be related to the fact that neonates in both studies underwent a much longer period of preoperative starvation without dextrose replacement and were not given routine dextrose infusions during or after surgery (Pinter 1973a; Elphick and Wilkinson, 1981), except for some neonates in the former study (Pinter, 1973a) who were given 10% dextrose for hypoglycaemia in the postoperative period. It was concluded that surgery had caused a temporary disturbance of glucose homeostasis, the cause or consequence of which was not clear (Pinter, 1973a; Elphick and Wilkinson, 1981).

The mechanism of perioperative surgical hyperglycemia can be considered in relation to the concurrent changes in insulin and the counter-regulatory hormones measured in this study (Shamoon et al, 1981; DeFronzo et al, 1980). At the end of surgery, the strong correlation of blood glucose values with plasma adrenaline values implies that the hyperglycemic response may have been precipitated by adrenaline release during surgery. Experimental work has shown that adrenaline not only stimulates hepatic glucose production (Exton and Park, 1966) and causes a sustained decrease in glucose utilisation (Deibert and DeFronzo, 1980; Kerr et al, 1981), but also stimulates glucagon secretion and suppresses the release of insulin (Bagdade et al, 1967; Unger and Orci, 1981). The end-operative correlation of blood glucose with plasma glucagon implies that patients who had relatively
higher circulating glucagon concentrations were able to mount a greater hyperglycaemic response, although a causal relationship may be suspected in patients who had distinct increases in glucagon concentration (DeFronzo et al., 1980). In this context, it is interesting to note that total gluconeogenic substrates were also increased significantly at the end of surgery and that the magnitude of increase was correlated with the degree of hyperglycaemia. The role of adrenaline in stimulation of the Cori cycle (Kusaka and Ui, 1977) and that of glucagon in utilisation of gluconeogenic substrates is well-known (Kraus-Friedman, 1984).

The relationship of blood glucose with plasma insulin and with the insulin/glucagon ratio at the end of surgery is not unexpected since it may represent a reflex secretion of insulin in response to the surgical hyperglycaemia. It is proposed however, that insulin secretion in response to the hyperglycaemia during surgery was not appropriate. This is evident from a significant decrease in the insulin/glucose molar ratio at the end of surgery, thereby implying inappropriately low insulin levels for the circulating glucose concentrations (Soltesz and Aynsley-Green, 1984), an effect which may have been caused by the adrenaline release during surgery (Sperling et al., 1984).

Six hours post-operatively, blood glucose concentrations were not significantly raised, possibly due to the marked increase in plasma insulin concentrations immediately following surgery. Further evidence for this is obtained from the strong correlations of blood glucose values with plasma insulin concentrations and the insulin/glucagon ratio at that time. In addition, at 6 hours postoperatively the insulin/glucose ratio had reverted to values which were not significantly different from the preoperative value. At this time plasma adrenaline concentrations had also returned to
their preoperative value and plasma insulin was found to be significantly increased, thereby implying the removal of inhibition of islet B-cells and superimposition of the hyperglycaemic stimulus to insulin secretion (Sperling, 1984). Thus, blood glucose values were only poorly related to plasma adrenaline, but were closely related to plasma glucagon, a correlation which achieved statistical significance even though glucagon was measured in only a few patients. According to these data therefore, it may be proposed that post-surgical hyperglycaemia in neonates is initiated by adrenaline release and is maintained into the post-operative period as a result of glucagon secretion.

The rapid return of the insulin/glucose ratio to preoperative values by 6 hours after surgery and its significant increase at 24 hours after surgery may indicate that the catabolic stimulus of surgical stress in newborn infants is short-lived in comparison with adult patients. It is tempting to speculate that this characteristic may explain the rapid post-surgical recovery of neonates even after moderate or major trauma.

At 12 hours postoperatively, plasma insulin concentrations were not significantly different from the preoperative values and blood glucose was significantly raised; but by 24 hours following surgery plasma insulin was again significantly raised and blood glucose concentrations were returning to the preoperative base-line, thereby causing a significant increase in the insulin/glucose ratio. Thus, it is evident that circulating blood glucose may be controlled by changes in insulin secretion, except at the end of surgery, when this control is lost probably due to sympathoadrenal activation (Halter et al, 1984). Turnover studies using stable isotopes (Kalhan et al, 1980) would be required to clarify the mechanism of these changes.
GLUCONEOGENIC SUBSTRATES

Concurrent with post-operative hyperglycemia, significant increases in blood lactate, pyruvate and alanine concentrations were observed at the end of surgery; these metabolites had reverted to preoperative values by 6 hours after surgery, although blood lactate concentrations were again elevated at 12 hours postoperatively. The magnitude of rise in blood lactate and pyruvate at the end of surgery was much greater than the increase documented in adult patients undergoing similar types of surgery (Horrelt et al, 1969; Kehlet et al, 1979; Walsh et al, 1983).

There are published data on changes in blood lactate but no information relating to blood pyruvate and alanine concentrations in newborn infants subjected to surgery. In the study of surgical neonates by Pinter (1973), blood lactate concentration increased from a mean value of 2.6 mmol/l to 3.6 mmol/l at the end of surgery, which is comparable to the increase observed in this study (from 1.6 to 2.7 mmol/l), although both preoperative and end-operative concentrations were higher in the previous study. No consistent change in blood lactate was documented in the neonates studied by Elphick (Elphick, 1972).

Pinter found that changes in blood lactate were strongly correlated with a metabolic acidosis during and after surgery (Pinter, 1972, 1973 and 1974). An earlier study (Borresen and Knutrud, 1967), had shown that there was no significant change in acid-base parameters before and after surgery in newborn infants. Acid-base changes in neonatal surgical patients were also studied by Scott and Inkster (1973), who found that hyperventilation during anaesthesia caused a respiratory alkalosis which usually masked an underlying metabolic acidosis in these patients at the end of surgery.
Borresen and Knutrud (1967) proposed that the acid-base changes during surgery were a part of normal variation in newborn infants, whereas Pinter (1973) proposed that the rise in blood lactate was probably due to tissue hypoxia during surgery; Scott and Inkster (1973) related metabolic acidosis to the changes in tissue-perfusion caused by hyperventilation and the stress of surgery.

Bunker et al (1952) studied the effects of diethyl ether anaesthesia in older infants (mean age 6 months) undergoing non-cardiac surgery and documented that all infants developed a severe metabolic acidosis during the operation which was regularly accompanied by a rise in serum lactate. On the basis of experimental work in dogs (Brewster et al, 1953), they proposed that the release of adrenaline caused by diethyl ether was responsible for the mobilisation of muscle glycogen as lactic acid in the blood. However, since there was no correlation between the duration of anaesthesia and production of an acidosis, they dismissed these metabolic changes as harmless and of no practical importance (Bunker et al, 1952). In infants (mean age 7 months) undergoing cardiac surgery, Baum et al (1968) found that blood lactate increased slowly during deep hypothermia and markedly during rewarming after circulatory arrest. They concluded that lactic acidosis in these patients may be related to hepatic dysfunction during hypothermia (Baum et al, 1968).

In this study, blood lactate and pyruvate concentrations were significantly correlated with plasma adrenaline values at the end of surgery and 6 hours after surgery. Arteriovenous catheterisation studies in adult patients undergoing abdominal surgery have shown that there is a markedly increased production of lactate and pyruvate in skeletal muscles during and after surgery (Stjernstrom et al, 1981). This is probably due to the mobilisation
of muscle glycogen stores caused by the adrenaline release during surgery (Stjernstrom et al, 1981; Elliot and Alberti, 1983). In addition, it has been demonstrated that injured tissues around the surgical wound derive their energy mainly from glycolysis (Im and Hoores, 1979; Wilmore, 1981) and thus may contribute towards increased lactate production after surgery.

A possible mechanism for the increase in blood alanine during surgery may be through the effects of cortisol and adrenaline on amino acid metabolism in skeletal muscle which are known to stimulate proteolysis and cause a redirection of carbon flow from glutamine toward alanine formation (Karl et al, 1976). Since blood alanine concentration is a poor indicator of alanine flux in newborn infants and since the gluconeogenic pathway is known to be completely functional within a few hours after birth (Frazer et al, 1981; Delamater et al, 1974), the small increase in blood alanine during surgery does not rule out an increased alanine turnover during and after surgery. In this context, the strong correlation of blood alanine with plasma glucagon values at the end of surgery and with plasma insulin concentrations at 6 and 12 hours after surgery is not unexpected, since the secretion of both hormones may be influenced by an increase in alanine concentrations (Sperling, 1982).

In preterm neonates exposed to birth asphyxia, Schultz et al (1977) have found that alanine concentrations were markedly raised in blood samples obtained between 1-12 hours after birth. In addition, Schultz et al (1980) have shown that alanine concentrations in term and preterm neonates may be decreased with an early onset of septicaemia and unchanged in neonates presenting with septicaemia after 1 week of age. It is proposed that the stress of asphyxia or septicaemia in newborn infants may have different effects on amino acid metabolism compared with those of surgical stress.
The circulating concentrations of total gluconeogenic substrates were increased markedly at the end of surgery and had reverted to preoperative values by 6 hours after surgery; probably due to rapid utilisation for gluconeogenesis which has been demonstrated by turnover studies in normal neonates (Frazer et al., 1981; Kalhan et al., 1980). Total gluconeogenic substrates were found to be strongly correlated with adrenaline values at the end of surgery thereby, indicating a primary role for this hormone in the stimulation of substrate mobilisation following surgical stress (Elliot and Alberti, 1983). At 6 hours after surgery, total gluconeogenic substrates were significantly correlated with plasma insulin and plasma glucagon values, which may denote a stimulatory effect on the secretion of both hormones after surgery. This effect may have resulted in decreased substrate mobilisation mediated by insulin release and increased hepatic uptake mediated by glucagon secretion, thus bringing down the concentration of these substrates 6 hours after surgery (Kraus-Friedman, 1984).

LIPID METABOLISM

The significant increase in non-esterified fatty acids, glycerol and total ketone bodies at the end of surgery may be indicative of lipolysis and ketogenesis mediated by the intra-operative hormonal changes. It is interesting to note that the blood concentrations of acetoacetate did not change during or after surgery, whereas hydroxybutyrate concentrations increased at the end of surgery and at 12 hours postoperatively, showing parallel alterations with the pattern of change in blood lactate concentrations. The return to normal of total ketone body levels by 6 hours postoperatively, may denote the sensitivity of ketone body production to an increase in plasma insulin concentration (Williamson, 1982; McGarry and Foster, 1977). Evidence for catecholamine-dependent lipolysis in this study
is also provided by a correlation of blood glycerol concentrations with plasma adrenaline at the end of surgery. Studies of glycerol turnover in normal neonates have shown that 75% of glycerol thus formed enters the gluconeogenic pathway in the neonatal liver and contributes to 5% of hepatic glucose production (Bougneres et al, 1982).

There are no published data available on changes in the blood ketone bodies or blood glycerol in neonates undergoing surgery. In adult patients, the circulating total ketone bodies may be increased, decreased or unchanged following surgery, burns or accidental trauma (Foster et al, 1979; Harris et al, 1982; Williamson and Smith, 1980). Williamson (1981) has proposed that the production of ketone bodies may be suppressed by vasopressin release during trauma, which increases the terminal oxidation of acetyl-CoA as well as the esterification of free fatty acids (Williamson, 1981).

Plasma concentrations of non-esterified fatty acids (NEFA) were measured in only a small number of cases and were found to increase significantly at the end of surgery, but by 6 hours postoperatively had reverted to their preoperative values. This pattern of change, although similar to that of blood glycerol and total ketone bodies in this study, is different from the pattern obtained by Pinter (1973), who found a significant rise in plasma NEFA at the end of surgery, but also found a further increase at 6 and 12 hours postoperatively (Pinter, 1973). The latter finding, however, may be an effect of the lack of nutrient supply rather than that of the operation itself, since the neonates studied by Pinter did not receive dextrose infusion up to 12 hours after surgery. Thereafter, dextrose was given only to neonates who were hypoglycaemic. Elphick and Wilkinson (1981) did not find consistent changes in plasma NEFA during or after surgery, although a significant decrease in triglycerides was documented and was presumed to
indicate an increased utilization of NEFA together with the decreased production of lipoproteins. In older infants undergoing surgery, Talbert et al (1967) found a significant increase in plasma NEFA concentrations at the end of surgery; this study was not extended into the postoperative period. In adult patients undergoing surgery, Kinney et al (1970) have shown that upto 75-90% of energy requirements may be met by oxidation of fats.

As may be expected from the physiological control of ketogenesis, the preoperative concentration of total ketone bodies was correlated strongly with plasma glucagon values. However, at the end of surgery and thereafter total ketone body values were not correlated with concentrations of any of the hormones measured. In the postoperative period, oxidation of free fatty acids in the neonatal liver may stimulate gluconeogenesis by the generation of extra ATP to support gluconeogenesis, the production of acetyl-CoA which activates pyruvate carboxylase, and the provision of reducing equivalents for glyceraldehyde 3-phosphate dehydrogenase (Williamson, 1982).

Utilisation of ketone bodies in peripheral tissues, through the formation of citrate and inhibition of phosphofructokinase, may inhibit the peripheral utilisation of glucose and further contribute towards postoperative hyperglycaemia (Williamson, 1982).

4.5.3 Urinary nitrogenous constituents :-

NITROGEN EXCRETION

Following the pioneering study by Rickham in 1957, several studies have shown that neonates undergoing surgery have a negative nitrogen balance in the postoperative period, the magnitude and duration of which are related to the extent of surgical trauma and to the form of nutritional support in the postoperative period (Rickham, 1957; Colle and Paulsen, 1959; Peonides et al, 1963; Hughes et al, 1965; Knutrud, 1965; Wilkinson et al, 1965; Suzuki

These results were confirmed in the present study, from the measurement of total nitrogen excretion during the 3 days following surgery. The neonates studied were not enterally fed during this period, and did not receive parenteral nutrition or blood transfusions during or after surgery. Thus, it may be assumed that nitrogen excretion in the postoperative period may represent the net nitrogen loss caused by postoperative protein catabolism.

The values measured on the second and third days after surgery were compared to those measured on the first postoperative day, although ideally the comparison should have been to control values obtained on the day before surgery. However, this was not practically feasible due to the emergency nature of several operations. Furthermore, several previous studies have found that nitrogen loss following surgery in neonates is evident on the second and subsequent postoperative days (Colle and Paulsen, 1959; Wilkinson et al, 1965; Greenall et al, 1983).

The significant increase in nitrogen excretion on the second postoperative day probably indicates that increased protein breakdown reached a maximum in these neonates during 24-48 hours after surgery; total nitrogen excretion on the third postoperative day was lower than on the previous day and was not significantly raised as compared to the first 24 hours after surgery. This pattern is in keeping with the short duration of other metabolic changes that have been documented in this study. The magnitude of nitrogen excretion and the pattern of changes found in this study are similar to the results obtained by Colle and Paulsen (1959), Wilkinson et al (1965), Hughes et al (1965) and Greenall et al (1983).
3-METHYLHISTIDINE/CREATININE RATIO

The molar 3-methylhistidine/creatinine ratio, measured on the urine samples of 20 patients, was found to be raised significantly on the second and third postoperative days, as compared to the first 24 hours after surgery. The 3-methylhistidine/creatinine ratio in urine has been proposed as a measure of the fractional rate of myofibrillar protein breakdown occurring in skeletal muscle (Ballard and Tomas, 1983; Young and Munro, 1978, Elia et al, 1981), although it is controversial whether skeletal muscle is the primary source of 3-methylhistidine in urine (Rennie and Millward, 1983; Wassner and Li, 1982). It is, however, not disputed that the excretion of 3-methylhistidine is associated with increased rates of protein catabolism from the breakdown of intracellular actin and myosin mainly from the skin, skeletal muscle and gastrointestinal tract (Rennie and Millward, 1983). Thus, the 3-methylhistidine/creatinine ratio is increased in some adults, after major trauma (Williamson et al, 1977), after surgery (Gross et al, 1978) and in several other clinical conditions associated with increased protein breakdown (Elia et al, 1981).

In neonatal muscle from several species, it has been shown that myosin fibres do not contain 3-methylhistidine residues (Young and Munro, 1978) and thus, intracellular actin would be the primary source of 3-methylhistidine in urine. The efficacy of this measurement has been validated in newborn infants by Burgoyne et al (1982) who found an increased fractional rate of myofibrillar protein breakdown in preterm neonates as compared to term neonates. In preterm neonates, the 3-methylhistidine/creatinine ratio has been related to the degree of nitrogen loss and the rate of weight gain (Seashore et al, 1980; Ballard et al, 1979). Seashore et al (1980) found that preterm neonates who were clinically 'stressed' due to the problems associated with prematurity had a significantly greater ratio as compared
to healthy preterm neonates. The neonates with raised 3-MH/creatinine ratios in that study had a poorer rate of growth, a negative nitrogen balance and inadequate caloric intakes when compared to the control group of healthy babies (Seashore et al, 1980). Therefore, in the present study, the finding of an increase in nitrogen excretion and the 3-methylhistidine/creatinine ratio during the 3 days following surgery may further confirm that the increased loss of nitrogen following surgery is due to endogenous protein breakdown.

Thus, from these data, it may be concluded that stress-related hormonal changes in newborn infants undergoing surgery may precipitate a catabolic state characterised by glycogenolysis, mobilisation of gluconeogenic substrates, lipolysis and endogenous protein breakdown in the postoperative period. It is possible that these hormonal and metabolic changes may have important clinical implications for the peri-operative clinical management of newborn infants undergoing surgery.

4.5.4 Clinical implications of the results :-

The clinical implications of a metabolic stress response may either relate to its direct effects on catabolism and substrate mobilisation in the postoperative period (eg, metabolic acidosis, negative nitrogen balance, delayed growth, etc) or to associated features of the stress response (eg, cardio-pulmonary instability, decreased immune resistance, gastric stress ulcers, hypercoagulability, etc).

In addition, the rapid development of hyperglycaemia during surgery has a special significance in the neonatal patient in view of its effect on plasma osmolality (Gennari, 1984). In newborn infants, an increase in plasma osmolality of greater than 25 mOsmols/Kg H₂O during a period of 4
hours or less can have detrimental effects on the renal cortex and cerebral vasculature (Finberg, 1967) and may even lead to intraventricular haemorrhage (Arant and Gooch, 1978). Thus, deleterious effects in term and preterm neonates from the development of a hyperosmolar state during surgery would not only be related to the magnitude, but to the speed of change in osmolarity as well (Finberg, 1967).

4.5.5 Questions to be answered:

From the analysis of data pertaining to all neonates included in this study, the general pattern of the endocrine and metabolic stress response in newborn infants undergoing surgery was defined. Further analysis was considered necessary in order to obtain preliminary answers to the following questions:

1. Is the stress response altered in neonates who receive inadequate anaesthesia during surgery?
2. Are there any specific effects of the different anaesthetic agents used on the pattern of the neonatal stress response?
3. Is the pattern of hormonal and metabolic changes in preterm neonates any different from that of term neonates?
4. What are the other components of peri-operative management that may alter the response of newborn infants to anaesthesia and surgery?

4.6 Effects of Anaesthetic Management:

As described in section 4.5.2, a wide variety of anaesthetic techniques were used for neonates included in this study. In order to answer the question of whether potent anaesthesia is required during surgery in newborn infants, the patients included in this study were divided into groups that received 'adequate' and 'inadequate' anaesthesia for the degree
and duration of the surgical trauma that they had undergone. This assessment was performed by an experienced anaesthetist who was not involved in the management of any patient in this study and was 'blind' to all other patient characteristics and the results of the study. A loss of awareness during surgery (Saunders, 1981) was taken as the primary criterion for this assessment. It is emphasized that this was a retrospective and subjective evaluation of the anaesthetic management of neonates included in this study, though care had been taken to remove any bias by obtaining the help of an independent anaesthetist.

On the basis of this assessment, 13 patients were included in the group receiving 'adequate' anaesthesia and 16 patients were found to receive 'inadequate' anaesthesia. The patient characteristics and results from these two groups were compared using the Mann-Whitney U test.

4.6.1 Description of patients and clinical management :-

The age, gestation, record at birth and details of the peri-operative management of patients in both groups are presented in Table 4.9.

The age and preoperative management of patients in the two groups were comparable. Moreover, the two groups of patients had undergone similar degrees of surgical stress (as measured by the stress score) and the duration of surgery, volume of blood transfused, temperature loss during surgery and postoperative analgesic therapy were also similar. It was found that all preterm neonates had received inadequate anaesthesia during surgery, and thus, the gestation, birth weight and weight at the time of surgery were significantly lower in the inadequate anaesthesia group. In addition, it was found that neonates in the inadequate anaesthesia group had received a lower rate of dextrose infusion during surgery; whereas
during the 24 hours postoperatively, they received a significantly higher rate of dextrose infusion as compared to the adequate anaesthesia group.

4.6.2 Hormonal changes :-

The hormonal changes in the two groups are presented in Table 4.10. Plasma adrenaline concentrations increased significantly during surgery in both groups, but the magnitude of increase was greater in the group of patients receiving inadequate anaesthesia, resulting in significantly higher values (p<0.05) at the end of surgery.

Plasma noradrenaline concentrations increased markedly in the group of patients receiving inadequate anaesthesia and did not change in the adequate anaesthesia group, thus at the end of surgery plasma noradrenaline values were significantly different (p<0.02) between the two groups.

Plasma insulin concentrations increased significantly during surgery in the adequate anaesthesia group but did not change in the inadequate anaesthesia group. However, plasma insulin values were not significantly different between the two groups at the end of surgery or in the postoperative period. Plasma glucagon concentrations were not compared between the two groups due to lack of sufficient data.

4.6.3 Metabolic changes :-

Metabolic changes in the two anaesthetic groups are presented in Table 4.11 and Table 4.12. There were no significant differences between the two groups in the blood concentrations of glucose, lactate, pyruvate, acetoacetate, hydroxybutyrate and glycerol before or after surgery. Blood alanine concentrations were significantly higher in the adequate anaesthesia group before surgery (p<0.01), at the end of surgery (p<0.01)
and at 24 hours postoperatively ($p<0.02$). Furthermore, there were no significant differences between the two groups in the preoperative or postoperative values of total ketone bodies, total gluconeogenic substrates, the lactate/pyruvate ratio, the insulin/glucose ratio or the hydroxybutyrate/acetoacetate ratio. The preoperative alanine/pyruvate ratio was not significantly different between the two groups; however, it was decreased in the inadequate anaesthesia group, giving rise to significant differences at the end of surgery ($p<0.005$), at 6 ($p<0.05$) and 24 hours after surgery ($p<0.05$).

4.6.4 Urinary nitrogenous constituents

There were no significant differences in the 3-methyl histidine/creatinine ratios between the anaesthetic groups during the 3 postoperative days (Table 4.13). Total nitrogen excretion was not compared between the two anaesthetic groups due to lack of sufficient data.

4.6.5 Discussion

From a review of the recent literature, it was concluded that anaesthetic techniques for neonatal patients had developed along empirical lines during the past few years. For example, the need for anaesthesia at all has been questioned (Betts and Downes, 1984; Shaw, 1982; Lipmann et al, 1976) and it was widely recommended that neonatal patients should receive little or no anaesthesia during surgery. After discussions with anaesthetic colleagues in the John Radcliffe Hospital and anaesthetists from other leading hospitals, it was learnt that major surgery in critically ill or preterm neonates was usually performed under the effect of muscle relaxants alone.

Therefore, the data collected in the preliminary study were used to test the hypothesis that newborn infants do not require potent anaesthetic
agents during surgery. The neonates included in this study were divided into groups that received 'adequate' or 'inadequate' anaesthesia for the type of surgery they had undergone. This classification, although based on a subjective criterion (that if there was a possibility of 'awareness' during surgery, the depth of anaesthesia was probably inadequate), was performed by an experienced anaesthetist who was 'blind' to all other patient details. As stated previously, the purpose of this analysis was to decide whether it was justified to start a more formal investigation of the above hypothesis, rather than to draw firm conclusions about the endocrine and metabolic effects of anaesthetic management.

The analysis of hormonal data showed no difference between the two anaesthetic groups except for plasma adrenaline and noradrenaline concentrations at the end of surgery, which were found to be significantly higher in the patients who received inadequate anaesthesia. This finding could be a result of centrally decreased sympathoadrenal activation from the surgical stress in neonates who received adequate anaesthesia, or may be due to direct sympathoadrenal suppression caused by the anaesthetic agents used. The majority of neonates in the adequate anaesthesia group received either halothane or sodium thiopentone or both; from studies on adult patients these agents are known to suppress the adrenal medulla directly (Derbyshire and Smith, 1984) and thereby may have obtunded the sympathoadrenal responses to surgical stress. Additional mechanisms may be responsible for this effect and have been discussed in Chapter VI.

The absence of major differences in the metabolic response may be due to differences in the clinical management during and after surgery. For example, significant differences in the rate of dextrose infusion were found during surgery as well as in the postoperative period (Table 4.9). It
is interesting to note that the increase in blood glucose concentrations at the end of surgery was greater in the group of neonates given inadequate anaesthesia, although this group had a significantly lower dextrose infusion rate during the surgical procedure. On the other hand, differences in the metabolic response may have been obscured by the fact that the two patient populations were not comparable in gestation, birth weight or weight at the time of operation. However, it is also likely that the minor differences in the hormonal response that have been documented were probably not sufficient to cause distinct metabolic alterations in the two groups of neonates.

The significant difference in alanine concentrations preoperatively, at the end of surgery and at 24 hours postoperatively, was probably related to the presence of preterm neonates in the inadequate anaesthesia group (Table 4.11). It is well-known that preterm neonates have lower circulating concentrations of alanine as compared to term neonates (Schultz et al, 1980; Soltesz et al, 1978) and this comparison has been further examined in the following section.

On the other hand, alanine/pyruvate ratios were similar in the two groups preoperatively and decreased during surgery in patients receiving inadequate anaesthesia, giving rise to significant differences at the end of surgery and at 6 hours and 24 hours postoperatively (Table 4.12). This effect could be due to the higher plasma adrenaline concentrations in the inadequate anaesthesia group at the end of surgery (Garber et al, 1976; DeLamater et al, 1974).

Thus, it is possible that the lack of adequate anaesthesia in newborn infants undergoing surgery may be associated with an accentuation of the
hormonal stress response with respect to changes in plasma catecholamine concentrations. However, further detailed studies will be required in order to investigate whether potent anaesthesia during surgery can decrease the hormonal and metabolic response of neonates undergoing surgery.

4.6.6 Comparison of halothane and thiopentone anaesthesia:

In the group of term neonates receiving adequate anaesthesia, 6 neonates received an anaesthetic regimen of halothane (0.5-2.0 %), nitrous oxide and d-tubocurarine, whereas 5 other neonates received thiopentone (4-5 mg/Kg) with nitrous oxide and d-tubocurarine. Both groups of neonates were subjected to Grade II surgical stress (stress score 6-10). The hormonal and metabolic results of these two groups were therefore compared to examine the specific effect of these anaesthetic agents.

End-operative blood glucose concentrations of neonates who received thiopentone anaesthesia were significantly greater than those of neonates who received halothane anaesthesia (p<0.025). There were no significant differences between the two groups in the blood levels of the other metabolites measured. Furthermore, no difference was found in the plasma insulin levels of the two groups before or after surgery. The greater hyperglycaemia in the thiopentone anaesthesia group could be due to a stimulation of hepatic glycogen phosphorylase by thiopentone as has been shown by Brunner and Haugaard (1965) in the rat liver. The hyperglycaemic effect of thiopentone anaesthesia has also been documented in adult patients (Clarke, 1970).

Thus, it is possible that that anaesthetic management during surgery may have a distinct influence on the hormonal and metabolic responses of newborn infants undergoing surgery. However, further studies may be
required to define the specific effects of potent anaesthetic agents on hormonal and metabolic parameters of the neonatal stress response.

4.7 EFFECTS OF PREMATURITY :-

Since hormonal and metabolic regulation in preterm neonates are known to be very different from term neonates at birth (Winter, 1982; Girard and Ferre, 1982) it is possible that the responses to surgical stress may also be altered in the infant born prematurely and of low birth weight. The responses of 8 preterm and 8 term neonates in the 'inadequate' anaesthesia group were compared in order to identify the pattern of these differences.

4.7.1 Description of patients and clinical management :-

The characteristics of preterm and term neonates are presented in Table 4.14. As expected, the birth weight, gestation and weight at the time of surgery were significantly lower (p<0.001) in the preterm neonates. However, the age of patients at the time of surgery was found to be significantly greater in preterm neonates as compared to the term neonates (p<0.01).

There were no significant differences between the two groups in the dextrose infusion rate, duration of preoperative starvation, the severity of surgical stress, temperature loss during surgery or in the postoperative analgesic therapy.

4.7.2 Hormonal changes :-

Hormonal changes in preterm and term neonates are listed in Table 4.15. From the limited data available, no significant differences were found between the two groups of neonates in plasma adrenaline and noradrenaline
concentrations before and after surgery. Plasma insulin concentrations increased during surgery and postoperatively in term neonates, whereas the levels did not change in preterm neonates. Hence, significant differences were found between the two groups at 6 hours (p<0.05) and 12 hours (p<0.05) after surgery. Changes in plasma glucagon were not compared between the two groups due to lack of sufficient data.

4.7.3 Metabolic changes:–

The metabolic data of preterm and term neonates are presented in Tables 4.16 and 4.17. There were no significant differences in the blood glucose concentrations between preterm and term neonates during or after surgery. Blood lactate concentrations increased during surgery in both groups of patients, but in the postoperative period blood lactate values were consistently higher in the term neonates at 6 hours (p<0.005), 12 hours (p<0.02) and 24 (p<0.05) hours following surgery. Similarly, blood pyruvate concentrations at 6 hours postoperatively were significantly higher in term neonates (p<0.05) than in preterm neonates. Blood alanine concentrations were found to be significantly lower in preterm neonates at the end of surgery (p<0.05) and at 6 hours after surgery (p<0.005). There was no significant difference between the blood glycerol values of preterm and term neonates before or after surgery. The values of total gluconeogenic substrates were decreased significantly in preterm neonates at 6 hours (p<0.005), 12 hours (p<0.025) and 24 hours (p<0.05) after surgery.

There were no significant differences in the blood concentrations of ketone bodies between the two groups before or after surgery. The insulin/glucose ratio in preterm neonates was significantly lower than that of term neonates at 6, 12 and 24 hours after surgery (p<0.05). Preterm neonates also had a significantly lower lactate/pyruvate ratio than term neonates.
preoperatively and at 6 (p<0.005) and 12 hours (p<0.05) after surgery.

4.7.4 Changes in plasma amino acids :-

The plasma concentrations of amino acids were measured in the same groups of term and preterm neonates. Prominent differences were found between the two groups with regard to the peri-operative changes in gluconeogenic amino acids and these are presented in Figure 4.1.

Before surgery, the plasma concentrations of valine (p<0.05) and glutamine (p<0.05) were significantly lower in the preterm neonates as compared to the term neonates. At the end of surgery, plasma concentrations of all the gluconeogenic amino acids were found to be decreased in the preterm neonates. Thus, the plasma concentrations of alanine (p<0.05), glutamine (p<0.01), glycine (p<0.025), valine (p<0.01), proline (p<0.025) and lysine (p<0.05) in preterm neonates were significantly lower than corresponding values in term neonates. Similar differences were maintained between preterm and term neonates at 6 hours postoperatively with respect to the plasma concentrations of alanine (p<0.005), glutamine (p<0.01), glycine (p<0.05) and proline (p<0.025); whereas at 12 hours after surgery, plasma glutamine concentrations were significantly lower in the preterm neonates (p<0.025) as compared to the values in term neonates. Total gluconeogenic amino acids were calculated from these data and, as expected, were found to be significantly lower in the preterm neonates at the end of surgery (p<0.01) and at 6 hours postoperatively (p<0.025), as compared to the corresponding values in term neonates.

As a result of these differences, the plasma concentration of total amino acids was also decreased at the end of surgery in preterm neonates, and was significantly lower than that of term neonates at the end of surgery.
(p<0.01) and at 6 hours postoperatively (p<0.05).

4.7.5 Urinary nitrogenous constituents :-
No significant differences were found between preterm and term neonates in the 3-methylhistidine/creatinine ratios during the three days following surgery (Table 4.18). Total nitrogen excretion was not compared between the two groups due to lack of sufficient data.

4.7.6 Discussion :-
The preterm neonate may be particularly ill-equipped to withstand a prolonged catabolic state due of immature hormonal and metabolic regulation and poorer reserves of fat, protein and carbohydrate than the term neonate (Girard and Ferre, 1982; Aynsley-Green, 1982). It was therefore, considered necessary to define the effects of prematurity by comparing the endocrine and metabolic responses of preterm and term neonates undergoing similar degrees of surgical stress with a similar anaesthetic management.

The postnatal age of preterm neonates was found to be significantly greater than that of term neonates at the time of surgery. This is because all neonates undergoing surgery were eligible for entry into the study till they reached a post-conceptional age of 44 weeks. Thus, preterm neonates upto the age of 59 days were entered into the study whereas term neonates beyond the age of 28 days were not considered eligible.

From the limited data available in this study, no significant differences were found in the catecholamine responses of preterm and term neonates to surgical stress. A distinct difference however, cannot be excluded since the number of patients compared was very small.
The primary difference in the hormonal response of preterm neonates was the lack of an insulin response to surgical hyperglycaemia during and after surgery. This finding may be due to a decreased responsiveness of islet beta-cells in the premature pancreas which has been documented previously (Soltesz and Aynsley-Green, 1984), or may be related to a prolonged inhibition of insulin secretion by the adrenaline release during surgery (Sperling, 1984). However, the most common surgical procedure performed in preterm neonates was ligation of a patent ductus arteriosus (PDA) and it is not known whether handling of the vagus nerve which occurs during surgery has an influence on postoperative insulin secretion. This is be unlikely, since the insulin responses of the three preterm neonates not undergoing PDA ligation were also suppressed in the postoperative period. However, further studies are required to establish this observation.

Possibly due to the lack of an insulin response, preterm neonates had a tendency towards greater hyperglycaemia at the end of surgery, but this was not significantly different from the term neonates. The insulin/glucose ratio in preterm neonates however, was found to be significantly lower than that of term neonates during the entire postoperative period; thereby confirming inappropriate secretion of insulin for the circulating glucose concentrations in preterm neonates.

Blood lactate and pyruvate concentrations were significantly lower in preterm neonates during the postoperative period, resulting in major differences from the term neonates in the circulating concentrations of total gluconeogenic substrates. These substrates were probably used for postoperative gluconeogenesis in order to maintain the surgical hyperglycaemia in preterm neonates (Frazer et al, 1981; Patel et al, 1982). An additional cause, however, could be the relatively smaller reserves of
muscle glycogen in preterm neonates as compared to the term neonates (Shelley, 1961) which may be responsible for production of lactate and pyruvate in smaller amounts following adrenaline-stimulated glycogenolysis.

Data from the measurement of plasma amino acids demonstrated a similar pattern of differences between preterm and term neonates in the peri-operative period. It was found that the gluconeogenic amino acids were decreased in preterm neonates at the end of surgery and at 6 hours postoperatively, whereas they remained unaltered in the term neonates. It is possible that this pattern of changes may also result from the lack of insulin secretion in preterm neonates during and after surgery. Thus, it is possible that the low insulin concentrations would cause a shift in the insulin/glucagon ratio favouring the utilization of amino acids and other substrates for gluconeogenesis in the postoperative period (Patel et al, 1982; Sperling, 1982). Furthermore, from these data it is tempting to suggest that surgical hyperglycaemia in preterm neonates is derived mainly from gluconeogenesis, whereas in term neonates it may be derived primarily from glycogenolysis. This hypothesis is also in keeping with the decreased glycogen reserves in preterm neonates, but would need to be confirmed by stable-isotope turnover studies of the gluconeogenic substrates in preterm and term neonates during the postoperative period.

Thus, it may be concluded that the endocrine and metabolic response of preterm infants undergoing surgery has specific and distinctive features compared to the response of term neonates subjected to surgical stress.

4.8 EFFECTS OF THE SEVERITY OF SURGICAL STRESS :-

In order define the efficacy of the scoring method developed for this
study, effects of the severity of surgical stress were examined by analysing the limited data obtained in the preliminary study. However, it was decided that this analysis would only be of a very preliminary nature, and that a more definitive analysis would be carried out later using the data obtained from neonates included in the subsequent clinical trials.

4.8.1 Method of analysis :
According to this scoring method, 9 neonates were subjected to Grade I surgical stress (score 0-5), 18 neonates to Grade II surgical stress (score 6-10) and 2 neonates to Grade III surgical stress (score 11-20). The values from neonates in these groups were analysed by the Kruskal-Wallis analysis of variance in order to identify overall differences between the three groups in the metabolic variables measured. Since hormonal variables were not measured in all the neonates studied, the hormonal data were not subjected to this analysis.

4.8.2 Results :
There were no significant differences between the minor, moderate and severe stress groups in the gestation, birth weight, Apgar scores at birth, post-natal age, duration of preoperative starvation, temperature loss during surgery, or the dextrose infusion rates before, during and after surgery.

There were no significant differences between the 3 stress groups in the preoperative concentrations of any metabolic variable.

At the end of surgery, significant differences were found between the minor, moderate and severe stress groups in the concentrations of blood glucose (p<0.025) and blood lactate (p<0.05). At 6 hours after surgery, the
values for blood glucose (p<0.01) and the lactate/pyruvate ratio (p<0.05) were significantly different between the 3 stress groups. At 12 hours postoperatively however, blood lactate concentrations (p<0.05) and the lactate/pyruvate ratio (p<0.01) were found to significantly different between the 3 stress groups. At 24 hours after surgery, no further significant differences remained between the three surgical stress groups.

The blood levels of pyruvate, ketone bodies, alanine and glycerol were found to be poor indicators of the degree of surgical stress.

4.8.3 Conclusions :-

From this analysis, it was evident that the newborn infant was capable of responding to different degrees of surgical stress. Peri-operative changes in blood glucose and blood lactate, which were the most prominent features of the neonatal stress response, were found to be modulated by the severity of surgical stress.

Since these differences were identified even in the presence of such small numbers of patients the three stress groups, it was possible to conclude that the proposed scoring method had a high efficacy in differentiating between the neonates who had been subjected to the different grades of surgical stress.

4.9 Overall Summary and Conclusions :-

1. The human newborn infant is capable of mounting a substantial endocrine and metabolic stress response to anaesthesia and surgery.

2. The hormonal changes associated with the stress response are an increase
in the plasma concentrations of adrenaline and noradrenaline during surgery, a postoperative increase in insulin secretion, together with a decrease in plasma glucagon concentrations by 24 hours after surgery.

3. The metabolic changes documented are characterised by hyperglycaemia, hyperlactataemia, together with transient increases in the blood concentrations of pyruvate, alanine, non-esterified fatty acids, glycerol and ketone bodies, and the increased urinary excretion of nitrogenous products.

4. The characteristic features of the neonatal response in contrast to the adult stress response are the short duration of the hormonal and metabolic changes, despite a greater magnitude of metabolic alterations in neonates following comparable degrees of surgical trauma.

5. It is possible that the endocrine and metabolic response may be modified by the provision of adequate anaesthesia during surgery and may also be affected by the specific effects of anaesthetic agents.

6. The hormonal and metabolic response of preterm neonates was found to be distinctly different from that of term neonates, mainly characterised by the lack of an insulin response in preterm neonates, together with lower circulating concentrations of the gluconeogenic amino acids and other gluconeogenic substrates in the postoperative period.

7. Neonates undergoing different grades of surgical stress may respond with an alteration in the magnitude of their peri-operative hyperglycaemia and hyperlactataemia.
Table 6.1: PRELIMINARY STUDY: Hormonal changes.

<table>
<thead>
<tr>
<th>Time</th>
<th>Insulin pmol/L</th>
<th>Glucagon pmol/L</th>
<th>Adrenaline nmol/L</th>
<th>Noradrenaline nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative</td>
<td>28</td>
<td>86 ± 16</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>6 hours</td>
<td>152 ± 29*</td>
<td>29 ± 5</td>
<td>10</td>
<td>0.36 ± 0.10</td>
</tr>
<tr>
<td>12 hours</td>
<td>123 ± 25</td>
<td>26 ± 3</td>
<td>8</td>
<td>0.31 ± 0.06</td>
</tr>
<tr>
<td>24 hours</td>
<td>157 ± 31*</td>
<td>17 ± 5*</td>
<td>8</td>
<td>0.29 ± 0.19</td>
</tr>
</tbody>
</table>

Changes in the plasma hormone concentrations of newborn infants undergoing surgery. Values measured at the end of surgery and post-operatively were compared to pre-operative values using Wilcoxon’s matched-pairs test. * p<0.05, ** p<0.025.

All values = Mean ± SEM.
Changes in the blood metabolite concentrations of newborn infants undergoing surgery. Values measured at the end of surgery and post-operatively were compared to pre-operative values using Wilcoxon's matched-pairs test. * p<0.05; ** p<0.025; *** p<0.005; **** p<0.0001.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Glucose mmol/L</th>
<th>Lactate mmol/L</th>
<th>Pyruvate mmol/L</th>
<th>Acetoacetate mmol/L</th>
<th>Hydroxybutyrate mmol/L</th>
<th>Alanine mmol/L</th>
<th>Glycerol mmol/L</th>
<th>Non-esterified fatty acids mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative</td>
<td>4.9±0.4</td>
<td>1.6±0.1</td>
<td>0.10±0.01</td>
<td>0.10±0.01</td>
<td>0.13±0.04</td>
<td>0.23±0.02</td>
<td>0.16±0.02</td>
<td>0.38±0.10</td>
</tr>
<tr>
<td>End-operative</td>
<td>10.4±0.6***</td>
<td>2.7±0.2*****</td>
<td>5.15±0.1****</td>
<td>0.13±0.02</td>
<td>0.24±0.07***</td>
<td>0.23±0.02**</td>
<td>0.21±0.02****</td>
<td>5.63±0.07**</td>
</tr>
<tr>
<td>24 hours</td>
<td>5.8±0.6</td>
<td>1.9±0.7</td>
<td>0.12±0.01</td>
<td>0.11±0.02</td>
<td>0.14±0.06</td>
<td>0.23±0.02</td>
<td>0.15±0.01</td>
<td>6.05±0.17</td>
</tr>
<tr>
<td>48 hours</td>
<td>6.3±0.6***</td>
<td>1.8±0.2**</td>
<td>0.11±0.01</td>
<td>0.10±0.02</td>
<td>0.09±0.04*</td>
<td>0.23±0.02</td>
<td>0.16±0.02</td>
<td>6.35±0.08</td>
</tr>
<tr>
<td>72 hours</td>
<td>5.3±0.3</td>
<td>1.8±0.2</td>
<td>0.11±0.01</td>
<td>0.10±0.01</td>
<td>0.08±0.02</td>
<td>0.23±0.01</td>
<td>0.14±0.01</td>
<td>6.27±0.04</td>
</tr>
</tbody>
</table>

Changes in the blood metabolite concentrations of newborn infants undergoing surgery. Values measured at the end of surgery and post-operatively were compared to pre-operative values using Wilcoxon's matched-pairs test. * p<0.05; ** p<0.025; *** p<0.005; **** p<0.0001. 
All values = Mean ± SEM.
<table>
<thead>
<tr>
<th></th>
<th>Total Ketones</th>
<th>Lactate/Pyruvate</th>
<th>Insulin/Glucose</th>
<th>Alanine/Pyruvate</th>
<th>Methylmalonyl/Acetoacetate</th>
<th>Total Gluconeogenic Substrates</th>
<th>Insulin/Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/L</td>
<td>Ratio (mg/mmol)</td>
<td>Ratio (pg/mmol)</td>
<td>Ratio (mg/mmol)</td>
<td>Ratio (mg/mmol)</td>
<td>(nmol/mmol)</td>
<td>Ratio (pg/mmol)</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>29</td>
<td>0.23 ± 0.05</td>
<td>17.3 ± 1.1</td>
<td>18 ± 4</td>
<td>2.5 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>Intra-operative</td>
<td>25</td>
<td>0.36 ± 0.08</td>
<td>19.1 ± 1.1</td>
<td>10 ± 2</td>
<td>2.0 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>6 hours</td>
<td>24</td>
<td>0.23 ± 0.08</td>
<td>16.2 ± 1.0</td>
<td>25 ± 5</td>
<td>2.1 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>12 hours</td>
<td>23</td>
<td>0.19 ± 0.05</td>
<td>19.8 ± 3.3</td>
<td>20 ± 3</td>
<td>2.4 ± 0.3</td>
<td>0.8 ± 0.1</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>24 hours</td>
<td>28</td>
<td>0.18 ± 0.05</td>
<td>21.8 ± 3.1</td>
<td>28 ± 5</td>
<td>2.9 ± 0.4</td>
<td>0.8 ± 0.1</td>
<td>2.3 ± 0.3</td>
</tr>
</tbody>
</table>

Changes in the derived hormonal-metabolic variables in newborn infants undergoing surgery. Values obtained at the end of surgery and post-operatively were compared to the pre-operative values using Wilcoxon's matched-pairs test. * p<0.05, ** p<0.025, *** p<0.005, **** p<0.0001. All values = Mean ± SEM.
Table 4.4 HORMONAL-METABOLIC CORRELATIONS: - Blood Glucose.

<table>
<thead>
<tr>
<th></th>
<th>Adrenaline</th>
<th>Insulin</th>
<th>Glucagon</th>
<th>Insulin/ Glucagon Ratio</th>
<th>Total Gluconeogenic Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>End-operative</td>
<td>0.77</td>
<td>0.47</td>
<td>0.68</td>
<td>0.66</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>N = 9</td>
<td>N = 28</td>
<td>N = 7</td>
<td>N = 7</td>
<td>N = 28</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>6 hr Post-operative</td>
<td>X</td>
<td>0.50</td>
<td>0.77</td>
<td>0.83</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N = 23</td>
<td>N = 6</td>
<td>N = 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
<td>p&lt;0.025</td>
<td></td>
</tr>
<tr>
<td>12 hr Post-operative</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>24 hr Post-operative</td>
<td>X</td>
<td>0.47</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N = 24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation of blood glucose values with hormonal and metabolic variables before and after surgery. Spearman rank correlation coefficients were calculated from values measured in the same blood sample; only the significant correlations are shown.
Correlation of blood lactate values with hormonal variables before and after surgery. Spearman rank correlation coefficients were calculated from values measured in the same blood sample; only the significant correlations are shown.

<table>
<thead>
<tr>
<th></th>
<th>Adrenaline</th>
<th>Insulin</th>
<th>Glucagon</th>
<th>Insulin/Glucagon Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>End-operative</td>
<td>0.87</td>
<td>0.35</td>
<td>X</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>N = 9</td>
<td>N = 28</td>
<td>N = 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.05</td>
<td>p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>6 hr Post-operative</td>
<td>0.61</td>
<td>0.60</td>
<td>X</td>
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<td></td>
<td>N = 8</td>
<td>N = 23</td>
<td>N = 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td>p&lt;0.001</td>
<td>p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>12 hr Post-operative</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>24 hr Post-operative</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Table 4.6 HORMONAL-METABOLIC CORRELATIONS: - Blood Pyruvate.

<table>
<thead>
<tr>
<th></th>
<th>Adrenaline</th>
<th>Insulin</th>
<th>Glucagon</th>
<th>Insulin/ Glucagon Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>End-operative</td>
<td>0.65</td>
<td>0.39</td>
<td>0.72</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>N = 9</td>
<td>N = 28</td>
<td>N = 7</td>
<td>N = 6</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td>p&lt;0.025</td>
<td>p&lt;0.05</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>6 hr Post-operative</td>
<td>0.61</td>
<td>0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N = 8</td>
<td>N = 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.05</td>
<td>p=0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 hr Post-operative</td>
<td></td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N = 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hr Post-operative</td>
<td></td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N = 24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation of blood pyruvate values with hormonal variables before and after surgery. Spearman rank correlation coefficients were calculated from values measured in the same blood sample; only the significant correlations are shown.
Table 4.7 HORMONAL-METABOLIC CORRELATIONS: Blood Alanine and Total Gluconeogenic Substrates

<table>
<thead>
<tr>
<th></th>
<th>Blood Alanine</th>
<th>Total Gluconeogenic Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adrenaline</td>
<td>Insulin</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>End-operative</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 hr Post-operative</td>
<td>X</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N = 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.001</td>
</tr>
<tr>
<td>12 hr Post-operative</td>
<td>X</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N = 21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.001</td>
</tr>
<tr>
<td>24 hr Post-operative</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Correlation of blood alanine values and of total gluconeogenic substrates with hormonal variables before and after surgery. Spearman rank correlation coefficients were calculated from values measured in the same blood sample; only the significant correlations are shown.
Table 4.8 PRELIMINARY STUDY: Urinary nitrogenous constituents

<table>
<thead>
<tr>
<th>Post-operative Urine</th>
<th>3-methylhistidine/creatinine ratio</th>
<th>Total Nitrogen excretion mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>20</td>
<td>B</td>
</tr>
<tr>
<td>Post-operative day 1</td>
<td>0.032 ± 0.003</td>
<td>101 ± 14</td>
</tr>
<tr>
<td>Post-operative day 2</td>
<td>0.044 ± 0.003*</td>
<td>198 ± 13*</td>
</tr>
<tr>
<td>Post-operative day 3</td>
<td>0.043 ± 0.004*</td>
<td>129 ± 3</td>
</tr>
</tbody>
</table>

Changes in urinary 3-methylhistidine/creatinine ratios and total nitrogen excretion in the post-operative period. Values measured on post-operative day 2 and day 3 were compared to values measured on post-operative day 1 using the Wilcoxon's matched-pairs test. *p<0.05.
All values = Mean ± SEM.
Table 4.9  EFFECTS OF ANAESTHETIC MANAGEMENT: - Comparison of patient groups.

<table>
<thead>
<tr>
<th></th>
<th>Adequate Anaesthesia</th>
<th>Mann-Whitney U Test</th>
<th>Inadequate Anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRE-OPERATIVE:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>13</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td>Age, days</td>
<td>18 ± 5</td>
<td>p&lt;0.005</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>Gestation, weeks</td>
<td>39.1 ± 0.4</td>
<td>p&lt;0.001</td>
<td>33.8 ± 1.3</td>
</tr>
<tr>
<td>Birthweight, kg</td>
<td>3.0 ± 0.2</td>
<td>p&lt;0.01</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Apgar at 1 minute</td>
<td>7.9 ± 0.5</td>
<td>n.s.</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>Apgar at 5 minutes</td>
<td>9.4 ± 0.2</td>
<td>n.s.</td>
<td>8.6 ± 0.3</td>
</tr>
<tr>
<td>Dextrose infusion rate, mg/kg/min.</td>
<td>3.0 ± 0.7</td>
<td>n.s.</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>Starvation, hours</td>
<td>5.7 ± 0.3</td>
<td>n.s.</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td>TPN = No of patients</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Days pre-operative</td>
<td>2.0 ± 0.0</td>
<td>n.s.</td>
<td>10.0 ± 7.0</td>
</tr>
<tr>
<td>TPN stopped, hours pre-operative</td>
<td>3.5</td>
<td>n.s.</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>INTRA-OPERATIVE:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight at operation, kg</td>
<td>3.3 ± 0.3</td>
<td>p&lt;0.005</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Dextrose infusion rate, mg/kg/min</td>
<td>6.0 ± 0.5</td>
<td>p&lt;0.005</td>
<td>4.7 ± 0.5</td>
</tr>
<tr>
<td>Surgical stress score</td>
<td>7.2 ± 0.8</td>
<td>n.s.</td>
<td>7.4 ± 0.4</td>
</tr>
<tr>
<td>Duration of surgery</td>
<td>52 ± 9</td>
<td>n.s.</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>Blood transfusion:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>3</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Volume of blood</td>
<td>36 ± 9</td>
<td>n.s.</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Temperature loss, °C</td>
<td>0.7 ± 0.1</td>
<td>n.s.</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td><strong>POST-OPERATIVE:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose infusion rate, mg/kg/min 0 - 24 hours</td>
<td>3.4 ± 0.6</td>
<td>p&lt;0.05</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>Diamorphine, total dose, mg/24 hours 0 - 24 hours</td>
<td>0.42</td>
<td>n.s.</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Comparison of patient characteristics and peri-operative clinical management between neonates given adequate and inadequate anaesthesia, using the Mann-Whitney U Test. All values = Mean ± SEM.
### Table 4.10  EFFECTS OF ANAESTHETIC MANAGEMENT: - Comparison of Hormonal Changes.

<table>
<thead>
<tr>
<th></th>
<th>Adequate Anaesthesia</th>
<th>Mann-Whitney U Test</th>
<th>Inadequate Anaesthesia</th>
<th>Mann-Whitney U Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>N</td>
</tr>
<tr>
<td><strong>Insulin pmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate Anaesthesia</td>
<td>13</td>
<td>80 ± 17</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>124 ± 38</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>155 ± 90</td>
<td>n.s.</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>125 ± 25</td>
<td>n.s.</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>181 ± 54</td>
<td>n.s.</td>
<td>14</td>
</tr>
<tr>
<td><strong>Glucagon pmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate Anaesthesia</td>
<td>5</td>
<td>30 ± 6</td>
<td>n.s.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>35 ± 9</td>
<td>n.s.</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>26 ± 3</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>22 ± 5</td>
<td>n.s.</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>19 ± 6</td>
<td>n.s.</td>
<td>1</td>
</tr>
<tr>
<td><strong>Adrenaline nmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate Anaesthesia</td>
<td>4</td>
<td>0.18 ± 0.05</td>
<td>n.s.</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.68 ± 0.45</td>
<td>p&lt;0.05</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.25 ± 0.12</td>
<td>n.s.</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>n.s.</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.11 ± 0.01</td>
<td>n.s.</td>
<td>6</td>
</tr>
<tr>
<td><strong>Noradrenaline nmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate Anaesthesia</td>
<td>4</td>
<td>2.98 ± 0.69</td>
<td>n.s.</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.25 ± 1.01</td>
<td>p&lt;0.05</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.08 ± 0.98</td>
<td>n.s.</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>n.s.</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.70 ± 1.81</td>
<td>n.s.</td>
<td>6</td>
</tr>
</tbody>
</table>

Comparison of changes in plasma hormone concentrations between neonates given adequate and inadequate anaesthesia, using the Mann-Whitney U Test.
Table 4.11 EFFECTS OF ANAESTHETIC MANAGEMENT: Comparison of Metabolic Changes

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Adequate Anaesthesia</th>
<th>Mann-Whitney U Test</th>
<th>Inadequate Anaesthesia</th>
<th>Mann-Whitney U Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mmol/L</td>
<td>N 13, Mean ± SEM = 5.0 ± 0.5, n.s.</td>
<td>N 16, Mean ± SEM = 4.9 ± 0.5, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 10.3 ± 1.1, n.s.</td>
<td>15 10.5 ± 1.1, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 5.3 ± 0.2, n.s.</td>
<td>14 6.2 ± 0.9, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 5.7 ± 0.4, n.s.</td>
<td>15 6.6 ± 0.9, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 5.2 ± 0.3, n.s.</td>
<td>16 5.4 ± 0.5, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate mmol/L</td>
<td>N 13, Mean ± SEM = 1.6 ± 0.2, n.s.</td>
<td>N 16, Mean ± SEM = 1.5 ± 0.1, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 2.5 ± 0.3, n.s.</td>
<td>15 2.9 ± 0.4, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 1.8 ± 0.2, n.s.</td>
<td>14 2.0 ± 0.4, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 1.9 ± 0.2, n.s.</td>
<td>15 1.8 ± 0.2, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 1.9 ± 0.2, n.s.</td>
<td>16 1.8 ± 0.2, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvate mmol/L</td>
<td>N 13, Mean ± SEM = 0.10 ± 0.01, n.s.</td>
<td>N 16, Mean ± SEM = 0.09 ± 0.01, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 0.13 ± 0.02, n.s.</td>
<td>15 0.16 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 0.12 ± 0.02, n.s.</td>
<td>14 0.12 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 0.10 ± 0.01, n.s.</td>
<td>15 0.11 ± 0.01, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 0.10 ± 0.01, n.s.</td>
<td>16 0.11 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetoacetate mmol/L</td>
<td>N 13, Mean ± SEM = 0.09 ± 0.01, n.s.</td>
<td>N 16, Mean ± SEM = 0.11 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 0.10 ± 0.02, n.s.</td>
<td>15 0.15 ± 0.03, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 0.09 ± 0.02, n.s.</td>
<td>14 0.12 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 0.08 ± 0.01, n.s.</td>
<td>15 0.10 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 0.11 ± 0.02, n.s.</td>
<td>16 0.09 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxybutyrate mmol/L</td>
<td>N 13, Mean ± SEM = 0.10 ± 0.03, n.s.</td>
<td>N 16, Mean ± SEM = 0.15 ± 0.07, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 0.13 ± 0.05, n.s.</td>
<td>15 0.32 ± 0.12, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 0.07 ± 0.02, n.s.</td>
<td>14 0.19 ± 0.10, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 0.04 ± 0.01, n.s.</td>
<td>15 0.12 ± 0.06, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 0.08 ± 0.02, n.s.</td>
<td>16 0.09 ± 0.03, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine mmol/L</td>
<td>N 13, Mean ± SEM = 0.26 ± 0.02, p&lt;0.01</td>
<td>N 16, Mean ± SEM = 0.20 ± 0.02, p&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 0.29 ± 0.02, p&lt;0.01</td>
<td>15 0.21 ± 0.03, p&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 0.25 ± 0.02, n.s.</td>
<td>14 0.21 ± 0.03, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 0.25 ± 0.02, n.s.</td>
<td>15 0.22 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 0.27 ± 0.02, p&lt;0.02</td>
<td>16 0.20 ± 0.02, p&lt;0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol mmol/L</td>
<td>N 13, Mean ± SEM = 0.15 ± 0.02, n.s.</td>
<td>N 16, Mean ± SEM = 0.16 ± 0.03, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 0.18 ± 0.02, n.s.</td>
<td>15 0.23 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 0.16 ± 0.02, n.s.</td>
<td>14 0.14 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 0.18 ± 0.03, n.s.</td>
<td>15 0.15 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 0.14 ± 0.02, n.s.</td>
<td>16 0.14 ± 0.02, n.s.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of changes in blood metabolite concentrations between neonates given adequate and inadequate anaesthesia, using the Mann-Whitney U Test.
Table 4.12 EFFECTS OF ANAESTHETIC MANAGEMENT: Comparison of hormonal metabolic variables

<table>
<thead>
<tr>
<th></th>
<th>Adequate Anaesthesia</th>
<th>Mann-Whitney U Test</th>
<th>Inadequate Anaesthesia</th>
<th>Mann-Whitney U Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SEM</td>
<td>U Test</td>
<td>N</td>
</tr>
<tr>
<td>Total Gluconeogenic Substrates mmol/L</td>
<td>13</td>
<td>2.2 ± 0.2</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>3.1 ± 0.3</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.3 ± 0.3</td>
<td>n.s.</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2.5 ± 0.1</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2.4 ± 0.2</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td>Alanine/Pyruvate Ratio mmol/mmol</td>
<td>13</td>
<td>2.7 ± 0.2</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>2.5 ± 0.3</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.5 ± 0.3</td>
<td>p&lt;0.005</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2.9 ± 0.7</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.7 ± 0.8</td>
<td>p&lt;0.005</td>
<td>16</td>
</tr>
<tr>
<td>Hydroxybutyrate/Acetoacetate Ratio mmol/mmol</td>
<td>13</td>
<td>1.2 ± 0.3</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>1.3 ± 0.2</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.6 ± 0.2</td>
<td>n.s.</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.4 ± 0.1</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.7 ± 0.1</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td>Total Ketones mmol/L</td>
<td>13</td>
<td>0.19 ± 0.04</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.23 ± 0.06</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.16 ± 0.04</td>
<td>n.s.</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.12 ± 0.02</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.19 ± 0.03</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td>Lactate/Pyruvate Ratio mmol/mmol</td>
<td>13</td>
<td>16.3 ± 1.4</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>20.3 ± 2.2</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>16.3 ± 1.8</td>
<td>n.s.</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>25.8 ± 8.8</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>26.3 ± 6.8</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td>Insulin/Glucose Ratio pmol/mmol</td>
<td>13</td>
<td>17 ± 4</td>
<td>n.s.</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>11 ± 3</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>20 ± 8</td>
<td>n.s.</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>23 ± 5</td>
<td>n.s.</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>33 ± 9</td>
<td>n.s.</td>
<td>14</td>
</tr>
</tbody>
</table>

Comparison of changes in derived hormonal-metabolic variables between neonates given adequate and inadequate anaesthesia, using the Mann-Whitney U Test.
Table 4.13: EFFECTS OF ANAESTHETIC MANAGEMENT: Urinary nitrogenous constituents.

<table>
<thead>
<tr>
<th></th>
<th>Adequate Anaesthesia</th>
<th>Mann-Whitney U Test</th>
<th>Inadequate Anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td></td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>3-Methylhistidine/creatinine ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>0.033 ± 0.003</td>
<td>n.s.</td>
<td>0.032 ± 0.006</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.043 ± 0.004</td>
<td>n.s.</td>
<td>0.045 ± 0.005</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.038 ± 0.004</td>
<td>n.s.</td>
<td>0.048 ± 0.006</td>
</tr>
</tbody>
</table>

Comparison of changes in the urinary 3-methylhistidine/creatinine ratios between neonates given adequate and inadequate anaesthesia, using the Mann-Whitney U Test.
### Table 4.14  EFFECTS OF PREMATURITY: - Comparison of patient groups.

<table>
<thead>
<tr>
<th></th>
<th>Preterm Neonates</th>
<th>Mann-Whitney U Test</th>
<th>Term Neonates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td>8</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td><strong>PRE-OPERATIVE:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, days</td>
<td>30 ± 5</td>
<td>p&lt;0.01</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>Gestation, weeks</td>
<td>29.3 ± 0.9</td>
<td>p&lt;0.001</td>
<td>38.3 ± 0.5</td>
</tr>
<tr>
<td>Birthweight, kg</td>
<td>1.3 ± 0.1</td>
<td>p&lt;0.001</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>Apgar at 1 minute</td>
<td>6.3 ± 0.9</td>
<td>n.s.</td>
<td>7.6 ± 0.7</td>
</tr>
<tr>
<td>Apgar at 5 minutes</td>
<td>8.4 ± 0.6</td>
<td>n.s.</td>
<td>9.1 ± 0.4</td>
</tr>
<tr>
<td>Dextrose infusion rate, mg/kg/min.</td>
<td>4.2 ± 0.8</td>
<td>n.s.</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td>Starvation, hours</td>
<td>5.3 ± 0.5</td>
<td>n.s.</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>TPN: - No. of patients</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Days pre-operative</td>
<td>10 ± 7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TPN stopped, hrs pre-operative</td>
<td>3 ± 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>INTRA-OPERATIVE:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight at operation, kg</td>
<td>1.4 ± 0.1</td>
<td>p&lt;0.001</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>Dextrose infusion rate, mg/kg/min.</td>
<td>4.5 ± 0.9</td>
<td>n.s.</td>
<td>4.8 ± 0.7</td>
</tr>
<tr>
<td>Surgical stress score</td>
<td>7.8 ± 0.4</td>
<td>n.s.</td>
<td>7.2 ± 1.2</td>
</tr>
<tr>
<td>Blood transfusion:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volume of blood</td>
<td>13 ± 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Temperature loss, °C</td>
<td>1.3 ± 0.4</td>
<td>n.s.</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td><strong>POST-OPERATIVE:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose infusion rate, mg/kg/min.</td>
<td>5.3 ± 0.8</td>
<td>n.s.</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>Diamorphine, Total dose mg/kg/day.</td>
<td>0.20 ± 0.02</td>
<td>n.s.</td>
<td>0.32 ± 0.10</td>
</tr>
</tbody>
</table>

Comparison of patient characteristics and peri-operative clinical management between preterm and term neonates undergoing surgery, using the Mann-Whitney U Test.
All values = Mean ± SEM.
Table 4.15  EFFECTS OF PREMATURITY: - Comparison of hormonal changes.

<table>
<thead>
<tr>
<th></th>
<th>Preterm neonates</th>
<th>Mann-Whitney U Test</th>
<th>Term neonates</th>
<th>Mann-Whitney U Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SEM</td>
<td>U Test</td>
<td>N</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>8</td>
<td>115 ± 49</td>
<td>n.s.</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>69 ± 22</td>
<td>n.s.</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>96 ± 27</td>
<td>≤0.05</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>55 ± 13</td>
<td>≤0.05</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>99 ± 41</td>
<td>n.s.</td>
<td>7</td>
</tr>
<tr>
<td>Adrenaline (nmol/L)</td>
<td>3</td>
<td>0.67 ± 0.15</td>
<td>n.s.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.13 ± 0.71</td>
<td>n.s.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.24 ± 0.07</td>
<td>n.s.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.13 ± 0.05</td>
<td>n.s.</td>
<td>3</td>
</tr>
<tr>
<td>Nor-adrenaline (nmol/L)</td>
<td>3</td>
<td>3.96 ± 0.15</td>
<td>n.s.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.51 ± 0.47</td>
<td>n.s.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.92 ± 0.82</td>
<td>n.s.</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.46 ± 0.82</td>
<td>n.s.</td>
<td>3</td>
</tr>
</tbody>
</table>

Comparison of changes in plasma hormone concentrations between preterm and term neonates undergoing surgery, using the Mann-Whitney U Test.
<table>
<thead>
<tr>
<th></th>
<th>Preterm neonates</th>
<th>Mann-Whitney U Test</th>
<th>Term neonates</th>
<th>Mann-Whitney U Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Mean ± SEM</td>
<td>N</td>
<td>Mean ± SEM</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5.4 ± 0.8</td>
<td>n.s.</td>
<td>8</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>7</td>
<td>12.1 ± 2.2</td>
<td>n.s.</td>
<td>8</td>
<td>9.2 ± 0.8</td>
</tr>
<tr>
<td>8</td>
<td>6.2 ± 0.8</td>
<td>n.s.</td>
<td>6</td>
<td>6.2 ± 2.0</td>
</tr>
<tr>
<td>8</td>
<td>6.5 ± 0.8</td>
<td>n.s.</td>
<td>7</td>
<td>6.7 ± 1.7</td>
</tr>
<tr>
<td>8</td>
<td>5.9 ± 0.9</td>
<td>n.s.</td>
<td>8</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td>Lactate mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Mean ± SEM</td>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.5 ± 0.2</td>
<td>n.s.</td>
<td>8</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>2.7 ± 0.4</td>
<td>n.s.</td>
<td>8</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>8</td>
<td>1.3 ± 0.2</td>
<td>p&lt;0.005</td>
<td>6</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>8</td>
<td>1.3 ± 0.2</td>
<td>p&lt;0.02</td>
<td>7</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>8</td>
<td>1.4 ± 0.2</td>
<td>p&lt;0.05</td>
<td>8</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Pyruvate mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Mean ± SEM</td>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.10 ± 0.02</td>
<td>n.s.</td>
<td>8</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.15 ± 0.03</td>
<td>n.s.</td>
<td>8</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>0.10 ± 0.01</td>
<td>p&lt;0.05</td>
<td>6</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>0.10 ± 0.02</td>
<td>n.s.</td>
<td>7</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>8</td>
<td>0.10 ± 0.02</td>
<td>n.s.</td>
<td>8</td>
<td>0.12 ± 0.03</td>
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<td>Acetoacetate mmol/L</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>N</td>
<td>Mean ± SEM</td>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.10 ± 0.03</td>
<td>n.s.</td>
<td>8</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>0.13 ± 0.03</td>
<td>n.s.</td>
<td>8</td>
<td>0.16 ± 0.05</td>
</tr>
<tr>
<td>8</td>
<td>0.11 ± 0.02</td>
<td>n.s.</td>
<td>6</td>
<td>0.14 ± 0.07</td>
</tr>
<tr>
<td>8</td>
<td>0.09 ± 0.02</td>
<td>n.s.</td>
<td>7</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>8</td>
<td>0.06 ± 0.01</td>
<td>n.s.</td>
<td>8</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>Hydroxybutyrate mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Mean ± SEM</td>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.10 ± 0.04</td>
<td>n.s.</td>
<td>8</td>
<td>0.20 ± 0.15</td>
</tr>
<tr>
<td>7</td>
<td>0.28 ± 0.13</td>
<td>n.s.</td>
<td>8</td>
<td>0.36 ± 0.20</td>
</tr>
<tr>
<td>8</td>
<td>0.13 ± 0.05</td>
<td>n.s.</td>
<td>6</td>
<td>0.28 ± 0.23</td>
</tr>
<tr>
<td>8</td>
<td>0.09 ± 0.05</td>
<td>n.s.</td>
<td>7</td>
<td>0.16 ± 0.12</td>
</tr>
<tr>
<td>8</td>
<td>0.06 ± 0.02</td>
<td>n.s.</td>
<td>8</td>
<td>0.11 ± 0.05</td>
</tr>
<tr>
<td>Alanine mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Mean ± SEM</td>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.17 ± 0.02</td>
<td>n.s.</td>
<td>8</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>0.15 ± 0.03</td>
<td>p&lt;0.05</td>
<td>8</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>8</td>
<td>0.15 ± 0.02</td>
<td>p&lt;0.005</td>
<td>6</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>8</td>
<td>0.19 ± 0.02</td>
<td>n.s.</td>
<td>7</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>8</td>
<td>0.19 ± 0.02</td>
<td>n.s.</td>
<td>8</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>Glycerol mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Mean ± SEM</td>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.14 ± 0.04</td>
<td>n.s.</td>
<td>8</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>0.22 ± 0.04</td>
<td>n.s.</td>
<td>8</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>0.12 ± 0.02</td>
<td>n.s.</td>
<td>6</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>0.13 ± 0.02</td>
<td>n.s.</td>
<td>7</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>0.11 ± 0.02</td>
<td>n.s.</td>
<td>8</td>
<td>0.18 ± 0.03</td>
</tr>
</tbody>
</table>

Comparison of changes in blood metabolite concentrations between preterm and term neonates undergoing surgery, using the Mann-Whitney U Test.
Table 4.17  EFFECTS OF PREMATURITY: - Comparison of hormonal-metabolic variables

<table>
<thead>
<tr>
<th></th>
<th>Preterm neonates</th>
<th>Mann-Whitney U Test</th>
<th>Term neonates</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SEM</td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Total Ketones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mmol/L</td>
<td>8</td>
<td>0.21 ± .05</td>
<td>n.s.</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>7</td>
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<td>P&lt;0.05</td>
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<td></td>
<td>7</td>
<td>18.5 ± 1.1</td>
<td>n.s.</td>
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<td>6</td>
<td>13.3 ± 0.8</td>
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<td></td>
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<td>14.0 ± 2.3</td>
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<td>16.1 ± 1.8</td>
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<td>23 ± 11</td>
<td>n.s.</td>
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<td>7</td>
<td>6 ± 2</td>
<td>n.s.</td>
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<td>8</td>
<td>15 ± 4</td>
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<td>10 ± 3</td>
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<td>16 ± 5</td>
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<td>3.2 ± 0.5</td>
<td>n.s.</td>
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<td>1.7 ± 0.2</td>
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<td>1.3 ± 0.4</td>
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<td>1.6 ± 0.3</td>
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<td>0.9 ± 0.2</td>
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<td></td>
<td>8</td>
<td>1.1 ± 0.3</td>
<td>n.s.</td>
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Comparison of changes in the derived hormonal-metabolic variables between preterm and term neonates undergoing surgery, using the Mann-Whitney U Test.
Figure 4.1: Comparison of peri-operative changes in the plasma concentrations of gluconeogenic amino acids between term and preterm neonates undergoing surgery. Differences between groups were analysed by the Mann-Whitney U Test, * p<0.05, ** p<0.025, *** p<0.005.
EFFECTS OF PREMATURITY: Changes in plasma gluconeogenic amino acids.
Table 4.18  EFFECTS OF PREMATURITY: Urinary nitrogenous constituents.

Comparison of changes in the urinary 3-methylhistidine/creatinine ratios between preterm and term neonates undergoing surgery, using the Mann-Whitney U Test.
CHAPTER V : DESIGN OF THE RANDOMISED CLINICAL TRIALS
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5.1 INTRODUCTION

The preliminary study was planned as an observational exercise in a group of patients whose endocrine and metabolic responses to surgical stress had not been investigated before. There is reason to believe that, as a direct result of this lack of knowledge, the anaesthetic management of newborn infants has developed along empirical lines. By default, it has been assumed that the human newborn infant does not respond to the stress of surgical trauma and therefore, does not require potent anaesthesia during surgery. These assumptions have been readily accepted, particularly in view of the limited cardiovascular and respiratory reserves of newborn infants during and after anaesthesia.

On the basis of data obtained in the preliminary study, it was evident that newborn infants were capable of mounting a substantial response to surgical stress. On a retrospective test, the hypothesis that there is no difference in the endocrine and metabolic response of neonates receiving 'adequate' or 'inadequate' anaesthesia had been rejected. In order to investigate further the initial evidence obtained from this analysis, it was decided to test the hypothesis with prospectively planned, randomised controlled trials. It was decided to maintain a similar format for these trials as in the preliminary study. However, experience gained during the preliminary study was used to justify specific changes in the study protocol.

5.2 PROBLEMS ENCOUNTERED DURING THE PRELIMINARY STUDY

This section outlines the main problems which arose during the course of the preliminary study. The modifications in the study protocol based on these difficulties are described in the following section.
5.2.1 Variable dextrose infusion rate:

In the preliminary study it was found that the dextrose infusion rate before, during and after surgery varied from 1.8 to 9.8 mg/Kg/min for individual cases. A high dextrose infusion rate was typically given to neonates who had borderline or hypoglycaemic blood glucose values in the preoperative period. During surgery, when a higher fluid infusion rate was required to compensate for blood loss, the dextrose content was not changed and a grossly increased dextrose infusion rate thus resulted.

A low dextrose infusion rate was generally given to preterm neonates undergoing fluid restriction for control of congestive heart failure or in term neonates on the first day after birth, when fluid requirements were not high enough to provide a sufficient amount of dextrose when an intravenous fluid containing 5% dextrose was given.

5.2.2 Standardisation of anaesthetic management:

During the preliminary study, the anaesthetic management was found to be extremely variable for different groups of neonates undergoing surgery (section 4.2.5). It was observed typically that the anaesthesia given during surgery was inadequate if the patient was a preterm neonate or had been sick in the preoperative period. After several discussions with members of the Anaesthetic Department an agreement could not be reached even on the broad guidelines for standardisation of neonatal anaesthetic techniques. Thus, the attempt at standardisation was abandoned for patients included in the preliminary study and an assurance was obtained for standardised anaesthetic protocols to be followed in subsequent studies.

5.2.3 Postoperative urine collection:

Due to shortage of nursing staff, adequate attention could not be provided
to obtaining a 24-hour urine collection even from the 13 neonates who were not fed during the 72 hours following surgery. Thus, the pooled urine samples from 5 neonates had to be discarded due to losses during the period of collection. Furthermore, some neonates developed a rash in the nappy area on the second or third day of collection, possibly as an allergic reaction to the sticking material on the urine bags. In these neonates, the collection of urine was terminated immediately. Some neonates undergoing elective surgery, who were likely to be discharged from hospital within 24 or 48 hours after the operation, were not included in this aspect of the study. Thus, the hospital stay of no patients was prolonged solely for the purposes of this study.

5.2.4 Postoperative analgesic therapy :-

It was suggested that a single drug (eg, diamorphine) be used for all neonates included in the preliminary study, and that some form of analgesia be provided to all neonates undergoing moderate or major surgery; it was also proposed that postoperative analgesia should not be given during the 2 hours preceding a postoperative blood sample. These recommendations were only partially followed by the clinical staff, since only 8 neonates received postoperative analgesia, of which 6 were given diamorphine and 2 were given morphine. However, a uniform policy was observed with regard to the clinical indications for analgesic therapy; thus, analgesia was only given if clinical signs such as excessive irritability, tachycardia or hypertension were evident.

5.2.5 Venous blood sampling :-

The preterm neonates included in the preliminary study were found to be clinically unstable during the postoperative period and, in some cases, their clinical condition was affected by the handling and pain associated
with venous blood sampling. Thus, in some instances, the blood samples at 6, 12 and 24 hours postoperatively were not obtained if the clinical condition of the neonate was considered to be unstable at that time. A few of these neonates had an intra-arterial catheter in situ which was suggested by the clinical staff as an alternative site for sampling of blood; however, arterial sampling was not performed from neonates included in the preliminary study. Similar considerations were considered to be applicable to the postoperative clinical state of neonates undergoing cardiac surgery.

Thus, a variety of difficulties were identified during execution of the preliminary study which prompted certain changes in the study protocol, these changes were incorporated in the protocols used for the subsequent randomised controlled trials.

5.3 CHANGES IN STUDY PROTOCOL FOR THE RANDOMISED TRIALS :

5.3.1 Patient entry :-

The preliminary study was composed of a patient population which was heterogenous with respect to the gestation, age, weight, clinical status and other characteristics of the neonates studied. In order to reduce this heterogeneity it was decided to design two separate clinical trials : (a) the halothane trial, which included all neonates subjected to surgery under general anaesthesia, and (b) the fentanyl trial, which included preterm neonates undergoing ligation of a patent ductus arteriosus (PDA). The latter group was selected out, since PDA ligation was found to be the commonest operation in preterm neonates and their gestation, postnatal age, body weight, preoperative and postoperative clinical state and other characteristics were found to be different from the corresponding features
of term neonates undergoing other types of surgery.

Furthermore, these neonates were not suitable for administration of halothane anaesthesia, since they were usually in congestive cardiac failure at the time of PDA ligation and the cardiovascular depression caused by halothane anaesthesia (Gregory, 1982) may have been detrimental to their clinical outcome. In addition, all neonates were ventilated for more than 24 hours after PDA ligation and thus, were suitable for the use of fentanyl anaesthesia, which is a strong respiratory depressant drug. On the other hand, neonates undergoing other types of surgery were not suitable for receiving fentanyl anaesthesia, since the majority were not ventilated in the postoperative period.

5.3.2 Blood sampling:
In order to minimize the handling and pain associated with blood sampling in critically ill neonates, it was decided to obtain blood samples from the in situ arterial catheter which had been inserted for clinical monitoring purposes in preterm neonates undergoing PDA ligation and in neonates subjected to open-heart surgery. Since neonates undergoing elective surgery usually did not have intra-arterial catheters, venous blood sampling was continued in neonates included in the halothane trial.

5.3.3 Intravenous dextrose therapy:
The rate of dextrose infusion before, during and after surgery was controlled more closely in neonates included in the randomised trials than for patients in the preliminary study. Neonates who were found to be hypoglycaemic in the preoperative period and were receiving an increased rate of dextrose before surgery were not operated upon till blood glucose values had returned to normal and the concentration of dextrose being
infused could be reduced appropriately. In term and preterm neonates whose fluid requirement was restricted, the concentration of dextrose was increased in the intravenous solution such that the rate of dextrose infusion could be maintained at 4-6 mg/kg/min.

5.3.4 Postoperative urine collection:
Since the randomised control trials were planned to include a much larger number of patients than in the preliminary study, in order to reduce the extra workload on nursing staff it was decided to collect urine for only 12 hours during the latter half of each postoperative day from the neonates included in these trials. Thus, it was decided that total nitrogen excretion would not be measured in these neonates, and that the urinary 3-methylhistidine/creatinine ratio would be used for indicating the extent of endogenous protein breakdown in these patients.

5.3.5 Postoperative analgesic therapy
Although the guidelines for giving postoperative analgesia were not changed from those proposed in the preliminary study, a consensus was reached for the use of morphine in all neonates included in the randomised trials.

In addition, in order to remove clinical bias from the prescription of analgesia after surgery, it was decided to seal the anaesthetic notes of each patient for a period of 24 hours postoperatively and the anaesthetic teams were requested not to discuss the anaesthetic management of any patients with the paediatric team responsible for their postoperative care. However, the sealed anaesthetic notes were always at hand and could be consulted, if necessary, by the clinical staff. Since the clinical criteria for giving analgesia were more or less uniform, it was proposed that the amount of analgesia prescribed and the timing of the first postoperative
analgesic dose would serve as additional criteria for comparing the clinical responses of neonates between the two randomised anaesthesia groups in each of the clinical trials.

5.3.6 Anaesthetic management :-
The anaesthetic protocols used for randomisation were prepared with the advice of senior members of the Anaesthetic Department and were regarded as official policy for the neonates included in the randomised trials. The detailed protocols for the anaesthetic management of each group are included in the respective chapters.

5.4 DESIGN OF RANDOMISED CLINICAL TRIALS :

5.4.1 Outcome measures :-
The variables selected as outcome measures were those hormonal and metabolic variables considered to be most 'responsive' to surgical stress: adrenaline, noradrenaline, glucose and the 3-methylhistidine/creatinine ratio. The preliminary study had shown significant differences in the plasma adrenaline and noradrenaline concentrations between neonates receiving adequate and inadequate anaesthesia and these data were used to calculate the required sample size for the halothane trial. Similar data were not available for blood glucose concentrations and the urinary 3-methylhistidine/creatinine ratios; therefore, a clinically relevant difference was assumed for the calculation of sample size.

The order of priority decided was plasma adrenaline, plasma noradrenaline, blood glucose and urinary 3-methylhistidine/creatinine ratio; following from the premise that the hormonal changes would precede the metabolic response characterised by an increase in blood glucose which may be
followed by changes in the excretion of urinary nitrogenous constituents as denoted by changes in 3-methylhistidine/creatinine ratio.

5.4.2 Sample size :-
The required sample size was calculated in order to provide an 80% chance of identifying a significant difference, if a true difference existed, at the \( P < 0.05 \) level. The calculation of sample size was based on the difference between the mean values of the inadequate and adequate anaesthesia groups in the index variables selected. The 'standardised difference' between the two groups was calculated by dividing the difference between means with the standard deviation of that variable in the whole population. Thereafter, the required sample size to give an 80% power for the randomised trial, was read from a nomogram prepared by Altman (1982). Thus, a sample size of 40 neonates for the halothane trial, 24 neonates for the low-dose fentanyl trial and 24 neonates for the high-dose fentanyl trial was obtained.

5.4.3 Technique of randomisation :-
Randomisation was carried out with sealed envelopes which contained details of the anaesthetic management for each group of patients. A schedule for balanced randomisation in blocks was prepared by Diana Elbourn at the National Perinatal Epidemiology Unit, Oxford. The randomisation code was retained by her until the three trials had been completed.

Randomisation was not carried out until just before the anaesthesia was about to begin and the anaesthetist had agreed that the neonate was suitable for both types of anaesthetic management. Sequential patients in each trial were randomised according to sequentially numbered envelopes and the randomisation card, the anaesthesia record sheet and anaesthetic notes
201

of each patient were sealed again at the end of surgery.

5.4.4 **Statistical analysis** :-

Since the purpose of these trials was to compare 'the policy of giving an
anaesthetic drug' as against 'a policy of not giving that drug', it was
decided that all patients who were randomised would be included in the
statistical analysis whether or not the anaesthetic protocol was followed
strictly for each specific patient (for methodological considerations, see
Peto et al, 1976). Since most of the metabolic and hormonal data would be
obtained in large batches towards the end of each trial, it was proposed
that no interim analysis of the data would be possible or desirable
(McPherson, 1974).

As in the preliminary study, it was decided to continue the use of
non-parametric tests for comparing the responses of neonates in the
randomised anaesthesia groups. Since the change in hormonal and metabolic
variables from the preoperative concentration in each neonate would be
representative of the 'response' of that neonate, it was decided that
comparison of the hormonal and metabolic responses between neonates in
the two randomised anaesthesia groups would be performed by statistical
analysis between delta values of the hormonal and metabolic parameters. The
delta values for each neonate were calculated by subtracting the
preoperative concentrations of each variable from the concentrations
measured at the end of surgery and at 6, 12 and 24 hours after surgery.

Furthermore, it may be argued that the statistical comparison of absolute
values of the hormonal and metabolic parameters between neonates in the two
randomised groups would represent a comparison of their hormonal and
metabolic 'state' before and after surgery, rather than a comparison of
their 'responses'. Since the neonates have been assigned to the anaesthetic groups on the basis of random allotment, and therefore, are expected to be comparable in all other respects (Silverman, 1981); thus, it would be logical to investigate the effects of a specific anaesthetic technique by changes caused in the surgical stress 'response' of neonates in the two randomised groups.

On the other hand, a similar method of analysis would not be applicable to the preliminary study (Chapter IV) since, in that study, the basic pattern of the stress response in neonates was investigated by comparison of the changes in hormonal and metabolic variables to their respective preoperative values. Furthermore, delta values could not be used to compare neonates in the 'adequate' and 'inadequate' anaesthesia groups, (or the preterm and term neonates) since (a) these groups were not strictly comparable in their characteristics, (b) their perioperative management had not been standardised as for neonates in the randomised trials, (c) the anaesthetic management within each group was widely variable, and (d) neonates had been placed into their respective groups on the basis of a retrospective and subjective assessment, rather than by random allotment.

5.5 HYPOTHESES TO BE TESTED:

5.5.1 Halothane trial:–
Anaesthesia given with halothane (0.5-2%) and nitrous oxide (50%) to newborn infants undergoing surgery does not decrease their endocrine and metabolic response as compared to that of neonates anaesthetised with nitrous oxide (50%) alone.

5.5.2 Low-dose fentanyl trial:–
Anaesthesia given with fentanyl (10-20 µg/kg) and nitrous oxide to preterm neonates undergoing thoracotomy for ligation of a patent ductus arteriosus does not decrease their endocrine and metabolic response as compared to that of preterm neonates who receive nitrous oxide alone.

5.5.3 High-dose fentanyl trial

Anaesthesia given with fentanyl (80-100 µg/kg) to neonates undergoing cardiac surgery, cardiopulmonary bypass, deep hypothermia and circulatory arrest does not decrease their hormonal and metabolic response as compared to that of neonates anaesthetised with papaveretum (0.5-1.0 mg/kg); other aspects of the anaesthetic and peri-operative management being comparable between the two groups.

5.5.4 Discussion of the hypotheses

The question being addressed in these trials is whether the use of a particular anaesthetic drug (given in a particular dose range) during surgery is likely to decrease the magnitude of the endocrine and metabolic responses of newborn infants. The questions of whether such changes in the endocrine and metabolic response will be beneficial or clinically important are not being investigated in these trials. The tacit assumption is that a decrease in the magnitude of the endocrine and metabolic changes would signify a decrease in postoperative catabolism, and, because of the reasons explained previously (Chapter II), this would be deemed as beneficial for the newborn infant undergoing surgery.

Furthermore, whether these endocrine-metabolic effects are a consequence of 'pain relief' during surgery or whether they are pharmacological effects of the anaesthetic drug itself, would be an unanswerable question from these studies since the two effects cannot be separated reliably. For this
reason, particular care was taken in selection of the anaesthetic agents to be investigated. Halothane and fentanyl were selected for the following reasons:

1. Both drugs can be safely given to neonates undergoing surgery, with minimal side-effects (Robinson and Gregory, 1981; Gregory et al, 1983).

2. Halothane and fentanyl are recommended as the drugs of first choice for non-opiate and opiate anaesthesia in paediatric patients, and hence are widely used in current anaesthetic practice (Warner et al, 1984; Wark, 1983; Hickey and Hansen, 1984; Robinson and Gregory, 1981). A beneficial effect of using these drugs for neonatal patients would be therefore readily acceptable to paediatric anaesthetists, who have been trained in their use.

3. In comparison with the endocrine and metabolic effects of surgery, halothane and fentanyl have relatively minor endocrine and metabolic effects. In this context, it is important to note that halothane has been shown to decrease oxygen uptake, gluconeogenesis, glycolysis and urea synthesis in hepatic cells, associated with a marked increase in lactate production (Biebuyck et al, 1972a).

Before starting each clinical trial, the benefits of this investigation were considered even if the result of the trial was negative, i.e., if the anaesthetic drugs used had no significant effect on the endocrine and metabolic parameters measured. Assuming such a result, the trials would still show that: (a) Halothane and fentanyl can/cannot be used safely in the doses described for the types of patients admitted to these trials; (b) If small differences exist, they were not marked enough to be detected by the numbers of neonates entered into these trials (trends detected in these trials could be used to predict the sample size required for a larger
clinical trial and also to identify those outcome measures which may be likely to produce fruitful results in future trials); and (c) If differences do not exist, it would suggest that these drugs (in the dosage and manner used) were not capable of altering the stress response of neonates and that other dosage schedules or other anaesthetic drugs or some non-anaesthetic means should be investigated for achieving the desired outcome. The decision of a positive or negative outcome of each trial would be based on a statistically significant difference in one or more of the selected outcome measures, whereas differences in other variables would be accepted as descriptive but not conclusive.

Thus, each randomised control trial was designed to answer a specific question; the criteria required for, and the implications of a positive or negative answer were outlined before the start of the trial.
CHAPTER VI: RANDOMISED TRIAL OF HALOTHANE ANAESTHESIA
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   6.3.5 Hypothesis
6.4 CONCLUSION
6.1 INTRODUCTION :-

The randomised trial of halothane anaesthesia was planned in order to investigate whether newborn infants require potent anaesthetic agents during surgery or not. The techniques currently used for neonatal anaesthesia were found to be based on principles and percepts evolved by Jackson Rees and his co-workers in the 1950s (Jackson Rees, 1950; Inkster, 1977). Since then, it was accepted that newborn infants were not capable of responding to the pain and stress of surgical trauma (Rackow et al, 1961; Bush and Stead, 1962; Calverley and Johnston, 1972; Downes and Raphaely, 1973; Ryan, 1975; Mircea and Balaban, 1975; Bennett et al, 1976; Vivori and Bush, 1977; Inkster, 1977; Brown and Fisk, 1979; Shaw, 1982; Betts and Downes, 1984). On the other hand, the use of halothane in neonates was documented in some reports (Ward et al, 1970; Yamamoto et al, 1972; Ryan, 1973; Goudsouzian et al, 1976; Steward et al, 1974; Salanitre and Rackow, 1977; Salem and Bennett, 1980; Tay, 1981; Dierdorf and Krishna, 1981); and the use of other agents such as ketamine or cyclopropane was also proposed on the basis of personal preference (Mohri et al, 1969; Steven et al, 1973; Radnay et al, 1974).

The reasons for the current practice of giving little or no anaesthesia to newborn infants undergoing surgery are mainly twofold:

(1) It was proposed that the neonatal brain is not capable of recognising and discriminating the painful stimuli during surgery. This concept arose from the observation that responses to painful cutaneous stimulation were attenuated in neonates (McGraw, 1963), and this was attributed to the lack of myelination in the central nervous system and to an absence of the memory of pain (McGraw, 1963; Dargassies, 1977).

(2) It was proposed that the margin of safety for the use of anaesthetic agents in newborn infants was narrow since, (a) it was found that neonates
and infants less than 6 months of age required greater amounts of halothane (Gregory et al, 1969; Nicodemus et al, 1969) or ketamine (Lockhart and Nelson 1974) to be effective against the same stimulus (skin incision) as compared to older children or adults; (b) it was found that the uptake of halothane was much faster in infants as compared to adults (Salanitre and Rackow, 1969; Eger et al, 1971) due to a larger ratio of alveolar ventilation to functional residual capacity, the delivery of a greater fraction of the cardiac output to highly perfused organs, and a greater cardiac output per kilogram body mass in neonates and infants; and (c) it was observed that halothane anaesthesia was more likely to cause myocardial depression (Diaz and Lockhart, 1979; Brandom et al, 1983), hypotension (Gregory, 1982; Brandom et al, 1983) and cardiac arrest (Rackow et al, 1961) in newborn infants. Thus, it was proposed that the greater requirement of anaesthetic agents in newborn infants, their faster uptake into the circulation and the increased susceptibility of neonates to potentially dangerous side effects, were all combined to produce a narrow margin of safety for the use of anaesthetic agents in neonates.

However, several of these concepts have been challenged in the light of recent observations. Firstly, Gregory et al (1983) found that newly born lambs had a 71% lower anaesthetic requirement when compared to lambs who were older than 12 hours of age (Gregory et al, 1983). These observations were confirmed subsequently in the human neonate. Neonates were found to have a significantly lower anaesthetic requirement than infants between 1 and 6 months of age and infants between 1 and 6 months of age were shown to have the highest anaesthetic requirement of any age group (Lerman et al, 1983). This data invalidated the previous studies by Gregory et al (1969) and Nicodemus et al (1969) since very few neonates were included in these studies and thus, the anaesthetic requirement for neonates had been
overestimated since it was assumed to be the same as that of older infants (Lerman et al, 1983).

As a consequence of this assumption, previous studies on the circulatory responses of neonates to halothane anaesthesia (Diaz et al, 1979; Rackow et al, 1961; Brandom et al, 1983) were performed at anaesthetic concentrations much in excess of their requirement. Thus, it was proposed that the cardiovascular depression documented in newborn infants during halothane anaesthesia was due to overdosage (Lerman et al, 1983).

It is suggested that the lower anaesthetic requirement of neonates (as compared to older infants) may be related to elevated plasma concentrations of β-endorphin and β-lipotropin that have been documented during the neonatal period (Moss et al, 1982; Facchinetti et al, 1982). However, there is no information to suggest that circulating concentrations of endogenous opiates in newborn infants would be sufficient to obviate the need for anaesthesia during prolonged and major surgery. Furthermore, a controlled comparison between neonates undergoing surgery with and without potent anaesthesia has not been attempted previously. Even in the absence of such information, most anaesthetists maintain that minimal anaesthesia is sufficient to relieve any pain or awareness that a neonate may perceive.

In order to resolve this controversy, it was considered necessary to measure the hormonal and metabolic responses of newborn infants who were randomly allotted to comparable anaesthetic regimens with or without the addition of a potent anaesthetic agent such as halothane.

**Trial protocol** :- The randomised trial of halothane anaesthesia was planned to include a total of 40 neonates undergoing surgery and was
expected to take approximately 1 year for completion. However, the rate of patient entry was lower than expected and the trial had to be terminated after the inclusion of 36 patients. The lower number of patients resulted in lowering the power of the trial from 80% to 77%. Thus, on the basis of outcome measures defined in Chapter V (plasma adrenaline, plasma noradrenaline, blood glucose and urinary 3-methylhistidine/creatinine ratio), the trial had a 77% chance of identifying a significant difference between the halothane and non-halothane anaesthesia groups, provided that the actual difference between the two groups was as large as that used to calculate the sample size. However, this degree of change in the power of the trial was not considered to be of major importance and it was decided that the null hypothesis would be accepted or rejected on the basis of the criteria outlined in Chapter V. Statistical comparison of the hormonal and metabolic responses was performed according to the randomised grouping of neonates, using the data from all neonates entered in this trial. As discussed in Chapter V, the 5 changes in hormonal and metabolite concentrations were considered to represent the "response" of the neonate to anaesthesia and surgery and these responses were compared between the two anaesthesia groups. (For reference, the absolute values of hormone and metabolite concentrations in neonates from the two anaesthesia groups are included in Appendix I.)

6.2 RESULTS OF THE HALOTHANE TRIAL

6.2.1 Description of patients and preoperative management :-

The characteristics of neonates in the randomised halothane and non-halothane anaesthesia groups are described in Table 6.1.

27 term and 9 preterm neonates undergoing surgery were included in the
halothane trial. Of the preterm neonates, 5 were randomly allocated to the halothane anaesthesia group and 4 to the non-halothane anaesthesia group. The gestation and birth weight of neonates in the halothane and non-halothane anaesthesia groups were similar and there were no significant differences in the post-natal age and weight of neonates in the two anaesthesia groups (Table 6.1).

The preoperative dextrose infusion rate was 3.5 ±0.4 mg/kg/min (mean ±SEM) in the halothane anaesthesia group and 3.3 ±0.5 mg/kg/min in the non-halothane anaesthesia group. The duration of starvation before surgery was identical (6 ±0.4 hours) in the two groups. Four neonates received parenteral nutrition during the days before surgery, all of whom were randomly allocated to the non-halothane anaesthesia group. Parenteral nutrition was not infused from 5 hours preoperatively up to the end of the study period.

6.2.2 Anaesthesia and clinical management during surgery :-

There were surprisingly few deviations from the anaesthetic protocol (Figures 6.1 and 6.2) amongst the neonates entered into the halothane trial. All neonates randomised to the halothane group received halothane 0.5-2% for induction and 0.5-1% for maintenance, which was given for three-quarters of the duration of the operative procedure in 83% patients. No patients in the non-halothane group received halothane.

However, there were two neonates in each of the randomised anaesthetic groups in whom the anaesthetic protocol was not followed strictly. In the halothane anaesthesia group, one neonate received pancuronium and another received atracurium as muscle relaxants during surgery; whereas in the non-halothane anaesthesia group both neonates received sodium thiopentone
for induction of anaesthesia, one of whom also received suxamethonium to facilitate endotracheal intubation. Nitrous oxide (50%) was given to neonates in both groups during surgery. The total dose of d-tubocurarine given to neonates in the halothane anaesthesia group (0.43 ± 0.06 mg/kg) was found to be significantly lower (p<0.025) than that for neonates in the non-halothane anaesthesia group (0.66 ± 0.07 mg/kg).

In the halothane anaesthesia group, the severity of surgical stress was Grade I (score 0-5) in 6 patients, Grade II (score 6-10) in 9 patients and Grade III (score 11-20) in 3 patients; whereas in the non-halothane anaesthesia group 6 patients were subjected to Grade I stress, 10 patients to Grade II stress and 2 patients to Grade III stress. There was no significant difference in the surgical stress scores obtained by neonates in the halothane and non-halothane anaesthesia groups (Table 6.1).

The mean dextrose infusion rate given during surgery was identical for neonates in the halothane and non-halothane anaesthesia groups. A blood transfusion during surgery was given to 5 neonates in the non-halothane anaesthesia group and to one neonate in the halothane anaesthesia group. The mean temperature loss during surgery was identical in neonates from the two anaesthesia groups (Table 6.1).

Thus, the preoperative condition, clinical management before and during surgery and the degree of surgical stress experienced by patients in both randomised anaesthetic groups were similar.

6.2.3 Hormonal changes :-

The hormonal responses of neonates in the halothane and non-halothane anaesthesia groups are compared in Tables 6.2 and 6.3.
Plasma adrenaline concentrations increased during surgery in the halothane and non-halothane anaesthesia groups, but the magnitude of intra-operative increase was significantly greater in the non-halothane anaesthesia group (p<0.05) in comparison with the response of neonates in the halothane anaesthesia group. At 6, 12 and 24 hours after surgery, there were no significant differences in the responses of neonates in the two groups.

Plasma noradrenaline concentrations increased during surgery in neonates from both anaesthesia groups. However, the response of neonates in the non-halothane anaesthesia group was more than three times greater than that of neonates in the halothane anaesthesia group; this difference between the two groups was highly significant (p<0.005). After surgery, noradrenaline values had decreased below the preoperative value in both groups and there was no significant difference between the two groups of neonates.

Plasma insulin concentrations increased during surgery and were maintained above preoperative concentrations at 6, 12 and 24 hours after surgery in neonates from both anaesthesia groups. However, the increase in plasma insulin concentrations at 6 hours after surgery was significantly greater in neonates from the non-halothane anaesthesia group (p<0.05) as compared to neonates in the halothane anaesthesia group.

Plasma glucagon concentrations were increased slightly at the end of surgery in the halothane and the non-halothane anaesthesia groups and were found to be decreased below the preoperative concentrations in both groups at 12 and 24 hours after surgery. There were no significant differences in plasma glucagon changes between neonates in the two anaesthesia groups.
At the end of surgery, the insulin/glucagon ratio was unaltered in the halothane anaesthesia group and was found to be substantially decreased in the non-halothane anaesthesia group, thus giving rise to a significant difference (p<0.05) between the responses of neonates in the two groups. Postoperative changes in the insulin/glucagon ratio were not significantly different between the two groups.

Plasma aldosterone concentrations were increased above preoperative values at the end of surgery and during the postoperative period in the halothane and non-halothane anaesthesia groups. There were no significant differences in the responses of neonates in the two anaesthesia groups.

Plasma corticosterone concentrations were increased substantially in both groups of neonates at the end of surgery and remained elevated at 6 and 12 hours after surgery; by 24 hours after surgery plasma corticosterone had returned to the respective preoperative value in both anaesthesia groups; there were no significant differences in the responses of neonates in the two anaesthesia groups.

Plasma 11-deoxycorticosterone (DOC) concentrations increased during surgery in the halothane and non-halothane anaesthesia groups but had returned to preoperative values by 12 hours after surgery. There were no significant differences in the plasma DOC responses of neonates in the two anaesthesia groups during or after surgery.

Plasma progesterone concentrations were found to be significantly lower before the start of surgery (p<0.05) in the halothane anaesthesia group as compared to the non-halothane anaesthesia group. In the halothane anaesthesia group, plasma progesterone concentrations increased during and
after surgery, whereas they were decreased in the non-halothane anaesthesia group; these responses were significantly different at the end of surgery (p<0.02) and at 6 hours after surgery (p<0.005). There were no significant differences in the progesterone responses of neonates in the two groups at 12 and 24 hours after surgery.

Plasma 17-hydroxyprogesterone concentrations increased during surgery in both anaesthesia groups, but remained elevated at 6 hours after surgery in the halothane anaesthesia group and decreased below the preoperative values in the non-halothane anaesthesia group; this difference in responses was significant (p<0.02). The changes in plasma 17-hydroxyprogesterone values were not significantly different between neonates in the two anaesthesia groups at 12 and 24 hours after surgery.

No significant differences were found between the responses of neonates in the halothane and non-halothane anaesthesia groups with respect to changes in plasma 11-deoxycortisol concentrations at the end of surgery or in the postoperative period.

Plasma cortisol concentrations increased during surgery in neonates from both anaesthesia groups; but this response was significantly greater in the non-halothane anaesthesia group (p<0.05) compared to the halothane anaesthesia group. A similar difference between the cortisol responses of neonates in the two anaesthesia groups was maintained after surgery, but this was significant only at 12 hours after surgery (p<0.05). By 24 hours following surgery, plasma cortisol concentrations had decreased below preoperative values in both groups of neonates.

Plasma cortisone concentrations decreased during surgery in the halothane
and non-halothane anaesthesia groups and remained below the respective preoperative values at 6, 12 and 24 hours after surgery. There were no significant differences between the responses of neonates in the two anaesthesia groups at the end of or after surgery.

Thus, the hormonal response of neonates in the halothane anaesthesia group was diminished with respect to changes in plasma adrenaline, noradrenaline and cortisol concentrations in comparison with the response of neonates in the non-halothane anaesthesia group. In addition, there were significant differences between the responses of neonates in the two anaesthesia groups in plasma insulin, progesterone, 17-hydroxyprogesterone and the insulin/glucagon ratio changes during and after surgery. It is probable that these differences in the hormonal response may be responsible for altering the metabolic response of neonates exposed to surgical stress.

6.2.4 Metabolite changes :-

The changes in metabolic variables are compared between neonates in the halothane and non-halothane anaesthesia groups in Tables 6.4, 6.5 and 6.6.

Blood glucose concentrations increased during surgery in both anaesthesia groups, but the hyperglycaemic response of neonates in the non-halothane anaesthesia group was significantly greater (p<0.025) than that of neonates in the halothane anaesthesia group. At 6, 12 and 24 hours after surgery, there were no significant differences in the blood glucose changes of neonates in the two anaesthesia groups.

Blood lactate and pyruvate concentrations were increased at the end of surgery in the halothane and non-halothane anaesthesia groups and there were no significant differences in the responses of neonates in the two
groups. In the postoperative period, blood lactate and pyruvate concentrations decreased in the non-halothane anaesthesia group and remained elevated in the halothane anaesthesia group, but these differences in the responses of neonates from the two anaesthesia groups were not significant.

Blood concentrations of 3-hydroxybutyrate increased during surgery in the halothane and non-halothane anaesthesia groups and there was no significant difference in the responses of the two groups at the end of or after surgery. Blood acetoacetate concentrations did not change from preoperative values in the halothane anaesthesia group during surgery, but were increased slightly in the non-halothane anaesthesia group at the end of surgery; the difference in these responses was significant (p<0.02). Similarly, total ketone bodies increased during surgery in both groups and the intra-operative increase in the non-halothane anaesthesia group was greater (p<0.05) than that of the halothane anaesthesia group.

Blood alanine concentrations were increased in response to surgery in the halothane anaesthesia group and remained unchanged in the non-halothane anaesthesia group, with significant differences between the responses of neonates in the two anaesthesia groups at the end of surgery (p<0.01) and at 6 hours postoperatively (p<0.02). At 12 and 24 hours after surgery, there was no significant difference in the blood alanine changes of neonates in the halothane and non-halothane anaesthesia groups.

The blood glycerol changes during and after surgery were identical in neonates from the two anaesthesia groups; blood glycerol concentrations were increased at the end of surgery and had returned to the preoperative values at 6, 12 and 24 hours after surgery in both groups.
Plasma non-esterified fatty acids were increased in response to surgery in both anaesthesia groups, but the response of neonates in the non-halothane group was found to be markedly greater than that of neonates in the halothane anaesthesia group at the end of surgery (p<0.01) and at 6 hours postoperatively (p<0.05). At 12 and 24 hours after surgery there were no significant differences in the plasma non-esterified fatty acid changes between neonates in the two anaesthesia groups.

Plasma triglyceride concentrations were decreased below the respective preoperative value in both anaesthesia groups at the end of surgery and postoperatively; no significant differences were found between the responses of neonates in the halothane and non-halothane anaesthesia groups before or after surgery.

The insulin/glucose ratio remained unaltered during and after surgery in the halothane and non-halothane anaesthesia groups and there were no significant differences between the responses of neonates in the two anaesthesia groups.

Total gluconeogenic substrates were increased at the end of surgery in the halothane and non-halothane anaesthesia groups. At 6 hours postoperatively however, total gluconeogenic substrates were substantially decreased in the non-halothane anaesthesia group, whereas they remained elevated in the halothane anaesthesia group. This difference was significant (p<0.05) between neonates in the two anaesthesia groups.

Peri-operative changes in the lactate/pyruvate ratio, the hydroxybutyrate/acetocetate ratio and the alanine/pyruvate ratio were not significantly
different between neonates in the halothane and non-halothane anaesthesia groups. The hydroxybutyrate/acetoacetate ratio increased during surgery from the respective preoperative values in both groups of neonates (p<0.05) but had reverted to the preoperative values by 6 hours after surgery. The alanine/pyruvate ratio was found to be decreased at 6 hours after surgery (p<0.01) in the halothane anaesthesia group, but was not significantly different from that of the non-halothane group.

Thus, the metabolic response of neonates in the halothane anaesthesia group was decreased with respect to changes in concentrations of blood glucose, ketone bodies and plasma non-esterified fatty acids at the end of surgery as compared to the response of neonates in the non-halothane anaesthesia group. In the postoperative period, concentrations of total gluconeogenic substrates and some individual gluconeogenic substrates such as alanine, lactate and pyruvate were elevated in the halothane anaesthesia group as compared to corresponding changes in the non-halothane anaesthesia group.

6.2.5 Urinary nitrogenous constituents:

The 3-methylhistidine/creatinine (3-MH/Cr) ratios measured in urine samples collected during the three days following surgery from neonates in the two anaesthesia groups are compared in Table 6.7.

The urinary 3-MH/Cr molar ratio was not changed during the postoperative period in urine collected from neonates in the halothane anaesthesia group. In the non-halothane anaesthesia group, the urinary 3-MH/Cr ratio was increased significantly on the second (p<0.05) and third (p<0.01) postoperative days compared to values obtained on the first postoperative day. There were no significant differences between the urinary 3-MH/Cr ratios measured in neonates from the two anaesthesia groups.
6.2.6 Clinical observations :-
During the surgical procedure, all neonates included in the halothane trial had routine monitoring of heart rate, respiration, ECG and rectal temperature.

The preoperative heart rate was found to be significantly higher in the halothane anaesthesia group (p<0.01) as compared to the non-halothane anaesthesia group. The heart rate increased with the start of surgery in both anaesthesia groups, but the maximum heart rate during surgery was significantly higher (p<0.0001) in neonates from the non-halothane group as compared to neonates in the halothane anaesthesia group. In addition, a larger number of neonates in the non-halothane anaesthesia group responded to noxious stimulation with bradycardia and thus the minimum heart rate during surgery was significantly lower in the non-halothane anaesthesia group as compared to the halothane anaesthesia group (p<0.05). The temperature changes during surgery in the two anaesthesia groups were identical.

In the postoperative period, it was found that the clinical state of neonates in the halothane anaesthesia group was relatively more stable than that of neonates in the halothane anaesthesia group. This was evident from the incidence of intra-operative and postoperative complications documented in the two anaesthetic groups, which are listed in Table 6.8.

6.2.7 Postoperative clinical management :-
Some aspects of the postoperative clinical management of neonates in the halothane and non-halothane anaesthesia groups is compared in Table 6.7.
The clinical staff responsible for the postoperative care of these neonates were 'blind' to the anaesthetic management allocated to each neonate. There was no significant difference in the rates of dextrose infusion given to neonates in the halothane and non-halothane anaesthesia groups during the three postoperative days. Postoperative analgesia was prescribed to 14, 9 and 2 neonates on each of the 3 postoperative days respectively. It was found that a larger number of patients in the non-halothane anaesthesia group required analgesia and received a relatively larger total dose of morphine sulphate on each postoperative day as compared to neonates in the halothane anaesthesia group. Furthermore, the timing of first analgesic dose was significantly earlier in the non-halothane anaesthesia group as compared to neonates in the halothane anaesthesia group (p<0.05).

6.3 DISCUSSION:

Apart from differences in the anaesthetic management, the neonates randomly allocated to the halothane and non-halothane anaesthesia groups were similar in their characteristics, they had undergone similar degrees of surgical stress and their pre-operative and intra-operative clinical management was identical with respect to those factors which may influence their hormonal and metabolic response to surgical stress.

The primary difference in their anaesthetic management was the inclusion of halothane in the anaesthetic regimen for one group of neonates and not for neonates in the other group. It was found that neonates in the non-halothane anaesthesia group responded to surgical stimulation with muscular movement or a generalised increase in muscular tension, thereby making performance of the surgical procedures more difficult. This problem was usually resolved by injecting supplementary doses of d-tubocurarine
during the surgical procedure. Thus, a secondary difference between the two
groups appeared, since neonates in the non-halothane anaesthesia group
received a significantly higher total dose of d-tubocurarine during surgery
as compared to neonates he halothane anaesthesia group.

In newborn infants, muscular activity during anaesthesia with nitrous oxide
alone has been observed previously (Inkster, 1977; Salem and Bennett, 1980)
and it is proposed that this clinical response was due to the incomplete
loss of awareness in neonates who were not given halothane anaesthesia
(Saunders, 1981). This response, in itself, may point towards the need for
potent anaesthesia in newborn infants undergoing surgery. On the other
hand, it could be argued that differences in the hormonal and metabolic
responses observed in this trial were not only due to the effect of
halothane, but were also contributed to by giving excess d-tubocurarine to
the group of neonates who did not receive halothane. However, in such a
clinical situation, the safe and efficient performance of surgery is the
most important consideration for any neonatal patient and it would be
difficult and ethically unjustified to avoid the use of muscle relaxants
whenever required.

Furthermore, this randomised trial was designed to compare 'the policy of
giving potent anaesthesia' (halothane and nitrous oxide) as against 'a
policy of not giving potent anaesthesia' (only nitrous oxide) to newborn
infants undergoing surgery (for theoretical considerations on clinical
trial methodology, see Peto et al (1976)). Thus, if the policy of not
giving potent anaesthetic agents to newborn infants is associated with
the use of relatively larger amounts of muscle relaxant drugs, it follows
that an investigation of the overall hypothesis should not be affected by
the occurrence of such a difference.
Therefore, it was decided that: (1) the conclusion based on rejection or acceptance of the hypothesis would not be affected by differences in the dosage of muscle relaxants during surgery, and (2) speculation on the mechanisms responsible for the hormonal and metabolic changes documented would take in account the effects of halothane, nitrous oxide and d-tubocurarine in one group of neonates and the effects of nitrous oxide and a greater amount of d-tubocurarine in the other group of neonates.

6.3.1 **Hormonal changes** :-

**CATECHOLAMINES**

The most prominent difference between the hormonal responses of neonates in the halothane and non-halothane anaesthesia groups was in the magnitude of the intra-operative catecholamine response. Neonates in the non-halothane anaesthesia group mounted an adrenaline response that was approximately twice that of neonates in the halothane group and a noradrenaline response which was three times that of neonates in the halothane group. These differences could be either due to the effects of halothane to given neonates in the halothane anaesthesia group or, less likely, may be related to the larger total dose of d-tubocurarine given to neonates in the non-halothane anaesthesia group.

It is probable that the pain relief and loss of awareness provided during surgery by halothane anaesthesia to the neonates may be responsible for these differences. In this context, it may be noted that surgical skin incision under halothane anaesthesia produced an 'EEG activation response' characterised by low-voltage fast waves in adults and high-voltage slow waves in young children, but produced no response in infants less than 1 year of age (Oshima et al, 1981). Since the EEG activation was associated
with increases in the heart rate, arterial pressure and pupillary dilatation (Oshima et al, 1981), this evidence may indirectly suggest that the lack of EEG activation in neonates and infants may also represent a central inhibition of sympatho-adrenal activation in response to surgery.

On the other hand, there is evidence to suggest three related mechanisms by which halothane anaesthesia may inhibit the catecholamine response to surgical stress: (1) as a result of direct inhibition of catecholamine release from chromaffin cells in the adrenal medulla and elsewhere, (2) by the central inhibition of opioid receptors or (3) by the release of endogenous opioids by halothane anaesthesia.

The direct inhibition of catecholamine secretion by halothane may be inferred from experimental studies as well as studies on adult patients undergoing surgery. Perry et al (1974) studied the effects of halothane on the sympatho-adrenal responses of dogs and found significant decreases in the catecholamine concentrations and blood pressure during halothane anaesthesia. Roizen et al (1974) studied the effect of halothane anaesthesia on changes in plasma catecholamines in rats and found that halothane decreased the concentrations of adrenaline and noradrenaline in a dose-related manner. In a subsequent study on adult patients, Roizen et al (1981) found that halothane, enflurane or morphine in appropriate doses could be used to abolish the catecholamine response to surgical incision. In several studies, halothane anaesthesia in adult patients was found to decrease plasma catecholamine concentrations after induction of anaesthesia and before the start of surgery (Halter et al, 1977; Hoar et al, 1980, Kono et al, 1981; Russell et al, 1981; Philbin et al, 1981). On the other hand, conflicting results were reported by Joyce et al (1982) who found an increase in plasma noradrenaline concentrations stimulated by
halothane anaesthesia. Some studies using a fluorimetric assays have failed
to find any increases in plasma adrenaline and noradrenaline concentrations
in adult patients undergoing surgery (Butler et al, 1977; Kehlet et al,

These conflicting results may result from the direct and indirect effects
of halothane anaesthesia on catecholamine secretion. In addition to the
direct suppressive effect on catecholamine secretion, halothane anaesthesia
may cause a decrease in blood pressure due to the reduction of myocardial
contractility (Smith 1981) and inhibition of baroreceptor responses (Duke
et al, 1977). The resulting hypotension may reflexly stimulate the release
of catecholamines. The opposing direct and indirect effects of anaesthetic
induction with halothane may be further confounded by the stimulation of
catecholamine secretion due to tracheal intubation (Russell et al, 1981;
Cummings et al, 1983; Derbyshire et al, 1983).

Alternative mechanisms responsible for the effect of halothane on
catecholamine release may be through the central inhibition of opioid
receptors or the release of endogenous opioids by halothane anaesthesia.
This was first proposed by Finck et al, who found that the analgesic
effects of halothane, enflurane or cyclopropane in rats were antagonised by
naloxone (Finck et al, 1977). Similar results had been found with nitrous
oxide (Berkowitz et al, 1976; Berkowitz et al, 1977). In dogs, it was found
that the cardiovascular and hypnotic effects of halothane anaesthesia were
inhibited when naloxone was perfused through the 4th ventricle (Arndt and
Freye, 1979a and 1979b). From this study, it was concluded that opiate
receptors in the structures bordering the 4th ventricle may mediate the
anaesthetic effects of halothane (Arndt and Freye, 1979).
Recently, Inoki et al (1983) have found that halothane decreases the binding affinity of δ-opioid receptors in the rat brain, but has no effect on the μ-, κ- or σ-opioid receptors; the binding affinities and density of which were altered by methoxyflurane anaesthesia. Although the anti-nociceptive responses induced by stress are widely believed to be a function of μ₁-opioid receptors (Fleuvry, 1983), evidence for the involvement of opioid δ-receptors has been provided by a recent study in mice (Hart et al, 1983).

However, some conflicting results have also been reported from comparable studies. For example, it was found that naloxone had little influence on the anaesthetic effects of halothane in rats (Harper et al, 1978; Bennett et al, 1978) or mice (Smith et al, 1978) or dogs (Pace and Wong, 1979). In addition, Way et al (1982) failed to observe an increase of beta-endorphin concentrations in the cerebrospinal fluid following halothane anaesthesia in human subjects (Way et al, 1982). In addition, it was found that intraventricular administration of human β-endorphin to rats causes a marked stimulation of central sympathetic outflow from the hypothalamic centers and the release of catecholamines (Van Loon et al, 1981).

Alternatively, the possible release of β-endorphin into the peripheral circulation may be responsible for suppression of the catecholamine response by halothane. Evidence for this mechanism has been provided by the recent study of Maiewski et al (1984), who found that halothane anaesthesia caused a 3-fold increase in the plasma β-endorphin immunoreactivity in rats which returned to control values by 30 minutes after induction of anaesthesia (Maiewski et al, 1984). Several studies have shown that circulating β-endorphin binds to the μ₁-opioid receptors on chromaffin cells in the adrenal medulla and elsewhere, resulting in an inhibition of
catecholamine release (Kumakura et al, 1980; Costa et al, 1980; Lemaire et al, 1980).

On the other hand, it is possible that the difference in dosage of d-tubocurarine between the two anaesthesia groups was responsible for these differences in the catecholamine response. Moss et al (1981) found that d-tubocurarine stimulated histamine release in adult patients undergoing surgery and this was a dose-related effect. However, in this study it was also documented that the raised histamine concentrations had returned to control values within 5 min after the injection of d-tubocurarine and did not cause any consistent changes in the heart rate or blood pressure (Moss et al, 1981). In addition, differences in the dose of d-tubocurarine which were similar to the difference in dosage between the halothane and non-halothane anaesthesia groups in this study were found to cause only a slight and insignificant difference in the histamine release of adult patients (Moss et al, 1981). Therefore, it is highly unlikely that hypotension stimulated by histamine release could be responsible for the increased catecholamine response of neonates in the non-halothane anaesthesia group.

Thus, the decreased adrenaline and noradrenaline responses of neonates included in the halothane anaesthesia group may be due either to a direct inhibition of the sympathoadrenal response by halothane or an indirect effect mediated through the central inhibition of opioid receptors or the release of endogenous opioids into the peripheral circulation.

INSULIN

Plasma insulin concentrations increased in response to the hyperglycaemia during surgery in both groups and there was no difference in the responses
of neonates in the halothane and non-halothane anaesthesia groups at the end of surgery. However, it is of interest to note that the insulin/glucose molar ratio had decreased in both groups at the end of surgery, implying the inhibition of an adequate insulin response to the degree of hyperglycaemia in both the anaesthesia groups. It may be speculated that this effect may be due to the direct inhibition of insulin secretion by the substantial adrenaline release in the non-halothane anaesthesia group (Sperling et al. 1984), whereas in the halothane anaesthesia group, insulin secretion may be decreased either due to a direct effect of halothane on insulin secretion as has been demonstrated in the perfused rat pancreas (Aynsley-Green et al. 1973) or due to the smaller adrenaline release in this group of neonates.

At 6 hours postoperatively, raised plasma insulin concentrations were maintained in neonates in the non-halothane anaesthesia group, whereas they had returned towards preoperative values in the halothane anaesthesia group. It is possible that at 6 hours after surgery, the inhibition of insulin secretion was overcome by the stimulatory effect of the marked surgical hyperglycaemia found in neonates from the non-halothane anaesthesia group (Sperling et al. 1984).

GLUCAGON

The changes in plasma glucagon concentration were not significantly different between the neonates in the two anaesthesia groups. Although an increase in glucagon values during surgery was observed in neonates from the non-halothane anaesthesia group, this was not sufficient to give rise to significant differences between the two groups. In neonates from both anaesthesia groups, a decrease in plasma glucagon concentrations was found at 12 and 24 hours after surgery, which confirmed the pattern obtained in
the preliminary study. This feature of the neonatal response is in contrast to the response of adult patients undergoing surgery, in whom a marked increase in plasma glucagon concentrations has been documented between 12 and 24 hours postoperatively (Russell et al, 1975).

In contrast to the individual hormonal changes, changes in the insulin/glucagon ratio were substantially different between neonates in the halothane and non-halothane anaesthesia groups. Probably as a result of intra-operative adrenaline secretion, the insulin/glucagon ratio was decreased in the non-halothane anaesthesia group, whereas it was unchanged in the halothane anaesthesia group. It has been proposed that changes in this ratio may be of greater importance in the control of glucose homeostasis in newborn infants (Sperling, 1982) than changes in the individual hormones themselves; and a decrease in the insulin/glucagon ratio would favour the development of a marked hyperglycaemic response (Patel et al, 1982; Sperling, 1982).

STEROID HORMONES
Since the pattern of adrenocorticoid responses in neonates undergoing surgery may be altered by the presence of a large fetal zone and a less developed definitive adrenal cortex, it was considered necessary to measure most of the glucocorticoids, mineralocorticoids and precursor hormones in the present study. Since similar data from newborn infants undergoing surgery have not been published previously, these responses have been compared to unpublished data collected by Golder (MD Thesis, 1982) from the study of older infants (mean age 8.6 months, N = 7) undergoing abdominal surgery and to the stress response of adult patients undergoing surgery; in addition, data from neonates exposed to other forms of stressful stimuli have been reviewed.
Glucocorticoids: In both anaesthesia groups, cortisol and corticosterone increased markedly during surgery and remained elevated at 6 hours after surgery. On the other hand, plasma cortisone concentrations were decreased substantially in both groups after surgery whereas plasma concentrations of 11-deoxycortisol did not change from the respective preoperative values in both groups of neonates.

At the end of surgery, the plasma cortisol response of neonates in the non-halothane anaesthesia group was significantly greater than neonates in the halothane anaesthesia group. Similar differences between the two groups were found at 12 hours postoperatively, since cortisol values had decreased below the preoperative concentration in the halothane anaesthesia group and remained elevated in the non-halothane anaesthesia group. These differences are in keeping with the catecholamine responses of neonates in the two anaesthesia groups and may be a consequence of the loss of pain and awareness provided by halothane anaesthesia during surgery.

Recently, similar findings from older infants undergoing surgery (mean age 7.3 months) have been reported by Obara et al (1984) who found that plasma cortisol concentrations during surgery and at the end of surgery were significantly greater in infants given nitrous oxide and pancuronium during surgery as compared to those given halothane, nitrous oxide and pancuronium (Obara et al, 1984). In adult patients, Werder et al (1970) found that the plasma concentrations of cortisol were decreased during prolonged halothane anaesthesia without surgery. Furthermore, Nishioka et al (1968) found that the increase in plasma corticosteroids in response to an injection of tetracosactrin was reduced during halothane anaesthesia.
Changes in the plasma concentration of the other glucocorticoids, namely, corticosterone, 11-deoxycortisol and cortisone were similar in neonates from the halothane and non-halothane anaesthesia groups.

Colle et al (1960) measured 17-hydroxycorticosteroids (17-OHCS) in the urine of 9 neonates undergoing surgery and found that those operated within one week after birth did not respond to surgery with a change in urinary 17-OHCS excretion, whereas of the 4 neonates operated after the first week of post-natal life, 3 responded to surgery with an increase in the urinary excretion of 17-OHCS. They concluded that neonates were not capable of responding to surgical trauma in the first week after birth (Colle et al, 1960). These results were contradicted by Haugen et al (1967) who, in a single neonate operated on the first day after birth, found that the plasma 17-OHCS concentration was markedly increased after surgery and the urinary excretion of 17-OHCS was increased from the 3rd to the 6th postoperative day. Steenberg et al (1966) measured 11-hydroxycorticosteroids in the plasma of 4 newborn infants undergoing surgery and found distinct increases during surgery which reverted to normal within 8 hours after surgery in 3 neonates, but they were elevated in one neonate at 10 hours after surgery.

In the recent study by Obara et al (1984), plasma cortisol concentrations in 7 neonates undergoing surgery were found to be raised during the surgical procedure and at the end of surgery, although these increases were not statistically significant due to a wide variation in their observations. They proposed that the hypothalamic-pituitary system or the adrenal cortex of neonates less than one week of age were not capable of responding to surgical stimuli (Obara et al, 1984).

There is incontrovertible evidence in the literature to refute this
conclusion of Obara et al (1984). Several studies have shown that the neonatal adrenal cortex at birth is capable of responding to ACTH (Winter, 1982) as well as to the stimuli of birth, respiratory distress, etc. (Sippell et al, 1979; Baden et al, 1973) and also has a normal cortisol production rate (Kenny et al, 1963).

In the neonates studied by Obara et al (1984), mean plasma cortisol increased from 494 nmol/l before surgery to 1330 nmol/l during surgery and 930 nmol/l at the end of surgery. In comparison, mean plasma cortisol increased from 342 nmol/l before surgery to 988 nmol/l at the end of surgery in the non-halothane group of neonates from the present study. The anaesthetic management of neonates studied by Obara et al (1984) was similar to that of neonates in the non-halothane anaesthesia group. Thus, although the changes in plasma cortisol were similar, the lack of significant findings in the former study were probably due to the small number of patients studied and a wide variation in the data obtained.

In older infants undergoing surgery, Golder (1982) found that plasma cortisol concentrations increased markedly during surgery and remained elevated for more than 72 hours after surgery. A substantial increase in plasma corticosterone concentrations which was also observed, had reverted to preoperative values by 24 hours after surgery.

In adult patients, it is well-known that surgical trauma causes a marked increase in plasma 11-hydroxycorticosteroids (Johnston, 1964; Lush et al, 1972; Clarke et al, 1974; Cosgrove and Jenkins, 1974), which is mainly due to an increase in the plasma concentrations of cortisol (Bromage et al, 1971; Bowen and Richardson, 1974; Engquist et al, 1981; Cooper et al, 1981; Moore et al, 1981; Haxholdt et al, 1981; Cowen et al, 1982; Bent et al, 1984).
The magnitude and duration of the raised cortisol concentrations have been related to the severity of injury (Nistrup-Madsen et al, 1976; Alberti et al, 1980; Foster et al, 1979; Batstone et al, 1976) in the absence of postoperative complications.

The increase in plasma cortisol concentrations in neonatal patients undergoing surgery documented in the present study was greater in magnitude than the response of adult patients undergoing similar degrees of surgical stress (Cooper et al, 1981; Haxholdt et al, 1981; Bent et al, 1984). However, peak cortisol concentrations in adult patients undergoing surgery are reached in the postoperative period and usually exceed the levels documented in newborn infants (Bromage et al, 1971; Engquist et al, 1981; Cooper et al, 1981; Moore et al 1981, Cowen et al, 1982). In addition, the response of newborn infants was very short-lived as compared to the adult response, in whom elevated cortisol levels may persist for more than 48 hours following surgery (Bromage et al, 1971; Kehlet and Binder, 1973; Oyama et al, 1977). This pattern of changes in neonates undergoing surgery has been observed with respect to several features of the hormonal and metabolic stress response (see Chapter IV).

The metabolic effects of elevated glucocorticoid levels may in fact be much greater than expected since the relative proportion of the unbound and physiologically active hormones is also elevated. This effect has been demonstrated in adult patients undergoing surgery (Uozumi et al, 1972) but may be further accentuated in newborn infants due to the decreased transcortin levels found in neonatal plasma (Hadjian et al, 1975).

Mineralocorticoids: Plasma aldosterone and 11-deoxycorticosterone (DOC) concentrations increased in response to surgery and there was no difference
between the responses of neonates in the halothane and non-halothane groups. From these responses it would appear that the secretion of aldosterone and DOC during and after surgery may be responsible for the postoperative sodium and water retention and potassium excretion that have been documented in several studies on newborn infants undergoing surgery (Rickham, 1957; Colle and Paulsen, 1959; Wilkinson et al, 1965; Knutrud, 1965; Suzuki et al, 1968; Bennett et al, 1970). Furthermore, these changes may be related to the studies of Kotchen et al (1972) who found that plasma renin activity and angiotensin II concentrations were elevated in newborn infants as compared to adult control values.

In some studies on neonates undergoing surgery, changes in water and electrolyte excretion have been related to the measurement of 17-hydroxycorticosteroids in urine (Colle et al, 1960; Haugen et al, 1967) and, of a single case, in plasma (Haugen et al, 1967). Bennett et al (1971) measured the urinary excretion of aldosterone on the third postoperative day in 15 neonates undergoing surgery and on the basis of insubstantial evidence, concluded that the neonate responds to sodium depletion with an increased secretion of aldosterone and can regulate urinary osmolality, excrete electrolytes and conserve water as required within wide limits (Bennett et al, 1971; Bennett et al, 1970; Bennett, 1975). As discussed earlier (see Chapter I) these conclusions are not justified from the data obtained and are not considered valid due to several flaws in this study.

Golder (1982) found an increase in plasma aldosterone concentrations during surgery in some cases, which was maintained up to 12 hours after surgery. Similarly, Enquist et al (1978) found a significant increase in the plasma aldosterone concentration of adult patients undergoing surgery which was maintained for 6 hours postoperatively. In addition, Moore et al (1985) and
Fragen et al (1984) have shown recently that plasma aldosterone concentrations in adult patients undergoing major abdominal surgery were increased during surgery and remained elevated for up to 4 hours after surgery. Although the pattern of changes in older infants and adults undergoing surgery is similar to that documented from newborn infants in the present study, the magnitude of the neonatal aldosterone response is much greater than the responses of older infants and adult patients undergoing surgery. An activation of the renin-angiotensin system during surgery in adult patients has been shown in several studies (Bevan et al., 1975; Jacubowski and Taube, 1974; Robertson and Michelakis, 1972), and is thought to be responsible for increases in plasma aldosterone values.

**Precursor Hormones**: Plasma 17-hydroxyprogesterone concentrations (17-OHP) increased marginally during surgery in both anaesthesia groups, but at 6 hours after surgery 17-OHP values remained elevated in the halothane anaesthesia group and had decreased below the preoperative concentration in the non-halothane anaesthesia group. A possible explanation for this difference could be the rapid conversion of 17-OHP into the further products of steroid biosynthesis, the concentrations of which were found to be elevated to a greater extent in neonates from the non-halothane group as compared to the halothane group.

Plasma 17-OHP values in neonates born by emergency caesarian section after fetal distress were not altered in comparison with neonates born by elective caesarian section (Sippell et al., 1979). Recently, Murphy et al (1983) have found that plasma 17-OHP concentrations were significantly raised in sick term and preterm neonates as compared to the values obtained from normal term and preterm controls; they concluded that the raised plasma OHP values, particularly in the preterm neonates, represented a
response to the stress of illness (Murphy et al, 1983).

The lack of a distinct increase in plasma 17-OHP during surgery in this study is contrary to these findings, probably since the responses studied by Murphy et al (1983) were due to the chronic stress of continuing illness, whereas the present study is concerned with the response of newborn infants to an acute and well-defined stressful stimulus: surgical trauma. It is likely that there would be major qualitative differences in the responses to these two clinical situations. Furthermore, in the study by Murphy et al (1983), longitudinal measurements were not obtained from the same neonate when stressed and unstressed and the comparison of randomly obtained cross-sectional measurements from separate groups of neonates may be influenced by a variety of factors apart from the stress of chronic illness. However, Golder (1982) found a marked increase in plasma 17-OHP during surgery and a further increase in the early postoperative period. In adult patients undergoing surgery (Moore et al, 1985), plasma 17-OHP values increased markedly during surgery and remained elevated for more than 10 hours after surgery. Thus, the proposal of Murphy et al (1983) that changes in plasma 17-OHP are responsive to stress may need further investigation in neonates undergoing greater degrees of surgical stress.

Plasma progesterone concentrations before surgery were significantly higher in the non-halothane anaesthesia group as compared to the halothane group. This finding was probably related to the inclusion of 7 neonates less than 3 days of age in the non-halothane anaesthesia group, whereas 4 neonates in the halothane anaesthesia group were less than 3 days of age. Sippell et al (1978) have shown that newborn infants at birth have a high circulating concentration of plasma progesterone, which is believed to be mainly of
placental origin. Progesterone concentrations decrease rapidly by about 3 orders of magnitude soon after birth and are stable thereafter (Sippell et al., 1978). Thus, the presence of progesterone of placental origin would be the most likely reason for this difference before surgery in neonates from the two anaesthesia groups.

Furthermore, the decrease of progesterone concentrations after surgery in the non-halothane anaesthesia group probably denotes a steady excretion of the placental hormone during the postoperative period, whereas the minor increase in neonates from the halothane group may represent the changes in response to surgery. The secretion of ACTH in response to surgical stress which has been documented in adult patients (Newsome and Rose, 1971) may occur in newborn infants as well and would stimulate the secretion of progesterone and other corticosteroids from the adrenal cortex (Kenny et al., 1963; Steenberg et al., 1966). In older infants undergoing surgery, Golder (1982) found that plasma progesterone increased during surgery and in three infants, but remained unchanged in the other cases.

Thus, the hormonal stress response to surgery was diminished with respect to changes in plasma adrenaline, noradrenaline and cortisol concentrations and altered with respect to changes in plasma insulin and the insulin/glucagon ratio in neonates who were given halothane anaesthesia during surgery. It is likely that these characteristic hormonal changes were responsible for differences in the metabolic response of neonates from the halothane and non-halothane anaesthesia groups.

6.3.2 Metabolite changes :-

GLUCOSE

Probably as a result of differences in the hormonal response of neonates in
the halothane and non-halothane groups, the hyperglycaemic response of neonates in the non-halothane anaesthesia group was significantly greater than that of neonates in the halothane anaesthesia group. It is possible that the mechanism of surgical hyperglycaemia for neonates in the halothane trial was the same as that discussed for neonates included in the preliminary study. Thus, as in the previous study, surgical hyperglycaemia in neonates undergoing surgery may have been precipitated by the release of adrenaline during surgery, which would be potentiated synergistically (Bessey et al, 1984; Shamoon et al, 1981) by the increases in plasma concentrations of glucagon and cortisol at the end of surgery that have been documented from both groups of neonates in the present study.

Furthermore, it is likely that differences in the hyperglycaemic response of neonates in the two anaesthesia groups were primarily as a result of the greater increases in plasma adrenaline and glucocorticoid concentrations in the non-halothane anaesthesia group as compared to corresponding changes in the halothane anaesthesia group, which may have stimulated an increased glucose production and/or decreased glucose utilization during surgery (Deibert and DeFronzo, 1980; Kerr et al, 1981). In addition, differences with respect to changes in the insulin/glucagon ratio between neonates in the two anaesthesia groups may also be important (Patel et al, 1982; Sperling, 1982) in mediating this difference in the hyperglycaemic response.

GLUCONEOGENIC SUBSTRATES
Blood lactate and pyruvate concentrations increased in both anaesthesia groups during surgery and, as shown by catheterisation studies in adult patients (Stjernstrom et al, 1981), this increase may be either due to the excessive production of lactate and pyruvate from glycogenolysis in skeletal muscles or may arise from glycolysis in injured tissues (Wilmore,
Although the magnitude of increase in blood lactate and pyruvate concentrations during surgery was similar in neonates from the halothane and non-halothane anaesthesia groups; at 6 hours postoperatively it was found that blood lactate and pyruvate concentrations remained elevated in the halothane anaesthesia group, but had decreased below preoperative values in the non-halothane anaesthesia group. Similar differences were seen between the two anaesthesia groups with respect to blood alanine changes at the end of surgery and 6 hours postoperatively.

Due to a combination of these differences, total gluconeogenic substrates at 6 hours after surgery were elevated in the halothane anaesthesia group and had decreased substantially below the preoperative values in the non-halothane anaesthesia group. This difference may be either due to the increased production of gluconeogenic substrates or, more likely due to the decreased utilization of gluconeogenic substrates in the neonates given halothane anaesthesia. Since hepatic gluconeogenesis (Frazer et al, 1981; Kalhan et al, 1980; Bougneres et al, 1982) is mainly responsible for the clearance of circulating lactate, pyruvate and alanine in neonates, the latter mechanism may be mediated either by a decreased rate of postoperative gluconeogenesis in the liver cells or a decreased hepatic blood flow during and after surgery.

Biebuyck et al (1972a) have shown that halothane directly inhibits gluconeogenesis and urea production in the perfused rat liver and this effect is associated with a marked increase in the rate of lactate production. They also found that the inclusion of a fatty acid (oleate) in the perfusion medium exerts a protective effect on the liver, and the rate
of gluconeogenesis is restored to control values (Biebuyck et al., 1972b). Based on the latter finding, and the observation that succinate oxidation in isolated liver mitochondria was not affected (Harris et al., 1971; Miller and Hunter, 1971), they proposed that halothane blocks electron transfer between NADH and the flavoproteins at the NADH dehydrogenase stage (Biebuyck, 1973). On the other hand, Gelman et al. (1984) have shown that the portal blood flow in dogs is decreased by anaesthetic concentrations of halothane and, at higher concentrations, it also reduces the hepatic artery blood flow. Thus, if these experimental findings are applicable to newborn infants, gluconeogenesis may be either inhibited directly by halothane anaesthesia or indirectly by a decrease in the hepatic blood flow.

Since halothane hepatotoxicity is almost unknown in paediatric patients (Smith, 1978; Wark, 1983; Warner et al., 1984), it is unlikely that these metabolic changes are associated with hepatocellular hypoxia (Shingu et al., 1982; Van Dyke, 1982) or have any prolonged effect in the neonatal patient. In this context, it may be noted that the blood concentrations of lactate, pyruvate and alanine in the halothane anaesthesia group had reverted to preoperative values by 12 hours after surgery.

FAT METABOLISM
Plasma concentrations of non-esterified fatty acids increased substantially during surgery in the non-halothane anaesthesia group and remained elevated at 6 hours postoperatively, whereas only a marginal response was observed in the halothane group. These differences may indicate a greater degree of lipolysis in the non-halothane group of neonates, probably mediated by the marked adrenaline release together with a decrease in the insulin/glucagon ratio during surgery (Williamson, 1982).
During surgery there was a slight increase in blood acetoacetate and total ketone bodies in the non-halothane anaesthesia group, whereas no change was recorded in the halothane anaesthesia group, giving rise to significantly different responses at the end of surgery. The greater increase in ketone bodies in neonates from the non-halothane group may again be related to the greater catecholamine and glucagon changes during surgery in these neonates as compared to the responses of neonates in the halothane anaesthesia group (Williamson, 1982).

There were no significant differences in the response of neonates in the two anaesthesia groups with respect to changes in the blood concentrations of glycerol, 3-hydroxybutyrate or triglycerides during and after surgery. Furthermore, changes in molar lactate/pyruvate, insulin/glucose, alanine/pyruvate and hydroxybutyrate/acetoacetate ratios during and after surgery were similar in the halothane and non-halothane anaesthesia groups.

Triglycerides were not significantly altered in both the anaesthesia groups during or after surgery. These findings are in contradiction to those obtained by Elphick and Wilkinson (1981) who had found significantly decreased triglyceride concentrations at 16 hours after surgery, the cause of which was not clear (Elphick and Wilkinson, 1981). However, it may be speculated that the postoperative decrease of plasma triglyceride values in their study was due to a lack of nutrient supply, since the neonates studied were not given dextrose infusions during or after surgery and underwent a much longer duration of preoperative starvation than neonates included in the present study. Moreover, Elphick and Wilkinson (1981) found no consistent change in non-esterified fatty acids during or after surgery, which may also be related to an increased postoperative consumption of NEFA
in the absence of dextrose supply.

On the other hand, blood triglycerides in neonates exposed to fetal
distress or birth asphyxia were found to be elevated in cord blood (Tsang
et al, 1974; Andersen and Friis-Hansen, 1976) and it was proposed that
elevated triglyceride levels may be due to the release of catecholamines by
intrapartum hypoxia, stimulation of lipolysis and the subsequent conversion
of excess NEFA into VLDL-lipoproteins, which carry the major part of cord
blood triglycerides (Andersen and Friis-Hansen, 1976).

Similar changes may not be found in neonates undergoing surgery due to the
marked hyperglycaemia and significantly raised plasma insulin during and
after surgery. Thus, it may be speculated that the raised plasma insulin
during and after surgery found in the present study may limit the degree of
lipolysis to some extent, whereas the concomitant release of glucagon and
adrenaline during surgery would favour oxidation of fatty acids and
production of ketone bodies (elevations of which have been documented in
the present study), rather than esterification and the production of
VLDL-lipoproteins (Bougneres et al, 1982; Williamson, 1982).

6.3.3 Urinary nitrogenous constituents :-

The 3-methylhistidine/creatinine ratio (3MH/Cr) increased significantly in
urine collected from neonates in the non-halothane anaesthesia group
whereas it remained unchanged in the halothane anaesthesia group; however,
differences between the two groups were not significant. An increase in the
urinary 3MH/Cr ratio in neonates from the non-halothane anaesthesia group
may possibly indicate an increased endogenous protein breakdown in the
postoperative period (Burgoyne et al, 1982); however, this finding needs to
be confirmed by means of simultaneous nitrogen balance studies during the
postoperative period. It is likely that the primary site of protein breakdown is the skeletal muscle (Ballard and Tomas, 1983), although an increased protein turnover in smooth muscle tissues has not been excluded (Rennie and Millward, 1983).

Thus, it is concluded that the metabolic response of neonates in the halothane anaesthesia group was characterised by a decrease in the hyperglycaemia and lipid mobilisation during surgery and a transient reduction in the clearance of gluconeogenic substrates postoperatively as compared to the response of neonates in the non-halothane anaesthesia group. It is possible that these changes in the metabolic stress response were associated with a decrease in endogenous protein breakdown in neonates given halothane anaesthesia during surgery.

6.3.4 Clinical observations :-
Although neonates in the halothane anaesthesia group had a significantly higher heart rate before the start of surgery, during the operation neonates in the non-halothane anaesthesia group had a much greater increase in the heart rate than neonates in the halothane anaesthesia group. This effect was presumably due to pain and awareness during surgery, and may have been mediated by the greater intra-operative release of catecholamines that was documented in the non-halothane anaesthesia group. Similar to the responses of neonates in the halothane anaesthesia group, Kissin and Green (1984) have recently shown that increasing concentrations of halothane can cause a proportional decrease of the cardiac acceleration response to somatic nerve stimulation in dogs.

Furthermore, it was observed that neonates in the non-halothane anaesthesia group were more prone to the development of reflex bradycardia in response
to the nociceptive stimulation of surgery as compared to neonates in the halothane anaesthesia group; thus, the minimum heart rate documented during surgery was significantly lower than in the latter group.

During the postoperative period, it was found that a larger number of neonates in the non-halothane anaesthesia group required narcotic analgesia, which was prescribed in larger doses as compared to neonates in the halothane anaesthesia group and the first analgesic dose was required at a significantly earlier time following surgery as compared to neonates in the halothane anaesthesia group. Since postoperative analgesia was prescribed by the clinical staff without knowledge of the anaesthetic management of each neonate and since the clinical criteria for prescription of analgesia were generally similar for both groups of neonates, the greater and earlier requirement of analgesia provides further evidence for a lack of adequate pain relief during the surgical operation. The hormonal changes documented in the non-halothane anaesthesia group would point towards a similar conclusion.

From the postoperative complications documented by nursing and clinical staff in neonates in the non-halothane anaesthesia group, it was clear that the clinical state of these neonates was relatively more unstable than that of neonates in the halothane anaesthesia group (Table 6.8).

6.3.5 Hypothesis :-

In Chapter V, the hypothesis was proposed that anaesthesia with halothane and nitrous oxide does not decrease the hormonal and metabolic response of newborn infants undergoing surgery as compared to neonates anaesthetised with nitrous oxide alone. It was proposed that this hypothesis would be rejected if there was a significant difference (at the p<0.05 level) with
respect to changes in plasma adrenaline, noradrenaline and blood glucose concentrations at the end of surgery and the urinary 3-methylhistidine/creatinine ratios on the 3 days following surgery between neonates who were randomly allotted to a halothane or a non-halothane anaesthetic regimen.

On analysis of the data relating to the two randomised groups, significant differences were found between the responses of neonates in the halothane and non-halothane anaesthesia groups with respect to plasma adrenaline \((p<0.05)\), plasma noradrenaline \((p<0.005)\), and blood glucose \((p<0.025)\) changes at the end of surgery. The urinary 3-methylhistidine/creatinine ratio was found to be increased on the second \((p<0.05)\) and third \((p<0.01)\) postoperative days in neonates from the non-halothane anaesthesia group, but remained unchanged in neonates who were given halothane anaesthesia during surgery.

On the basis of these results, it is possible to summarily reject the above hypothesis. Thus, we may conclude that halothane anaesthesia given to newborn infants during surgery is associated with a significant decrease in the magnitude of their hormonal and metabolic stress response.

6.4 CONCLUSION :-

Lack of potent anaesthesia during surgery may be undesirable for newborn infants due to an accentuation of their endocrine and metabolic stress response and its contribution towards an unstable clinical state.
Figure 6.1: Anaesthetic protocol for neonates randomly allocated to the Non-halothane anaesthesia group.
HALOTHANE TRIAL: ANAESTHETIC PROTOCOL

NON-HALOTHANE GROUP

1. Preoxygenation (2-3 minutes)
2. Intubation: Awake
3. Intravenous fluids: 4% Dextrose + 0.18% saline 6-9 ml/kg/hr
4. Relaxant: d-Tubocurarine 0.2-0.4 mg/kg
5. Maintenance: (a) Nitrous Oxide + Oxygen = 66:33%.
   (NB. Nitrous Oxide should not be used in a concentration higher than 66%. For patients with an increased oxygen requirement, lower concentrations may be used).
   (b) Supplements of d-Tubocurarine 0.1-0.2 mg/kg IV
6. Reversal of relaxation: Atropine 0.02 mg/kg IV
   Neostigmine 0.05 mg/kg IV
   (NB. Reversal may or may not be given).
Figure 6.2: Anaesthetic protocol for neonates randomly allocated to the Halothane anaesthesia group.
HALOTHANE TRIAL: ANAESTHETIC PROTOCOL

HALOTHANE GROUP

1. Pre-oxygenation (2-3 minutes)

2. Induction: HALOTHANE 1-2% conc.
   Nitrous oxide + Oxygen = 66:33%

3. Intubation: Semi-awake. (After Halothane 1% has been given for 1-3 mins).

4. Intravenous fluids: 4% Dextrose + 0.18% saline 6-9 ml/kg/hr

5. Maintenance: (a) Nitrous oxide + Oxygen 66:33%
   (b) d-Tubocurarine 0.2-0.4 mg/kg IV
       Supplements 0.1-0.2 mg/kg IV.
   (c) HALOTHANE 0.5%-1.0%.

6. Reversal of relaxation: Atropine 0.02 mg/kg IV
   Neostigmine 0.05 mg/kg IV

   (NB. Reversal may or may not be given).

CRITERIAL FOR USE OF HALOTHANE: -

(A) Halothane in a concentration of at least 1% should be given before intubation and again before surgical incision (minimum 1 minute).

(B) For maintenance, Halothane should not be used in a concentration of less than 0.5%

(C) Halothane 0.5% should be administered for at least three-quarters of the duration of the surgical procedure.
### Table 6.1 HALOTHANE TRIAL: Description of patients.

<table>
<thead>
<tr>
<th>PRE-OPERATIVE</th>
<th>HALOTHANE</th>
<th>Mann-Whitney U Test</th>
<th>NON-HALOTHANE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>18</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Age, days</td>
<td>24 ± 5</td>
<td>n.s.</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>Gestation, weeks</td>
<td>37 ± 1</td>
<td>n.s.</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>Birthweight, kg</td>
<td>2.8 ± 0.3</td>
<td>n.s.</td>
<td>2.8 ± 0.2</td>
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<tr>
<td>Dextrose infusion rate, mg/kg/min</td>
<td>3.5 ± 0.4</td>
<td>n.s.</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Starvation, hours pre-operative</td>
<td>6.0 ± 0.4</td>
<td>n.s.</td>
<td>6.0 ± 0.4</td>
</tr>
<tr>
<td>TPN: Number of patients</td>
<td>0</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Days pre-operative</td>
<td>-</td>
<td>-</td>
<td>8 ± 6</td>
</tr>
<tr>
<td>Stopped, hours pre-operative</td>
<td>-</td>
<td>-</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Antibiotics: Number of patients</td>
<td>4</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Days pre-operative</td>
<td>4 ± 3</td>
<td>n.s.</td>
<td>6 ± 2</td>
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<table>
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<th>INTRA-OPERATIVE</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Weight at operation, kg</td>
<td>3.1 ± 0.3</td>
<td>n.s.</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Dextrose infusion rate, mg/kg/min</td>
<td>4.8 ± 0.4</td>
<td>n.s.</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>Surgical Stress Score</td>
<td>6.9 ± 0.8</td>
<td>n.s.</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td>Blood transfusion:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>1</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Volume, ml</td>
<td>40</td>
<td>n.s.</td>
<td>41 ± 8</td>
</tr>
<tr>
<td>Temperature loss, °C</td>
<td>0.7 ± 0.2</td>
<td>n.s.</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Dose of d-Tubocurarine, mg/kg</td>
<td>0.43 ± 0.06</td>
<td>p&lt;0.025</td>
<td>0.66 ± 0.07</td>
</tr>
<tr>
<td>Heart rate: Base line</td>
<td>149 ± 3</td>
<td>p&lt;0.01</td>
<td>139 ± 2</td>
</tr>
<tr>
<td>Maximum</td>
<td>174 ± 3</td>
<td>p&lt;0.0001</td>
<td>204 ± 4</td>
</tr>
<tr>
<td>Minimum</td>
<td>131 ± 7</td>
<td>p&lt;0.05</td>
<td>116 ± 8</td>
</tr>
</tbody>
</table>

Characteristics of the patient material in halothane and non-halothane anaesthesia groups and details of pre-operative and intra-operative management. All values = Mean ± SEM.
Table 6.2 HALOTHANE TRIAL: Hormonal changes.

<table>
<thead>
<tr>
<th></th>
<th>HALOTHANE</th>
<th>Mann-Whitney U Test</th>
<th>NON-HALOTHANE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SEM</td>
<td>p</td>
</tr>
<tr>
<td>Adrenaline Pre-operative</td>
<td>15</td>
<td>0.43 ± 0.11</td>
<td>n.s.</td>
</tr>
<tr>
<td>△ Adrenaline nmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-op</td>
<td>14</td>
<td>0.86 ± 0.34</td>
<td>pc&lt;0.05</td>
</tr>
<tr>
<td>6 hr post-op</td>
<td>14</td>
<td>0.09 ± 0.09</td>
<td>n.s.</td>
</tr>
<tr>
<td>12 hr post-op</td>
<td>11</td>
<td>-0.27 ± 0.11</td>
<td>n.s.</td>
</tr>
<tr>
<td>24 hr post-op</td>
<td>11</td>
<td>-0.30 ± 0.12</td>
<td>n.s.</td>
</tr>
<tr>
<td>Noradrenaline Pre-operative</td>
<td>16</td>
<td>7.60 ± 0.83</td>
<td>n.s.</td>
</tr>
<tr>
<td>△ Noradrenaline nmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-op</td>
<td>14</td>
<td>1.05 ± 2.31</td>
<td>pc&lt;0.005</td>
</tr>
<tr>
<td>6 hr post-op</td>
<td>13</td>
<td>-0.14 ± 0.79</td>
<td>n.s.</td>
</tr>
<tr>
<td>12 hr post-op</td>
<td>13</td>
<td>-0.29 ± 0.79</td>
<td>n.s.</td>
</tr>
<tr>
<td>24 hr post-op</td>
<td>12</td>
<td>1.99 ± 1.36</td>
<td>n.s.</td>
</tr>
<tr>
<td>Insulin Pre-operative</td>
<td>17</td>
<td>44 ± 14</td>
<td>n.s.</td>
</tr>
<tr>
<td>△ Insulin pmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-op</td>
<td>16</td>
<td>30 ± 19</td>
<td>n.s.</td>
</tr>
<tr>
<td>6 hr post-op</td>
<td>15</td>
<td>14 ± 5</td>
<td>pc&lt;0.05</td>
</tr>
<tr>
<td>12 hr post-op</td>
<td>14</td>
<td>61 ± 28</td>
<td>n.s.</td>
</tr>
<tr>
<td>24 hr post-op</td>
<td>12</td>
<td>10 ± 15</td>
<td>n.s.</td>
</tr>
<tr>
<td>Glucagon Pre-operative</td>
<td>10</td>
<td>10.0 ± 1.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>△ Glucagon pmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-op</td>
<td>10</td>
<td>1.7 ± 0.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>6 hr post-op</td>
<td>7</td>
<td>-2.4 ± 1.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>12 hr post-op</td>
<td>7</td>
<td>-0.7 ± 2.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>24 hr post-op</td>
<td>6</td>
<td>-1.5 ± 2.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Insulin/glucagon Pre-operative</td>
<td>10</td>
<td>2.1 ± 0.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>△ Insulin/glucagon ratio</td>
<td>9</td>
<td>0.9 ± 0.7</td>
<td>pc&lt;0.05</td>
</tr>
<tr>
<td>6 hr post-op</td>
<td>7</td>
<td>7.4 ± 5.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>12 hr post-op</td>
<td>7</td>
<td>7.9 ± 4.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>24 hr post-op</td>
<td>6</td>
<td>3.2 ± 1.4</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Comparison of changes in plasma hormone concentrations between neonates in the halothane and non-halothane anaesthesia groups. Delta values at the end of surgery and post-operatively were obtained by subtraction of the pre-operative value in each neonate.
### Table 6.3 HALOTHANE TRIAL: - Hormonal changes

<table>
<thead>
<tr>
<th></th>
<th>HALOTHANE</th>
<th>Mann-Whitney U Test</th>
<th>NON-HALOTHANE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SEM</td>
<td>p</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>16</td>
<td>0.84 ± 0.17</td>
<td>n.s.</td>
<td>1.15 ± 0.26</td>
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<td>△ Aldosterone nmol/L</td>
<td>15</td>
<td>1.34 ± 0.56</td>
<td>n.s.</td>
<td>1.63 ± 0.55</td>
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<tr>
<td></td>
<td>13</td>
<td>1.09 ± 0.57</td>
<td>n.s.</td>
<td>1.15 ± 0.81</td>
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<tr>
<td></td>
<td>11</td>
<td>1.52 ± 1.18</td>
<td>n.s.</td>
<td>0.29 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.26 ± 0.63</td>
<td>n.s.</td>
<td>0.53 ± 0.61</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>16</td>
<td>12.8 ± 6.2</td>
<td>n.s.</td>
<td>13.9 ± 5.8</td>
</tr>
<tr>
<td>△ Corticosterone nmol/L</td>
<td>15</td>
<td>52.7 ± 15.0</td>
<td>n.s.</td>
<td>68.7 ± 22.6</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>25.4 ± 9.4</td>
<td>n.s.</td>
<td>10.5 ± 5.8</td>
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<tr>
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<td>11</td>
<td>7.9 ± 11.0</td>
<td>n.s.</td>
<td>8.9 ± 8.3</td>
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<tr>
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<td>11</td>
<td>-7.0 ± 9.9</td>
<td>n.s.</td>
<td>-0.7 ± 4.0</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>16</td>
<td>0.29 ± 0.07</td>
<td>n.s.</td>
<td>0.39 ± 0.10</td>
</tr>
<tr>
<td>△ 11-Deoxycorticosterone nmol/L</td>
<td>15</td>
<td>0.20 ± 0.12</td>
<td>n.s.</td>
<td>0.19 ± 0.08</td>
</tr>
<tr>
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<td>13</td>
<td>0.16 ± 0.10</td>
<td>n.s.</td>
<td>0.02 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.03 ± 0.05</td>
<td>n.s.</td>
<td>-0.06 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>-0.11 ± 0.14</td>
<td>n.s.</td>
<td>-0.14 ± 0.09</td>
</tr>
<tr>
<td>Progesterone</td>
<td>16</td>
<td>6.35 ± 3.15</td>
<td>p&lt;0.05</td>
<td>8.73 ± 4.10</td>
</tr>
<tr>
<td>△ Progesterone nmol/L</td>
<td>15</td>
<td>3.30 ± 3.27</td>
<td>p&lt;0.02</td>
<td>-0.41 ± 2.20</td>
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<tr>
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<td>13</td>
<td>2.15 ± 2.07</td>
<td>p&lt;0.003</td>
<td>-3.10 ± 1.67</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.08 ± 5.29</td>
<td>n.s.</td>
<td>-2.58 ± 1.73</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>-2.67 ± 2.88</td>
<td>n.s.</td>
<td>-5.71 ± 3.60</td>
</tr>
<tr>
<td>17-Hydroxyprogesterone</td>
<td>16</td>
<td>3.16 ± 0.62</td>
<td>n.s.</td>
<td>4.17 ± 1.05</td>
</tr>
<tr>
<td>△ 17-Hydroxyprogesterone nmol/L</td>
<td>15</td>
<td>1.01 ± 0.48</td>
<td>n.s.</td>
<td>0.95 ± 1.21</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>1.11 ± 0.62</td>
<td>p&lt;0.02</td>
<td>-1.63 ± 1.56</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>-0.37 ± 0.94</td>
<td>n.s.</td>
<td>-0.37 ± 0.96</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.09 ± 0.51</td>
<td>n.s.</td>
<td>-2.03 ± 1.30</td>
</tr>
<tr>
<td>11-Deoxycortisol</td>
<td>16</td>
<td>0.96 ± 0.15</td>
<td>n.s.</td>
<td>1.34 ± 0.38</td>
</tr>
<tr>
<td>△ 11-Deoxycortisol nmol/L</td>
<td>15</td>
<td>0.87 ± 0.37</td>
<td>n.s.</td>
<td>-0.15 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.56 ± 0.48</td>
<td>n.s.</td>
<td>-0.36 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.05 ± 0.37</td>
<td>n.s.</td>
<td>-0.78 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>-0.20 ± 0.30</td>
<td>n.s.</td>
<td>-0.37 ± 0.35</td>
</tr>
<tr>
<td>Cortisol</td>
<td>16</td>
<td>321 ± 93</td>
<td>n.s.</td>
<td>342 ± 73</td>
</tr>
<tr>
<td>△ Cortisol nmol/L</td>
<td>15</td>
<td>423 ± 90</td>
<td>p&lt;0.05</td>
<td>634 ± 108</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>95 ± 87</td>
<td>n.s.</td>
<td>176 ± 87</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>-174 ± 101</td>
<td>p&lt;0.05</td>
<td>173 ± 85</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>-98 ± 124</td>
<td>n.s.</td>
<td>-68 ± 94</td>
</tr>
<tr>
<td>Cortisone</td>
<td>16</td>
<td>221 ± 60</td>
<td>n.s.</td>
<td>208 ± 30</td>
</tr>
<tr>
<td>△ Cortisone</td>
<td>15</td>
<td>-38 ± 17</td>
<td>n.s.</td>
<td>-36 ± 23</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>-28 ± 36</td>
<td>n.s.</td>
<td>-21 ± 19</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>-38 ± 27</td>
<td>n.s.</td>
<td>-22 ± 26</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>-98 ± 26</td>
<td>n.s.</td>
<td>-15 ± 47</td>
</tr>
</tbody>
</table>

Comparison of changes in plasma hormone concentrations between neonates in the halothane and non-halothane anaesthesia groups. Delta values at the end of surgery and post-operatively were obtained by subtraction of the pre-operative value in each neonate.
Table 6.4 HALOTHANE TRIAL: Metabolite changes

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Pre-operative</th>
<th>End-op</th>
<th>6 hr post-op</th>
<th>12 hr post-op</th>
<th>24 hr post-op</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>N = 18</td>
<td>5.0 ± 0.3</td>
<td>5.4 ± 0.8</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lactate</td>
<td>N = 18</td>
<td>1.9 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>0.2 ± 0.3</td>
<td>0.2 ± 0.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>N = 18</td>
<td>0.10 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.03 ± 0.03</td>
<td>n.s.</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>N = 18</td>
<td>0.17 ± 0.03</td>
<td>0.08 ± 0.03</td>
<td>0.02 ± 0.03</td>
<td>0.06 ± 0.04</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hydroxybutyrate</td>
<td>N = 18</td>
<td>0.22 ± 0.03</td>
<td>0.05 ± 0.01</td>
<td>0.00 ± 0.02</td>
<td>0.01 ± 0.02</td>
<td>n.s.</td>
</tr>
<tr>
<td>Glycerol</td>
<td>N = 18</td>
<td>0.17 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.02 ± 0.03</td>
<td>0.04 ± 0.02</td>
<td>n.s.</td>
</tr>
<tr>
<td>NEFA</td>
<td>N = 5</td>
<td>0.52 ± 0.15</td>
<td>0.17 ± 0.09</td>
<td>0.17 ± 0.05</td>
<td>0.08 ± 0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>N = 5</td>
<td>0.88 ± 0.22</td>
<td>-0.08 ± 0.07</td>
<td>-0.12 ± 0.11</td>
<td>-0.26 ± 0.13</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Comparison of changes in blood metabolite concentrations between neonates in the halothane and non-halothane anaesthesia groups. Delta values at the end of surgery and post-operatively were obtained by subtraction of the pre-operative value in each neonate.
Table 6.5 HALOTHANE TRIAL: Derived hormonal-metabolic variables.

<table>
<thead>
<tr>
<th></th>
<th>HALOTHANE</th>
<th>Mann-Whitney U Test</th>
<th>NON-HALOTHANE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SEM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Ketones</td>
<td>Pre-operative</td>
<td>18</td>
<td>0.23 ± 0.04</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>End-operative</td>
<td>17</td>
<td>0.09 ± 0.04</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>6hr post-operative</td>
<td>16</td>
<td>0.04 ± 0.07</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>12hr post-operative</td>
<td>15</td>
<td>-0.06 ± 0.03</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>24hr post-operative</td>
<td>13</td>
<td>-0.02 ± 0.06</td>
<td>n.s.</td>
</tr>
<tr>
<td>△ Total Ketones mmol/L</td>
<td>Pre-op</td>
<td>18</td>
<td>2.4 ± 0.2</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>End-operative</td>
<td>17</td>
<td>0.8 ± 0.3</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>6hr post-operative</td>
<td>16</td>
<td>0.2 ± 0.3</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>12hr post-operative</td>
<td>15</td>
<td>0.3 ± 0.4</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>24hr post-operative</td>
<td>13</td>
<td>-0.3 ± 0.2</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Comparison of changes in derived hormonal-metabolic variables between neonates in the halothane and non-halothane anaesthesia groups. Delta values at the end of surgery and post-operatively were obtained by subtraction of the pre-operative value in each neonate.
Comparison of changes in derived hormonal-metabolic variables between neonates in the halothane and non-halothane anaesthesia groups. (Differences between the two groups are analysed by the Mann-Whitney U Test, whereas changes from pre-operative values within each group are analysed by the Wilcoxon test.)
Comparison of post-operative clinical management, body weight and urinary 3-methylhistidine/creatinine ratios between neonates in the halothane and non-halothane anaesthesia groups by Mann-Whitney U test. Changes in body weight and urinary 3-methylhistidine/creatinine ratios were also compared within each group to values obtained on the first post-operative day by Wilcoxon's matched-pairs test.

<table>
<thead>
<tr>
<th></th>
<th>HALOTHANE</th>
<th></th>
<th>NON-HALOTHANE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wilcoxon Test</td>
<td>Mean ± SEM</td>
<td>Number of Patients</td>
<td>Mann-Whitney U Test</td>
</tr>
<tr>
<td>Dextrose infusion mg kg⁻¹ min⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>4.7 ± 0.2</td>
<td>18</td>
<td>18</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Day 2</td>
<td>4.3 ± 0.3</td>
<td>16</td>
<td>n.s.</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Day 3</td>
<td>4.5 ± 0.2</td>
<td>7</td>
<td>n.s.</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Dose of Morphine mg kg⁻¹ day⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>0.23 ± 0.06</td>
<td>6</td>
<td>8</td>
<td>0.33 ± 0.04</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.10 ± 0.03</td>
<td>2</td>
<td>7</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.06 ± 0.03</td>
<td>0</td>
<td>0</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>First dose, hours post-operative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.5 ± 1.6</td>
<td>6</td>
<td></td>
<td>1.9 ± 0.03</td>
</tr>
<tr>
<td>Height, cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>3.1 ± 0.2</td>
<td>16</td>
<td>18</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Day 2</td>
<td>3.3 ± 0.3</td>
<td>18</td>
<td>n.s.</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Day 3</td>
<td>3.6 ± 0.3</td>
<td>18</td>
<td>p&lt;0.05</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>3-methylhistidine</td>
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<td></td>
<td></td>
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<tr>
<td>Creatinine ratio, umol umol⁻¹</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>0.043 ± 0.003</td>
<td>7</td>
<td>n.s.</td>
<td>0.044 ± 0.003</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.046 ± 0.003</td>
<td>7</td>
<td>n.s.</td>
<td>0.049 ± 0.003</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.041 ± 0.003</td>
<td>5</td>
<td>n.s.</td>
<td>0.052 ± 0.003</td>
</tr>
</tbody>
</table>

Comparison of post-operative clinical management, body weight and urinary 3-methylhistidine/creatinine ratios between neonates in the halothane and non-halothane anaesthesia groups by Mann-Whitney U test. Changes in body weight and urinary 3-methylhistidine/creatinine ratios were also compared within each group to values obtained on the first post-operative day by Wilcoxon's matched-pairs test.

*Comparison of post-operative clinical management, body weight and urinary 3-methylhistidine/creatinine ratios between neonates in the halothane and non-halothane anaesthesia groups by Mann-Whitney U test. Changes in body weight and urinary 3-methylhistidine/creatinine ratios were also compared within each group to values obtained on the first post-operative day by Wilcoxon's matched-pairs test.*
Table 6.8  HALOTHANE TRIAL: - Peri-operative complications

<table>
<thead>
<tr>
<th>INTRA-OPERATIVE COMPLICATIONS</th>
<th>HALOTHANE</th>
<th>NON-HALOTHANE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hypothermia</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>2. Excessive blood loss</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>3. Hypotension</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4. Cyanosis and bradycardia</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>5. Persistent tachycardia</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>6. Severe pulmonary hypertension (persistent fetal circulation)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7. Difficult intubation</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>POST-OPERATIVE COMPLICATIONS</th>
<th>HALOTHANE</th>
<th>NON-HALOTHANE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Respiratory instability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Increased oxygen requirements</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>(b) Increased ventilation requirements</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2. Hypotension</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3. Vomiting</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>4. Frequent spontaneous bradycardias</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>5. Severe pulmonary hypertension (persistent fetal circulation)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6. Gastric bleeding (stress ulcers?)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>7. Fever</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>8. Excessive irritability</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>9. Temperature instability</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>10. Persistent metabolic acidosis</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>11. Paralytic ileus</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>12. Peripheral circulatory 'shut-down'</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>13. Post-operative oliguria</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>14. Septicaemia and multiple abscesses</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>15. Cardiac arrest and death</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

Clinical complications observed during surgery and in the post-operative period in neonates from the halothane and non-halothane anaesthesia groups.
CHAPTER VII: RANDOMISED TRIAL OF FENTANYL ANAESTHESIA
7.1 INTRODUCTION

7.2 RESULTS OF THE FENTANYL TRIAL
   7.2.1 Description of patients and preoperative management
   7.2.2 Anaesthesia and clinical management during surgery
   7.2.3 Hormonal changes
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7.3 DISCUSSION
   7.3.1 Hormonal changes
   7.3.2 Metabolic changes
   7.3.3 Urinary nitrogenous constituents
   7.3.4 Clinical observations
   7.3.5 Hypothesis

7.4 CONCLUSION
7.1 INTRODUCTION


For the present study, fentanyl was considered to be the drug of choice in preference to morphine since it had been demonstrated that morphine anaesthesia in adult patients was associated with a marked cardiovascular instability (Philbin et al, 1981) due to the release of histamine at the time of anaesthetic induction (Philbin et al, 1981b; Rosow et al, 1982). On the other hand, fentanyl was associated with minimal side-effects in preterm and term neonates and its use was advocated even in critically ill
neonates because of the cardiovascular stability documented during fentanyl anaesthesia (Robinson and Gregory, 1981; Schweiss and Pennington, 1981; Haselby et al., 1982; Hickey and Hansen, 1984; Vacanti et al., 1984).

The randomised trial of fentanyl anaesthesia was planned to include only preterm neonates undergoing surgical ligation of a patent ductus arteriosus (PDA). This set of patients were selected for inclusion into a separate trial since, (1) they had many special characteristics with regard to their preoperative and postoperative clinical state, (2) all the neonates underwent a standardised surgical procedure performed by a single surgeon and, (3) after PDA ligation all the neonates were ventilated for at least 24 hours in the postoperative period. The latter characteristic was of particular importance, since fentanyl causes respiratory depression in adults (Andrews et al., 1983; Cartwright et al., 1983) and it was shown that neonates were particularly sensitive to the respiratory depressant effects of opiate drugs (Way et al., 1965; Evans et al., 1976). Thus, fentanyl anaesthesia could be given to only those neonates who were likely to be ventilated for at least 24 hours after surgery (Robinson and Gregory, 1981).

The dose of fentanyl used for inhibition of the stress response in adult patients was usually between 50-150 μg/kg (Cooper et al., 1981; Zurick et al., 1982). Although Haxholdt et al. (1981) found that the cortisol response and hyperglycaemia following upper abdominal surgery in adult patients were not affected by smaller doses of fentanyl (10 and 30 μg/kg); in a randomised and controlled trial, Campbell et al. (1984) have shown recently that fentanyl can inhibit the hyperglycaemic and catecholamine responses to upper abdominal surgery in doses of 15-20 μg/kg (Campbell et al., 1984). In preterm neonates undergoing PDA ligation, Robinson and Gregory (1981) have advocated the use of fentanyl 30-50 μg/kg; however, in view of the
increased sensitivity of newborn infants to opiate drugs and since fentanyl had rarely been used for preterm neonates in this hospital, it was decided that fentanyl would be given in a dose of 10 µg/kg to those neonates randomly allocated to the fentanyl anaesthesia group. It is well-known that surgical anaesthesia in adult patients can be obtained with much smaller doses of fentanyl (Viars, 1982; Hengstmann et al, 1980) and it was expected that this dose may be sufficient to inhibit the hormonal and metabolic response of preterm neonates to a relatively minor surgical stimulus such as PDA ligation. Furthermore, Stanley et al have shown experimentally that deep analgesia with narcotic agents is obtained even at a low opiate receptor occupancy in various parts of the brain. In rats, full saturation of opiate receptors was obtained at doses which were 8 times those required to produce surgical anaesthesia (Stanley et al, 1983).

As stated previously, the necessity of giving anaesthesia to preterm neonates undergoing surgery had been questioned and it was generally accepted by anaesthetists at the John Radcliffe Hospital and other leading hospitals that preterm neonates were not capable of the perception or interpretation of pain, presumably due to an immaturity of their central nervous system (Lipmann et al, 1976; Shaw, 1982; Betts and Downes, 1984). A review of the published literature was performed for reports on the clinical management of preterm neonates undergoing PDA ligation and it was found that in 76% cases the operation had been performed under the influence of nitrous oxide alone (given at the time of induction and stopped during the retraction of the left lung) or without the use of anaesthesia (Anand and Aynsley-Green, 1985).

The randomised trial was planned to include a total of 24 preterm neonates and was expected to take approximately 18 months for completion. During the
latter half of this period, however, the rate of patient entry was much lower than expected and the trial had to be terminated after the inclusion of only 16 patients. The lower number of patients resulted in lowering the power of the trial from 80% to 65%; thus, on the basis of the outcome measures defined earlier, the trial had only a 65% chance of identifying a significant difference between neonates in the fentanyl and non-fentanyl anaesthesia groups, provided that the actual difference between the two groups in these parameters was as large as that used to calculate the sample size. The decreased power of the trial was considered to be an important defect and it was decided that the hypothesis being tested by this trial would be rejected only if significant differences were found between the two anaesthetic groups in two or more of the outcome measures.

In contrast to the previous studies (Chapter IV and VI), it must be noted that arterial blood was obtained for the measurement of hormonal and metabolic variables from neonates included in this trial. Therefore, it was expected that the absolute blood concentrations of certain metabolites (eg, glucose, lactate) and hormones (eg, noradrenaline) would be different from those documented in the previous two studies. However, since the changes in each variable were calculated from the difference between the preoperative measurement and the end-operative and postoperative measurements obtained from each patient, it was expected that the direction of changes in the hormonal and metabolite variables would not be affected by their measurement in arterial blood.
7.2 RESULTS OF THE FENTANYL TRIAL:

7.2.1 Description of patients and preoperative management:

The patient characteristics and clinical management of neonates in the fentanyl and non-fentanyl anaesthesia groups are compared in Table 7.1. There were no significant differences between neonates randomly allocated to the fentanyl and non-fentanyl anaesthesia groups with respect to gestation and birth weight, as well as age and weight at the time of operation. There was no significant difference in the preoperative dextrose infusion rate, the duration of preoperative starvation, or with regard to any other aspects of preoperative clinical management. Premedication was not given to the preterm neonates entered in this trial.

7.2.2 Anaesthesia and clinical management during surgery:

There were no deviations from the anaesthetic protocol (Figures 7.1 and 7.2) for neonates included in the fentanyl or non-fentanyl anaesthesia groups. Neonates in the fentanyl anaesthesia group received fentanyl in a mean dose of 12.2 ± 1.5 μg/kg, whereas neonates in both anaesthesia groups received nitrous oxide (50%) and an identical mean dose of d-tubocurarine during surgery. Blood transfusions were required by 1 neonate in the fentanyl anaesthesia group and 2 neonates in the non-fentanyl anaesthesia group during the operation, the intra-operative dextrose infusion rate was not significantly different between the two groups.

As expected, the surgical stress scores obtained by neonates in the two anaesthesia groups were identical; the mean temperature loss during surgery was similar in the two anaesthesia groups. The base-line heart rate before the induction of anaesthesia was similar in the two anaesthesia groups;
however, during surgery the heart rate increased to a greater extent in the non-fentanyl anaesthesia group and the maximum heart rate was significantly higher (p<0.01) than in the fentanyl anaesthesia group.

Thus, apart from the anaesthetic management, there were no significant differences in the characteristics of the preterm neonates in the fentanyl and non-fentanyl anaesthesia groups. In addition, their clinical management before and during the surgical operation was identical.

7.2.3 Hormonal changes :

The changes in hormonal variables in preterm neonates undergoing PDA ligation are compared between the fentanyl and non-fentanyl anaesthesia groups in Tables 7.2 and 7.3.

Plasma adrenaline concentrations decreased during surgery in the fentanyl anaesthesia group and remained below the preoperative concentration at 6, 12 and 24 hours after surgery; whereas they had increased during surgery in the non-fentanyl anaesthesia group and remained elevated postoperatively. Thus, there were significant differences between the responses of preterm neonates in the fentanyl and non-fentanyl anaesthesia groups at the end of surgery (p<0.002), and at 6 hours (p<0.01), 12 hours (p<0.025) and 24 hours (p<0.01) after surgery.

Plasma noradrenaline concentrations increased during surgery in the fentanyl and non-fentanyl anaesthesia groups and although this increase was greater in the non-fentanyl anaesthesia group, differences between the two groups were not significant. Throughout the postoperative period, mean delta noradrenaline concentrations were decreased in the fentanyl anaesthesia group and remained elevated in the non-fentanyl anaesthesia
group; this difference between the two groups was significant at 24 hours after surgery (p<0.025).

Plasma insulin concentrations increased during surgery in the fentanyl anaesthesia group, whereas they had decreased at the end of surgery in the non-fentanyl anaesthesia group; however, there were no significant differences between the two groups at the end of surgery or in the postoperative period.

Plasma glucagon concentrations decreased slightly during surgery in the fentanyl anaesthesia group, whereas a distinct increase was observed in the non-fentanyl anaesthesia group; the difference in these responses was significant at the end of surgery (p<0.05). At 6, 12 and 24 hours after surgery, plasma glucagon values in both anaesthesia groups were decreased below their respective preoperative concentrations and there was no significant difference between the responses of neonates in the two groups.

As a result of these changes, the insulin/glucagon molar ratio increased substantially during surgery in the fentanyl anaesthesia group whereas it had decreased during surgery in the non-fentanyl anaesthesia group, giving rise to a significant difference between the two groups at the end of surgery (p<0.025). In the postoperative period, there was no significant difference between the changes in plasma insulin/glucagon molar ratios of the two groups.

Plasma concentrations of the steroid hormones were measured in 8 neonates from the fentanyl anaesthesia group and 4 neonates from the non-fentanyl anaesthesia group. The 6 concentrations of the steroid hormones in the two anaesthesia groups are compared in Table 7.3.
Plasma aldosterone concentrations were raised at the end of surgery in both groups and there was no significant difference in the responses of neonates in the fentanyl and non-fentanyl groups. After surgery, plasma aldosterone concentrations were decreased below the preoperative value in the fentanyl anaesthesia group and remained elevated in the non-fentanyl anaesthesia group, with significant differences at 6 hours (p<0.025) and 12 hours (p<0.01) after surgery between the two anaesthesia groups.

Plasma corticosterone concentrations increased during surgery in the fentanyl and non-fentanyl anaesthesia groups; however, the magnitude of intra-operative increase was significantly greater in the non-fentanyl anaesthesia group (p<0.025) as compared to the fentanyl anaesthesia group. At 6, 12 and 24 hours after surgery, there was no significant difference in the corticosterone changes between neonates in the two anaesthesia groups.

Similarly, although plasma 11-deoxycorticosterone concentrations increased during surgery in both the anaesthesia groups, the magnitude of this response was significantly greater in preterm neonates of the non-fentanyl anaesthesia group (p<0.05) as compared to those in the fentanyl anaesthesia group. At 6, 12 and 24 hours postoperatively, plasma 11-deoxycorticosterone concentrations had decreased to their respective preoperative values in both groups and were not significantly different between the fentanyl and non-fentanyl anaesthesia groups.

Plasma 11-deoxycortisol concentrations were decreased at the end of surgery in neonates in the fentanyl anaesthesia group, whereas they had increased substantially in the non-fentanyl anaesthesia group, giving rise to a significant difference between the two groups at the end of surgery.
(p<0.05). In the postoperative period, there was no significant difference in the plasma 11-deoxycortisol responses of preterm neonates in the fentanyl and non-fentanyl anaesthesia groups.

Changes in plasma progesterone, 17-hydroxyprogesterone, cortisol and cortisone concentrations during and after surgery were not significantly different between neonates in the two anaesthesia groups.

Thus, the hormonal responses of neonates in the fentanyl anaesthesia group were diminished with respect to changes in plasma adrenaline, noradrenaline, glucagon, aldosterone, corticosterone, 11-deoxycortisol and 11-deoxycorticosterone at the end of surgery and postoperatively as compared to the responses of neonates in the non-fentanyl anaesthesia group. It is probable that inhibition of the hormonal responses of preterm neonates by fentanyl anaesthesia was responsible for mediating changes in the metabolic adjustments following surgical stress.

7.2.4 Metabolite changes :-

The changes in blood metabolite concentrations from the respective preoperative concentrations are compared between neonates in the fentanyl and non-fentanyl anaesthesia groups in Table 7.4.

Blood glucose concentrations were increased at the end of surgery in preterm neonates of both anaesthesia groups, but the magnitude of this intra-operative increase was substantially greater in the non-fentanyl anaesthesia group (p<0.025) as compared to the fentanyl anaesthesia group. At 6 hours postoperatively, blood glucose values had decreased to the preoperative concentrations in the fentanyl anaesthesia group whereas they remained elevated in the non-fentanyl anaesthesia group, with significant
differences between the two groups (p<0.005). At 12 and 24 hours after surgery, blood glucose concentrations had reverted to the respective preoperative values in both anaesthesia groups.

Blood lactate and pyruvate concentrations in the fentanyl anaesthesia group were not altered from preoperative values during surgery or in the postoperative period. However, blood lactate and pyruvate concentrations increased during surgery in the non-fentanyl anaesthesia group were decreased substantially below the preoperative values at 24 hours after surgery, giving rise to significant differences between the two groups at the end of surgery (p<0.02) and at 24 hours postoperatively (p<0.01).

Blood acetoacetate concentrations were unchanged at the end of surgery in the fentanyl anaesthesia group and increased during surgery in the non-fentanyl anaesthesia group; this response was significantly different between neonates in the two groups (p<0.05). Postoperatively, there was no significant difference between the blood acetoacetate changes in the two anaesthesia groups.

Blood alanine concentrations were not significantly altered from the respective preoperative concentrations at the end of surgery and during the postoperative period in the fentanyl and non-fentanyl anaesthesia groups and there were no differences in the responses of the two groups. However, the preoperative concentrations of blood alanine were found to be significantly higher in the non-fentanyl anaesthesia group (p<0.05) as compared to the fentanyl anaesthesia group.

Blood glycerol concentrations did not change at the end of surgery or at 6 hours postoperatively from the respective preoperative values in the
fentanyl and non-fentanyl anaesthesia groups. At 12 hours after surgery, blood glycerol concentrations were decreased in the non-fentanyl anaesthesia group and this response was significantly different from that of the fentanyl anaesthesia group \((p<0.01)\). There was no significant difference in blood glycerol changes at 24 hours postoperatively between the two anaesthesia groups.

The peri-operative changes in the derived hormonal-metabolic variables are compared between the fentanyl and non-fentanyl anaesthesia groups in Tables 7.5 and 7.6.

Blood concentrations of total ketone bodies did not change during or after surgery in the fentanyl and non-fentanyl anaesthesia groups and there were no differences in the responses of neonates in the two groups. The values for total gluconeogenic substrates remained unaltered during and after surgery in the fentanyl anaesthesia group, but were increased markedly at the end of surgery in the non-fentanyl anaesthesia group and had decreased at 24 hours postoperatively; thus the responses of neonates in the two groups were significantly different at the end of surgery \((p<0.01)\) and 24 hours after surgery \((p<0.005)\).

The lactate/pyruvate ratio was unchanged at the end of surgery and in the postoperative period in neonates from the fentanyl and non-fentanyl anaesthesia groups. In the fentanyl anaesthesia group, the insulin/glucose and alanine/pyruvate ratios remained unchanged during surgery, whereas in the non-fentanyl anaesthesia group, both ratios were found to be decreased at the end of surgery \((p<0.025)\). However, there were no significant differences in the insulin/glucose or alanine/pyruvate ratios between the two anaesthesia groups during or after surgery. There were no significant
differences between the fentanyl and non-fentanyl anaesthesia groups in 3-hydroxybutyrate/acetoacetate ratios at the end of surgery or at 6 and 12 hours after surgery, but this ratio was significantly lower in the fentanyl anaesthesia group at 24 hours after surgery (p<0.05).

Thus, the metabolic response of neonates in the non-fentanyl anaesthesia was characterised by the development of a significantly greater hyperglycaemia during and after surgery, together with significant increases in blood lactate, pyruvate and acetoacetate concentrations during surgery; these metabolic changes were found to be inhibited in the fentanyl anaesthesia group. In addition, total gluconeogenic substrates increased at the end of surgery and the insulin/glucose molar ratio was found to decrease during surgery in the non-fentanyl anaesthesia group, whereas they remained unchanged in the fentanyl anaesthesia group. It is probable that fentanyl anaesthesia was responsible for inhibiting the hyperglycaemia and substrate mobilisation caused by surgical stress in preterm neonates.

7.2.5 Urinary nitrogenous constituents :-

The changes in urinary 3-methylhistidine/creatinine (3-MH/Cr) molar ratios, measured in urine collected from 7 neonates in the fentanyl anaesthesia group and 6 neonates in the non-fentanyl anaesthesia group, are compared in Table 7.7.

As compared to values obtained on the first postoperative day, the 3-MH/Cr ratio increased significantly in the fentanyl anaesthesia group on the second postoperative day (p<0.05), but values obtained on the third postoperative day were not significantly different. In the non-fentanyl anaesthesia group, the 3-MH/Cr ratio was significantly increased on the second (p<0.05) and third (p<0.05) postoperative days, as compared to
values obtained on the first day after surgery. The urinary 3-MH/Cr ratios were significantly greater in the non-fentanyl anaesthesia group on the second (p<0.05) and third (p<0.05) days after surgery compared to corresponding values in the fentanyl anaesthesia group.

7.2.6 Postoperative clinical management :-

The postoperative clinical management of neonates in the fentanyl and non-fentanyl anaesthesia groups is compared in Table 7.7.

During the postoperative period, there was no significant difference in the rate of dextrose infusion between neonates in the fentanyl and non-fentanyl anaesthesia groups. Postoperative analgesia was prescribed by clinical staff to the neonates included in this trial without knowledge of their anaesthetic management. It was found that a larger number of neonates in the non-fentanyl anaesthesia group required analgesia on each of the three postoperative days as compared to neonates in the fentanyl anaesthesia group. Furthermore, the timing of the first analgesic dose after surgery was found to be significantly earlier for neonates in the non-fentanyl anaesthesia group (p<0.002) as compared to neonates in the fentanyl anaesthesia group.

During the postoperative period, there was a decrease in the body weight of neonates in the non-fentanyl anaesthesia group, whereas it was found to be unchanged in neonates belonging to the fentanyl anaesthesia group, the changes in body weight were significantly different between the two groups (p<0.025) on the second postoperative day.

7.2.7 Clinical observations :-

The mean heart rate of neonates in the fentanyl and non-fentanyl
anaesthesia groups before the induction of anaesthesia was identical and increased in both groups during surgery. However, the maximum heart rate during surgery was significantly higher in the non-fentanyl anaesthesia group (p<0.01) as compared to the fentanyl anaesthesia group. During surgery, the mean temperature loss was found to be 0.6 °C in the fentanyl anaesthesia group and 0.8 °C in the non-fentanyl anaesthesia group; this difference was not significant.

Peri-operative complications in the fentanyl and non-fentanyl anaesthesia groups are described in Table 7.8. Intra-operative complications were found to be similar in the fentanyl and non-fentanyl anaesthesia groups; the commonest complication found during surgery was a cyanotic episode with bradycardia usually occurring when the left lung was retracted for dissection of the patent ductus arteriosus.

In the postoperative period, preterm neonates in the non-fentanyl anaesthesia group were found to be more clinically unstable than neonates in the fentanyl anaesthesia group. This was reflected by the higher rate of complications in the non-fentanyl anaesthesia group and the greater severity of the complications that were documented. The most frequent complications in the non-fentanyl anaesthesia group were related to the cardiorespiratory system and metabolic milieu of the preterm neonates. In the fentanyl anaesthesia group, however, the commonest complication was a certain degree of temperature variability during the postoperative period which was found in 6 of the 8 neonates given fentanyl anaesthesia.

Thus, it was found that inhibition of the hormonal and metabolic responses in preterm neonates given fentanyl anaesthesia was associated with a greater clinical stability in the postoperative period and a reduced
requirement of postoperative analgesia.

7.3 DISCUSSION:

The two groups of preterm neonates compared in this trial were similar in their characteristics, they had undergone an identical surgical operation, and the clinical management before and during surgery was also identical. An important short-coming in the fentanyl trial, however, was the smaller number of neonates in each anaesthesia group. Thus, the power of the trial to detect significant differences between the hormonal and metabolic responses of preterm neonates in the fentanyl and non-fentanyl anaesthesia groups was decreased according to the criteria defined in Chapter V.

7.3.1 Hormonal changes:

CATECHOLAMINES

In both anaesthesia groups, plasma adrenaline and noradrenaline were found to be raised before surgery, which may be due to the unstable clinical condition of preterm neonates undergoing PDA ligation (Mikhail et al., 1982). All neonates had hyaline membrane disease and were being ventilated prior to surgery, fluids had been restricted for a duration of 24-72 hours and all the neonates were receiving diuretics for the control of congestive heart failure secondary to the patent ductus, ten neonates had undergone a trial of indomethacin therapy (0.2 mg/kg given 12 hourly for 3 doses). In addition, noradrenaline values before and after surgery were found to be much higher than those documented in the preliminary study and halothane trial. This difference may be related to the measurement of noradrenaline concentrations in arterial blood (Christensen et al., 1984; Esler et al., 1984) since circulating noradrenaline is known to be extracted by the peripheral muscular tissues and by viscoeral organs (Esler et al., 1984).
The most prominent difference in the hormonal responses of preterm neonates with and without fentanyl anaesthesia during PDA ligation was in the pattern of changes in plasma adrenaline concentrations. In all the neonates given fentanyl anaesthesia, plasma adrenaline concentrations were decreased at the end of surgery and remained below preoperative concentrations at 6, 12 and 24 hours after surgery. On the other hand, the response of neonates in the non-fentanyl anaesthesia group was characterised by a marked increase in the plasma adrenaline at the end of surgery, which remained elevated throughout the postoperative period. Thus, there were clear differences between the adrenaline response of preterm neonates in the two anaesthesia groups at the end of surgery as well as postoperatively.

There was a trend towards similar differences in the noradrenaline responses of preterm neonates in the fentanyl and non-fentanyl anaesthesia groups during and after surgery. However, this was found to be significant only at 24 hours after surgery. The lack of consistent differences between the noradrenaline responses of preterm neonates in the fentanyl and non-fentanyl anaesthesia groups may be due to the variability of noradrenaline kinetics in the plasma (Cryer, 1984) or the measurement of these values in arterial blood (Christensen et al, 1984). However, it is tempting to suggest that suppression of the adrenaline response to surgery is mediated by the effect of fentanyl on high-affinity mu- and kappa-opiate receptors in the hypothalamus (Pleuvry, 1983) which decreases the sympathetic outflow to the adrenal medulla (Van Loon, 1981); on the other hand, this suppression may not be obtained in the lungs, kidneys or the peripheral sympathetic ganglia, which are known to be the primary sites of noradrenaline release in adult subjects (Esler et al, 1984).
Although there are no published data on the effects of fentanyl anaesthesia on the catecholamine responses of preterm neonates, although such an effect may be inferred from indirect evidence available in the literature. Thus, Robinson and Gregory (1981) found that fentanyl anaesthesia was associated with a remarkable degree of cardiovascular stability during surgery. In neonates and older infants undergoing cardiac surgery, Hickey and Hansen (1984) documented that fentanyl in doses of 50 and 75 μg/kg could block the cardiovascular responses, such as increases in heart rate, systolic, diastolic and mean arterial pressures associated with tracheal intubation and surgical trauma (Hickey and Hansen, 1984) which are known to be mediated by catecholamine release from studies in adult subjects (Cummings et al, 1983; Derbyshire et al, 1983; Stanley et al, 1980). Vacanti et al have shown recently that fentanyl blocks the hyperreactive pulmonary vasoconstriction in neonates with congenital diaphragmatic hernia, which may be triggered by a variety of stressful stimuli in the postoperative period (Vacanti et al, 1984).

In adult patients undergoing cardiac surgery, a suppression of the adrenaline and noradrenaline response to surgical stress before the start of cardiopulmonary bypass has been obtained with high-dose fentanyl anaesthesia in several studies (Stanley et al, 1980; Sebel et al, 1981; Kono et al, 1981; Zurick et al, 1982). The dose of fentanyl in these studies ranged from 60 μg/kg (Sebel et al, 1981) to 150 μg/kg (Zurick et al, 1982) and was found to inhibit the increase in plasma adrenaline and noradrenaline concentrations only during the surgical procedure before the start of cardiopulmonary bypass. Thereafter, the catecholamine responses to deep hypothermia and cardiopulmonary bypass were not suppressed (Kono et al, 1981; Zurick et al, 1982). In a well-controlled experimental study, Taborsky et al (1982) demonstrated that morphine anaesthesia not only
suppressed the plasma adrenaline and noradrenaline responses to laparotomy in dogs, but also caused a significant decrease in plasma catecholamines after surgery. Since the catecholamine response to the injection of a neuroglucopenic agent (2-deoxyglucose) was not affected by morphine anaesthesia, they concluded that morphine only blocks the responses to nociceptive stimulation probably due to its analgesic properties (Taborsky et al, 1982). This conclusion is in keeping with the specific binding of morphine with μ-opiate receptors on neurones in particular areas of the brain (Pleuvry, 1983). In adult patients undergoing upper abdominal surgery, Campbell et al (1984) have documented that the noradrenaline response to non-cardiac surgery could be inhibited even with the use of moderate doses of fentanyl (15-20 μg/kg). The results obtained from preterm neonates in the present study would therefore be comparable to those of Campbell et al (1984). The recent report by Pathak et al (1985), in which a suppression of the adrenaline response to major orthopaedic surgery was observed in patients who were given a much smaller induction dose of fentanyl (2.5 μg/kg) followed by a continuous low dose infusion during the surgical procedure (1.5-2.5 μg/kg/hr), may need further confirmation.

GLUCAGON
Plasma glucagon concentrations in the fentanyl anaesthesia group decreased slightly during surgery and remained below the preoperative concentrations at 6, 12 and 24 hours after surgery, whereas in the non-fentanyl group plasma glucagon increased during surgery, with significant differences in the responses of neonates in the two anaesthesia groups. This increase in the non-fentanyl anaesthesia group may have resulted from the stimulation of glucagon secretion by adrenaline release during surgery (Sperling et al, 1984). In the both the anaesthesia groups, there was a trend towards a
decrease in plasma glucagon concentrations by 24 hours after surgery, which was significant when the data for both groups were analysed together. This pattern is similar to the changes documented from the preliminary study and the halothane trial and also confirms differences in the plasma glucagon response of newborn infants compared to that of adult patients undergoing surgery (Russell et al, 1975).

INSULIN
The plasma insulin values documented in this trial were similar to the corresponding values obtained from preterm neonates included in the preliminary study. In the fentanyl anaesthesia group there was a trend towards an increase in plasma insulin concentrations during surgery, whereas in the non-fentanyl anaesthesia group there was a trend towards a decrease in plasma insulin concentrations at the end of surgery, but the difference in responses of the two anaesthesia groups was not significant. The lack of an increase in insulin concentrations despite the substantial hyperglycaemia documented from neonates in the non-fentanyl anaesthesia group may be related to the marked adrenaline release in these neonates during surgery (Sperling et al, 1984). However, it is not known whether the decreased responsiveness of beta-islet cells in the premature pancreas (Soltesz and Aysley-Green, 1984) or surgical handling of the vagus nerve which occurs during PDA ligation (Mikhail et al, 1982) have a role in suppression of the insulin response to surgical hyperglycaemia.

The difference in insulin responses of the preterm neonates in the fentanyl and non-fentanyl anaesthesia groups was characterised further by a comparison of changes in the insulin/glucagon ratio between the two groups, since it has been proposed (Sperling, 1982) that changes in this ratio may have a greater physiological significance than changes in the individual
hormones themselves. A substantial increase in the insulin/glucagon ratio was documented from neonates in the fentanyl anaesthesia group at the end of surgery, whereas neonates in the non-fentanyl anaesthesia group responded with a marked decrease in the insulin/glucagon ratio at the end of surgery. It is probable that these striking differences may have resulted from the respective adrenaline responses of neonates in the two anaesthesia groups and may, in turn, have mediated important differences in the metabolic response of preterm neonates in the fentanyl and non-fentanyl anaesthesia groups.

STEROID HORMONES

It was noted that there were certain characteristic features in the pattern of the adrenocortical response documented from preterm neonates in this trial, which were different from the corresponding changes documented in the (predominantly) term neonates included in the halothane trial. The response of preterm neonates was characterised by a lesser secretion of the final products of steroid synthesis, eg, cortisol and aldosterone, together with an increased secretion of precursor hormones, eg, corticosterone, 11-deoxycorticosterone, 11-deoxycortisol and 17-hydroxyprogesterone. These differences were evident on comparing the anaesthetic groups in this trial with the corresponding anaesthetic groups in the halothane trial. It is proposed that these differences may reflect a relative immaturity of the steroid biosynthetic pathway in preterm newborn infants. It is well-known that steroid hydroxylase enzymes in the fetal adrenal cortex mature from the proximal to the distal end of the steroid biosynthetic pathway (Solomon et al, 1967; Villee and Driscoll, 1965). Furthermore, it is believed that the synthesis of cortisol and aldosterone is confined primarily to the definitive zone of the adrenal cortex (Davies, 1982) which is likely to have been poorly developed in premature neonates.
Sippell et al (1981) have documented that concentrations of all corticosteroid hormones increase gradually in the amniotic fluid from 14-16 weeks of gestation until 36-38 weeks. From the molar ratio of each steroid to its precursor hormone, they found that the activity of mitochondrial hydroxylases increased substantially after approximately 30 weeks of gestation (Sippell et al, 1981). Furthermore, they have found that during the neonatal period, preterm neonates have lower circulating concentrations of the final biosynthetic products (eg, cortisol, cortisone, aldosterone) in comparison with term neonates, whereas circulating concentrations of the precursor hormones such as progesterone, 17-hydroxyprogesterone and 11-deoxycorticosterone are increased in preterm neonates (Sippell, 1985; Sippell et al, 1978c). Although Kenny et al (1963) found that the cortisol production rates of normal preterm and term neonates soon after birth were similar, the preterm neonates included in their study were considerably more mature than the neonates included in this study. Furthermore, it is likely that a relative immaturity of the steroid biosynthetic pathway may be evident only at the time of adrenocortical stimulation caused by stress, and may not have been identified in the unstressed premature neonates studied by Kenny et al (1963).

Glucocorticoids: Plasma cortisol concentrations increased during surgery in both groups of preterm neonates, and although the magnitude of increase was greater in the non-fentanyl anaesthesia group, this difference was not statistically significant. However, this finding may be unreliable since only a small number of neonates were included in the non-fentanyl anaesthesia group; thus, further studies with a larger sample size will be required to investigate whether fentanyl anaesthesia during surgery results in a true difference in the cortisol response of preterm neonates.
Plasma corticosterone concentrations were found to increase during surgery in both groups of preterm neonates, but the magnitude of increase in neonates from the non-fentanyl anaesthesia group was approximately three times the corresponding changes in the fentanyl anaesthesia group. The marked difference in this response may be related to the generalised suppression of the hormonal stress response caused by deep opiate analgesia through the hypothalamus. It is possible that the reduced adrenocortical stimulation may be mediated by a decreased secretion of corticotropin releasing factor, and consequently, that of ACTH.

In addition, plasma 11-deoxycortisol concentrations were decreased below the preoperative value at the end of surgery in the fentanyl anaesthesia group, whereas in the non-fentanyl anaesthesia group a substantial increase was recorded during surgery. These differences between the two anaesthesia groups provide further evidence for suppression of the hormonal response to surgery by fentanyl anaesthesia in preterm neonates.

Several studies in adult patients undergoing non-cardiac surgery have shown that the adrenocortical response was inhibited by fentanyl anaesthesia (George et al, 1974; Reier et al, 1973; Haxholdt et al, 1981; Cooper et al, 1981; Campbell et al, 1984). In these studies, the degree of surgical trauma was much greater than the amount of trauma associated with the ligation of a PDA and relatively larger doses of fentanyl were used to obtain suppression of the adrenocortical response to surgery.

Mineralocorticoids: Plasma aldosterone concentrations increased during surgery in both groups of preterm neonates undergoing PDA ligation and although the magnitude of increase was greater in the non-fentanyl group,
this difference was not significant at the end of surgery. During the postoperative period, however, the responses of neonates in the fentanyl and non-fentanyl anaesthesia groups were significantly different, since elevated aldosterone concentrations were maintained in preterm neonates in the non-fentanyl anaesthesia group, whereas they had declined to values below the preoperative concentrations in neonates who received fentanyl anaesthesia during surgery. Thus, it is likely that the decreased stress associated with surgery in preterm neonates who were given fentanyl anaesthesia was also responsible for a decrease in the aldosterone response in comparison with that of neonates in the non-fentanyl anaesthesia group.

In adult patients, it has been shown that the aldosterone response to surgery can be abolished by etomidate anaesthesia (Moore et al, 1985; Fragen et al, 1984) or by the administration of intravenous saline during surgery (Engquist et al, 1978), but the effect of fentanyl on this response has not been investigated.

Plasma 11-deoxycorticosterone concentrations increased during surgery in both anaesthesia groups. However, the changes observed in the non-fentanyl anaesthesia group were four times that of the corresponding changes in preterm neonates in the fentanyl anaesthesia group. These differences provide further evidence for suppression of the adrenocortical responses in preterm neonates who were given fentanyl anaesthesia.

**Precursor hormones:** No significant differences were obtained between the two anaesthesia groups with respect to changes in the plasma concentrations of progesterone and 17-hydroxyprogesterone during and after surgery. In view of the marked differences obtained in other parameters of the hormonal and metabolic stress response of preterm neonates, it is possible that the lack of any differences in plasma 17-hydroxyprogesterone between neonates
in the two groups provides further evidence against the proposal by Murphy et al (1983). Thus, it is unlikely that 17-hydroxyprogesterone is responsive to stress, particularly in preterm neonates exposed to acute and well-defined stressful stimuli such as surgery.

In this study, fentanyl anaesthesia during PDA ligation caused a suppression of the hormonal response of preterm neonates with respect to changes in plasma adrenaline, glucagon, mineralocorticoid and glucocorticoid concentrations at the end of surgery and postoperatively. In addition, the pattern of changes in the insulin/glucagon molar ratio was markedly altered in preterm neonates given fentanyl anaesthesia. It is likely that these differences in the hormonal response were responsible for alterations in the metabolic stress response of preterm neonates undergoing PDA ligation.

7.3.2 Metabolic changes

The circulating concentrations of metabolites were measured in arterial blood obtained from the preterm neonates included in this trial. As expected therefore, the blood glucose values before and after surgery were found to be higher (Harris, 1974), blood lactate concentrations were found to be lower (Koch and Wendel, 1968), whereas the concentrations of other blood metabolites were not significantly different from the corresponding values measured in venous blood in the halothane trial and the preliminary study.

GLUCOSE

The most prominent difference in the metabolic response of neonates in the fentanyl and non-fentanyl anaesthesia groups was found to be in the magnitude and duration of peri-operative hyperglycaemia stimulated by
surgical stress. Neonates in the non-fentanyl anaesthesia group responded with the development of a substantial hyperglycaemia at the end of surgery whereas neonates in the fentanyl anaesthesia group responded with a slight increase in blood glucose concentrations during surgery. This difference in the hyperglycaemic response was further accentuated in the postoperative period, since blood glucose concentrations had returned to preoperative values in the fentanyl anaesthesia group and were markedly elevated in the non-fentanyl anaesthesia group.

The mechanism of peri-operative hyperglycaemia in the non-fentanyl anaesthesia group was probably caused by the marked increases of adrenaline and glucagon concentrations during surgery, which were not observed in the neonates given fentanyl anaesthesia. The hyperglycaemic response mediated by these hormonal changes may have been synergistically potentiated by the greater release of glucocorticoid hormones in neonates in the non-fentanyl anaesthesia group (De Fronzo et al, 1980; Shamoon et al, 1981).

Although only a slight decrease in plasma insulin concentrations of preterm neonates in the non-fentanyl anaesthesia group was documented, the suppression of insulin secretion during surgery was clearly evident from the substantial decrease in the insulin/glucagon and the insulin/glucose ratios at the end of surgery. Whereas the insulin/glucagon ratio may have a prominent effect on control of glucose homeostasis in newborn infants (Sperling, 1982), a decrease in the insulin/glucose ratio denotes an inappropriately low insulin response to the circulating glucose concentrations at the end of surgery (Soltesz and Aynsley-Green, 1984). In direct contrast to these responses, preterm neonates in the fentanyl anaesthesia group demonstrated a marked increase in the insulin/glucagon ratio at the end of surgery and a distinct increase in the insulin/glucose
ratio; both of which denote an appropriate insulin response to the mild surgical hyperglycaemia documented in these neonates. The clinical significance of the changes in blood glucose concentration of preterm neonates in the non-fentanyl anaesthesia group is discussed below.

GLUCONEOGENIC SUBSTRATES

Concomitant with surgical hyperglycaemia, the responses of neonates in the two anaesthesia groups were significantly different with respect to changes in blood lactate and pyruvate concentrations at the end of surgery. Blood lactate and pyruvate concentrations increased in neonates in the non-fentanyl anaesthesia group, whereas they were unchanged in neonates given fentanyl anaesthesia during surgery. This difference is also probably derived from the lack of changes in plasma adrenaline in the fentanyl anaesthesia group, which may have resulted in lower gluconeogenic substrate mobilisation (Kraus-Friedman, 1984; Sperling et al, 1984). This effect was also evident from the changes in total gluconeogenic substrates, which were decreased slightly in the fentanyl anaesthesia group and increased substantially in the non-fentanyl anaesthesia group.

It is possible that the mobilisation of gluconeogenic substrates was required to support the postoperative hyperglycaemic response of neonates in the latter group through the stimulation of gluconeogenesis (Frazer et al, 1981; Kalhan et al, 1980; Bougneres et al, 1982). Similar findings have been documented from preterm neonates in the preliminary study (Chapter IV). In this context, it is not unexpected that changes in alanine concentration were not significantly different between neonates in the two anaesthesia groups, since blood alanine concentration is known to be a poor indicator of alanine turnover in term newborn infants (Frazer et al, 1981) and similar considerations may apply to the preterm neonate. During the
postoperative period, the blood concentrations of gluconeogenic substrates (lactate, pyruvate, alanine, glycerol) were found to be significantly lowered in preterm neonates in the non-fentanyl anaesthesia group which may indicate the utilisation of these substrates for glucose production by the gluconeogenic pathway (Patel et al., 1982).

In adult patients, a suppression of the hyperglycaemic response to surgical stress with fentanyl anaesthesia has been documented in several studies. This effect has been demonstrated in adult patients undergoing cardiac surgery with cardiopulmonary bypass (Walsh et al., 1981; Sebel et al., 1981; Brandt et al., 1978) as well as in patients undergoing non-cardiac surgery (Hall et al., 1978; Cooper et al., 1981; Campbell et al., 1984). The perioperative increase in plasma concentrations of gluconeogenic substrates, particularly relating to blood lactate, pyruvate and alanine values, was also suppressed with the use of fentanyl anaesthesia (Walsh et al., 1981; Cooper et al., 1981; Hall et al., 1978). In contrast, Haxholdt et al. (1981) found that fentanyl anaesthesia given to patients undergoing upper abdominal surgery did not have any effect on the hyperglycaemic response (Haxholdt et al., 1981). The findings from the latter study are atypical and difficult to explain, and could be related possibly to the difference in methods used for the measurement of blood glucose in this study and the subsequent investigations (Walsh et al., 1981; Campbell et al., 1984).

However, the findings from preterm neonates in the present study differ from the previous studies in adult patients with respect to the dose of fentanyl required to inhibit the metabolic stress response. The suppression of surgical hyperglycaemia and substrate mobilisation can be obtained in adult patients given fentanyl in dose of 50 μg/kg or more (Sebel et al., 1981; Walsh et al., 1981; Cooper et al., 1981; Hall et al., 1978); except in
the recent study by Campbell et al (1984), in which fentanyl was given in a mean dose of 17 μg/kg. In contrast, the mean dose of fentanyl given to preterm neonates in the present study was 12 μg/kg. It is tempting to speculate that this difference may related to the greater sensitivity of newborn infants to opiate analgesia during surgery, similar to their sensitivity documented with respect to respiratory depression (Way et al, 1965; Evans et al, 1976); or may be related to circulating endogenous opioids such as beta-endorphin, which are released during surgical stress (Dubois et al, 1981) and are known to have potent analgesic properties in adult patients (Foley et al, 1979), high concentrations of which have been documented in newborn infants (Wardlaw et al, 1979; Puolakka et al, 1982; Facchinetti et al, 1982; Panerai et al, 1983). Dubois et al (1982) have recently demonstrated that fentanyl in a dose of 10-20 μg/kg is capable of abolishing the beta-endorphin response of adult patients to abdominal surgery (Dubois et al, 1982).

KETONE BODIES

Probably due to the differences in the catecholamine and glucagon response blood acetoacetate concentrations were increased slightly at the end of surgery in neonates in the non-fentanyl anaesthesia group, whereas this change was not observed in neonates given fentanyl anaesthesia. On the other hand, there were no differences in the pattern of changes in total ketones bodies or 3-hydroxybutyrate concentrations as well as the 3-hydroxybutyrate/acetoacetate molar ratio between preterm neonates in the two anaesthesia groups. Furthermore, it was observed that the changes in ketone bodies documented during and after surgery in this trial were very slight in comparison to the corresponding changes obtained from neonates in the halothane trial. The lack of change in ketone body concentrations during surgery may be either due to lowered fatty acid mobilisation caused
by poor fat reserves in preterm babies (Girard and Ferre, 1982) or possibly related to the low levels of carnitine acyltransferase that have been found in the premature liver (Girard and Ferre, 1982).

In conclusion, the metabolic response of preterm neonates in the fentanyl anaesthesia group was substantially decreased in comparison with the responses obtained from neonates in the non-fentanyl anaesthesia group. These effects were observed with respect to the surgical hyperglycaemia and substrate mobilisation precipitated by the hormonal changes documented in the latter group, which were largely inhibited in the neonates given fentanyl anaesthesia during the surgical stress.

7.3.3 Urinary nitrogenous constituents :-

As in the previous studies, changes in urinary 3-methylhistidine/creatinine (3-MH/Cr) ratios were compared to the values obtained from urine collected within 24 hours after surgery in the fentanyl and non-fentanyl anaesthesia groups. In the fentanyl anaesthesia group, the 3-MH/Cr ratio increased slightly but significantly on the second day after surgery, but was not raised on the third postoperative day. In the non-fentanyl anaesthesia group, significant increases in 3-MH/Cr values were obtained on both the postoperative days and these values were significantly greater than the 3-MH/Cr ratios documented from neonates in the fentanyl anaesthesia group. Although these differences may indicate a greater degree of endogenous protein breakdown in the neonates who did not receive fentanyl anaesthesia during surgery, this finding would need to be confirmed by the measurement of postoperative nitrogen balance in preterm neonates undergoing surgery.

The magnitude of these increases in 3-MH/Cr ratios were greater than the changes documented from neonates in the preliminary study and the halothane
trial, and may represent an increased rate of endogenous protein breakdown in preterm neonates subjected to surgical stress. From measurement of urinary 3-MH/Cr ratios in normal preterm and term neonates, it has been documented that the rate of myofibrillar protein degradation is greater in preterm neonates as compared to term neonates (Tomas et al., 1979; Burgoyne et al., 1982). In addition, stable isotope turnover studies have shown that the rates of protein synthesis, protein breakdown and whole body protein turnover are much higher in preterm neonates as compared to term neonates or any other age group (Pencharz et al., 1977; Nissim et al., 1983; De Benoist et al., 1984). Using similar measurements in newborn guinea pigs, Ogata and Holliday (1976) showed that changes in the rate of muscle protein catabolism may influence net muscle protein balance to a greater degree than changes in the rate of synthesis.

If these findings are applicable to the human preterm neonate, it follows that the catabolic stimulus of surgical stress may possibly produce a greater degree of endogenous protein breakdown in premature newborn infants than in the term neonate or older age groups. The magnitude of the changes in urinary 3-MH/Cr ratios obtained from neonates in the non-fentanyl anaesthesia group point towards a similar conclusion. In addition, the significantly greater postoperative weight loss found in preterm neonates from the non-fentanyl anaesthesia group may be related to an increased rate of endogenous protein breakdown in these neonates.

Ballard et al. (1979) measured the urinary 3-MH excretion and calculated the rate of muscle protein degradation in normal and sick preterm neonates. They found that the rate of muscle protein degradation was raised in neonates who were losing weight at the time of the study and markedly raised in neonates who died within 2 weeks of the analysis. In a similar
study, Seashore et al (1980) found that preterm neonates who were clinically 'stressed' due to the multiple problems of prematurity, had a negative nitrogen balance and raised $3$-$\text{MH}/\text{Cr}$ ratios as compared to clinically stable preterm neonates who were gaining weight at the time of study. It is interesting to note that the urinary $3$-$\text{MH}/\text{Cr}$ ratios measured from neonates in the non-fentanyl anaesthesia group were more than twice the values documented by Seashore et al (1980) from clinically stressed neonates, which may indicate that the additional stress of surgery under minimal anaesthesia may precipitate a greater loss of protein in sick preterm neonates.

Thus, in the preterm neonates given fentanyl anaesthesia during surgery inhibition of the hormonal stress response may result in a decreased postoperative catabolism of carbohydrate and protein reserves in preterm neonates.

7.3.4 Clinical observations :-

The heart rate of all preterm neonates increased with the start of surgery, but the maximum heart rate achieved during surgery by neonates in the non-fentanyl anaesthesia group was significantly greater than that of neonates in the fentanyl anaesthesia group. This difference was evidently due to the catecholamine release during surgery in the former group, which was found to be attenuated in the neonates given fentanyl anaesthesia.

The intra-operative complications were found to be similar in the two anaesthesia groups, but during the post-operative period it was found that the clinical state of neonates in the non-fentanyl anaesthesia group was more unstable than that of neonates in the fentanyl anaesthesia group. This was particularly observed from the incidence of cardiorespiratory
complications such as frequent spontaneous bradycardias, hypotension and poor peripheral circulation, which in some cases required treatment with an increase in the ventilatory requirements during the postoperative period. Metabolic complications related to the severe degree of hyperglycaemia were observed in a few cases, e.g., glycosuria and a metabolic acidosis which persisted for more than 12 hours postoperatively in 2 neonates, despite adequate ventilation and correction with THAM.

As discussed in Chapter IV, the change in blood glucose concentrations at the end of surgery, particularly in neonates from the non-fentanyl anaesthesia group, may have significant clinical implications (Finberg, 1967) due to its effect on plasma osmolality (Gennari, 1984). Although the degree of change in osmolality due to hyperglycaemia was not very marked, the rapidity of this change during the 15 minutes of surgery may be of greater clinical significance in the development of intraventricular haemorrhage (Arant and Gooch, 1978) or other effects (Finberg, 1967). In addition, Levene et al (1984) have found that metabolic acidosis is a prominent factor associated with the extension of an intra-ventricular haemorrhage in sick preterm neonates.

In this study, an intra-ventricular haemorrhage was documented in two preterm neonates for the first time in the postoperative period, both of whom belonged to the non-fentanyl anaesthesia group. Although no causal inferences can be made from this observation, it is proposed that a careful clinical and ultrasound examination before and after surgery in preterm newborn infants would provide further information in this direction.

Similar to the findings in the halothane trial, neonates in the fentanyl anaesthesia group were clinically more stable and were found to have a
lesser requirement for analgesia in the postoperative period than neonates in the non-fentanyl anaesthesia group. In addition, the first analgesic dose was required at a significantly greater duration after surgery than neonates in the non-fentanyl anaesthesia group. Thus, fentanyl anaesthesia during PDA ligation in preterm neonates may be associated with a relatively stable clinical condition during the period after surgery.

7.3.5 Hypothesis

The question to be answered by this trial was whether anaesthesia given with fentanyl to preterm neonates undergoing PDA ligation could decrease their hormonal and metabolic stress response. It was proposed that the null hypothesis would be rejected if there was a significant difference (at the p<0.05 level) in outcome measures such as plasma adrenaline, plasma noradrenaline and blood glucose changes at the end of surgery, and in the urinary 3-MH/Cr molar ratio during the three postoperative days.

On comparison of the responses of neonates in the fentanyl and non-fentanyl anaesthesia groups, a significant difference was found between the two groups in plasma adrenaline (p<0.002) and blood glucose (p<0.025) changes at the end of surgery, which was maintained into the postoperative period. In addition, significant differences were also documented between neonates in the fentanyl and non-fentanyl anaesthesia groups in the urinary 3-MH/Cr ratios on the second (p<0.05) and third (p<0.05) postoperative days.

On the basis of these results, it is possible to reject the null hypothesis and propose that fentanyl anaesthesia can decrease the hormonal and metabolic response of preterm neonates to surgical stress. This conclusion is justified since significant differences between the two groups were documented in 3 of the outcome measures defined for testing the hypothesis,
even though the power of the trial was lowered due to the entry of a smaller number of patients than had been proposed in the design of the trial. The fact that the significance level of these differences was much higher than expected implies that the actual magnitude of the effect was greater than that hypothesized for calculation of the sample size. Furthermore, this evidence was corroborated with the identification of significant differences in other hormonal and metabolic variables which are known to be responsive to surgical stress, but were not included as definitive outcome measures.

7.4 CONCLUSION

Anaesthesia given with fentanyl in a dose of 10–20 mcg/kg to preterm neonates undergoing ligation of a patent ductus arteriosus causes a decrease in their hormonal and metabolic response stimulated by the surgical stress. This effect may be associated with a decreased breakdown of body tissues in the postoperative period and an improved clinical outcome following surgery in preterm neonates.
Figure 7.1: Anaesthetic protocol for preterm neonates randomly allocated to the Non-fentanyl anaesthesia group.
FENTANYL TRIAL: ANAESTHETIC PROTOCOL

NON-FENTANYL GROUP

1. Preoxygenation (2-3 minutes)

2. Intubation: Done pre-operatively for R.D.S.

3. Intravenous fluids: 4% Dextrose + 0.18% saline 6-9 ml/kg/hr

4. Relaxant: d-Tubocurarine 0.2-0.4 mg/kg

5. Maintenance: (a) Nitrous Oxide + Oxygen = 66:33%.
   (NB. Nitrous Oxide should not be used in concentrations higher than 66%. For patients with an increased oxygen requirement, lower concentrations may be used).
   (b) Supplements of d-Tubocurarine 0.1-0.2 mg/kg IV.

6. Reversal of relaxation: Atropine 0.02 mg/kg IV
   Neostigmine 0.05 mg/kg IV
   (NB. Reversal may or may not be given).
Figure 7.2: Anaesthetic protocol for preterm neonates randomly allocated to the Fentanyl anaesthesia group.
FENTANYL TRIAL: ANAESTHETIC PROTOCOL

FENTANYL GROUP

1. Preoxygenation: (2-3 minutes)

2. Intubation: Done pre-operatively for RDS.

3. Induction: INTRAVENOUS FENTANYL 10-20 μg/kg (given slowly).
   Nitrous Oxide + Oxygen = 66:33%

4. Intravenous fluids: 4% Dextrose + 0.18% Saline = 6-9 ml/kg/hr
   OR
   5% Dextrose = 5-7 ml/kg/hr.

5. Maintenance: (a) Nitrous Oxide + Oxygen = 66:33%
   (b) d-Tubocurarine 0.2-0.4 mg/kg IV
   (c) FENTANYL: Supplements 3-5 μg/kg IV

   (NB. 1. For patients with an increased oxygen requirement,
   lower concentrations of Nitrous oxide may be used.

   2. Supplements of d-Tubocurarine or Fentanyl may or may not be given, depending on the length of the surgical procedure.)

6. Reversal of relaxation: - Atropine 0.02 mg/kg IV
   Neostigmine 0.05 mg/kg IV

   (NB. Reversal may or may not be given).
### Table 7.1 Fentanyl Trial: Description of Patients and Clinical Management

<table>
<thead>
<tr>
<th></th>
<th>Fentanyl Anaesthesia</th>
<th>Mann-Whitney U Test</th>
<th>Non-fentanyl Anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td>8</td>
<td>-</td>
<td>8</td>
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<tr>
<td><strong>Age, days</strong></td>
<td>13 ± 1</td>
<td>n.s.</td>
<td>17 ± 3</td>
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<tr>
<td><strong>Gestation, weeks</strong></td>
<td>28.6 ± 0.7</td>
<td>n.s.</td>
<td>28.3 ± 0.3</td>
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<tr>
<td><strong>Birth weight, kg</strong></td>
<td>1.1 ± 0.1</td>
<td>n.s.</td>
<td>1.0 ± 0.1</td>
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<tr>
<td><strong>PRE-OPERATIVE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dextrose infusion rate, mg/kg/min</strong></td>
<td>4.4 ± 0.4</td>
<td>n.s.</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td><strong>Starvation, hours pre-operative</strong></td>
<td>4.9 ± 0.4</td>
<td>n.s.</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td><strong>TPN: No of patients pre-operative</strong></td>
<td>3</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td><strong>Antibiotics: No of patients Days pre-operative</strong></td>
<td>3.3 ± 0.3</td>
<td>n.s.</td>
<td>4.8 ± 1.8</td>
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<td></td>
<td>8.5 ± 4.2</td>
<td>n.s.</td>
<td>3.3 ± 1.6</td>
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<tr>
<td></td>
<td>6 ± 1</td>
<td>n.s.</td>
<td>5 ± 1</td>
</tr>
<tr>
<td><strong>INTRA-OPERATIVE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td><strong>Weight at operation, kg</strong></td>
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<td>n.s.</td>
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<tr>
<td><strong>Dextrose infusion rate, mg/kg/min</strong></td>
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<td>n.s.</td>
<td>5.7 ± 0.6</td>
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<td><strong>Surgical stress score</strong></td>
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<td>n.s.</td>
<td>8.3 ± 0.4</td>
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<tr>
<td><strong>Blood transfusion:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No of patients</strong></td>
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<td>-</td>
<td>2</td>
</tr>
<tr>
<td><strong>Volume, ml</strong></td>
<td>6 ± 1</td>
<td>n.s.</td>
<td>17 ± 3</td>
</tr>
<tr>
<td><strong>Temperature loss °C</strong></td>
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<td>n.s.</td>
<td>0.8 ± 0.3</td>
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<tr>
<td><strong>Dose of d-tubocurarine mg/kg</strong></td>
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<td>n.s.</td>
<td>0.57 ± 0.1</td>
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<td><strong>Dose of fentanyl, µg/kg</strong></td>
<td>12.2 ± 1.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Heart rate:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Base-line</strong></td>
<td>148 ± 5</td>
<td>n.s.</td>
<td>145 ± 3</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>170 ± 7</td>
<td>p&lt;0.01</td>
<td>199 ± 5</td>
</tr>
<tr>
<td><strong>Minimum</strong></td>
<td>130 ± 10</td>
<td>n.s.</td>
<td>134 ± 3</td>
</tr>
</tbody>
</table>

Comparison of patient characteristics and clinical management before and during surgery between neonates in the fentanyl and non-fentanyl anaesthesia groups. (Differences between the two groups are analysed by the Mann-Whitney U Test. All values = Mean ± SEM.)
Table 7.2 FENTANYL TRIAL: Hormonal changes

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Pre-operative</th>
<th>FENTANYL</th>
<th>Mann-Whitney U Test</th>
<th>NON-FENTANYL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline</td>
<td></td>
<td>N</td>
<td>Mean ± SEM</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>1.29 ± 0.43</td>
<td>1.11 ± 0.43</td>
</tr>
<tr>
<td>Δ Adrenaline</td>
<td>End-op</td>
<td>7</td>
<td>-0.41 ± 0.24</td>
<td>p&lt;0.002</td>
</tr>
<tr>
<td>mmol/L</td>
<td></td>
<td>7</td>
<td>-0.62 ± 0.27</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>7</td>
<td>-0.52 ± 0.20</td>
<td>p&lt;0.025</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>7</td>
<td>-0.88 ± 0.34</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>7</td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>Pre-operative</td>
<td>8</td>
<td>12.26 ± 2.78</td>
<td>n.s.</td>
</tr>
<tr>
<td>Δ Noradrenaline</td>
<td>End-op</td>
<td>8</td>
<td>5.19 ± 2.81</td>
<td>n.s.</td>
</tr>
<tr>
<td>mmol/L</td>
<td></td>
<td>8</td>
<td>-2.33 ± 1.18</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>8</td>
<td>-1.41 ± 1.48</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>8</td>
<td>-3.80 ± 1.32</td>
<td>p&lt;0.025</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>8</td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>Insulin</td>
<td>Pre-operative</td>
<td>5</td>
<td>39 ± 12</td>
<td>n.s.</td>
</tr>
<tr>
<td>Δ Insulin</td>
<td>End-op</td>
<td>4</td>
<td>45 ± 33</td>
<td>n.s.</td>
</tr>
<tr>
<td>pmol/L</td>
<td></td>
<td>5</td>
<td>40 ± 53</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>4</td>
<td>5 ± 13</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>5</td>
<td>22 ± 25</td>
<td>n.s.</td>
</tr>
<tr>
<td>Glucagon</td>
<td>Pre-operative</td>
<td>6</td>
<td>12.6 ± 4.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Δ Glucagon</td>
<td>End-op</td>
<td>6</td>
<td>-0.9 ± 2.5</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>pmol/L</td>
<td></td>
<td>6</td>
<td>-3.0 ± 2.2</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>5</td>
<td>-4.6 ± 2.9</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>6</td>
<td>-4.4 ± 2.1</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>6</td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>Insulin/glucagon ratio</td>
<td>Pre-operative</td>
<td>5</td>
<td>3.8 ± 1.9</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>End-op</td>
<td>4</td>
<td>7.6 ± 5.5</td>
<td>p&lt;0.025</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>5</td>
<td>-1.3 ± 0.9</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>4</td>
<td>4.8 ± 2.1</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>5</td>
<td>2.0 ± 1.8</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Comparison of changes in plasma hormone concentrations between neonates in the fentanyl and non-fentanyl anaesthesia groups. Delta values at the end of surgery and post-operatively were obtained by subtraction of the pre-operative value in each neonate.
Table 7.3 FENTANYL TRIAL: - Hormonal changes

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Pre-operative</th>
<th>6hr post-op</th>
<th>12hr post-op</th>
<th>24hr post-op</th>
<th>Pre-operative</th>
<th>6hr post-op</th>
<th>12hr post-op</th>
<th>24hr post-op</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.025</td>
<td>p&lt;0.01</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-op</td>
<td>0.08 ± 1.12</td>
<td></td>
<td></td>
<td></td>
<td>0.07 ± 0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12hr post-op</td>
<td>-0.18 ± 0.21</td>
<td></td>
<td></td>
<td></td>
<td>-0.31 ± 0.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hr post-op</td>
<td>-0.23 ± 0.46</td>
<td></td>
<td></td>
<td></td>
<td>-0.32 ± 0.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosterone</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>n.s.</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-op</td>
<td>22.2 ± 11.0</td>
<td></td>
<td></td>
<td></td>
<td>23.7 ± 11.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12hr post-op</td>
<td>-13.3 ± 12.2</td>
<td></td>
<td></td>
<td></td>
<td>-13.3 ± 12.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hr post-op</td>
<td>-17.2 ± 11.8</td>
<td></td>
<td></td>
<td></td>
<td>-17.2 ± 11.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxy-corticosterone</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.025</td>
<td>p&lt;0.01</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-op</td>
<td>0.34 ± 0.12</td>
<td></td>
<td></td>
<td></td>
<td>0.33 ± 0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12hr post-op</td>
<td>0.23 ± 0.16</td>
<td></td>
<td></td>
<td></td>
<td>0.23 ± 0.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hr post-op</td>
<td>-0.13 ± 0.14</td>
<td></td>
<td></td>
<td></td>
<td>-0.21 ± 0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.05</td>
<td>p&lt;0.01</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-op</td>
<td>0.95 ± 0.41</td>
<td></td>
<td></td>
<td></td>
<td>0.95 ± 0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12hr post-op</td>
<td>1.20 ± 1.27</td>
<td></td>
<td></td>
<td></td>
<td>1.20 ± 1.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hr post-op</td>
<td>-1.24 ± 0.38</td>
<td></td>
<td></td>
<td></td>
<td>-1.24 ± 0.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-OH-progesterone</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.025</td>
<td>p&lt;0.01</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-op</td>
<td>2.78 ± 1.47</td>
<td></td>
<td></td>
<td></td>
<td>2.78 ± 1.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12hr post-op</td>
<td>-0.89 ± 2.60</td>
<td></td>
<td></td>
<td></td>
<td>-0.89 ± 2.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hr post-op</td>
<td>-5.13 ± 4.34</td>
<td></td>
<td></td>
<td></td>
<td>-5.13 ± 4.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-Deoxycorticisol</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-op</td>
<td>1.88 ± 2.40</td>
<td></td>
<td></td>
<td></td>
<td>1.88 ± 2.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12hr post-op</td>
<td>-2.45 ± 1.56</td>
<td></td>
<td></td>
<td></td>
<td>-2.45 ± 1.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hr post-op</td>
<td>-2.23 ± 1.92</td>
<td></td>
<td></td>
<td></td>
<td>-2.23 ± 1.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-op</td>
<td>159 ± 89</td>
<td></td>
<td></td>
<td></td>
<td>159 ± 89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12hr post-op</td>
<td>-2 ± 65</td>
<td></td>
<td></td>
<td></td>
<td>-2 ± 65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hr post-op</td>
<td>-36 ± 54</td>
<td></td>
<td></td>
<td></td>
<td>-36 ± 54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>Mean ± SEM</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-op</td>
<td>-9 ± 12</td>
<td></td>
<td></td>
<td></td>
<td>-9 ± 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12hr post-op</td>
<td>-7 ± 18</td>
<td></td>
<td></td>
<td></td>
<td>-7 ± 18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hr post-op</td>
<td>22 ± 32</td>
<td></td>
<td></td>
<td></td>
<td>22 ± 32</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of changes in plasma hormone concentrations between neonates in the fentanyl and non-fentanyl anaesthesia groups. Delta values at the end of surgery and post-operatively were obtained by subtraction of the pre-operative value in each neonate.
Table 7.4 FENTANYL TRIAL: - Metabolite changes

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Pre-operative</th>
<th>FENTANYL</th>
<th>Mann-Whitney U Test</th>
<th>NON-FENTANYL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SEM</td>
<td>p (FENTANYL)</td>
<td>N</td>
</tr>
<tr>
<td>Glucose</td>
<td>8</td>
<td>8.9 ± 1.1</td>
<td>n.s.</td>
<td>8</td>
</tr>
<tr>
<td>Δ Glucose</td>
<td>8</td>
<td>2.6 ± 1.6</td>
<td>p&lt;0.025</td>
<td>7.6 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>0.6 ± 1.4</td>
<td>p&lt;0.005</td>
<td>7.3 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>2.1 ± 1.9</td>
<td>n.s.</td>
<td>1.4 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>0.2 ± 1.9</td>
<td>n.s.</td>
<td>2.3 ± 3.3</td>
</tr>
<tr>
<td>Lactate</td>
<td>8</td>
<td>0.9 ± 0.4</td>
<td>n.s.</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Δ Lactate</td>
<td>8</td>
<td>-0.1 ± 0.1</td>
<td>p&lt;0.02</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>-0.1 ± 0.1</td>
<td>n.s.</td>
<td>-0.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>-0.3 ± 0.2</td>
<td>n.s.</td>
<td>-0.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>0.1 ± 0.1</td>
<td>p&lt;0.005</td>
<td>-0.6 ± 0.1</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>8</td>
<td>0.12 ± 0.02</td>
<td>n.s.</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Δ Pyruvate</td>
<td>8</td>
<td>0.00 ± 0.01</td>
<td>p&lt;0.02</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>0.00 ± 0.02</td>
<td>n.s.</td>
<td>-0.01 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>-0.02 ± 0.01</td>
<td>n.s.</td>
<td>-0.02 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>0.03 ± 0.02</td>
<td>p&lt;0.01</td>
<td>-0.03 ± 0.01</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>8</td>
<td>0.10 ± 0.01</td>
<td>n.s.</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Δ Acetoacetate</td>
<td>8</td>
<td>0.00 ± 0.01</td>
<td>p&lt;0.05</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>0.00 ± 0.01</td>
<td>n.s.</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>0.00 ± 0.01</td>
<td>n.s.</td>
<td>-0.01 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>-0.01 ± 0.01</td>
<td>n.s.</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Hydroxybutyrate</td>
<td>8</td>
<td>0.02 ± 0.01</td>
<td>n.s.</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Δ Hydroxybutyrate</td>
<td>8</td>
<td>-0.01 ± 0.01</td>
<td>n.s.</td>
<td>0.00 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>0.01 ± 0.02</td>
<td>n.s.</td>
<td>0.01 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>-0.01 ± 0.01</td>
<td>n.s.</td>
<td>-0.01 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>0.00 ± 0.00</td>
<td>n.s.</td>
<td>0.05 ± 0.06</td>
</tr>
<tr>
<td>Alanine</td>
<td>8</td>
<td>0.12 ± 0.04</td>
<td>p&lt;0.05</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Δ Alanine</td>
<td>8</td>
<td>-0.02 ± 0.02</td>
<td>n.s.</td>
<td>0.00 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>-0.01 ± 0.02</td>
<td>n.s.</td>
<td>-0.01 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>-0.03 ± 0.03</td>
<td>n.s.</td>
<td>-0.02 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>-0.02 ± 0.04</td>
<td>n.s.</td>
<td>-0.04 ± 0.06</td>
</tr>
<tr>
<td>Glycerol</td>
<td>8</td>
<td>0.14 ± 0.04</td>
<td>n.s.</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>Δ Glycerol</td>
<td>8</td>
<td>0.02 ± 0.03</td>
<td>n.s.</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>6hr post-op</td>
<td>0.01 ± 0.02</td>
<td>n.s.</td>
<td>0.01 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>12hr post-op</td>
<td>0.01 ± 0.02</td>
<td>p&lt;0.01</td>
<td>-0.04 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>24hr post-op</td>
<td>0.02 ± 0.03</td>
<td>n.s.</td>
<td>-0.02 ± 0.01</td>
</tr>
</tbody>
</table>

Comparison of changes in blood metabolite concentrations between neonates in the fentanyl and non-fentanyl anaesthesia groups. Delta values at the end of surgery and post-operatively were obtained by subtraction of the pre-operative value in each neonate.
Table 7.5  FENTANYL TRIAL: Derived hormonal-metabolic variables.

<table>
<thead>
<tr>
<th></th>
<th>FENTANYL</th>
<th>Mann-Whitney U Test</th>
<th>NON-FENTANYL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SEM</td>
<td>N</td>
</tr>
<tr>
<td>Total Ketones: Pre-operative</td>
<td>8</td>
<td>0.12 ± 0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>End-operative</td>
<td>8</td>
<td>-0.01 ± 0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-operative</td>
<td>8</td>
<td>0.01 ± 0.03</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>12hr post-operative</td>
<td>7</td>
<td>0.00 ± 0.01</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>24hr post-operative</td>
<td>8</td>
<td>-0.02 ± 0.01</td>
<td>0.08 ± 0.08</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>0.11 ± 0.01</td>
<td>n.s.</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of changes in derived hormonal-metabolic variables between neonates in the fentanyl and non-fentanyl anaesthesia groups. Delta values at the end of surgery and post-operatively were obtained by subtraction of the pre-operative value in each neonate.
Table 7.6  FENTANYL TRIAL: - Derived hormonal-metabolic variables.

<table>
<thead>
<tr>
<th></th>
<th>FENTANYL</th>
<th></th>
<th>Mann-Whitney U Test</th>
<th>NON-FENTANYL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test</td>
<td>Mean ± SEM</td>
<td>N</td>
<td>Test</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Lactate/Pyruvate ratio</td>
<td>-</td>
<td>12.6 ± 2.1</td>
<td>8</td>
<td>n.s.</td>
<td>12.7 ± 1.3</td>
</tr>
<tr>
<td>mmol/mmol</td>
<td>n.s.</td>
<td>11.3 ± 2.2</td>
<td>8</td>
<td>n.s.</td>
<td>12.4 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>12.2 ± 2.2</td>
<td>7</td>
<td>n.s.</td>
<td>10.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>13.0 ± 3.1</td>
<td>7</td>
<td>n.s.</td>
<td>8.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>11.3 ± 2.3</td>
<td>8</td>
<td>p&lt;0.05</td>
<td>13.0 ± 1.1</td>
</tr>
<tr>
<td>Insulin/Glucose ratio</td>
<td>-</td>
<td>5.9 ± 2.4</td>
<td>5</td>
<td>n.s.</td>
<td>8.7 ± 2.7</td>
</tr>
<tr>
<td>pmol/mmol</td>
<td>n.s.</td>
<td>5.9 ± 1.9</td>
<td>4</td>
<td>n.s.</td>
<td>4.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>7.4 ± 4.8</td>
<td>5</td>
<td>n.s.</td>
<td>7.9 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>6.1 ± 2.5</td>
<td>5</td>
<td>n.s.</td>
<td>9.5 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>6.6 ± 2.5</td>
<td>5</td>
<td>n.s.</td>
<td>7.5 ± 2.6</td>
</tr>
<tr>
<td>Alanine/Pyruvate ratio</td>
<td>-</td>
<td>1.0 ± 0.3</td>
<td>8</td>
<td>n.s.</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>mmol/mmol</td>
<td>n.s.</td>
<td>0.8 ± 0.2</td>
<td>8</td>
<td>n.s.</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>1.0 ± 0.3</td>
<td>8</td>
<td>n.s.</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.9 ± 0.3</td>
<td>8</td>
<td>p&lt;0.05</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>Hydroxybutyrate/Acetoacetate ratio mmol/mmol</td>
<td>-</td>
<td>0.30 ± 0.11</td>
<td>8</td>
<td>n.s.</td>
<td>0.58 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.27 ± 0.11</td>
<td>8</td>
<td>n.s.</td>
<td>0.40 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.28 ± 0.19</td>
<td>8</td>
<td>n.s.</td>
<td>0.41 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.24 ± 0.14</td>
<td>7</td>
<td>n.s.</td>
<td>0.33 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.26 ± 0.08</td>
<td>8</td>
<td>p&lt;0.05</td>
<td>0.85 ± 0.37</td>
</tr>
</tbody>
</table>

Comparison of changes in derived hormonal-metabolic variables between neonates in the fentanyl and non-fentanyl anaesthesia groups. (Differences between the two groups are analysed by the Mann-Whitney U Test, whereas changes from pre-operative values within each group are analysed by the Wilcoxon test.)

N = Number of patients
Table 7.7: FENTANYL TRIAL: - Post-operative data.

<table>
<thead>
<tr>
<th></th>
<th>FENTANYL</th>
<th>Mann-Whitney U Test</th>
<th>NON-FENTANYL</th>
<th>Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>N</td>
<td>Mean ± SEM</td>
<td>N</td>
</tr>
<tr>
<td>Dextrose infusion rate, mg/kg/min:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>4.7 ± 0.4</td>
<td>8</td>
<td>n.s.</td>
<td>6</td>
</tr>
<tr>
<td>Day 2</td>
<td>4.9 ± 0.4</td>
<td>8</td>
<td>n.s.</td>
<td>8</td>
</tr>
<tr>
<td>Day 3</td>
<td>5.5 ± 0.5</td>
<td>8</td>
<td>n.s.</td>
<td>8</td>
</tr>
<tr>
<td>Dose of Morphine mg/kg:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>0.23 ± 0.02</td>
<td>5</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.09 ± 0.00</td>
<td>2</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Day 3</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>First dose, hours post-operative</td>
<td>9.6 ± 1.4</td>
<td>p&lt;0.002</td>
<td>2.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>1.1 ± 0.1</td>
<td>8</td>
<td>n.s.</td>
<td>8</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.1 ± 0.1</td>
<td>8</td>
<td>p&lt;0.05</td>
<td>8</td>
</tr>
<tr>
<td>Day 3</td>
<td>1.1 ± 0.1</td>
<td>8</td>
<td>n.s.</td>
<td>8</td>
</tr>
<tr>
<td>3-methylhistidine/creatinine ratio, umol/umol:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>0.033 ± 0.002</td>
<td>6</td>
<td>n.s.</td>
<td>6</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.042 ± 0.004</td>
<td>7</td>
<td>p&lt;0.05</td>
<td>6</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.044 ± 0.004</td>
<td>7</td>
<td>p&lt;0.05</td>
<td>6</td>
</tr>
</tbody>
</table>

Comparison of post-operative data between neonates in the fentanyl and non-fentanyl anaesthesia groups. Differences between the two groups were analysed by the Mann-Whitney U Test, whereas changes from Day 1 post-operative values within each group were analysed by the Wilcoxon test.

N = Number of patients.
Table 7.8  FENTANYL TRIAL: - Peri-operative complications

<table>
<thead>
<tr>
<th>INTRA-OPERATIVE COMPlications</th>
<th>NUMBER OF PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HALOTHANE</td>
</tr>
<tr>
<td>1. Cyanotic episode with bradycardia</td>
<td>3</td>
</tr>
<tr>
<td>2. Pneumothorax</td>
<td>1</td>
</tr>
<tr>
<td>3. Excessive blood loss</td>
<td>1</td>
</tr>
<tr>
<td>4. Persistent tachycardia</td>
<td>-</td>
</tr>
<tr>
<td>5. Hypothermia</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>POST-OPERATIVE COMPLICATIONS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Respiratory instability:-</td>
<td></td>
</tr>
<tr>
<td>(a) Increased oxygen requirements</td>
<td>2</td>
</tr>
<tr>
<td>(b) Increased ventilation requirements</td>
<td>1</td>
</tr>
<tr>
<td>2. Pneumothorax</td>
<td>1</td>
</tr>
<tr>
<td>3. Cyanotic episode with bradycardia</td>
<td>3</td>
</tr>
<tr>
<td>4. Spontaneous bradycardias: -</td>
<td></td>
</tr>
<tr>
<td>(a) Occasional</td>
<td>1</td>
</tr>
<tr>
<td>(b) Frequent</td>
<td>4</td>
</tr>
<tr>
<td>5. Hypotension</td>
<td>-</td>
</tr>
<tr>
<td>6. Poor peripheral circulation</td>
<td>-</td>
</tr>
<tr>
<td>7. Glycosuria</td>
<td>-</td>
</tr>
<tr>
<td>8. Persistent metabolic acidosis</td>
<td>-</td>
</tr>
<tr>
<td>9. Intra-ventricular haemorrhage</td>
<td></td>
</tr>
<tr>
<td>(a) Grade II</td>
<td>-</td>
</tr>
<tr>
<td>(b) Grade III</td>
<td>-</td>
</tr>
<tr>
<td>10. Temperature variability</td>
<td>6</td>
</tr>
</tbody>
</table>

Clinical complications observed during surgery and in the post-operative period in neonates from the fentanyl and non-fentanyl anaesthesia groups.
CHAPTER VIII : PRELIMINARY STUDY OF NEONATES UNDERGOING CARDIAC SURGERY
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   8.2.4 Responses of neonates given routine anaesthesia
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   8.3.1 The neonatal stress response to cardiac surgery
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8.4 CONCLUSION
8.1 INTRODUCTION

The study of newborn infants subjected to cardiac surgery, cardiopulmonary bypass, deep hypothermia and circulatory arrest was considered to be an important line of investigation, since it would not only illustrate the neonatal response to maximal degrees of surgical stress but also could provide some evidence to explain the unusually high morbidity and mortality following cardiac surgery in newborn infants (Mohri et al, 1969; Mustard et al, 1970; Caldwell and Almond, 1973; Gattiker, 1972; Steward et al, 1974; Wong et al, 1974; Tharion et al, 1982). This was considered to be likely since several reports on the outcome of neonates undergoing cardiac surgery have documented ill-defined causes for some of the postoperative mortality, such as, 'biochemical deaths' or 'metabolic acidosis of unknown origin' (Mohri et al, 1969; Mustard et al, 1970; Steward et al, 1974; Wong et al, 1974). On the other hand, the hormonal and metabolic responses of adult patients undergoing similar procedures has been extensively investigated and it is well-known that, in addition to the surgical trauma, the unphysiological state characterised by non-pulsatile cardiopulmonary bypass and deep hypothermia provid a marked stimulus to the hormonal and metabolic stress response (Elliott and Alberti, 1983).

Due to these findings from adult patients, specific anaesthetic techniques have been investigated recently for manipulation of the severe hormonal and cardiovascular stress response stimulated by cardiac surgery. The most popular of these have been the use of opiate analgesic drugs in high doses, particularly following (1) the observations of Lowenstein et al (1969) that large doses of morphine provided cardiovascular stability, (2) those of Stanley et al (1974) who documented that it inhibited the catecholamine response, and (3) those of George et al (1974) who found that the cortisol
and growth hormone responses were also blocked. Thereafter, subsequent studies found that many other hormonal-metabolic parameters of the adult stress response were also inhibited by morphine anaesthesia (Brandt et al., 1978; Philbin and Coggins, 1978) and it was proposed that a decreased metabolic response would be desirable in these patients (Hall et al., 1978).

The use of fentanyl was recommended initially on the basis of experimental work by Liu et al. (1976) in dogs, who found that cardiovascular stability was maintained even with doses larger than those equivalent to the doses of morphine used in adult patients. This was confirmed in adult patients by Stanley et al., who also found that the hormonal stress response with respect to changes in catecholamines, cortisol (Stanley et al., 1980) and vasopressin (Stanley et al., 1979) were attenuated by fentanyl anaesthesia in patients undergoing coronary bypass surgery. Thereafter, several studies have found that surgical hyperglycaemia and substrate mobilisation in addition to the changes in plasma catecholamines, cortisol, growth hormone, insulin, plasma renin activity, vasopressin, aldosterone as well as changes in renal and cardiovascular function may be inhibited or abolished by high-dose fentanyl anaesthesia (Sebel et al., 1981; Walsh et al., 1981; Kono et al., 1981; Zurick et al., 1982; Crone et al., 1982). In addition, the use of fentanyl provided a greater cardiovascular stability as compared to morphine anaesthesia due to the lack of histamine release during induction (Philbin et al., 1981; Rosow et al., 1982). Currently, therefore, the use of high-dose fentanyl anaesthesia for adult patients undergoing cardiac surgery is widely preferred (Savarese and Lowenstein, 1985). As stated previously, Hickey and Hansen (1984) have found that high-dose fentanyl anaesthesia in neonates undergoing open-heart surgery is associated with minimal side-effects, whereas Vacanti et al. (1984) have shown that it can be used to decrease the hyperreactive pulmonary
vasoconstriction in neonates operated for congenital diaphragmatic hernia. Apart from these reports the use of high-dose fentanyl anaesthesia in newborn infants has not been documented previously.

Since these reports were not available at the start of this project, it was decided to examine the effects of high-dose fentanyl anaesthesia in this study from a comparison of the hormonal and metabolic responses of two groups of neonates: those given the routine anaesthetic management for neonates undergoing cardiac surgery and those given high doses of fentanyl (100 mcg/kg) during cardiac surgery. For these studies and the subsequent investigations on the effects of high-dose fentanyl anaesthesia on the neonatal stress response, collaboration was obtained with the Cardio-thoracic Surgical Units at Harefield Hospital and The Brompton Hospital.

A preliminary clinical trial was carried out to determine the safety of fentanyl anaesthesia in doses of 50, 75 and 100 µg/kg given intravenously to neonates undergoing cardiac surgery. It was found that intravenous injection of these doses were associated with a minimal haemodynamic changes, which were characterised by a slight decrease in the heart rate and blood pressure of neonates at the time of anaesthetic induction. These effects were transient and did not give rise to any changes in the infant's condition (as assessed by clinical signs, monitoring of cardiovascular parameters and arterial oxygenation) or the need for supportive therapy.

Thus, it was proposed that high-dose fentanyl anaesthesia does not decrease the hormonal and metabolic response of newborn infants undergoing cardiac surgery and, on the basis of estimated differences in the outcome measures defined in Chapter V, it was calculated that a total of 24 patients would be required to investigate the validity of this hypothesis.
The neonates included in this study were randomly allotted to the routine anaesthesia or the high-dose fentanyl anaesthesia groups and randomisation was carried out in separate strata for the two centres. However, due to the lack of standardisation of anaesthetic techniques and peri-operative clinical management, the branch of the clinical trial in Brompton Hospital, London had to be terminated after the study of a single patient (the results from this patient have not been included in the data analysis). In Harefield Hospital, the rate of patient entry was found to be much lower than expected and thus, the trial had to be terminated after the inclusion of only 13 patients. As a result of such small numbers, the power of this trial was reduced to less than 50%; that is, on the basis of previously defined criteria, the clinical trial had less than a 50% chance of identifying significant differences between the hormonal and metabolic responses of neonates in the two anaesthesia groups. Furthermore, partly due to the small numbers in each group, it was found that there were distinct differences in the extent of surgical stress and the clinical management of neonates in the two anaesthesia groups.

In view of these circumstances and to guard against the identification of chance differences between the hormonal and metabolic responses of neonates in the two anaesthesia groups, it was decided that only a provisional comparison of the effects of routine anaesthesia and high-dose fentanyl anaesthesia would be possible. Thus, the data from neonates randomised to the routine anaesthesia group were analysed separately in order to identify the basic characteristics of the neonatal response to cardiac surgery and its associated procedures. Thereafter, major differences between the pattern of responses of neonates in the two anaesthesia groups were identified in order to generate hypotheses and criteria, which could be
used for planning definitive clinical trials in the future. These decisions regarding the methods for data analysis were made at the termination of the trial, and before the blood samples obtained from neonates in the two anaesthesia groups had been analysed.

8.2 RESULTS OF THE PRELIMINARY CARDIAC STUDY:

8.2.1 Description of patients and preoperative management:

The patient characteristics and preoperative clinical management of neonates undergoing cardiac surgery are described in Table 8.1.

In this study, 7 neonates were randomised to the routine anaesthesia group and 6 neonates to the high-dose fentanyl anaesthesia group. The gestation, birth weight and post-natal age weight at the time of surgery of neonates in the two anaesthesia groups were similar. Preoperative clinical management with respect to intravenous dextrose therapy and duration of preoperative starvation were not significantly different between the two anaesthesia groups.

During the 24 hours prior to surgery, the drugs given to neonates in the two anaesthesia groups are listed in Table 8.1. All neonates received premedication with atropine and a mixture of pethidine, chlorpromazine and promethazine by intramuscular injection at 30 minutes before induction of anaesthesia.

8.2.2 Anaesthesia and clinical management during surgery:

The characteristics of the surgical procedure and clinical management in the two anaesthesia groups are described in Table 8.2. The anaesthetic protocols for the two groups are described in Figures 8.1 and 8.2.
Anaesthesia was induced with halothane, nitrous oxide and pancuronium in all neonates and after tracheal intubation, artificial ventilation was started at a rate of 40-50/min. Thereafter, a peripheral venous catheter, two central venous catheters and an arterial catheter were inserted in all patients and the pre-operative blood sample was obtained. The mean interval from the induction of anaesthesia to the preoperative blood sample was 47.5 min in neonates from the routine anaesthesia group and 38.8 min in neonates from the high-dose fentanyl anaesthesia group. All neonates were surface-cooled to a temperature of 32-34 °C before the start of surgery. Thus, it may be noted that these neonates had undergone a significant period of stress before the preoperative blood sample was obtained.

In the routine anaesthesia group, 5 neonates were operated for anatomical correction of transposition of the great vessels and 2 neonates were operated for pulmonary valvotomy and repair of an atrial septal defect. In the high-dose fentanyl anaesthesia group, 4 neonates were operated for anatomical correction of transposition of the great vessels and 2 neonates for the correction of total anomalous pulmonary venous drainage (TAPVD).

It was found that the surgical procedures performed on neonates in the high-dose fentanyl anaesthesia group required a longer operating time, a longer duration of cardiopulmonary bypass and of circulatory arrest during the procedure; and, according to the surgical stress score, were of a slightly greater severity. Due to the longer duration of surgery, the total dose of pancuronium required in the fentanyl anaesthesia group was slightly greater than in the routine anaesthesia group. Apart from heparin, no other drugs were given to the neonates till the start of cardiopulmonary bypass.
In the CPB pump prime, fresh heparinised blood was used without the addition of any other fluid medium. However, it was retrospectively found that dexamethasone (5 mg) had been added into the bypass pump prime of 4 neonates in the routine anaesthesia group and 1 neonate in the high-dose fentanyl anaesthesia group; sodium bicarbonate and potassium chloride were added to the pump prime of all neonates. During CPB, all neonates were cooled to a temperature of 10-14 °C and were then subjected to a period of circulatory arrest.

After the start of cardiopulmonary bypass and upto the end of surgery, a variety of drugs were given for supportive or adjuvant therapy; these are listed in Table 8.2. Neonates in the high-dose fentanyl anaesthesia group required a greater amount of sympathomimetic drugs after the termination of CPB, presumably due to the greater severity of their surgical procedures or the longer duration of circulatory arrest. The rate of intravenous dextrose infusion before and after CPB was similar in the two groups.

Thus, the neonates included in the two anaesthesia groups were similar with respect to their characteristics, but there were distinct differences in their peri-operative management and in the severity of surgical stress experienced by them. However, since these were a group of critically ill neonates, it was expected that certain differences would be found in their clinical management before and during the surgical procedure.

8.2.3 Postoperative clinical management :-

The postoperative drug therapy of neonates in the two anaesthesia groups is summarised in Table 8.3.

All neonates were ventilated for more than 24 hours after the surgical
procedure and received a similar intravenous dextrose therapy in the two anaesthesia groups. During the 24 hours following surgery, diuretic therapy was given with frusemide to all neonates (total dose 4.9 ± 0.8 mg/kg in the routine anaesthesia group and 3.3 ± 0.9 mg/kg in the fentanyl anaesthesia group), analgesia and sedation were provided with papaveretum and diazepam or small doses of fentanyl, whereas antibiotic cover was provided with flucloxacillin and gentamicin. Analgesic and sedative therapy were adjusted so that these drugs were not injected during the 2 hours preceding a postoperative blood sample. A large number of drugs were required for supportive therapy during the 24 hours following surgery, these are listed in Table 8.3. It was noted that neonates in the routine anaesthesia group required a greater amount of diuretic therapy in the postoperative period.

Thus, it is likely that the hormonal and metabolic changes found during the postoperative period may have been influenced, to some extent, by the variable drug therapy and clinical condition of neonates during the postoperative period.
8.2.4 Responses of neonates given routine anaesthesia:

8.2.4.1 Hormonal changes:

The plasma hormone concentrations measured in neonates receiving routine anaesthetic management are listed in Tables 8.4 and 8.5.

Plasma adrenaline concentrations increased markedly during surgery up to the start of CPB (p<0.05) and increased further at the end of surgery (p<0.02), at 6 hours (p<0.05) and at 12 hours (p<0.05) postoperatively. Plasma noradrenaline concentrations also increased significantly during surgery up to the start of CPB (p<0.05), and were increased markedly at the end of surgery (p<0.02), at 6 hours (p<0.05) and at 12 hours (p<0.05) after surgery.

Plasma insulin concentrations were not significantly altered during or at the end of surgery, or in the postoperative period. Plasma glucagon concentrations did not change significantly during surgery up to the start of CPB or at the end of surgery. However, a significant increase in plasma glucagon concentrations was recorded at 6 hours (p<0.05) and 12 hours (p<0.05) postoperatively. The insulin/glucagon molar ratio increased significantly during the surgical procedure before the start of CPB (p<0.05), but had reverted to preoperative values at the end of surgery and during the postoperative period.

Plasma progesterone and 17-hydroxyprogesterone concentrations were not significantly altered during surgery or in the postoperative period. The plasma concentrations of aldosterone and 11-deoxycorticosterone did not change significantly from their respective preoperative values during surgery, at the end of surgery or in the postoperative period; however, a
significant decrease was recorded in plasma aldosterone concentrations at 24 hours after surgery (p<0.05).

A significant increase from preoperative values was recorded just before the start of CPB in the plasma concentrations of cortisol (p<0.05) and corticosterone (p<0.05), but at the end of surgery and in the postoperative period the plasma concentrations of both hormones had reverted to the respective preoperative values. Plasma concentrations of 11-deoxycortisol and cortisone were not significantly changed from their preoperative values during the entire study period.

Thus, the hormonal response of neonates undergoing cardiac surgery was characterised by a marked release of catecholamines and glucocorticoids during surgery, together with an increase in the insulin/glucagon ratio. In the postoperative period, the increased plasma concentrations of catecholamines were maintained and plasma glucagon concentrations were raised. These hormonal changes may be responsible for mediating the metabolic response of newborn infants undergoing cardiac surgery.

8.2.4.2 Metabolite changes :-

The blood concentrations of metabolites measured in neonates given routine anaesthesia during cardiac surgery are presented in Tables 8.6 and 8.7. The concentrations of metabolites measured in the CPB pump prime are presented in Table 8.8.

Blood glucose concentrations were increased massively in response to surgical stress before the start of CPB (p<0.02) and at the end of surgery (p<0.02); however, at 6, 12 and 24 hours postoperatively blood glucose values, although still raised, were not significantly different from the
preoperative concentrations.

Blood lactate concentrations increased significantly during the surgical procedure before the start of CPB (p<0.02) and were increased further at the end of surgery (p<0.02). The concentrations of blood lactate were increased significantly at 6 hours (p<0.05) and 12 hours (p<0.05) after surgery, but had returned to preoperative values at 24 hours following the operation. Blood pyruvate concentrations were found to be increased significantly at the end of surgery (p<0.02), at 6 hours (p<0.05) and at 12 hours (p<0.05) following surgery. The blood concentrations of alanine increased significantly during the surgical procedure before the start of CPB (p<0.05) and were raised at the end of surgery (p<0.05). Although blood alanine concentrations were raised at 6 and 12 hours after surgery, no significant differences were found from the preoperative value.

Blood glycerol concentrations were significantly raised at the end of surgery (p<0.05), but had reverted to the preoperative values at 6, 12 and 24 hours after surgery. No significant changes were found in the blood concentrations of acetoacetate and 3-hydroxybutyrate separately, the total ketone bodies, or in the hydroxybutyrate/acetoacetate molar ratio during or after surgery.

The lactate/pyruvate molar ratio increased significantly before the start of CPB (p<0.05), was increased further at the end of surgery (p<0.02) and remained elevated at 6 hours (p<0.05) and 12 hours (p<0.05) after surgery. By 24 hours postoperatively, the lactate/pyruvate molar ratio had reverted to the preoperative values. The alanine/pyruvate molar ratio was not altered before the start of CPB, but was found to be significantly decreased below the preoperative value at the end of surgery (p<0.02) and
The blood concentrations of total gluconeogenic substrates were increased significantly before the start of CPB (p<0.05), at the end of surgery (p<0.02), at 6 (p<0.05) and 12 (p<0.05) hours after surgery, and had returned to the preoperative values at 24 hours postoperatively. There was a trend towards a decrease in the insulin/glucose molar ratio during surgery but this was not significant.

Since fresh heparinised blood was used for priming the CPB pump without the addition of any other fluid medium, the concentrations of metabolites were also measured in the pump prime before the start of cardiopulmonary bypass (Table 8.6). The concentrations of glucose in the prime were found to be lower than the preoperative values documented from neonates, whereas the lactate concentrations measured in the CPB pump prime were substantially higher than blood lactate values measured in the neonates before surgery. All other metabolites in the CPB pump prime were found to be similar to the blood concentrations measured before the start of surgery.

Thus, the metabolic response of neonates undergoing cardiac surgery was characterised by a massive surgical hyperglycaemia and lactic acidaemia, together with an increase in the blood concentrations of the other gluconeogenic substrates. These effects reached a maxima at the end of surgery, but were maintained also into the postoperative period.

8.2.5 Effects of high-dose fentanyl anaesthesia :-

As in the previous trials, the effects of high-dose fentanyl anaesthesia were identified by a comparison of the delta changes in hormonal and metabolic variables from the preoperative value measured in each case,
between neonates in the two anaesthesia groups. Since the number of patients in the two groups were much smaller than those required to make a valid comparison, it was decided that only the striking differences between the hormonal and metabolic responses of neonates in the routine and high-dose fentanyl anaesthesia groups would be presented and discussed. For reference, the complete data from the two groups is included in Appendix I.

8.2.5.1 **Comparison of hormonal changes** :-

Salient differences between the hormonal response of neonates in the routine anaesthesia group and the high-dose fentanyl anaesthesia group are described in Figures 8.3 and 8.4.

Plasma adrenaline concentrations were found to decrease from the respective preoperative values in all neonates given fentanyl anaesthesia during the surgical procedure upto the start of cardiopulmonary bypass (CPB), whereas a marked increase had been observed in all neonates who received the routine anaesthetic management. Despite the small number of neonates in the two groups, this difference in the pattern of responses was highly significant \((p<0.01)\). A similar decrease was observed with regard to plasma noradrenaline concentrations in three neonates given high-dose fentanyl anaesthesia, but an increase during surgery was observed in one neonate. There were no significant differences in the plasma noradrenaline responses of neonates in the two anaesthesia groups during or after surgery.

The increases in plasma insulin concentrations during surgery upto the start of CPB, at the end of surgery and in the postoperative period were much greater in the fentanyl anaesthesia group, although a significant difference \((p<0.025)\) as compared to the response of neonates in the routine anaesthesia group was obtained only at 6 hours after surgery. The changes
in plasma glucagon concentration and the insulin/glucagon ratio were similar in neonates from the two anaesthesia groups.

The increase in plasma cortisol concentrations at the end of surgery was found to be significantly greater \((p<0.05)\) in neonates from the fentanyl anaesthesia group as compared to the response of neonates in the routine anaesthesia group; there were no significant differences between the two groups postoperatively. Except at the end of surgery, plasma corticosterone concentration was increased to a greater extent in the routine anaesthesia group throughout the study period as compared to the response of neonates in the fentanyl anaesthesia group. However, differences between the two groups were not significant.

Plasma aldosterone concentrations were decreased substantially in the fentanyl anaesthesia group during surgery and in the postoperative period. On the other hand, plasma aldosterone concentrations were found to be increased above preoperative values in the routine anaesthesia group during surgery before the start of CPB and at 6 hours after surgery; however, these differences between the two groups were not significant.

8.2.5.2 Comparison of metabolite changes :-

Salient differences between the metabolic response of neonates in the routine and high-dose fentanyl anaesthesia groups are described in Figures 8.5 and 8.6.

The hyperglycaemic responses of neonates in the routine anaesthesia group during surgery and at the end of surgery were found to be greater than the response of neonates in the high-dose fentanyl anaesthesia group; however, these differences were not statistically significant. In the postoperative
period, the change in blood glucose concentrations was similar in neonates from the two anaesthesia groups.

The insulin/glucose molar ratio increased during surgery in the fentanyl anaesthesia group whereas it had decreased from the preoperative values in neonates from the routine anaesthesia group. Thus, the response of neonates in the high-dose fentanyl anaesthesia group was significantly different from that of neonates in the routine anaesthesia group at the end of surgery (p<0.02) and at 6 hours postoperatively (p<0.05).

Changes in the blood concentrations of gluconeogenic substrates were significantly greater in neonates from the fentanyl anaesthesia group with respect of changes in blood pyruvate (p<0.025) and blood alanine (p<0.05) concentrations at the end of surgery, and with respect to changes in blood pyruvate (p<0.05) and blood glycerol (p<0.005) concentrations at 6 hours postoperatively. However, the responses of neonates in the two anaesthesia groups with respect to changes in total gluconeogenic substrates were not significantly different, although small differences were observed between the two groups.

Thus, the effects of high-dose fentanyl anaesthesia on the hormonal and metabolic responses of neonates undergoing cardiac surgery and CPB were characterised by a reversal of the catecholamine response to surgery before the start of CPB and an increase of the insulin response postoperatively; these changes were associated with greater changes in blood concentrations of pyruvate, alanine and glycerol after surgery. The cortisol response of neonates in the routine anaesthesia group at the end of surgery was found to be lower than neonates in the fentanyl anaesthesia group, but this was believed to be due to the addition of dexamethasone in the CPB pump prime
rather than due to the effects of high-dose fentanyl anaesthesia.

8.2.5.3 Peri-operative complications :-

Clinical assessments were made on the basis of physiological monitoring and the clinical state of the neonates during the postoperative period. The ECG, respiration, central and peripheral temperature, arterial gases and arterial blood pressure, central venous pressure, left atrial pressure and transcutaneous oxygen were monitored in all neonates during the 24 hours after surgery. The complications documented during surgery and in the postoperative period in neonates from the two anaesthesia groups are listed in Table 8.9.

The most frequent complication encountered during surgery and in the postoperative period was systemic hypotension, which required treatment with inotropic agents such as dopamine and digoxin, as well as transfusions with blood or plasma. Before the start of surgery, a fall in blood pressure and bradycardia were observed in two neonates who were given high-dose fentanyl anaesthesia; however, the heart rate and blood pressure reverted to the pre-anaesthetic values immediately after skin incision and did not require any definitive treatment.

In the routine anaesthesia group, other cardiovascular complications noted were: frequent spontaneous bradycardias, prolonged supraventricular tachycardia, ventricular extrasystoles and cardiac arrest, which were successfully treated. Apart from these, there were two patients who died within 48 hours after cardiac surgery; one neonate developed ventricular fibrillation and the other developed a persistent metabolic acidosis and anuria, followed by cardiac arrest. A greater degree of oliguria was observed in the routine anaesthesia group, as reflected by the greater
amounts fo diuretic therapy required in the postoperative period.

In the high-dose fentanyl anaesthesia group, hypotension was observed in fewer neonates during the postoperative period. As expected, the neonates operated for correction of TAPVD showed evidence of severe pulmonary hypertension, one of whom died in the postoperative period. Another neonate in this group died due to acute left ventricular failure secondary to the development of aortic incompetence.

Thus, a total of 4 neonates included in this study died giving a postoperative mortality rate of 31%. During the postoperative period, the clinical condition of neonates in the high-dose fentanyl anaesthesia group was found to be more stable than that of neonates in the routine anaesthesia group.

8.3. DISCUSSION :-

Although the hormonal and metabolic responses of neonates undergoing cardiac surgery have not been studied previously, the responses of adult patients to open-heart surgery, cardiopulmonary bypass and deep hypothermia have been investigated in several studies. This preliminary study, therefore, was planned in order to document the pattern of endocrine and metabolic changes presumably in response to the maximal degree of surgical stress in newborn infants and compare the findings in newborn infants with the response of adult patients from the published literature.

In addition, most of these neonates were cyanosed and clinically unwell preoperatively and were critically ill after surgery; a high morbidity and mortality rate has been documented from similar neonates in previous
reports on their outcome following cardiac surgery. (Caldwell and Almond, 1973; Steward et al, 1974; Wong et al, 1974; Tharion et al, 1982).

In this study, 9/13 neonates were subjected to the 'arterial switch' operation for transposition of the great vessels. This surgical procedure was described by Jatene et al (1976) and early attempts to perform it on neonates met with little success (Williams et al, 1981; Ebert, 1981). It was thus proposed that the neonatal age group of patients was not capable of surviving after such major surgery (Williams et al, 1981). The clinical outcome of neonates undergoing the switch procedure in Harefield Hospital has been reported recently (Radley-Smith and Yacoub, 1984) and the mortality rate was found to be only 8%; the only other centre which has reported the results of this operation in neonates found a mortality rate of 18%, even though neonates and infants upto the age of 7 months were included in this analysis (Hougen et al, 1984).

The neonates included in this study were of similar age, gestation and birth weight; they were starved for a similar duration preoperatively and received the same premedication prior to anaesthetic induction. However, a marked variability was observed between individual neonates and between neonates in the two anaesthesia groups with respect to the preoperative drug therapy and the dextrose infusion rate before surgery.

Although the neonates in both groups were subjected to a similar degree of surgical trauma and procedures for deep hypothermia and cardiopulmonary bypass (CPB) were standardised, it was noted that the degree of surgical stress was greater in neonates randomised to the fentanyl anaesthesia group. This was evidenced by the greater duration of surgery, CPB and circulatory arrest in the fentanyl anaesthesia group, as well as a higher
surgical stress score. The total dose of pancuronium given during surgery was found to be higher in the fentanyl anaesthesia group; this may be due to the longer duration of surgery in the latter group or, less likely, may be related to the 'tight chest' syndrome which was documented during fentanyl anaesthesia in adult patients, but was found to be eliminated by the use of pancuronium (Hill et al, 1981; Christian et al, 1983; Jaffe and Ramsey, 1983).

The CPB pump prime was composed of only fresh heparinised blood without the addition of any glucose containing solutions. The metabolite concentrations measured in it were similar to the values obtained from the preoperative blood samples of neonates, with the exception of glucose values (which were lower) and lactate values (which were higher). However, an important defect in the study arose from the addition of dexamethasone (5 mg) to the pump prime of 4 neonates in the routine anaesthesia group and 1 neonate in the fentanyl anaesthesia group. Although it had been decided that no drugs, apart from electrolyte solutions, would be added to the CPB pump prime; retrospectively, it was found that this recommendation had been disregarded in the case of some neonates in the cardiac study. It is likely that this could have been a deliberate decision, in order to influence the clinical outcome of those neonates who were given routine anaesthesia. Thus, all neonates were subjected to an identical intra-operative clinical management up to the start of CPB; thereafter, the clinical management of neonates in the two anaesthesia groups differed with respect to the addition of dexamethasone in the CPB pump prime, the severity of surgical stress and the clinical management of individual patients.

During the postoperative period, all patients received similar artificial ventilation, the timing of analgesic therapy and the rate of dextrose
infusion were also standardised. As expected, there was a substantial variability in the clinical management of individual neonates after surgery, mainly due to the different postoperative complications documented during the 24 hours following surgery. In particular, it was noted that neonates in the routine anaesthesia group were found to be clinically less stable than neonates in the fentanyl anaesthesia group.

It is likely that these differences in perioperative clinical management may have significantly influenced the hormonal and metabolite changes which were measured at the end of surgery and postoperatively. However, it is difficult to formulate strict criteria for the clinical management of critically ill neonates without jeopardising the safety of individual patients; thus, the perioperative management of these neonates was expected to be variable. The effects of this variability can be decreased to some extent by inclusion of large numbers of similar patients in the study population, but for reasons discussed previously, this was not feasible in the present study.

Thus, the hormonal and metabolic response of neonates in the routine anaesthesia group were examined separately (sections 8.3.1 and 8.3.2), and only the prominent effects of high-dose fentanyl anaesthesia could be commented upon from a comparison of the two groups (section 8.3.3).

8.3.1 The neonatal stress response to cardiac surgery :

8.3.1.1 Hormonal changes :

CATECHOLAMINES

The plasma concentrations of adrenaline and noradrenaline were found to be moderately raised in the preoperative blood sample, the latter more so than
the former. At the time of designing the study, it had been suggested that an arterial catheter for sampling purposes be placed on the day previous to the operation, but this was not considered feasible by the collaborating clinical team. The plasma noradrenaline values were raised to a greater extent presumably since the stimulus was non-visceral in origin (Roizen et al., 1981; Cryer, 1984).

Thereafter, the marked increase in plasma concentrations of both catecholamines during the surgical procedure before CPB denotes a response to the surface cooling employed during this period (to 34°C) and to the surgical trauma of thoracotomy, exposure of the heart and positioning of the CPB cannulae. It is interesting to note that the response to these stimuli was characterised by a relatively greater release of adrenaline than of noradrenaline. Furthermore, the magnitude of this response is much greater than has been observed even after severe degrees of surgical trauma in newborn infants undergoing non-cardiac surgery. It is likely that these features of the catecholamine response may be related to the rich innervation of the sternal periosteum and mediastinal structures; as well as to the somatic and sympathetic innervation of the pericardium and the heart (Davson and Segal, 1976; Warwick and Williams, 1973). The handling of the heart and incisions into the great vessels and the right atrium for insertion of the CPB cannulae may stimulate the sympathetic nervous system via neural pathways relaying in the cardiac plexuses and stellate ganglia (Davson and Segal, 1976).

In adult patients undergoing cardiac surgery, Hine et al. (1976) found an increase in plasma adrenaline concentrations during surgery before the start of CPB, but the noradrenaline concentrations were unchanged; whereas Butler et al. (1977) found that the noradrenaline values increased during
surgery before the start of CPB and the adrenaline values were unchanged. On the other hand, a significant increase in the plasma concentrations of both catecholamines has been observed during the period of surgery preceding CPB in several studies (Philbin et al, 1979; Watkins et al, 1979; Hoar et al, 1980; Kono et al, 1981; Sebel et al, 1981). The magnitude of increase in plasma adrenaline concentrations found in the present study are much greater than the corresponding responses documented from adult patients. The reasons for this difference are not clear, but this pattern is in keeping with the accentuated hormonal-metabolic response of neonates which has been documented in Chapters IV, VI and VII, and possibly may be related to the 'light' anaesthesia given to neonates in the routine anaesthesia group.

After the termination of CPB and before the end of surgery, a dopamine infusion was started in 4 neonates and an isoprenaline infusion was started in another neonate. It has been recently shown that these drugs directly stimulate the adrenal medulla and significantly increase the secretion of adrenaline and noradrenaline in infants and children (Zaritsky et al, 1984). Although the plasma concentrations of adrenaline and noradrenaline were markedly raised at the end of surgery and in the postoperative period, it is not possible to attribute the extent to which this increase was caused by the surgical stress or by the infusion of sympathomimetic drugs. However, it may be pointed out that whichever reason the catecholamine release may be attributed to, these hormones would still have their effects on metabolism in the postoperative period.

INSULIN

There was a trend towards an increase in the plasma insulin concentrations during surgery before the start of CPB which was reflected by a significant
increase in the insulin/glucagon molar ratio during this period of surgery. Despite the substantial hyperglycaemia, insulin concentrations remained unchanged after surgery which could be partly due to a suppression of insulin secretion by the markedly elevated adrenaline concentrations at the end of surgery and postoperatively (Sperling et al, 1984) or partly due to a decreased uptake of insulin from the beta-islet cells caused by a decrease in the splanchnic circulation during deep hypothermia and CPB (Waldhausen et al, 1959; Halley et al, 1959). In adult patients undergoing cardiac surgery, no significant changes in plasma insulin concentrations have been documented during surgery and CPB, or during the postoperative period (Walsh et al, 1981; Sebel et al, 1981).

Baum et al (1968) found that plasma insulin concentrations were decreased during cardiac surgery under deep hypothermia in 9 infants (mean age 6.8 months) and increased during the rewarming phase at the end of surgery in some of the infants. One neonate was also included in this study, in whom the plasma insulin concentrations did not change during or after surgery (Baum et al, 1968).

GLUCAGON
The plasma glucagon concentrations were not changed during or at the end of surgery, but were found to be significantly increased at 6 and 12 hours after surgery. This pattern of changes may be caused by the stimulation of glucagon secretion by the elevated plasma adrenaline concentrations during the postoperative period (Sperling, 1982). However, the lack of change in plasma glucagon values during surgery may be related to the decreased uptake of glucagon from the pancreatic islets as a result of a poor splanchnic circulation during deep hypothermia and CPB.
In adult patients, an increase in plasma glucagon values after cardiac surgery and CPB has been observed after 12-18 hours following the end of the operation and the elevated concentrations are maintained for up to 48 hours postoperatively (Kobayashi et al, 1980; Teramoto et al, 1980). Thus, in comparison to adult response, the changes in plasma glucagon documented from neonates in the present study occurred earlier and were short-lived. This pattern of responses may be related to an immaturity of the glucagon-secretory mechanism in the neonatal pancreas, as has been documented from experimental work on sheep (Sperling et al, 1980) and also has been suggested from the hormonal responses of newborn infants to hypoglycaemia (Soltesz and Aynsley-Green, 1985).

Due to these changes in the individual concentrations of plasma insulin and glucagon, the insulin/glucagon molar ratio was found to be significantly raised during the period of surgery before the start of CPB. This change probably denotes that the massive hyperglycaemia developed during the surgical procedure before CPB period had a greater influence on insulin and glucagon secretion than the effect of catecholamine release. From adult studies, it has been shown that although adrenaline modifies the secretion of insulin and glucagon at any given blood glucose concentration, the effects of hyperglycaemia can not be completely overcome by catecholamine release (Halter et al, 1984).

STEROID HORMONES

There were no significant changes in the plasma concentrations of progesterone, 17-hydroxyprogesterone, aldosterone, 11-deoxycorticosterone, 11-deoxycortisol and cortisol during or after the surgical procedure. However, there was a tendency towards an intra-operative increase in the concentrations of plasma aldosterone, DOC and 11-deoxycortisol but these
changes were not significant.

As documented in previous studies, the most prominent changes in the adrenocortical hormones were found in the glucocorticoids: cortisol and corticosterone. These hormones increased significantly in response to the surgical stimulus prior to the start of CPB, but had reverted to the preoperative values at the end of surgery. This pattern of change may be related partly to the decreased uptake of adrenocortical hormones as a result of decreased splanchnic circulation at the end of surgery and postoperatively and partly to haemodilution caused by the CPB pump prime, the effect of which would be greater in neonates than in adults due to the disparity between the neonatal blood volume (~ 275 ml) and the volume of the CPB pump prime (~ 1000 ml). On the other hand, it is more likely that an inhibition of the adrenocortical response was caused by the addition of dexamethasone to the CPB pump prime of four neonates. This is also evident from a comparison of the plasma cortisol responses of neonates in the routine and high-dose fentanyl anaesthesia groups, since the latter did not receive dexamethasone in the pump prime (Fig 8.2).

In adult patients subjected to cardiac surgery and CPB under a similar anaesthetic management, the peak plasma cortisol changes were obtained before the start of CPB, as in this study. Similarly, during the period of CPB the plasma cortisol concentrations were found to be reduced (Uozumi et al, 1972; Yokota et al, 1977; Walsh et al, 1981; Sebel et al, 1981; Kono et al, 1981) and thereafter, increased gradually during the postoperative period to reach a secondary peak on the day after surgery (Yokota et al, 1977; Walsh et al, 1981). Thus, the pattern of changes in plasma cortisol found in newborn infants undergoing cardiac surgery are similar to those documented from adult patients. Uozumi et al (1972) have shown that there
is a marked decrease in the plasma cortisol-binding capacity during CPB. Although plasma cortisol concentrations may decrease, the concentration of non-protein-bound cortisol, which is the physiologically active hormone, increases further during CPB and these elevated levels persist into the postoperative period (Uozumi et al, 1972).

Thus, the hormonal response of newborn infants subjected to open-heart surgery under routine anaesthesia is characterised mainly by a marked increase in the plasma concentrations of catecholamines and glucocorticoids before the start of cardiopulmonary bypass. During the subsequent surgical procedure and post-operatively, the catecholamine response was obscured by the intravenous infusion of sympathomimetic drugs to the majority of neonates and the corticosteroid response at the end of surgery was blocked by the addition of dexamethasone to the CPB pump prime. In the postoperative period, a short-lasting increase in plasma glucagon was also observed. These hormonal changes may be responsible for mediating the peri-operative metabolic adjustments documented from these newborn infants in response to cardiac surgery.

8.3.1.2 Metabolic changes :-

As in the previous study, the metabolite concentrations were measured in arterial blood samples drawn from the neonates included in this study. The most prominent feature of the metabolic response documented from neonates undergoing cardiac surgery was the severe lactic acidemia observed during surgery and in the postoperative period. This feature is at variance from the responses of neonates undergoing non-cardiac surgery, in whom the most prominent feature of the peri-operative metabolic changes was seen in the hyperglycaemic responses to surgical stress.
GLUCOSE

A marked hyperglycaemic response was also documented from newborn infants undergoing cardiac surgery, which reached its peak before the start of cardiopulmonary bypass and was maintained at the end of surgery. Although still raised, blood glucose values at 6, 12 and 24 hours after surgery were not significantly different from the preoperative concentrations. This may be partly due to comparison with blood glucose values which were already raised when the preoperative blood sample was obtained; or may be partly related to depletion of the limited carbohydrate reserves in these neonates due to the severe hyperglycaemic response during the surgical procedure (Shelley, 1961). Alternatively, the rapid utilisation of glucose due to glycolysis in the extensive regions of injured tissue (Wilmore, 1981) and the loss of glucose in urine may also contribute to the decreased blood glucose values of neonates in the postoperative period.

The mechanism of development of massive hyperglycaemia during the surgical procedure may be partly explained by the increased glucose production and decreased peripheral utilisation stimulated by the release of adrenaline and glucocorticoids (Sperling, 1982), and possibly may be contributed to by lowering the body temperature of the neonate to 10-14°C, which would not only provide an additional stimulus to the hormonal stress response, but may also decrease peripheral glucose utilisation.

The insulin/glucagon molar ratio was found to increase in response to the hyperglycaemia before the start of CPB, implying the stimulation of insulin secretion and/or suppression of glucagon secretion (Sperling, 1982). On the other hand, it was found that the insulin/glucose molar ratio had decreased during the surgical procedure before the start of CPB. These opposite changes, may give indirect evidence of two opposing factors in the control
of insulin and glucagon secretion during surgery, that of hyperglycaemia and that of intra-operative adrenaline release (Halter et al., 1984); but would need to be confirmed in future studies.

GLUCONEOGENIC SUBSTRATES

The increase in blood concentrations of the gluconeogenic substrates, particularly with respect to blood lactate concentrations, was found to be much greater than the response of neonates undergoing non-cardiac surgery. The marked increase in blood lactate concentrations during surgery before the start of CPB may be related to the adrenaline release in response to surgical stress. From adult studies, it has been shown that glycogenolysis stimulated by adrenaline forms the main source of lactate production during surgery (Kusaka et al., 1977; Stjernstrom et al., 1981; Wilmore, 1981).

At the end of surgery, however, the massive increase in blood lactate concentrations may be related to the anaerobic metabolism during the period of circulatory arrest. In addition, alterations in regional circulation during cardiopulmonary bypass, the increased lactate content of blood which was used for the CPB pump prime and other factors such as peripheral vasoconstriction due to hypothermia, may also contribute to the grossly raised blood lactate concentrations at the end of surgery. Furthermore, the clearance of circulating lactate by the liver will be decreased not only due to the decreased activity of metabolic pathways, as has been demonstrated by studies on the perfused rat liver (Zimmermann et al. 1976); but also due to the splanchnic vasoconstriction caused by CPB and deep hypothermia (Halley et al., 1959). It is likely that the combined effect of all these factors would not only increase blood lactate to the very high concentrations which were documented, but these effects may also persist during the postoperative period and would be responsible for the continued
elevation of blood lactate concentrations postoperatively.

In this context, it must be noted that lactate concentrations in the pre-operative blood samples were measured in cyanosed arterial blood, whereas the lactate values in blood samples obtained at the end of surgery and in the postoperative period were measured in well-oxygenated arterial blood. Thus, the significance of the increase in blood lactate concentrations may be much greater than is evident from these changes.

The concentrations of blood pyruvate were also increased at the end of surgery and remained elevated during the postoperative period. Changes in blood pyruvate values may also be related to the adrenaline-stimulated-glycogenolysis and decreased clearance by liver cells during cardiac surgery, CPB and deep hypothermia (Zimmermann et al, 1976; Wilmore, 1981).

Despite the increase in blood pyruvate concentrations, it was observed that the lactate/pyruvate molar ratio was raised significantly during surgery, at the end of surgery, at 6 and 12 hours after surgery; thereby implying the effects of impaired circulation during hypothermia and tissue hypoxia during the period of circulatory arrest. Probably due to a gradually improving circulation even after the termination of circulatory arrest and CPB, this anaerobic deficit was maintained at 6 and 12 hours after surgery. By 24 hours after surgery, the effects of anaerobic metabolism were no longer evident and lactate/pyruvate ratio had returned to preoperative values.

The blood concentrations of alanine were also found to be increased during surgery before the start of CPB, which may be due either to the effects of cortisol and corticosterone secretion on alanine production in skeletal
muscle, as shown by experimental studies (Garber et al, 1976; Karl et al, 1976; Muhlbacher et al, 1984), or may be related to the decreased utilisation of alanine for gluconeogenesis (Zimmermann et al, 1976; Frazer et al, 1981; Sperling et al, DeLamater et al, 1974; Kraus-Friedman, 1984). Although alanine concentrations remained elevated at the end of surgery, a decrease in the alanine/pyruvate molar ratio was observed, which may be mediated by the effect of raised adrenaline values (Garber et al, 1976).

Similar to the other gluconeogenic substrates, blood glycerol values were also raised at the end of surgery. These changes in blood glycerol may either be due to catecholamine-stimulated lipolysis (Williamson, 1982) or more probably, may be related to the decreased utilisation of glycerol by the gluconeogenic pathway in liver cells due to the effects of deep hypothermia and CPB (Zimmermann et al, 1976). Thus, the metabolic response of neonates undergoing cardiac surgery was characterised by a marked elevation of total gluconeogenic substrates during surgery before the start of CPB, at the end of surgery and in the postoperative period.

No changes were observed in the blood concentrations of ketone bodies during or after cardiac surgery and CPB. This pattern of response may be due either to a suppression of the ketogenic pathway in the liver cells caused by deep hypothermia, or may result from a decreased uptake of ketone bodies due to splanchnic vasoconstriction during CPB. On the other hand, this response may represent the marked sensitivity of ketogenesis to an increased insulin/glucagon ratio during surgery (Williamson, 1982).

Therefore, it may be concluded that cardiac surgery and its associated procedures stimulate a severe metabolic response in newborn infants, which is characterised by a marked lactic acidaemia and hyperglycaemia and may
have a direct bearing on their clinical outcome following cardiac surgery. The severity of the hormonal and metabolic changes documented in neonates subjected to cardiac surgery under routine anaesthesia may justify the investigation of specific therapeutic measures to inhibit the stress responses to surgery. The techniques most commonly employed to achieve this therapeutic goal in adult patients undergoing cardiac surgery are the use of opiate drugs in high doses for anaesthetic management. A similar approach was investigated in a small number of newborn infants undergoing cardiac surgery, cardiopulmonary bypass, deep hypothermia and circulatory arrest. These neonates were randomly selected and, to some extent, received a similar clinical management during and after surgery as neonates in the routine anaesthesia group.

8.3.2 The effects of high-dose fentanyl anaesthesia

Differences in the hormonal and metabolic response of neonates in the routine and high-fentanyl anaesthesia groups may be primarily due to the effect of differences in anaesthetic management, but may also be influenced by the variable peri-operative management and clinical condition of neonates in the two anaesthesia groups. For these reasons and due to the comparison of small numbers of patients in the two groups, these differences would need to be confirmed in larger and more standardised clinical trials. However, data obtained from the present study can be used to generate hypotheses as well as to establish the criteria required for planning future trials.

8.3.2.1 Hormonal changes

CATECHOLAMINES

The most prominent difference between the hormonal responses of neonates in
the routine and high-dose fentanyl anaesthesia groups was observed with respect to changes in plasma adrenaline concentration during the surgical procedure prior to the start of cardiopulmonary bypass. The plasma adrenaline concentrations were found to be decreased in all neonates given fentanyl anaesthesia, whereas they were markedly raised in neonates from the routine anaesthesia group. The suppressive effect of fentanyl on catecholamine secretion also has been documented in preterm neonates undergoing PDA ligation (Chapter VII). Similar findings in adult patients undergoing cardiac surgery have been documented in several studies (Stanley et al, 1980; Sebel et al, 1981; Kono et al, 1981; Zurick et al, 1982). The mechanism of this effect may be either the binding of fentanyl to mu-opioid receptors in the hypothalamus causing a competitive block of the sympathetic outflow from hypothalamic centres during surgery (Cohen et al, 1983; Van Loon et al, 1981) or could be caused by the direct and non-competitive inhibition of catecholamine release from the binding of fentanyl to opioid receptors on chromaffin cells in the adrenal medulla and elsewhere (Lemaire et al, 1980; Costa et al, 1980).

There was a trend towards similar differences in the noradrenaline response of neonates given fentanyl anaesthesia. However, this was not consistent. It is tempting to suggest that the lack of a clear distinction between the noradrenaline responses of neonates in the fentanyl and routine anaesthesia groups may be due to the variability of noradrenaline kinetics in plasma (Cryer, 1984; Christensen et al, 1984). The catecholamine responses at the end of surgery and postoperatively were not significantly different between the two anaesthesia groups. These responses may have been obscured by the stimulatory effects of dopamine or isoprenaline infusions given to neonates in the two anaesthesia groups (Zaritsky et al, 1984) and therefore, would not be representative of the hormonal response to cardiac surgery.
INSULIN

In response to surgical hyperglycaemia, it was noted that plasma insulin concentrations increased during surgery in the routine and fentanyl anaesthesia groups and remained elevated in the postoperative period. The response of neonates in the high-dose fentanyl anaesthesia was greater than that of neonates in the routine anaesthesia group, but a significant difference between the two groups was obtained only at 6 hours after surgery. This difference could be due to the suppressive effect of adrenaline on insulin secretion in the routine anaesthesia group (Sperling et al, 1984), but such an explanation is unlikely since the plasma adrenaline values at the end of surgery and postoperatively were similar in the two anaesthesia groups. On the other hand, the results of in vivo animal studies (Ipp et al, 1980) and some studies on adult humans (Guigliano, 1984; Feldman et al, 1983) have shown that the intravenous injection of large doses of opiates and opioid peptides stimulate insulin and glucagon secretion by a direct action on islet cells. It is possible that the effects of surgical hyperglycaemia and fentanyl were combined at the end of surgery and postoperatively to cause a marked stimulation of insulin secretion and a relative inhibition of glucagon secretion in neonates from the high-dose fentanyl anaesthesia group.

STEROID HORMONES

There were no major differences in the corticosteroid responses of neonates in the fentanyl and routine anaesthesia groups, except for a smaller cortisol response at the end of surgery in the neonates from the routine anaesthesia group. It is likely that this difference, and the trend towards a similar difference in the plasma corticosterone changes, was due to the addition of dexamethasone in the CPB pump prime of neonates in the routine
anaesthesia group. Dexamethasone was added to the CPB pump prime of 4 neonates in the routine anaesthesia group (all of whom were included in the measurement of plasma corticosteroids) and 1 neonate in the high-dose fentanyl anaesthesia group (in whom the plasma corticosteroids were not measured due to insufficient plasma samples). Thus, these differences may not be relevant for the comparison of hormonal responses between neonates in the two anaesthesia groups, although they may possibly influence the respective metabolic responses of neonates in the two groups.

8.3.2.2 Metabolic changes :-

Although the hyperglycaemic responses of neonates given routine anaesthesia or high-dose fentanyl anaesthesia were not significantly different, it was observed that the degree of hyperglycaemia during the surgical procedure prior to CPB and at the end of surgery was decreased in neonates who were given high-dose fentanyl anaesthesia. The magnitude of this difference in responses was considered to be clinically important and, if confirmed in a larger clinical trial, may be a noteworthy effect of high-dose fentanyl anaesthesia in neonates undergoing cardiac surgery. It is likely that this may be mediated either by the inhibition of adrenaline secretion during surgery (which has been documented in neonates given fentanyl anaesthesia) or may be a result of the stimulation of insulin secretion as discussed above. The latter mechanism may be suggested by the striking difference between neonates in the routine and high-dose fentanyl anaesthesia groups with respect to changes in the insulin/glucose ratio during and after surgery (Figure 8.3).

Another prominent feature of the metabolic response of neonates in the high-dose fentanyl anaesthesia group was the increased blood concentrations of gluconeogenic substrates documented at the end of surgery and 6 hours
postoperatively with regard to changes in blood concentrations of pyruvate, alanine and glycerol. The cause of these differences is not clear and may possibly be related to the increased cortisol and corticosterone responses documented at the end of surgery in neonates from the fentanyl anaesthesia group. From in vitro experimental studies (Garber et al, 1976; Karl et al, 1976), it has been shown that the glucocorticoids stimulate the rate of proteolysis in skeletal muscle and markedly increase the production of alanine, although the formation of pyruvate is not affected. Alternatively, these differences may be caused by a decreased utilisation by the liver cells for gluconeogenesis; which may result either from a decrease of the hepatic blood flow or from a direct suppression of the gluconeogenic pathway by fentanyl. It has been documented that low doses of fentanyl do not alter the hepatic blood flow (Tornetta and Boger, 1964; Wiklund, 1975), whereas a suppressive effect on hepatic gluconeogenesis seems unlikely on the basis of studies in adult patients (Hall et al, 1978). In addition, the lack of any significant differences between neonates in the two anaesthesia groups with regard to changes in blood lactate or total gluconeogenic substrates indicates that these effects may not be consistent.

Thus, it may be provisionally concluded that high-dose fentanyl anaesthesia given to neonates undergoing cardiac surgery causes a suppression of the catecholamine responses and an increased secretion of insulin, which may be associated with a tendency towards a decrease in the hyperglycaemic response to cardiac surgery and its associated procedures.

8.3.3 Hypotheses for further investigation:

On the basis of these responses, it is proposed that the use of high-dose fentanyl anaesthesia in neonates undergoing cardiac surgery may inhibit
some aspects of the hormonal and metabolic stress response. From the severity of the metabolic changes documented in neonates receiving routine anaesthetic management, it is possible that a suppression of the stress response to cardiac surgery may be clinically beneficial in these patients and may even lead to an improvement of the postoperative morbidity and mortality.

Thus, the following hypotheses may be proposed on the basis of the present study:

(A) Anaesthesia given with fentanyl (100 μg/kg) to neonates undergoing cardiac surgery, cardiopulmonary bypass, deep hypothermia and circulatory arrest does not decrease their hormonal and metabolic stress response as compared to that of neonates given non-narcotic anaesthesia and subjected to similar surgical procedures.

(B) A suppression of the hormonal and metabolic stress response in newborn infants undergoing cardiac surgery does not decrease the postoperative morbidity and mortality of these patients as compared to that of neonates with a uninhibited hormonal and metabolic stress response to similar surgical procedures.

It is suggested that the outcome measures used for planning the first clinical trial (A) and for the definition of its criteria may be: adrenaline, insulin, glucose and lactate; in that order of priority. On the basis of changes documented in the present study, a total of 36 neonates would be required to prove or disprove the stated hypothesis at a significance level of $P<0.05$.

The outcome measures required to investigate the second trial (B) need to
be defined from the clinical outcome of neonates undergoing cardiac surgery and correlated to the hormonal and metabolic variables measured. It is suggested that the second clinical trial may be planned on the basis of the following measures: mortality rate calculated from deaths within four weeks of the surgical procedure, complication rates from the documented complications within one week of the surgical procedure, duration of stay in intensive care, duration of stay in hospital, duration of postoperative ventilation. Except the mortality rate, the priority of the remaining outcome measures cannot be defined from the present information. On the basis of the current mortality rate of neonates undergoing cardiac surgery, it is estimated that a total of 240 neonates will be required to prove or disprove the stated hypothesis at a significance level of $P<0.05$. Thus, the present study can be used to plan and define the criteria for future clinical trials which would investigate the effects of high-dose fentanyl anaesthesia on the hormonal-metabolic stress response and the effects of these changes in the stress response on the morbidity and mortality of newborn infants undergoing cardiac surgery.

8.4 CONCLUSION:

The hormonal and metabolic changes documented from neonates undergoing cardiac surgery were of much greater magnitude than has been observed from the previous studies on neonates undergoing non-cardiac surgery, as well as in comparison to the response of adult patients undergoing cardiac surgery. The provisional effects of high-dose fentanyl anaesthesia documented from a small number of neonates in this study are of sufficient importance to merit further investigation. The data obtained from this study thus, can be used to plan further clinical trials in order to improve the outcome of newborn infants subjected to cardiac surgery.
Figure 8.1: - Anaesthetic protocol for neonates randomly allocated to the routine anaesthesia group.
HIGH-DOSE FENTANYL TRIAL: ANAESTHETIC PROTOCOL

NON-NARCOTIC ANAESTHETIC TECHNIQUE

1. Premedication: - Atropine 0.02-0.04 mg/kg

2. Induction: - Halothane 1-2%
   - Nitrous oxide + Oxygen 50:50
   - IV Pancuronium 0.1-0.2 mg/kg

3. Intubation: insertion of arterial and CVP lines

4. Maintenance: - Omnopon 0.1-0.5 mg/kg
   - Nitrous Oxide + Oxygen 50:50
   - IV Pancuronium 0.1-0.2 mg/kg (as often as required)

5. Additional drugs: - Heparin, Soda bicarb, Protamine, Dopamine, Phenoxymethylamine,
   - Potassium chloride, Calcium chloride, etc.

6. Intravenous fluids: - 5% Dextrose = 4.8-7.2 ml/kg/min, or
   - 10% Dextrose = 2.4-3.6 ml/kg/min, or
   - 15% Dextrose = 1.6-2.4 ml/kg/min.
   - 0.9% Heparinised saline as flush solution.

COOLING PROCEDURE: -

1. Surface cooling to 34°C.

2. Bypass cooling to 15°C.

BYPASS PROCEDURE: -

   - F.F.P. (for dilution, if necessary)

2. Perfusion technique: - (a) Non-pulsatile flow
   (b) Flow rate 2-2.4 litres/min./Meters², which may be
      reduced in accordance with the cooling procedure.
   (c) Perfusion pressure 40-50 mmHg when temperature is above
      28°C and 30-40 mmHg when temperature is below 28°C.
   (d) Circulatory arrest below 15°C.
Figure 8.2: Anaesthetic protocol for neonates randomly allocated to the high-dose fentanyl anaesthesia group.
HIGH-DOSE FENTANYL TRIAL: ANAESTHETIC PROTOCOL

NARCOTIC ANAESTHETIC TECHNIQUE

1. Premedication: - Atropine 0.02-0.04 mg/kg

2. Induction: - Halothane 1-2%
   Nitrous oxide = Oxygen 50:50%
   IV Pancuronium 0.1-0.2 mg/kg

3. Intubation; insertion of arterial and CVP lines.

4. NARCOTIC INDUCTION: - IV Fentanyl 50 ug/kg (given slowly BEFORE incision)

5. Maintenance: - Nitrous oxide + Oxygen 50:50%
   IV Pancuronium 0.1-0.2 mg/kg (as often as required)

6. Additional drugs: - Heparin, Soda bicarb, Protamine, Dopamine, Phenoxybenzamine, Potassium chloride, Calcium chloride, etc.

7. Post-CPB narcotic maintenance: - IV Fentanyl 10 ug/kg (given slowly at hourly intervals, as per condition of patient)

8. Intravenous fluids: - 5% Dextrose = 4.8-7.2 ml/kg/min.
   10% Dextrose = 2.4-3.6 ml/kg/min.
   15% Dextrose = 1.6-2.4 ml/kg/min.
   0.9% Heparinised saline as flush solution.

COOLING PROCEDURE

1. Surface cooling to 34°C.

2. Bypass cooling to 15°C.

BYPASS PROCEDURE:-

   F.F.P. (for dilution, if necessary)
   Add FENTANYL 40 ug/kg

2. Perfusion technique: - (a) Non-pulsatile flow
   (b) Flow rate 2-2.4 litres/min./Metres², which may be reduced in accordance with the cooling procedure.
   (c) Perfusion pressure 40-50 mmHg when temperature is above 28°C and 30-40 mmHg when temp. is below 28°C.
   (d) Circulatory arrest below 15°C.
Table 8.1 CARDIAC STUDY: Description of patients

<table>
<thead>
<tr>
<th>Description of patients</th>
<th>Routine Anaesthesia (Mean ± SEM)</th>
<th>High-dose Fentanyl Anaesthesia (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Age, days</td>
<td>18 ± 5</td>
<td>21 ± 7</td>
</tr>
<tr>
<td>Gestation, weeks</td>
<td>38.4 ± 0.6</td>
<td>38.1 ± 1.1</td>
</tr>
<tr>
<td>Birthweight, kg</td>
<td>3.4 ± 0.2</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>Dextrose infusion rate, mg/kg/min</td>
<td>3.2 ± 0.9</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>Starvation, hours pre-operative</td>
<td>7.1 ± 0.4</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>Weight at operation, kg</td>
<td>3.2 ± 0.2</td>
<td>3.3 ± 0.2</td>
</tr>
</tbody>
</table>

Pre-operative drug therapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Routine Anaesthesia</th>
<th>High-dose Fentanyl Anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin E2 infusion</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Frusemide</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Digoxin</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Dopamine infusion</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Chloral hydrate</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

Characteristics of neonates in the routine anaesthesia and high-dose fentanyl anaesthesia groups, and a list of drugs given during the 24 hours before surgery.
Table 8.2 - CARDIAC STUDY: - Clinical management during surgery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Routine Anaesthesia (Mean ± SEM)</th>
<th>High-dose Fentanyl Anaesthesia (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Surgical stress score</td>
<td>24.9 ± 1.5</td>
<td>26.7 ± 0.4</td>
</tr>
<tr>
<td>Dextrose infusion rate, mg/kg/min</td>
<td>6.9 ± 1.7</td>
<td>6.7 ± 1.4</td>
</tr>
<tr>
<td>Time for operation, min</td>
<td>289 ± 59</td>
<td>309 ± 33</td>
</tr>
<tr>
<td>Cardiopulmonary bypass, min</td>
<td>118 ± 31</td>
<td>137 ± 23</td>
</tr>
<tr>
<td>Circulatory arrest, min</td>
<td>48 ± 9</td>
<td>56 ± 5</td>
</tr>
<tr>
<td>Temperature loss, °C</td>
<td>24 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Dose of papaveretum, mg/kg</td>
<td>1.0 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>Dose of fentanyl, µg/kg</td>
<td>-</td>
<td>93 ± 6</td>
</tr>
<tr>
<td>Dose of pancuronium mg/kg</td>
<td>0.27 ± 0.05</td>
<td>0.37 ± 0.06</td>
</tr>
<tr>
<td>Dose of heparin mg/kg</td>
<td>3.9 ± 0.1</td>
<td>3.6 ± 0.2</td>
</tr>
</tbody>
</table>

Intra-operative drug therapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>NUMBER OF PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium bicarbonate</td>
<td>5</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
</tr>
<tr>
<td>Frusemide</td>
<td>2</td>
</tr>
<tr>
<td>Adrenaline, intracardiac</td>
<td>1</td>
</tr>
<tr>
<td>Dopamine infusion</td>
<td>3</td>
</tr>
<tr>
<td>Isoprenaline infusion</td>
<td>1</td>
</tr>
<tr>
<td>Phenoxybenzamine</td>
<td>3</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>3</td>
</tr>
<tr>
<td>Thiopentone sodium</td>
<td>1</td>
</tr>
<tr>
<td>Digoxin</td>
<td>-</td>
</tr>
</tbody>
</table>

Parameters of the surgical procedure and list of drugs given to neonates in the routine anaesthesia and high-dose fentanyl anaesthesia groups during surgery.
Table 8.3  CARDIAC STUDY: - Post-operative drug therapy

<table>
<thead>
<tr>
<th>LIST OF DRUGS</th>
<th>Routine Anaesthesia</th>
<th>High-dose Fentanyl Anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digoxin</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Lasix</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Mannitol</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Calcium gluconate</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Dobamine infusion</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Isoprenaline infusion</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Adrenaline, intracardiac</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Diazepam</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Papaveretum</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Penicillin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

List of drugs given to neonates in the routine anaesthesia and non- fentanyl anaesthesia groups during the 24 hours after cardiac surgery.
Table 8.4  CARDIAC STUDY: - Hormonal changes in neonates given routine anaesthesia.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Time</th>
<th>Mean ± SEM</th>
<th>N</th>
<th>Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline</td>
<td>Pre-operative</td>
<td>1.27 ± 0.24</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>nmol/L</td>
<td>Pre-CPB</td>
<td>11.92 ± 4.30</td>
<td>6</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>End-operative</td>
<td>35.54 ± 14.41</td>
<td>7</td>
<td>p&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>6hr post-operative</td>
<td>22.38 ± 4.61</td>
<td>6</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>12hr post-operative</td>
<td>18.32 ± 6.47</td>
<td>6</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>24hr post-operative</td>
<td>11.94 ± 5.70</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>Pre-operative</td>
<td>17.09 ± 3.41</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>nmol/L</td>
<td>Pre-CPB</td>
<td>24.82 ± 6.76</td>
<td>6</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>End-operative</td>
<td>42.62 ± 11.57</td>
<td>7</td>
<td>p&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>6hr post-operative</td>
<td>41.00 ± 10.96</td>
<td>6</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>12hr post-operative</td>
<td>31.67 ± 5.29</td>
<td>6</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>24hr post-operative</td>
<td>24.62 ± 7.19</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Insulin</td>
<td>Pre-operative</td>
<td>117 ± 28</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>pmol/L</td>
<td>Pre-CPB</td>
<td>250 ± 71</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>End-operative</td>
<td>181 ± 59</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>6hr post-operative</td>
<td>115 ± 32</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>12hr post-operative</td>
<td>132 ± 29</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>24hr post-operative</td>
<td>124 ± 52</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Glucagon</td>
<td>Pre-operative</td>
<td>7.1 ± 0.8</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>pmol/L</td>
<td>Pre-CPB</td>
<td>5.8 ± 0.8</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>End-operative</td>
<td>9.3 ± 1.7</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>6hr post-operative</td>
<td>13.9 ± 2.7</td>
<td>5</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>12hr post-operative</td>
<td>12.7 ± 3.6</td>
<td>5</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>24hr post-operative</td>
<td>7.0 ± 2.9</td>
<td>4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Insulin/gluca</td>
<td>Pre-operative</td>
<td>15.0 ± 3.9</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>gon ratio</td>
<td>Pre-CPB</td>
<td>41.2 ± 13.7</td>
<td>5</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>End-operative</td>
<td>28.5 ± 11.1</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>6hr post-operative</td>
<td>10.0 ± 3.0</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>12hr post-operative</td>
<td>12.8 ± 3.2</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>24hr post-operative</td>
<td>18.4 ± 4.1</td>
<td>4</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Changes in plasma hormone concentrations of neonates given routine anaesthesia during cardiac surgery. Values measured during and after surgery were compared to pre-operative values using Wilcoxon's matched-pairs test.
<table>
<thead>
<tr>
<th>Hormone</th>
<th>Pre-operative</th>
<th>Pre-CPB</th>
<th>End-operative</th>
<th>6hr post-operative</th>
<th>12hr post-operative</th>
<th>24hr post-operative</th>
<th>Mean ± SEM</th>
<th>N</th>
<th>Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/L</td>
<td>4.72 ± 1.49</td>
<td>6.88 ± 2.37</td>
<td>3.49 ± 0.38</td>
<td>5.96 ± 3.36</td>
<td>4.33 ± 1.40</td>
<td>1.54 ± 0.41</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/L</td>
<td>28.2 ± 9.4</td>
<td>68.4 ± 20.1</td>
<td>22.8 ± 6.4</td>
<td>50.6 ± 33.7</td>
<td>50.1 ± 23.5</td>
<td>6.6 ± 2.5</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-Deoxycorticosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/L</td>
<td>0.20 ± 0.03</td>
<td>0.23 ± 0.06</td>
<td>0.33 ± 0.07</td>
<td>0.29 ± 0.07</td>
<td>0.27 ± 0.09</td>
<td>0.18 ± 0.03</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/L</td>
<td>3.16 ± 1.13</td>
<td>2.43 ± 0.67</td>
<td>1.82 ± 0.36</td>
<td>1.40 ± 0.31</td>
<td>1.86 ± 0.42</td>
<td>1.38 ± 0.31</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Hydroxyprogesterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/L</td>
<td>2.27 ± 0.46</td>
<td>2.71 ± 0.43</td>
<td>1.68 ± 0.35</td>
<td>2.55 ± 1.10</td>
<td>3.03 ± 1.45</td>
<td>1.45 ± 0.48</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-Deoxycortisol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/L</td>
<td>0.57 ± 0.19</td>
<td>0.88 ± 0.33</td>
<td>0.73 ± 0.22</td>
<td>1.11 ± 0.50</td>
<td>1.30 ± 0.81</td>
<td>0.23 ± 0.05</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/L</td>
<td>403 ± 105</td>
<td>805 ± 251</td>
<td>482 ± 96</td>
<td>733 ± 261</td>
<td>651 ± 157</td>
<td>305 ± 116</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/L</td>
<td>123 ± 31</td>
<td>82 ± 21</td>
<td>86 ± 17</td>
<td>122 ± 37</td>
<td>87 ± 27</td>
<td>127 ± 60</td>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Changes in plasma hormone concentrations of neonates given routine anaesthesia during cardiac surgery. Values measured during and after surgery were compared to pre-operative values using Wilcoxon's matched-pairs test.
### Table 8.6 CARDIAC STUDY: Metabolic changes in neonates given routine anaesthesia

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Pre-operative</th>
<th>Pre-CPB</th>
<th>End-operative</th>
<th>6hr post-operative</th>
<th>12hr post-operative</th>
<th>24hr post-operative</th>
<th>Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>8.2 ± 0.8</td>
<td>28.9 ± 4.4</td>
<td>24.1 ± 6.3</td>
<td>11.0 ± 2.4</td>
<td>13.1 ± 5.9</td>
<td>9.5 ± 1.9</td>
<td>--</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>2.1 ± 0.4</td>
<td>3.8 ± 0.8</td>
<td>7.1 ± 1.2</td>
<td>4.8 ± 1.2</td>
<td>3.4 ± 0.4</td>
<td>2.3 ± 0.3</td>
<td>--</td>
</tr>
<tr>
<td>Pyruvate (mmol/L)</td>
<td>0.16 ± 0.02</td>
<td>0.22 ± 0.05</td>
<td>0.33 ± 0.05</td>
<td>0.26 ± 0.04</td>
<td>0.22 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>--</td>
</tr>
<tr>
<td>Acetoacetate (mmol/L)</td>
<td>0.17 ± 0.04</td>
<td>0.18 ± 0.07</td>
<td>0.11 ± 0.05</td>
<td>0.13 ± 0.04</td>
<td>0.13 ± 0.04</td>
<td>0.10 ± 0.02</td>
<td>--</td>
</tr>
<tr>
<td>3-Hydroxybutyrate (mmol/L)</td>
<td>0.18 ± 0.06</td>
<td>0.41 ± 0.14</td>
<td>0.25 ± 0.12</td>
<td>0.10 ± 0.03</td>
<td>0.14 ± 0.09</td>
<td>0.07 ± 0.03</td>
<td>--</td>
</tr>
<tr>
<td>Alanine (mmol/L)</td>
<td>0.29 ± 0.04</td>
<td>0.35 ± 0.05</td>
<td>0.38 ± 0.04</td>
<td>0.37 ± 0.04</td>
<td>0.40 ± 0.09</td>
<td>0.20 ± 0.02</td>
<td>--</td>
</tr>
<tr>
<td>Glycerol (mmol/L)</td>
<td>0.36 ± 0.06</td>
<td>0.39 ± 0.07</td>
<td>0.61 ± 0.09</td>
<td>0.39 ± 0.07</td>
<td>0.33 ± 0.09</td>
<td>0.32 ± 0.11</td>
<td>--</td>
</tr>
</tbody>
</table>

Changes in blood metabolite concentrations of neonates given routine anaesthesia during cardiac surgery. Values measured during and after surgery were compared to pre-operative values using Wilcoxon's matched-pairs test.
Table 6.7 CARDIAC STUDY: Derived hormonal-metabolic variables in neonates given routine anaesthesia.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SEM</th>
<th>N</th>
<th>Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Ketones mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-operative</td>
<td>0.35 ± 0.08</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Pre-CPB</td>
<td>0.59 ± 0.18</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td>End-operative</td>
<td>0.36 ± 0.17</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-operative</td>
<td>0.23 ± 0.06</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>12hr post-operative</td>
<td>0.27 ± 0.13</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>24hr post-operative</td>
<td>0.16 ± 0.05</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Lactate/pyruvate ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-operative</td>
<td>12.3 ± 1.2</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Pre-CPB</td>
<td>17.9 ± 2.8</td>
<td>7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>End-operative</td>
<td>21.2 ± 1.9</td>
<td>7</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>6hr post-operative</td>
<td>17.4 ± 1.9</td>
<td>6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>12hr post-operative</td>
<td>15.6 ± 1.1</td>
<td>6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>24hr post-operative</td>
<td>11.8 ± 0.8</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Insulin/glucose ratio pmol/mmol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-operative</td>
<td>15.5 ± 4.7</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Pre-CPB</td>
<td>8.1 ± 1.2</td>
<td>6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>End-operative</td>
<td>8.7 ± 2.7</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-operative</td>
<td>11.2 ± 1.9</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>12hr post-operative</td>
<td>17.9 ± 6.8</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>24hr post-operative</td>
<td>19.7 ± 12.5</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Total gluconeogenic substrates mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-operative</td>
<td>2.9 ± 0.5</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Pre-CPB</td>
<td>4.7 ± 0.9</td>
<td>7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>End-operative</td>
<td>8.4 ± 1.3</td>
<td>7</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>6hr post-operative</td>
<td>5.9 ± 1.4</td>
<td>6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>12hr post-operative</td>
<td>4.2 ± 0.7</td>
<td>5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>24hr post-operative</td>
<td>2.9 ± 0.4</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Alanine/pyruvate ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-operative</td>
<td>1.9 ± 0.2</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Pre-CPB</td>
<td>1.8 ± 0.2</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td>End-operative</td>
<td>1.2 ± 0.2</td>
<td>7</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>6hr post-operative</td>
<td>1.5 ± 0.2</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>12hr post-operative</td>
<td>1.9 ± 0.4</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>24hr post-operative</td>
<td>1.1 ± 0.1</td>
<td>6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Hydroxybutyrate/acetoacetate ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-operative</td>
<td>1.45 ± 0.43</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Pre-CPB</td>
<td>2.38 ± 0.98</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td>End-operative</td>
<td>2.63 ± 0.68</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td>6hr post-operative</td>
<td>0.84 ± 0.18</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>12hr post-operative</td>
<td>0.74 ± 0.27</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td>24hr post-operative</td>
<td>0.54 ± 0.18</td>
<td>6</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Changes in derived hormonal-metabolic variables of neonates given routine anaesthesia during cardiac surgery. Values obtained during and after surgery were compared to pre-operative values using Wilcoxon's matched-pairs test.
Table 6.8  CARDIAC STUDY: Metabolite concentrations in CPB pump prime

<table>
<thead>
<tr>
<th></th>
<th>Glucose mmol/L</th>
<th>Lactate mmol/L</th>
<th>Pyruvate mmol/L</th>
<th>Acetoacetate mmol/L</th>
<th>Hydroxybutyrate mmol/L</th>
<th>Alanine mmol/L</th>
<th>Glycerol mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.33</td>
<td>3.92</td>
<td>0.06</td>
<td>0.05</td>
<td>0.05</td>
<td>0.45</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>4.67</td>
<td>3.39</td>
<td>0.08</td>
<td>0.02</td>
<td>0.03</td>
<td>0.38</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>4.08</td>
<td>4.01</td>
<td>0.06</td>
<td>0.08</td>
<td>0.10</td>
<td>0.42</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>3.91</td>
<td>4.26</td>
<td>0.09</td>
<td>0.06</td>
<td>0.14</td>
<td>0.34</td>
<td>0.09</td>
</tr>
<tr>
<td>5</td>
<td>5.03</td>
<td>3.97</td>
<td>0.12</td>
<td>0.09</td>
<td>0.02</td>
<td>0.49</td>
<td>0.05</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>4.41 ± 0.20</td>
<td>3.91 ± 0.14</td>
<td>0.08 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.07 ± 0.02</td>
<td>0.40 ± 0.04</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>

Metabolite concentrations measured in the fresh heparinised blood used for cardiopulmonary bypass of five neonates undergoing cardiac surgery.
Table 8.9 CARDIAC STUDY: Peri-operative complications

<table>
<thead>
<tr>
<th></th>
<th>NUMBER OF PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Routine Anaesthesia</td>
</tr>
<tr>
<td>INTRA-OPERATIVE COMPLICATIONS</td>
<td></td>
</tr>
<tr>
<td>1. Hypotension</td>
<td>4</td>
</tr>
<tr>
<td>2. Bradycardia</td>
<td>-</td>
</tr>
<tr>
<td>POST-OPERATIVE COMPLICATIONS</td>
<td></td>
</tr>
<tr>
<td>1. Hypotension</td>
<td>6</td>
</tr>
<tr>
<td>2. Prolonged supra-ventricular tachycardia</td>
<td>1</td>
</tr>
<tr>
<td>3. Frequent spontaneous bradycardias</td>
<td>2</td>
</tr>
<tr>
<td>4. Ventricular extrasystoles</td>
<td>1</td>
</tr>
<tr>
<td>5. Cardiac arrest</td>
<td>1</td>
</tr>
<tr>
<td>6. Pulmonary hypertension</td>
<td>-</td>
</tr>
<tr>
<td>7. Persistent metabolic acidosis</td>
<td>1</td>
</tr>
<tr>
<td>8. Post-operative deaths</td>
<td>2</td>
</tr>
</tbody>
</table>

Clinical complications observed in neonates from the routine anaesthesia and high-dose fentanyl anaesthesia groups during the 24 hours after cardiac surgery.
Figure 8.3: Comparison of peri-operative changes in plasma concentrations of adrenaline, noradrenaline and insulin between neonates given routine anaesthetic management (continuous lines) or high-dose fentanyl anaesthesia (interrupted lines) during cardiac surgery. Differences between groups were analysed by the Mann-Whitney U Test, * p<0.05, ** p<0.025, *** p<0.01.

{NB. (a) Changes in plasma adrenaline and noradrenaline concentrations before the start of cardiopulmonary bypass are shown for each patient. Catecholamine responses at the end of surgery and post-operatively have not been presented since they were obscured by infusion of sympathomimetic drugs.

(b) Changes in the mean plasma insulin values derived from neonates in the two anaesthesia groups are presented. The number in parenthesis at each data point denotes the number of patients in each group.}
CARDIAC SURGERY: Comparison of routine and high-dose fentanyl anaesthesia

**Adrenaline**

Routine Anaesthesia

Fentanyl Anaesthesia

**Noradrenaline**

Routine Anaesthesia

Fentanyl Anaesthesia

**Insulin**

Routine Anaesthesia

Fentanyl Anaesthesia

![Graph of Adrenaline, Noradrenaline, and Insulin changes during cardiac surgery](image)
Figure 8.4: - Comparison of peri-operative changes in plasma concentrations of cortisol, corticosterone and aldosterone between neonates given routine anaesthesia management (continuous lines) or high-dose fentanyl anaesthesia (interrupted lines) during cardiac surgery. Differences between groups were analysed by the Mann-Whitney U Test, * p<0.05.

{NB: - Changes in the mean values derived from neonates in the two anaesthesia groups are presented. The number in parenthesis at each data point denotes the number of patients in each group.}
CARDIAC SURGERY: Comparison of routine and high-dose fentanyl anaesthesia

**Δ Cortisol**
- Fentanyl Anaesthesia
- Routine Anaesthesia

**Δ Corticosterone**
- Fentanyl Anaesthesia
- Routine Anaesthesia

**Δ Aldosterone**
- Fentanyl Anaesthesia
- Routine Anaesthesia

Pre-op Pre-CPB End-op 6 hr 12 hr 24 hr
Figure 8.5: Comparison of peri-operative changes in blood glucose concentrations and the insulin/glucose ratio in neonates given routine anaesthetic management (continuous lines) or high-dose fentanyl anaesthesia (interrupted lines) during cardiac surgery. Differences between groups were analysed by the Mann-Whitney U Test, * p<0.05, ** p<0.025.

{NB: Changes in the mean values derived from neonates in the two anaesthesia groups are presented. The number in parenthesis at each data point denotes the number of patients in each group.}
CARDIAC SURGERY: Comparison of routine and high-dose fentanyl anaesthesia

**Δ Glucose**

**Δ Insulin/Glucose ratio**
Figure 8.6: Comparison of peri-operative changes in blood concentrations of pyruvate, alanine and glycerol in neonates given routine anaesthetic management (continuous lines) or high-dose fentanyl anaesthesia (interrupted lines) during cardiac surgery. Differences between groups were analysed by the Mann-Whitney U Test, * p<0.05, ** p<0.025, *** p<0.01.

{NB: Changes in the mean values derived from neonates in the two anaesthesia groups are presented. The number in parenthesis at each data point denotes the number of patients in each group.}
CARDIAC SURGERY: Comparison of routine and high-dose fentanyl anaesthesia

△Pyruvate

△Alanine

△Glycerol

Pre-op Pre-CPB End-op 6 hr 12 hr 24 hr
CHAPTER IX: MEASURING THE SEVERITY OF SURGICAL STRESS
CONTENTS

9.1 INTRODUCTION
   9.1.1 Construction of the Surgical Stress Score
   9.1.2 Methods for statistical analysis

9.2 RESULTS
   9.2.1 Correlation with hormonal and metabolic changes
   9.2.2 Differences in hormonal-metabolic responses of the stress groups
   9.2.3 Preliminary observations on postoperative outcome

9.3 DISCUSSION
   9.3.1 Hormonal changes
   9.3.2 Metabolic changes
   9.3.3 Use of the Surgical Stress Score

9.5 CONCLUSION
9.1 INTRODUCTION

Recent advances in surgical technique and neonatal intensive care, together with the development of sophisticated monitoring techniques, optimum ventilatory management, intravenous feeding and the availability of safe and effective antibiotics; all have combined to make major surgical procedures in term and preterm neonates feasible and common-place for their clinical management. Yet even within a single paediatric surgical unit, the type and extent of surgery, and other factors associated with the surgical operation may be very different for each patient.

It may be considered essential to take the differences of severity of surgery into account when comparing the morbidity and mortality of different groups of neonates undergoing surgery, either in the same centre or between different centres; when measuring the effects of differences in therapy or surgical approach; or for the purposes of evaluating requirements for intensive care, monitoring and subsequent therapy in the postoperative period.

Logically, there would be two basic research techniques for approaching this problem. The first would be to compare only those neonates who are subjected to a similar surgical procedure with a well-documented outcome. However, this approach is not always applicable to newborn infants since major variations may exist in the type of presentation and outcome of even the commonest congenital abnormalities (eg, tracheo-oesophageal fistula, diaphragmatic hernia) or acquired conditions (eg, patent ductus arteriosus, necrotising enterocolitis) that require surgery in the neonatal age group. Furthermore, the numbers of neonates undergoing a specific surgical procedure are often too small to support statistically valid conclusions.
Nevertheless, this approach has been used for planning the fentanyl trial (Chapter VII) and, to some extent, for the study of neonates undergoing cardiac surgery (Chapter VIII); and has also been used widely in studies of adult patients undergoing surgery (eg, Bormann et al, 1983).

The second approach may be to compare those neonates who are subjected to surgical trauma of comparable severity, though not necessarily the same operation for the same clinical condition. This approach, which has been used in the preliminary study (Chapter IV) and the halothane trial (Chapter VI), involved the use of a system by which the surgical trauma could be graded objectively to determine the degree of its 'stressfulness'. In contrast to the clinical situation of adult patients undergoing surgery, it was also necessary to evaluate and account for the effects of non-surgical stress factors, such as prematurity, hypothermia or infection, which could be associated with the operative procedure in a neonate and could possibly influence the response to surgical trauma.

Thus, the surgical stress score was based on 5 factors which contribute to the stress of surgical trauma directly and associated stress factors, which were specific for the neonatal age group, were added to it. The value judgements required for assigning the appropriate scores to each variable were entirely conjectural, despite their basis on the stress response of adult patients undergoing surgery as well as the advice of experienced paediatric and surgical colleagues (see section 4.2.2, Chapter IV).
## SURGICAL STRESS SCORE

1. **AMOUNT OF BLOOD LOSS:**
   - < 5% Blood Volume: 0
   - 5-10% Blood Volume: 1
   - 10-15% Blood Volume: 2
   - > 15% Blood Volume: 3

2. **SITE OF SURGERY:**
   - Superficial: 0
   - Intra-abdominal/Intra-cranial: 1
   - Intra-thoracic: 2

3. **AMOUNT OF SUPERFICIAL TRAUMA:** (skin, muscle, etc.)
   - Minimal: 1
   - Moderate: 2
   - Maximal: 3

4. **EXTENT OF VISCERAL TRAUMA:**
   - Brief visceral handling: 1
   - Prolonged visceral handling: 2
   - Minor resection: 3
   - Major resection: 4

5. **DURATION OF SURGERY:**
   - 0-30 minutes: 1
   - 30-90 minutes: 2
   - 90-180 minutes: 3
   - 180-300 minutes: 4
   - > 300 minutes: 5

6. **ASSOCIATED STRESS FACTORS:**
   - (a) Hypothermia 1.5 - 3°C: 1
     - Hypothermia > 3°C: 2
     - Deep hypothermia: 3
   - (b) Localised infection: 1
     - Generalised infection (NEC, septicemia, etc): 3
   - (c) Prematurity 30 - 34 weeks: 1
     - < 30 weeks: 2

7. **CARDIAC SURGERY:**
   - Cardiopulmonary bypass: 4
   - Circulatory arrest < 40 minutes: 2
     - > 40 minutes: 4

(NB: For patients undergoing cardiopulmonary bypass, scores for 'Amount of blood loss' are applied to the blood loss before the start of bypass.)

### SCORING PROCEDURE:
- Score 01-05: Grade I surgical stress
- Score 06-10: Grade II surgical stress
- Score 11-20: Grade III surgical stress
- Score 21-30: Grade IV surgical stress
9.1.1 Construction of the Surgical Stress Score:

The basis for including each of the variables assessed in the Surgical Stress Score and for allotting scores to these observations is discussed below.

The amount of blood loss during a surgical procedure would obviously reflect its severity and have an important influence on the degree of stress imposed by the operation. The values for the degree of blood loss were based on a study of 1787 paediatric patients (from premature neonates up to 16 years of age) undergoing surgery by Davenport and Barr (1963). This study showed that minor operations were usually associated with the loss of less than 10% blood volume, moderate operations with a loss of 10-15% blood volume and more than 15% of the blood volume could be lost during severe surgical operations. Based on these data, Davenport and Barr (1963) formulated the criteria used for replacement therapy in paediatric patients undergoing surgery.

The site of surgery was first shown to be a determinant of the stress response by Weddell and Gale in 1934, who found in adult patients that hyperglycaemia following intra-peritoneal operations was greater than after extra-peritoneal operations. As detailed in Chapter IV (section 4.2.1), several studies on adult patients (Clarke et al, 1970; Clarke, 1970; Wright and Johnston, 1975; Butler et al, 1977; Aarimaa et al, 1978; Bormann et al, 1983) have found similar differences, not only in blood glucose changes, but also with regard to changes in plasma concentrations of cortisol, growth hormone, insulin, catecholamines, vasopressin, glucagon, non-esterified fatty acids, and other measures of the stress response. Thus, the values given to the different anatomical sites of surgery were
obtained from these studies on the adult stress response.

The amount of superficial trauma was given scores on the basis of an empirical judgement of the surgeon rather than any objective measurement. Although a system based on the length of surgical incision could be devised for quantification of the degree of superficial trauma, it would be a source of error in operations where a large skin incision is required for operations with relatively minor trauma (eg, repair of meningocoele, repair of exomphalos major, etc.). However, immediately after an operation most experienced surgeons could make an accurate assessment of the amount of tissue dissection that was required and it is expected that there would be only slight discrepancies between the judgement of different surgeons. Nevertheless, it is acknowledged that this would be a subjective judgement in each case and may be a source of error in calculation of the score.

The extent of visceral trauma was included as a separate variable in the scoring method since it is known to provide a strong stimulus to the stress response, mainly through afferent vagal fibres which supply the upper abdominal and thoracic viscera (Kehlet et al, 1980). The criteria used for scoring the degree of visceral trauma were more objective and may be typified by common operations, eg, for pyloric stenosis (brief handling), intussusception, exomphalus (prolonged handling), Meckel's diverticulum (minor resection) and meconium ileus, volvulus (major resection).

The duration of surgery would be an obvious determinant of the severity of surgical stress and has been used to classify adult patients undergoing minor and major surgery (Wright and Johnston, 1975). This factor may be of additional importance in newborn infants, since it is recommended that neonates should be given little or no anaesthesia during the surgical
The selection of associated stress factors in neonates undergoing surgery was considered to be necessary in order to account for differences in the stress response of special groups of neonates, particularly those exposed to hypothermia during surgery, those who have localised or generalised infections in the preoperative period and the premature neonates, who are exposed to a number of stressful stimuli associated with the problems of prematurity. The value judgements for these factors were based on studies of the metabolic changes following hypothermia (Hey, 1972; Adamsons et al, 1965), infection (Seashore et al, 1980; Schultz et al, 1980), and the advice of experienced paediatric colleagues. Other factors pertaining to the clinical condition of neonates were not included in order to keep this method as simple as possible and since it was considered that other factors may not have a marked influence on the neonatal stress response to surgery.

In order to include the assessment of neonates undergoing cardiac surgery factors such as cardiopulmonary bypass and the duration of circulatory arrest were added to the scoring method. The scores given for these factors are in keeping with the findings from adult patients, which have shown that the procedures of cardiopulmonary bypass, deep hypothermia and circulatory arrest are the most potent stimuli known for triggering the stress response and are not inhibited by anaesthetic procedures which abolish the stress response to non-cardiac surgery (Butler et al, 1977; Stanley et al, 1980). In recent experimental studies, the duration of circulatory arrest during open-heart surgery has been identified as an important determinant of the outcome and the metabolic response to surgery (Treasure et al, 1983).

On an empirical basis, the total scores obtained were used to classify the
degree of surgical stress as grade I (score 0-5), grade II (score 6-11), grade III (11-20) and grade IV (21-30).

9.1.2 Methods for statistical analysis :-

The purpose of this analysis was, in the first instance, to identify those hormonal and metabolic parameters which were sensitive to differences in the degree of surgical stress, as quantified by the above scoring method. This objective was met by correlating the scores obtained with delta changes in the various hormonal and metabolic parameters measured at the end of surgery. In order to avoid the assumption of continuity in the stress scores obtained, or the assumption of linearity in the relationship between stress score and response, it was decided to use the Spearman rank correlation coefficient for this analysis (Seigel, 1956).

The analysis of these data was used subsequently to identify differences in the magnitude of the hormonal and metabolic changes between neonates in the different stress groups and the duration for which these differences persisted after surgery. This was obtained by the Kruskal-Wallis analysis of variance between the responses of neonates in the four stress groups (Seigel, 1956).

9.2 RESULTS :

9.2.1 Correlation with hormonal and metabolic changes:-

The results from rank correlation of the stress score obtained by each neonate with the delta changes in plasma hormone concentration at the end of surgery and postoperatively are presented in Table 9.1.

The stress scores were strongly correlated with changes in plasma
adrenaline concentration at the end of surgery (p<0.0001) and at 6 hours (p<0.0001), 12 hours (p<0.001) and 24 hours (p<0.05) postoperatively. A similar correlation was obtained with the plasma noradrenaline responses at the end of surgery (p<0.0001), but weaker correlations were observed at 6 hours (p<0.05) and 12 hours (p<0.025) after surgery.

The stress scores were also correlated with changes in plasma insulin concentrations at the end of surgery (p<0.001) and with changes in plasma glucagon concentration at 6 hours (p<0.005) and 12 hours (p<0.005) after surgery. The correlation of stress scores with plasma cortisol responses was found to be significant only at 6 hours (p<0.05) and 24 hours (p<0.02) postoperatively.

The correlations observed between the stress score and changes in blood metabolite concentration at the end of surgery and postoperatively are presented in Table 9.2.

The stress score was strongly correlated with the degree of hyperglycaemia at the end of surgery (p<0.0001), but this correlation was not maintained at 6, 12 and 24 hours after surgery. On the other hand, strong correlations were obtained between the stress scores and changes in blood lactate at the end of surgery (p<0.0001), at 6 hours (p<0.0001) and 12 hours (p<0.01) postoperatively. The stress scores were also correlated with changes in blood pyruvate concentration at the end of surgery (p<0.0001), 6 hours (p<0.0001), 12 hours (p<0.0001) and 24 hours (p<0.005) postoperatively; and with changes in blood alanine at the end of surgery (p<0.005) and 6 hours (p<0.005) after surgery. The stress scores obtained were negatively correlated with the changes in blood alanine concentration at 24 hours postoperatively (p<0.02) and with the changes in total ketone bodies at the
end of surgery (p<0.02). As expected from the correlations with blood lactate, pyruvate and alanine concentrations, the stress scores were strongly correlated with changes in total gluconeogenic substrates at the end of surgery (p<0.0001), 6 hours (p<0.0001) and 12 hours (p<0.01) postoperatively.

Thus, the hormonal and metabolic responses of neonates at the end of surgery and in the postoperative period were found to be strongly correlated with the stress scores obtained from an objective assessment of the severity of surgical stress that they had undergone. The strongest correlations were obtained with those hormones and metabolites which are well-known as indicators of stress, eg, adrenaline, noradrenaline, glucose and the gluconeogenic substrates.

9.2.2 Differences in hormonal-metabolic responses of the stress groups :-

According to the scores obtained, the neonates were classified into groups which had undergone the following degrees of surgical stress; the hormonal and metabolic changes in these groups were analysed in order to define overall differences in their response.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Score</th>
<th>Neonates</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0-5</td>
<td>22</td>
</tr>
<tr>
<td>II</td>
<td>6-10</td>
<td>49</td>
</tr>
<tr>
<td>III</td>
<td>11-20</td>
<td>12</td>
</tr>
<tr>
<td>IV</td>
<td>21-30</td>
<td>11</td>
</tr>
</tbody>
</table>

The hormonal and metabolic data from neonates undergoing different grades of surgical stress are presented in Figures 9.1, 9.2 and 9.3.

Significant differences were found between the hormonal responses of neonates in the four stress groups with respect to changes in plasma adrenaline concentrations at the end of surgery (p<0.0001), at 6 hours
(p<0.0001) and 12 hours (p<0.001) after surgery. The hormonal responses of neonates in the stress groups were also significantly different with respect to changes in plasma insulin concentrations at the end of surgery (p<0.001). There were no significant differences between the responses of neonates in these stress groups with regard to changes in the plasma concentration of glucagon or the steroid hormones during and after surgery.

The metabolic response of neonates undergoing the four grades of surgical stress was significantly different with respect to changes in blood glucose concentration at the end of surgery (p<0.001). The changes in blood lactate concentrations at the end of surgery (p<0.0001), at 6 hours (p<0.0002) and 12 hours (p<0.0005) postoperatively; the changes in blood pyruvate concentrations at the end of surgery (p<0.0001), at 6 hours (p<0.0001) and 12 hours (p<0.001) after surgery and the changes in blood alanine values at the end of surgery (p<0.001) were found to be significantly different between neonates in the two groups. Due to these differences, changes in the blood concentration of total gluconeogenic substrates was significantly different between the stress groups at the end of surgery (p<0.0001), 6 hours (p<0.0002) and 12 hours (p<0.001) postoperatively.

Thus, the responses of neonates in the four stress groups were substantially different with respect to changes in plasma adrenaline concentrations as well as the degree of hyperglycaemia and increase in the blood concentrations of the gluconeogenic substrates following surgery.

9.2.3 Preliminary observations on postoperative outcome :

As expected, neonates undergoing Grade III and Grade IV surgical stress were found to have a more unstable clinical condition during the 24 hours following surgery as compared to neonates undergoing less severe grades of
surgical stress. Following Grades I or II surgical stress, the majority of patients had an uneventful postoperative course and a remarkably rapid postoperative recovery.

There were no postoperative deaths in the groups of neonates undergoing Grade I or Grade II surgical stress. During the 48 hours after surgery two deaths were recorded from neonates undergoing Grade III surgical stress, whereas four deaths were recorded from the neonates subjected to Grade IV surgical stress. Thus, the postoperative mortality rate in neonates undergoing Grades III and IV surgical stress was 17% and 36% respectively.

9.3 DISCUSSION:

Apart from the effects of anaesthesia, prematurity and other factors which have been shown to influence the neonatal stress response in previous chapters, it is reasonable to expect that the degree of surgical stress would be the most important determinant of the hormonal and metabolic changes following surgery. Also, it would be expected that the variables which show the greatest degrees of change during surgery would be the ones most likely to differentiate between groups of neonates undergoing the different grades of surgical stress.

As detailed in Chapter IV, several studies have examined the hormonal and metabolic responses of adult patients undergoing anatomically different surgical operations. However, there are no published data on objective methods for grading the severity of surgical stress in either adult patients or paediatric patients undergoing surgery. Moore and Ball (1952) had proposed a 'quasi-quantitative scale of ten' for grading the degree of surgical or traumatic stress which, in their own words, involved a
'surgical guess' rather than any objective evaluation. This method, which is described in Table 9.3, has been criticised for being heavily dependent upon individual interpretation (Baue, 1974).

On the other hand, the Abbreviated Injury Scale and the Comprehensive Research Injury Scale were described by the Committee on Medical Aspects of Automotive Safety in 1971 and 1972 respectively, in order to describe the individual injuries incurred in road traffic accidents. Since these scoring methods were seldom applicable to the condition of patients with multiple injuries and had a poor relation to the subsequent morbidity and mortality in these patients, Baker et al (1974) developed the 'Injury Severity Score' based on the mortality data from 2128 patients with multiple injuries. This score was further validated by Bull (1975), who, using probit analysis, also suggested a correction for different the age groups evaluated by this scoring method. Thus, the Injury Severity Score was not only related to the morbidity and mortality following accidental trauma (Bull, 1975), but was also found to be related to the hormonal (Stoner et al, 1977) and metabolic (Oppenheim et al, 1980; Stoner et al, 1979) changes stimulated by accidental trauma.

A prognostic index for evaluating the clinical condition and predicting the survival of patients with accidental trauma was developed by Cowley et al (1974). Using Euclidean distance analysis on data from 350 patients, they found that the changes in serum creatinine, osmolality, blood pressure and haematocrit could be used to predict the survival of injured patients. The predictions made by this index were tested in a subsequent study of 688 patients and were found to be correct in 91% cases (Cowley et al, 1974).

Similar indices to predict the probability of survival in patients with
burns were proposed by McCoy et al (1968) and Moores et al (1975). McCoy et al (1968) used discriminant function analysis based on the age, sex and extent of burn to calculate the discriminant index for each patient which was then related to the probability of survival from a predicted probability curve based on the data of all patients. A similar method of analysis was used by Moores et al (1975), although calculations of the discriminant index were based not only on the age, sex or extent of burn of each patient, but also on whether the patient was likely to be infected or not. Batstone et al (1976) used the latter index to classify patients with burns into minor, moderate and severe groups, and found that the hormonal and metabolic changes in the three groups were significantly different.

A scoring method for grading the severity of sepsis has been proposed recently by Elebute and Stoner (1983), which is based on arbitrary scores allotted to the local effects of sepsis, the pyrexic manifestations, the secondary effects of sepsis and the laboratory data of septic patients. Although this method has not been validated, preliminary findings from 18 patients have shown that the sepsis score is related to randomly measured plasma cortisol concentrations and to the rate of fat oxidation in septic patients (Stoner et al, 1983).

Thus, several methods are presently available for objective measurement of the severity of accidental trauma, burn injury or sepsis. Of these methods, the Injury Severity Score is particularly useful, since it has been related to the responses and the outcome of patients with accidental trauma, and has therefore, provided a stimulus to detailed studies on several aspects of accidental trauma (Elebute and Stoner, 1983). It is proposed that the present paucity of studies on neonatal patients undergoing surgery may be overcome, to some extent, by the availability of an objective method for
measuring surgical stress in these patients.

9.3.1 **Hormonal changes** :-

The differences between the stress groups in plasma adrenaline responses at the end of surgery and postoperatively may be due to a greater degree of sympatho-adrenal activation by operations of greater severity. Similar differences between adult patients undergoing ophthalamic and abdominal surgery have been reported in the postoperative period by Nikki et al (1972); whereas Butler et al (1977) found that the stress of cardiac surgery caused a greater catecholamine response than non-cardiac surgery. The adrenaline responses of neonates undergoing Grades I and II surgical stress were similar, and it is likely that the significance of differences between neonates in the four stress groups were due to the marked responses of neonates undergoing Grades III and IV surgical stress.

Similar differences in response were obtained between the four grades of surgical stress with regard to changes in plasma insulin concentrations at the end of surgery. It is possible that differences in the insulin response resulted from the markedly greater degrees of hyperglycaemia observed in neonates undergoing Grades III and IV of surgical stress. Aarimaa et al (1978) also found that the adult patients subjected to major surgical trauma had greater increases in plasma insulin concentrations after surgery as compared to patients undergoing a moderate degree of surgical trauma.

9.3.2 **Metabolic changes** :-

Despite the different anaesthetic techniques used for inhibiting the metabolic response in different groups of neonates undergoing surgery, the hyperglycaemia stimulated by surgical stress was found to be distinctly different at the end of surgery between neonates in the four stress groups.
However, it was surprising that this difference was not maintained into the postoperative period. Differences in the hyperglycaemic response may be mediated by the adrenaline responses of neonates in the four stress groups, and may be, in turn, responsible for differences of the insulin response at the end of surgery. Several studies have documented similar differences between adult patients subjected to different surgical procedures (Clarke et al, 1970; Wright and Johnston, 1975; Aarimaa et al, 1978).

From the present analysis, it was evident that the magnitude of changes in blood concentrations of the gluconeogenic substrates, particularly lactate, pyruvate and alanine provided the most prominent differences between neonates subjected to the four different grades of surgical stress. It may be proposed that these differences were also due to the marked difference in adrenaline release, although the effects of changes in glucagon and glucocorticoid secretion cannot be excluded.

In the study of neonates undergoing surgery by Pinter (1973), blood lactate concentrations were found to be higher at 12 hours postoperatively in neonates undergoing operations on the alimentary tract as compared to neonates undergoing non-alimentary surgery. Although this difference has not been commented upon (Pinter, 1973), it could be possible that this was due to a greater degree of surgical stress in the former group of neonates.

In summary, therefore, neonates undergoing the four different grades of surgical stress (as quantified by the Surgical Stress Score) were found to have prominent differences in their hormonal and metabolic stress response. The differentiation between neonates undergoing Grades I and II surgical stress was only slight, whereas major differences in the magnitude of hormonal and metabolic changes were observed between Grades II and III, as
well as between Grades III and IV.

Furthermore, the postoperative recovery of neonates undergoing Grade I and Grade II degrees of surgical stress was found to be uneventful, whereas a variety of complications were documented after Grade III and Grade IV surgical stress. In addition, there were no postoperative deaths in neonates undergoing grades I and II of surgical stress whereas a mortality of 17% and 36% was documented after Grades III and IV of surgical stress respectively. However, this mortality may also be related to those clinical conditions for which severe grades of surgery were required, rather than to the effects of surgical stress per se.

The initial classification of the grades of surgical stress had been made entirely on an empirical basis. Thus, on the basis of these differences in the hormonal and metabolic response as well as the postoperative outcome, it is suggested that neonates undergoing Grades I and II of surgical stress could be classified into a single group:

Score 01-10 Minor stress
Score 11-20 Moderate stress
Score 21-30 Severe stress

9.3.3 Use of the Surgical Stress Score :-

It must be emphasized that the Surgical Stress Score is intended primarily as a research tool; it has not been designed as a predictive or prognostic index and should not be used as such. The main application of this scoring method may be to illustrate the homogeneity of patient material in studies of neonates undergoing surgery. This may be necessary for comparing different groups of neonates in the same centre or in different centres, particularly when the effects of changes in perioperative management or
surgical approach are under investigation. Since the postoperative morbidity and mortality seem to be different for neonates in the above three stress groups, comparisons of the postoperative outcome of neonates undergoing surgery should be made only between neonates belonging to the same stress group. Thus, the scoring method may prove to be useful for the epidemiological surveys of morbidity and mortality in neonates undergoing various surgical procedures (Kiely, 1984) or for evaluating the performance of different paediatric surgical centres with regard to the postoperative outcome of neonates undergoing surgery.

Furthermore, based on the findings from this study and after further validation (by correlation to physiological measurements and morbidity and mortality following surgery in newborn infants) it could be used also for evaluating the requirements for intensive care and therapy in the postoperative period. Thus, neonates in the minor stress group would require routine postoperative care, those in the moderate stress group would require intensive care for a short period after surgery, whereas neonates in the severe stress group would require life-supportive intensive care for a longer period after surgery. In addition, if it can be shown that therapeutic measures to decrease or abolish the stress response are beneficial for the outcome of neonates undergoing major surgery, then this scoring method may help to decide the degree and duration of such therapy.

9.5 CONCLUSION:

The Surgical Stress Score constructed at the start of this project was found to be an efficient research tool for measuring the severity of surgical stress in neonates undergoing surgery.
Table 9.1 STRESS SCORE: - Correlation between scores obtained and hormonal changes.

<table>
<thead>
<tr>
<th></th>
<th>End-operative</th>
<th>6hr post-operative</th>
<th>12hr post-operative</th>
<th>24hr post-operative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Adrenaline nmol/L</td>
<td>r_s</td>
<td>N</td>
<td>p&lt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>62</td>
<td>0.0001</td>
<td>0.55</td>
</tr>
<tr>
<td>Δ Noradrenaline nmol/L</td>
<td>r_s</td>
<td>N</td>
<td>p&lt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.43</td>
<td>66</td>
<td>0.0001</td>
<td>0.25</td>
</tr>
<tr>
<td>Δ Insulin pmol/L</td>
<td>r_s</td>
<td>N</td>
<td>p&lt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>83</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Δ Glucagon pmol/L</td>
<td>r_s</td>
<td>N</td>
<td>p&lt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.43</td>
<td>41</td>
<td>0.005</td>
<td>0.49</td>
</tr>
<tr>
<td>Δ Cortisol nmol/L</td>
<td>r_s</td>
<td>N</td>
<td>p&lt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.28</td>
<td>42</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

Spearman rank correlation coefficients (r_s) showing relationships between the stress score of each patient and the change in plasma hormone concentrations after surgery. (Only significant correlations shown).
Table 9.2 STRESS SCORE: Correlation between scores obtained and metabolic changes.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>End-operative</th>
<th>6 hr post-operative</th>
<th>12 hr post-operative</th>
<th>24 hr post-operative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Glucose mmol/L</td>
<td>rs</td>
<td>N 91</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Δ Lactate mmol/L</td>
<td>rs</td>
<td>N 91</td>
<td>0.0001</td>
<td>0.28 77</td>
</tr>
<tr>
<td>Δ Pyruvate mmol/L</td>
<td>rs</td>
<td>N 90</td>
<td>0.0001</td>
<td>0.38 76</td>
</tr>
<tr>
<td>Δ Alanine mmol/L</td>
<td>rs</td>
<td>N 91</td>
<td>0.005</td>
<td>-0.23 78</td>
</tr>
<tr>
<td>Δ Total Ketones mmol/L</td>
<td>rs</td>
<td>N 90</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Δ Total Gluconeogenic Substrates mmol/L</td>
<td>rs</td>
<td>N 90</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Spearman rank correlation coefficients (rs) showing relationships between the stress score of each patient and change in blood metabolite concentrations after surgery. (Only significant correlations shown).
Table 9.3: 'Scale of 10' for surgical or other trauma

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Third degree burn of 25% or more body surface area.</td>
</tr>
<tr>
<td>9, 8, or 7</td>
<td>Multiple wounds, including major or compound fractures, penetrating wounds of chest or abdomen, non-penetrating wounds with visceral injury, multivisceral operations for cancer.</td>
</tr>
<tr>
<td>5</td>
<td>Major anastomotic gastro-intestinal surgery, eg, colectomy or sub-total gastrectomy.</td>
</tr>
<tr>
<td>4, 5, or 6</td>
<td>Operations like lobectomy, radical mastectomy, open reduction of femoral shaft, prostatectomy, nephrectomy; depending on circumstances.</td>
</tr>
<tr>
<td>3 or 5</td>
<td>Operations like cholecystectomy, appendectomy, thyroidectomy, hysterectomy; depending on whether complicated or not.</td>
</tr>
<tr>
<td>2</td>
<td>Minor procedures (not illustrated).</td>
</tr>
<tr>
<td>1</td>
<td>Ankle sprain or its operative counterparts.</td>
</tr>
</tbody>
</table>

(Reproduced in tabular form, from Moore and Ball, 'The Metabolic Response to Surgery', pp. 10-12, Charles C Thomas, 1952, Springfield.)
Figure 9.1: - Comparison of changes in plasma adrenaline and insulin concentrations between neonates undergoing different grades of surgical stress. Differences between groups were analysed by the Kruskal-Wallis one-way analysis of variance, * p<0.001, ** p<0.0001.

{NB: - Changes in the mean values derived from neonates in the four surgical stress groups are presented.

Grade I stress  N = 22 neonates
Grade II stress N = 49 neonates
Grade III stress N = 12 neonates
Grade IV stress N = 11 neonates.}
SURGICAL STRESS SCORE: Hormonal changes.

**ADRENALINE**

△Adrenaline nmol/l

- p < 0.001 K-W
- **p < 0.0001** ANOVA

**INSULIN**

△Insulin pmol/l

- *p < 0.001* K-W ANOVA

Graphs showing changes in adrenaline and insulin levels over time post-surgery.
Figure 9.2: Comparison of changes in blood glucose and lactate concentrations between neonates undergoing different grades of surgical stress. Differences between groups were analysed by the Kruskal-Wallis analysis of variance, * $p<0.001$, ** $p<0.0002$, *** $p<0.0001$.

{NB: - Changes in the mean values derived from neonates in the four surgical stress groups are presented.

Grade I stress N = 22 neonates
Grade II stress N = 49 neonates
Grade III stress N = 12 neonates
Grade IV stress N = 11 neonates.}
SURGICAL STRESS SCORE: - Metabolic changes.

**Δ GLUCOSE**

* p < 0.001
K-W ANOVA

**Δ LACTATE**

* p < 0.0005
** p < 0.002
*** p < 0.0001

Pre-op  End-op  6 hrs  12 hrs  24 hrs
Figure 9.3: - Comparison of changes in blood concentrations of pyruvate, alanine and total gluconeogenic substrates between neonates undergoing different grades of surgical stress. Differences between groups were analysed by the Kruskal-Wallis analysis of variance, * p<0.001, ** p<0.0002, *** p<0.0001.

{NB: - Changes in the mean values derived from neonates in the four surgical stress groups are presented.}

Grade I stress N = 22 neonates
Grade II stress N = 49 neonates
Grade III stress N = 12 neonates
Grade IV stress N = 11 neonates.
SURGICAL STRESS SCORE: Metabolic changes.

**Pyruvate**
- **△Pyruvate mmol/l**
- 
  - **p < 0.001** K-W
  - **p < 0.0001** ANOVA

**Alanine**
- **△Alanine mmol/l**
- 
  - **p < 0.0005** K-W ANOVA

**Total Gluconeogenic Substrates**
- **△Total gluconeogenic substrates mmol/l**
- 
  - **p < 0.001** K-W
  - **p < 0.0002** K-W
  - **p < 0.0001** ANOVA

Graphs showing changes in metabolites over time: Pre-op, End-op, 6 hrs, 12 hrs, 24 hrs.
CHAPTER X : GENERAL DISCUSSION
CONTENTS

10.1 SPECIFIC FEATURES OF THE NEONATAL STRESS RESPONSE
10.2 EFFECTS OF PREMATURITY
10.3 OVERALL CONCLUSIONS
10.4 RECOMMENDATIONS FOR CLINICAL PRACTICE
10.1 SPECIFIC FEATURES OF THE NEONATAL STRESS RESPONSE:

The characteristic feature of the neonatal stress response was the remarkably short duration of the hormonal and metabolic changes in the postoperative period; although associated with a relatively increased magnitude of the changes documented, as compared to the response of adult patients undergoing similar degrees of surgical trauma. Even in neonates subjected to relatively severe trauma, it was found that most hormonal and metabolic alterations were returning towards preoperative values within 24 hours after surgery. These hormonal and metabolic trends were also associated with a characteristic improvement in the clinical condition of neonates by the end of the study period. The neonates who did not show this rapid rate of recovery in the postoperative period generally belonged to two categories: (a) neonates who had on-going clinical problems associated with either prematurity or the condition for which surgery was required, and (b) neonates who died within the 48-72 hours following surgery.

These patterns of the postoperative outcome would appear to reinforce the impressions which were voiced in the Lancet of 12 March, 1960:

The Bible, mythology, history, and even modern fiction abound with strange tales of infants abandoned on rocks, in the desert, at sea, and in forests, to be nursed into childhood by wolves, birds, apes, and giants. Not all of them become founders of city-empires or kings of the jungle, but even sceptics must concede that that newborn babies are singularly well fitted to survive such rigours. Their survival, as indeed our own, depends on a series of physical and chemical "steady states"; and the self-regulating systems which maintain them are already in a high state of efficiency at birth.

.....but we are becoming increasingly aware that even minor setbacks may have disastrous consequences. Physical trauma has long been recognised as a cause of permanent defects and deformities. Biochemical trauma can be no less catastrophic - though most of its after-effects, especially damage to the central nervous system, we are only now beginning to discern.

The substantial hormonal and metabolic responses of neonates who were subjected to minor or moderate grades of surgical stress were limited to a few hours postoperatively. Even in neonates subjected to severe surgical stress, most of the hormonal and metabolic changes documented were relatively short-lived. On the other hand, neonates who developed severe and life-threatening complications in the postoperative period, had a sustained increase in the concentrations of hormonal and metabolic parameters even after the end of surgery. The postoperative stress response of such neonates was characterised by a progressively increasing metabolic acidosis and severe hyperglycaemia, associated with a continued increase in the concentrations of catecholamines.

The clinical condition these patients was characterised primarily by a peripheral circulatory collapse, anuria and severe hypoxaemia secondary to pulmonary vasoconstriction. The poor postoperative outcome of such patients was responsible for the 17% mortality observed in neonates subjected to Grade III stress and 36% mortality in neonates subjected to Grade IV surgical stress. From these neonates, three blood samples were obtained at or near the terminal state and were found to contain extremely high concentrations of lactate, glucose and the catecholamines, whereas plasma cortisol concentrations were found to be decreased. Similar hormonal and metabolic derangements have been found in adult patients with circulatory shock due to trauma, haemorrhage or sepsis (Nishijima et al, 1973; Benedict and Grahame-Smith, 1978).

The two characteristic features of the neonatal stress response in comparison to that of the adult patient, an increased magnitude but shorter duration may be explained by various hypotheses. From the results obtained in the randomised anaesthetic trials, it is tempting to suggest that the
increased magnitude of the neonatal response was probably a feature of the inadequate anaesthesia generally given to the neonates undergoing surgery. On the other hand, the remarkably short duration of the stress response may be a more characteristic feature of the neonatal age group and several hypotheses can be advanced in order to explain this feature of the stress response in newborn infants.

First, it is likely that the psychological stress factors before and after surgery would be absent in the neonatal age group. Thus, the neonate would be blissfully unaware of the impending surgical experience, whereas in adult patients, the fear or apprehension before surgery may itself amplify the stress response. Even after surgery, the interpretation of the surgical experience by adult patients, postoperative pain and discomfort, or the anxiety caused by postoperative procedures, may partly explain the prolonged duration of the adult stress response.

Several studies in adult patients have documented raised plasma values of corticosteroids before surgery, which were found to decrease slightly after the induction of anaesthesia (Brunt and Ganong, 1963; Lush et al, 1972; Gordon et al, 1973; Cooper et al, 1979). An increase in plasma adrenaline concentrations before surgery has been documented by Derbyshire and Smith (1984), although these values were not related to linear analogue scores for anxiety. Plasma concentrations of the non-esterified fatty acids and glycerol were found to be elevated before surgery in adult patients (Hall et al, 1978; Kehlet et al, 1979) and it was proposed that these may be due to sympathetic activation caused by anxiety. In children undergoing adenoidectomy, Sigurdsson et al (1983) found that heavily sedated patients had a decreased sympathoadrenal response to surgery, as measured by plasma catecholamine concentrations and the incidence of ventricular arrhythmias,
than a control group of lightly sedated patients. This finding indicates that the level of sympathetic arousal before surgery may have a significant influence on the hormonal responses to surgical stress.

Similar conclusions have been suggested by Pickar et al (1983) from the measurement of plasma cortisol and beta-endorphin immunoreactivity in adult patients undergoing surgery. They found that the mean values of cortisol and beta-endorphin during surgery and in the postoperative period were both predicted by the preoperative plasma concentrations of the respective hormones; further supporting the hypothesis that the degree of arousal or 'biologic tone' prior to surgical stress may be an important factor in predicting the magnitude of the stress response. Thus, it is likely that the stress response of newborn infants is not influenced by psychological stress factors before and after surgery. The survival of 5 newborn infants following the recent earthquake in Mexico may be a case in point.

However, an alternative hypothesis for the short duration of the neonatal stress response could be due to an absence of the memory of pain, which may be found to develop during the period of early infancy. This hypothesis has been suggested particularly since the changes in plasma cortisol concentrations in neonates undergoing surgery were limited to the 12 or 24 hours after surgery, whereas infants subjected to surgery between 4 and 11 months of age were found to have elevated plasma cortisol concentrations for up to 72 hours following surgery (Golder, 1982). Similarly, it has been suggested that (Dargassies, 1977; McGraw, 1963) that both, the sensitivity to painful stimuli and the behavioural response to pain (localised withdrawal) develop during the first few months after birth. Thus, it is possible that the perception and interpretation of pain could be related to the previous experiences of pain and their memory, which may be absent in
some of the neonates subjected to surgery.

Third, differences between the neonatal and adult stress responses may be related to the relatively immature endocrine and metabolic regulation in newborn infants.

(1) It has been proposed that the secretion of insulin and glucagon in the fetal or neonatal pancreas is sluggishly responsive to the classical physiological stimuli due to an immaturity of the cAMP-generating system (Sperling, 1982). There is a predominantly anabolic drive during fetal life associated with an increased insulin/glucagon ratio and an increase in the number and affinity of the insulin receptors. In addition, a decreased number of glucagon receptors are present in fetal hepatic tissue, the majority of which are not functionally mature due to a lack of coupling to the adenylate cyclase system (Ganguli et al, 1984). Thus, the short-lived catabolic drive could be due to a predominance of insulin receptors, which has been found in several tissues at birth (Sperling, 1982).

(2) The catecholamine responses to hypoxia and hypoglycaemia have been documented even during fetal life (Phillippe, 1983). In newborn infants, the response to hypoxia is characterised by the secretion of noradrenaline from extra-medullary chromaffin tissue (Lagercrantz and Bisoletti, 1977; Hervonen and Korkala, 1972; Phillippe, 1983) whereas the response to hypoglycaemia is characterised mainly by the secretion of adrenaline from the adrenal medulla (Greenberg et al, 1960). It has been proposed that the adrenal medulla undergoes maturation in human infants for upto three years after birth (Sperling et al, 1984), although there is little evidence to substantiate this hypothesis. However, particularly in preterm neonates, it is likely that the sympathetic tract in the spinal cord, which innervates
adrenal medullary cells via the splanchnic nerves may not be completely
developed at birth (Dobbing, 1981).

Therefore, it is suggested that the intra-operative adrenaline response
could be related to a direct stimulation of the adrenal medulla via the
splanchnic nerves, which could be possible due to the relative lack of
inhibitory pathways through the sympathetic tract. It is reasonable to
expect that such stimulation would be limited to the duration of the
stressful stimulus. On the other hand, excessive release of endogenous
opioids into the peripheral circulation stimulated by surgical stress, may
inhibit the postoperative release of catecholamines from the adrenal
medulla (Costa et al, 1980).

From the changes documented it can be suggested that in newborn infants
undergoing surgery, the secretion of adrenaline is a primary feature of the
stress response, whereas the adult response is primarily characterised by
the changes in plasma cortisol concentrations (Alberti et al, 1980).

In addition, maturational differences in the metabolic regulation mediated
by catecholamines have been suggested by recent studies on neonatal and
adult rat liver cell membranes (Bendeck and Noguchi, 1985). The density of
β-adrenergic receptors decreases whereas that of α-adrenergic receptors
increases substantially during the neonatal period; this change in receptor
density may be responsible for changes in the control of glycogenolysis
from β- to predominantly α-adrenergic mechanisms during maturation
(Bendeck and Noguchi, 1985). Thus, the secretion of adrenaline during surgery
would be physiologically more appropriate since the liver cell membranes at
birth would be responsive to it.
The adrenal cortex undergoes well-documented maturational changes in the transition from fetal to adult life (Winter, 1982). However, apart from the maturation of steroid hydroxylases, functional differences have not been identified between isolated fetal and adult adrenal cortex cells in vitro (Fujieda et al., 1982). It is currently believed that in the term fetus, plasma cortisol concentrations are low due to the inhibition of 3β-hydroxysteroid dehydrogenase activity by placental oestrogens (Winter, 1982), adrenal hyperplasia is mainly due to increased plasma concentrations of ACTH in response to the low cortisol concentrations.

Thus, the cortisol response to exogenous ACTH is relatively decreased; but by 3 or 4 weeks of age, despite a marked involution of the adrenal cortex, the neonatal capacity to produce cortisol in response to ACTH is enhanced (Forest, 1978). On the other hand, the preterm neonate is likely to produce larger amounts of precursor hormones, since the steroid hydroxylase enzymes mature from the proximal to distal end of the steroid biosynthetic pathway (Solomon et al., 1967). Due to these factors, the secretion of cortisol in the neonate undergoing surgery soon after birth, particularly in preterm neonates, may be lower as compared to the adult cortisol responses.

The role of endogenous opioids at birth and their responses to birth asphyxia have been investigated recently. It has been documented that β-endorphin like immunoreactivity (PBE.ir) in the cord plasma of normal term neonates was much higher than corresponding values in resting adults (Wardlaw et al., 1979; Facchinetti et al., 1982, Puolakka et al., 1982; Panerai et al., 1983); the PBE.ir decreased during the 24 hours after birth to reach adult levels (Facchinetti et al., 1982), whereas in another study, plasma β-endorphin, β-lipotropin and met-enkephalin values remained elevated for up to 5 days after birth (Panerai et al., 1983). Marked
increases in plasma concentrations of β-endorphin and β-lipotropin have been observed in response to birth asphyxia (Wardlaw et al, 1979) or complicated delivery (Puolakka et al, 1982).

β-endorphin concentrations in plasma (Hindmarsh et al, 1984) and cerebrospinal fluid (Burnard et al, 1982) were found to be markedly raised in neonates with acute clinical illness; in addition, a 1000-fold increase was documented in infants of drug-addicted mothers (Panerai et al, 1983). These changes may be important since endogenous opioids are known to modulate the secretion of several hormones involved in the stress response, e.g., pancreatic hormones (Giugliano, 1984), catecholamines (Costa et al, 1980) and pituitary hormones (Foley et al, 1979). Thus, it could be hypothesized that the marked release of endorphins in the peripheral circulation caused by surgical stress may inhibit the postoperative release of catecholamines from the adrenal medulla (Costa et al, 1980).

Finally, developmental changes in metabolism after birth are likely to be responsible for some of the differences in the stress responses of neonates and adults. These changes would also be influenced by the rapid rate of growth in the neonatal age group and the limited reserves of fat, protein and carbohydrate. It is proposed that differences in ketone body metabolism and protein metabolism are the most prominent between neonates and adult patients.

The pathways for partial oxidation of non-esterified fatty acids and production of ketone bodies are not fully mature in newborn infants at birth, and may develop by 48 hours after birth (Hahn and Novak, 1985). This delay has been attributed to low carnitine levels at birth, which are probably derived from milk (Warshaw and Curry, 1980; Hahn and Novak, 1985). The
The utilisation of ketone bodies is known to be facilitated in the neonatal brain (Williamson, 1982), since they may be required not only for energy production, but for myelination and brain growth as well (Yeh and Sheehan, 1985; Williamson, 1982).

The neonatal period is characterised by a relative hyperketonaemia in several species (Williamson, 1982). It is possible that high circulating concentrations of ketone bodies may serve as a protective mechanism against excessive protein breakdown. In this context, it has been documented that in adult patients exposed to trauma the development of hyperketonaemia is associated with a decreased nitrogen loss as compared to those patients who remain normoketonaemic (Smith et al, 1975; Williamson et al, 1977).

(b) Although there are several specific differences between the protein metabolism in neonates and adults, the differences in overall protein turnover rates are likely to be of primary relevance for this study. Using stable isotope infusion techniques, it was documented that the rates of protein synthesis and breakdown, or whole body protein turnover are markedly greater in newborn infants as compared to adult values (Pencharz et al, 1977; Nissim et al, 1983; DeBenoist et al, 1984). In addition, it was found that the rate of protein turnover in preterm neonates was higher than that of term neonates (Pencharz et al, 1981; Nissim et al, 1983); there was a greater efficiency of protein synthesis as a function of protein intake in preterm neonates (Nissim et al, 1983); and the rate of skeletal muscle protein breakdown was found to be greater (Tomas et al, 1979; Pencharz et al, 1981; Nissim et al, 1983).

The two underlying factors that influence the regulation of protein metabolism in neonates, may also influence their stress response: (1) the
lower metabolic reserves of the newborn infant, particularly if it is premature, and (2) the rapid rate of growth in this period, which is presumably responsible for the rapid protein turnover of newborn infants and which, in turn, results in an increased protein requirement and a greater caloric demand during the neonatal period.

Thus, it is possible that the short-lived metabolic response is partly due to the low reserves of glycogen (Shelley, 1961), lipid stores (Melichar et al, 1965) and the metabolizable protein (Pencharz et al, 1981) in preterm and term newborn infants. The rapid rate of protein turnover documented above would be of particular concern in a prolonged catabolic reaction to surgical stress since a much greater negative nitrogen balance would result, compared to that of adults, if the rate of protein synthesis is depressed (Rennie and Millward, 1983) or, more likely, if the rate of protein breakdown is increased (Ogata and Holliday, 1976) in the postoperative period. Finally, it is tempting to suggest that the short duration of the neonatal stress response, mediated through the characteristic hormonal changes, is a physiological mechanism to protect protein metabolism and preserve growth in the newborn organism. It may be expected that the effect of these two basic metabolic factors, poor reserves and increased protein turnover, would be of greater significance in the preterm neonate.

10.2 EFFECTS OF PREMATURITY :-

Specific features of the neonatal stress response related to prematurity were identified by the comparison of preterm and term neonates in the preliminary study who had received a similar anaesthetic management, and by a comparison of neonates in the non-halothane and non-fentanyl groups from the two trials. These features are enumerated below:

(1) Suppression of the postoperative insulin response in preterm neonates,
as identified in the preliminary study, was confirmed in the subsequent trials (Tables 6.2 and 7.2).

(2) The intra-operative glucagon response of preterm neonates was greater than that of term neonates (Tables 6.2 and 7.2).

(3) Changes in the corticosteroid hormones in preterm neonates were characterised by greater increases in the plasma concentrations of the steroid precursors, eg, 11-deoxycorticosterone, 11-deoxycortisol and 17-hydroxyprogesterone; whereas the aldosterone, corticosterone and cortisol responses were reduced marginally, as compared to those of term neonates (Tables 6.3 and 7.3).

(4) The hyperglycaemic response of preterm neonates was similar to that of term neonates at the end of surgery, but was prolonged to 6 hours postoperatively in the preterm neonates and not in the term neonates (Figures 10.4 and 10.7).

(5) The gluconeogenic amino acids were found to decrease during and after surgery in the preterm neonates, whereas they were unchanged in the term neonates (Figure 4.1).

Difference in the insulin response postoperatively may also be related to the decreased responsiveness of beta-cells in the premature pancreas (Sperling, 1982) or to handling of the vagus nerve during PDA ligation. The adrenocortical responses of preterm neonates were probably due to the delayed maturation of hydroxylase enzymes involved in the steroid biosynthetic pathway (Solomon, 1967).

The prolonged hyperglycaemic response of preterm neonates was probably mediated by the greater glucagon response and, more important, the complete lack of changes in plasma insulin concentrations in the postoperative period (Sperling et al, 1984). Changes in the gluconeogenic amino acids
during and after surgery in the preterm neonates suggest that the surgical hyperglycaemia in preterm neonates is probably derived from hepatic or renal gluconeogenesis rather than glycogenolysis alone. This mechanism is in agreement with the greater glucagon response in preterm neonates and the presence of lower glycogen stores, as compared to the term neonates. Alternatively, this finding could be related to a decreased release of the gluconeogenic amino acids by extrahepatic tissues during surgery, which may occur in preterm neonates due to the decreased cortisol responses, and not in the term neonates. Further investigations to measure the turnover of gluconeogenic precursors in preterm and term neonates during the postoperative period will be required to clarify the mechanism of these changes.

In summary therefore, the pattern of the neonatal stress response was characterised by massive changes in the circulating concentrations of hormonal and metabolic parameters, which were limited to the immediate peri-operative period. In the majority of cases, postoperative recovery was rapid and there were only minor detrimental effects associated with the marked hormonal and metabolic alterations. However, those cases who did not recover soon after surgery were found to develop major complications in the postoperative period. It is also likely that a severe and prolonged catabolic reaction to surgery may affect the rapid growth occurring in preterm and term neonates.

With this background, the suppression of hormonal and metabolic changes observed in the three randomised controlled trials would appear to be relevant for the clinical management of newborn infants undergoing surgery.

10.3 OVERALL CONCLUSIONS:
1. The human newborn infant, whether born prematurely or at term, mounts a substantial endocrine and metabolic stress response to surgical trauma.

2. The hormonal and metabolic responses of neonates undergoing various types surgical procedures can be modified with the use of appropriate anaesthetic techniques.

3. Neonates subjected to different degrees of surgical stress, as measured by a scoring method, were found to have a correspondingly altered stress response.

10.4 RECOMMENDATIONS FOR CLINICAL PRACTICE:

At the start of this project it was observed that the peri-operative clinical management of preterm and term neonates undergoing surgery was based on empirical principles or personal preference. The greatest variation in clinical practice was obtained in the anaesthetic management during surgery and the postoperative analgesia given to neonates undergoing surgery. Based on the data obtained in this project, it is possible to make positive recommendations on these aspects of the clinical management of neonates undergoing surgery.

The randomised controlled trials on halothane and fentanyl anaesthesia have clearly shown that term and preterm neonates undergoing surgery should be given potent anaesthetic agents during the surgical procedure. For neonates who are likely to be ventilated in the postoperative period, it is recommended that opiate drugs like fentanyl should be given during surgery whereas halothane anaesthesia may be given to neonates who will not require
ventilation after surgery.

It is possible that high-dose fentanyl anaesthesia may be appropriate for neonates undergoing open-heart surgery and cardiopulmonary bypass; however, this should be regarded strictly as a research technique until further evidence is available.

Although a formal investigation of the effects of postoperative analgesia was not carried out as a part of this project, it may be reasonable to extrapolate these findings from the effects of surgery without adequate anaesthesia to the postoperative period, and to propose that effective analgesia should be provided during the postoperative period.

Thus, it is recommended that preterm and term newborn infants undergoing surgery should receive adequate pain relief during and after surgery.
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APPENDIX I : DATA OF HALOTHANE AND FENTANYL TRIALS
Comparison of hormonal changes between neonates in the halothane and non-halothane anaesthesia groups. (Differences between the two groups are analysed by the Mann-Whitney U Test, whereas changes from pre-operative values within each group are analysed by the Wilcoxon test).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Mean ± SEM</th>
<th>N</th>
<th>P &lt; 0.05</th>
<th>P &lt; 0.01</th>
<th>N.S.</th>
<th>P &lt; 0.05</th>
<th>P &lt; 0.01</th>
<th>N.S.</th>
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</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>7.9 ± 1.5</td>
<td>15</td>
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<tr>
<td>Glucagon</td>
<td>9.7 ± 2.1</td>
<td>15</td>
<td></td>
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<tr>
<td>Glucagon/Insulin ratio</td>
<td>1.1 ± 0.5</td>
<td>15</td>
<td></td>
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<tr>
<td>Adrenaline</td>
<td>21.3 ± 10.5</td>
<td>15</td>
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<tr>
<td>Noradrenaline</td>
<td>0.6 ± 0.1</td>
<td>15</td>
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</tbody>
</table>

Number of Patients:
- Halothane Anaesthesia: 17
- Non-halothane Anaesthesia: 10

Legend:
- U.S.: Not available
- N.S.: Not significant

Table 1. Maternal Hormones: Neonatal Changes.
<table>
<thead>
<tr>
<th>Test</th>
<th>HALOTHANE ANAESTHESIA (Mean ± SEM)</th>
<th>NON-HALOTHANE ANAESTHESIA (Mean ± SEM)</th>
<th>Wilcoxon Test</th>
<th>Mann-Whitney U Test</th>
<th>N</th>
<th>Wilcoxon Test</th>
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<tr>
<td>Aldosterone nmol/L</td>
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<tr>
<td>p&lt;0.05</td>
<td>0.84 ± 0.17</td>
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<td>p&lt;0.005</td>
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<tr>
<td>p&lt;0.025</td>
<td>2.04 ± 0.56</td>
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<tr>
<td>n.s.</td>
<td>2.04 ± 0.56</td>
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<tr>
<td>Corticosterone nmol/L</td>
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<tr>
<td>p&lt;0.01</td>
<td>63.5 ± 11.0</td>
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<tr>
<td>p&lt;0.005</td>
<td>35.9 ± 10.1</td>
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<tr>
<td>n.s.</td>
<td>14.3 ± 8.9</td>
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<tr>
<td>n.s.</td>
<td>9.5 ± 5.0</td>
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<td>17-hydroxyprogestosterone nmol/L</td>
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<tr>
<td>p&lt;0.05</td>
<td>4.21 ± 0.50</td>
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<tr>
<td>n.s.</td>
<td>2.62 ± 0.56</td>
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</tr>
<tr>
<td>n.s.</td>
<td>2.37 ± 0.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-deoxy-cortisol nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p&lt;0.05</td>
<td>1.68 ± 0.39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n.s.</td>
<td>1.51 ± 0.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n.s.</td>
<td>1.08 ± 0.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n.s.</td>
<td>0.93 ± 0.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p&lt;0.005</td>
<td>738 ± 99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>n.s.</td>
<td>430 ± 79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n.s.</td>
<td>187 ± 50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n.s.</td>
<td>239 ± 91</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisone nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p&lt;0.05</td>
<td>187 ± 35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n.s.</td>
<td>208 ± 37</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n.s.</td>
<td>178 ± 55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n.s.</td>
<td>141 ± 47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of hormonal changes between neonates in the halothane and non-halothane anaesthesia groups. Differences between the two groups are analysed by the Mann-Whitney U Test. Changes from pre-operative values within each group are analysed by the Wilcoxon test.

N = Number of patients.
<table>
<thead>
<tr>
<th>Metabolic Changes</th>
<th>Halothane Anaesthesia</th>
<th>Non-halothane Anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wilcoxon Test</td>
<td>Mann-Whitney U Test</td>
</tr>
<tr>
<td>glucose mmol/L</td>
<td>5.0 ± 0.3</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>p&lt;0.0001</td>
<td>10.4 ± 0.8</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td>6.0 ± 0.2</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>p&lt;0.005</td>
<td>6.3 ± 0.6</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>n.s.</td>
<td>4.8 ± 0.3</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>lactate mmol/L</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>p&lt;0.05</td>
<td>2.5 ± 0.3</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>n.s.</td>
<td>2.2 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>p&lt;0.01</td>
<td>2.3 ± 0.3</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>pyruvate mmol/L</td>
<td>0.13 ± 0.01</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>p&lt;0.01</td>
<td>0.16 ± 0.02</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>p&lt;0.05</td>
<td>0.17 ± 0.01</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.15 ± 0.01</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.19 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>acetacetate mmol/L</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.11 ± 0.01</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.11 ± 0.02</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.08 ± 0.01</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.13 ± 0.02</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>hydroxybutyrate mmol/L</td>
<td>0.13 ± 0.03</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>p&lt;0.025</td>
<td>0.22 ± 0.03</td>
<td>0.25 ± 0.06</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.14 ± 0.05</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.07 ± 0.02</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.07 ± 0.02</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>alginine mmol/L</td>
<td>0.22 ± 0.03</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td>0.27 ± 0.03</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.21 ± 0.03</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>n.s.</td>
<td>0.20 ± 0.01</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>p&lt;0.05</td>
<td>0.16 ± 0.03</td>
<td>0.19 ± 0.02</td>
</tr>
</tbody>
</table>

Comparison of metabolic changes between halothane and non-halothane anaesthetic groups by Mann-Whitney U Test. Within each group, the changes in metabolite concentrations from pre-operative values are analysed by Wilcoxon's test.

\( n \) = number of patients; sequence of values = pre-operative, end-operative, 6 hours post-operative, 12 hours post-operative, 24 hours post-operative.
Table IV: HALOTHANE TRIAL - Metabolic changes.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>HALOTHANE ANAESTHESIA</th>
<th>NON-HALOTHANE ANAESTHESIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wilcoxon Test</td>
<td>Mann-Whitney U Test</td>
</tr>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol (mmol/L)</td>
<td>0.17 ± 0.02</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.20 ± 0.03</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.20 ± 0.04</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.17 ± 0.03</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fatty Acids (mmol/L)</td>
<td>0.52 ± 0.15</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.69 ± 0.22</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.58 ± 0.14</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.50 ± 0.14</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.28 ± 0.07</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.88 ± 0.21</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.75 ± 0.16</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.66 ± 0.18</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.70 ± 0.13</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.78 ± 0.22</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Total Ketones (mmol/L)</td>
<td>0.23 ± 0.04</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.33 ± 0.06</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.23 ± 0.07</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.15 ± 0.02</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.20 ± 0.04</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of metabolic changes between halothane and non-halothane anaesthetic groups by Mann-Whitney U Test. Within each group, the changes in metabolite concentrations from pre-operative values are analysed by Wilcoxon's test.

N = Number of patients; sequence of values = pre-operative, end-operative, 6 hours post-operative, 12 hours post-operative, 24 hours post-operative.
Table I. V  FENTANYL TRIAL: - Hormonal changes.

<table>
<thead>
<tr>
<th>FENTANYL ANAESTHESIA</th>
<th>Mann-Whitney U Test</th>
<th>NON-FENTANYL ANAESTHESIA</th>
<th>Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>N</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Insulin pmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- n.s.</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>39 ± 12</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>80 ± 35</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>80 ± 62</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>44 ± 15</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>61 ± 36</td>
<td>n.s.</td>
</tr>
<tr>
<td>Glucagon pmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- n.s.</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>12.6 ± 4.7</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>9.6 ± 2.9</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>9.7 ± 3.9</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>8.2 ± 3.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Adrenaline nmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- n.s.</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>1.29 ± 0.43</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.87 ± 0.29</td>
<td>n.s. &lt;0.025</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.67 ± 0.26</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0025</td>
<td>0.77 ± 0.35</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.025</td>
<td>0.46 ± 0.18</td>
<td>n.s.</td>
</tr>
<tr>
<td>Noradrenaline nmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- n.s.</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>12.3 ± 2.8</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>11.5 ± 2.9</td>
<td>n.s. &lt;0.025</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>9.8 ± 2.9</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.025</td>
<td>10.9 ± 3.1</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.025</td>
<td>8.3 ± 2.4</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Comparison of hormonal changes between neonates in the fentanyl and non-fentanyl anaesthesia groups. Differences between the two groups are analysed by the Mann-Whitney U Test, whereas changes from pre-operative values within each group are analysed by the Wilcoxon test. N = Number of patients.
Comparison of changes in plasma concentrations of steroid hormones between neonates in the fentanyl and non-fentanyl anaesthesia groups. (Differences between the two groups are analysed by the Mann-Whitney U Test, whereas changes from pre-operative values within each group are analysed by the Wilcoxon test.)

\( N = \text{Number of patients.} \)

**Table I.VI FENTANYL TRIAL: - Hormonal changes.**

<table>
<thead>
<tr>
<th>Steroid Hormone</th>
<th>FENTANYL ANAESTHESIA</th>
<th>Mann-Whitney U Test</th>
<th>NON-FENTANYL ANAESTHESIA</th>
<th>Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wilcoxon Test</td>
<td>Mean ± SEM</td>
<td>N</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Aldosterone nmol/L</td>
<td>-</td>
<td>1.32 ± 0.42</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>2.14 ± 0.79</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.40 ± 0.22</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>1.33 ± 0.46</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>1.30 ± 0.42</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Corticosterone nmol/L</td>
<td>-</td>
<td>21.7 ± 10.4</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td>34.4 ± 11.0</td>
<td>6</td>
<td>p&lt;0.025</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>10.0 ± 1.5</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>44.3 ± 17.9</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>6.2 ± 1.6</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Deoxycorticosterone nmol/L</td>
<td>-</td>
<td>0.51 ± 0.15</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.64 ± 0.08</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.59 ± 0.19</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.84 ± 0.29</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.43 ± 0.11</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Progesterone nmol/L</td>
<td>-</td>
<td>2.04 ± 0.45</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>2.92 ± 0.71</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>2.02 ± 0.66</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>3.44 ± 1.54</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.91 ± 0.48</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>17-Hydroxy-Progesterone nmol/L</td>
<td>-</td>
<td>5.99 ± 3.77</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>9.74 ± 4.31</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>5.66 ± 3.82</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>6.97 ± 3.39</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>1.57 ± 0.22</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>11-Deoxycortisol nmol/L</td>
<td>-</td>
<td>3.48 ± 2.15</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>2.95 ± 1.38</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>2.38 ± 1.42</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>4.45 ± 1.55</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>1.81 ± 0.89</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Cortisol nmol/L</td>
<td>-</td>
<td>193 ± 99</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td>320 ± 146</td>
<td>6</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>217 ± 61</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>471 ± 170</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>179 ± 53</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Cortisone nmol/L</td>
<td>-</td>
<td>130 ± 20</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>129 ± 23</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>112 ± 23</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>135 ± 13</td>
<td>6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>156 ± 41</td>
<td>7</td>
<td>-</td>
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</tbody>
</table>
Table I. VII FENTANYL TRIAL: Metabolite changes.

<table>
<thead>
<tr>
<th></th>
<th>Mann-Whitney U Test</th>
<th>Mann-Whitney U Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>N</td>
</tr>
<tr>
<td>Glucose mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FENTANYL ANAESTHESIA</td>
<td>8.9 ± 1.1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.5 ± 1.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.5 ± 1.4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.9 ± 0.9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.0 ± 1.2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Lactate mmol/L</td>
<td>0.9 ± 0.4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.3 ± 0.2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.2 ± 0.1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.2 ± 0.2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5 ± 0.2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Pyruvate mmol/L</td>
<td>0.12 ± 0.02</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.12 ± 0.02</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.12 ± 0.02</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.11 ± 0.02</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.15 ± 0.02</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Acetoacetate mmol/L</td>
<td>0.10 ± 0.01</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02 ± 0.02</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10 ± 0.01</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.08 ± 0.01</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>3-Hydroxybutyrate mmol/L</td>
<td>0.02 ± 0.01</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02 ± 0.02</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02 ± 0.02</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02 ± 0.01</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02 ± 0.01</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Alanine mmol/L</td>
<td>0.12 ± 0.04</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10 ± 0.03</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.12 ± 0.04</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10 ± 0.03</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10 ± 0.02</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Glycerol mmol/L</td>
<td>0.14 ± 0.04</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.16 ± 0.06</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.14 ± 0.04</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.16 ± 0.05</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.17 ± 0.05</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
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</table>

Comparison of metabolite changes between neonates in the fentanyl and non-fentanyl anaesthesia group. Differences between the two groups are analysed by the Mann-Whitney U test, whereas changes from pre-operative values within each group are analysed by the Wilcoxon test. n = Number of patients.
### Table I. Fentanyl Trial - Hormonal-metabolic changes.

<table>
<thead>
<tr>
<th></th>
<th>Fentanyl Anaesthesia</th>
<th>Mann-Whitney U Test</th>
<th>Non-Fentanyl Anaesthesia</th>
<th>Wilcoxon Test</th>
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<tr>
<td></td>
<td>Test</td>
<td>Mean ± SEM</td>
<td>N</td>
<td>Test</td>
</tr>
<tr>
<td>Total Ketones</td>
<td>-</td>
<td>0.12 ± 0.01</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.11 ± 0.02</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.13 ± 0.03</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.11 ± 0.01</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.10 ± 0.01</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lactate/Pyrurate</td>
<td>-</td>
<td>12.6 ± 2.1</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td>ratio mmol/mmol</td>
<td>n.s.</td>
<td>11.3 ± 2.2</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>12.2 ± 2.2</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>13.0 ± 3.1</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>13.3 ± 2.3</td>
<td>8</td>
<td>p&lt;0.05</td>
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<tr>
<td>Insulin/Glucose</td>
<td>-</td>
<td>5.9 ± 2.4</td>
<td>5</td>
<td>n.s.</td>
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<tr>
<td>ratio pmol/mmol</td>
<td>n.s.</td>
<td>5.9 ± 1.9</td>
<td>4</td>
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<tr>
<td></td>
<td>n.s.</td>
<td>7.4 ± 4.8</td>
<td>5</td>
<td>n.s.</td>
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<tr>
<td></td>
<td>n.s.</td>
<td>6.1 ± 2.5</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>6.6 ± 2.5</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total Gluconeogenic</td>
<td>-</td>
<td>1.7 ± 0.2</td>
<td>8</td>
<td>n.s.</td>
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<tr>
<td>Substrates mmol/L</td>
<td>n.s.</td>
<td>1.6 ± 0.2</td>
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<td>p&lt;0.05</td>
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<tr>
<td></td>
<td>n.s.</td>
<td>1.6 ± 0.1</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>1.5 ± 0.2</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>1.9 ± 0.2</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td>Alanine/Pyrurate</td>
<td>-</td>
<td>1.0 ± 0.3</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td>ratio mmol/mmol</td>
<td>n.s.</td>
<td>0.8 ± 0.2</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>1.0 ± 0.3</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>1.0 ± 0.3</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>0.9 ± 0.3</td>
<td>8</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Acetoacetate/</td>
<td>-</td>
<td>5.6 ± 1.7</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hydroxybutyrate</td>
<td>n.s.</td>
<td>5.2 ± 1.1</td>
<td>7</td>
<td>n.s.</td>
</tr>
<tr>
<td>ratio mmol/mmol</td>
<td>n.s.</td>
<td>6.3 ± 2.2</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>7.3 ± 3.0</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>5.2 ± 0.8</td>
<td>8</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Comparison of changes in derived hormonal-metabolic variables between neonates in the fentanyl and non-fentanyl anaesthesia groups. Differences between the two groups are analysed by the Mann-Whitney U Test, whereas changes from pre-operative values within each group are analysed by the Wilcoxon test.

N = Number of patients.
APPENDIX II: EXAMPLES OF DATA SHEETS
Dear Parent,

You are aware that your newly born baby will need to have an operation in the near future. We would like to ask for your cooperation in allowing us to study your child during and after the operation so that we learn more of the way newborn babies respond to the stress of surgery. This information is important so that we can learn what is the best way to anaesthetize small babies, and what is the best way to give medicines to relieve pain.

We would like to take a small volume of blood (less than 1 teaspoonful) before and at the end of the operation, and three times during the days after the operation. The blood will be taken at times when other blood samples are needed for the routine measurements that are always done at this time, and no extra pain or discomfort will be caused.

This study has been approved by the Central Oxford Research Ethics Committee. However, we may include your baby in it only with your approval and consent. Although a relative may not, according to the strict letter of the law, allow a child to participate in research, in practice it is legally acceptable for research to be performed with parental consent particularly when, as in this case, the risks are negligible, and after informed discussion has taken place.

Yours sincerely,

[Signature]

Dr. A. Anand
University Lecturer and Honorary Consultant
Department of Paediatrics, JNHI

Dr. K. S. Anand
Research Fellow
Department of Paediatrics, JNHI
**NEONATAL DATA SHEET**

**Name:** ..........................  
**G.A.:** .... wks  
**Age:** ..... days  
**Sex:** M/F  
**D.O.B.:** ..........  
**Time:** ...... hrs  
**lrrs Hea:** ..........  
**Birth Wt.:** .......... grams  
**Type of Delivery:** .....  
**Group:** .....  

**Record at Birth:**  
**Age at 1 min:** .......  
**Age at 5 min:** ......  
**T.E.R.:** ..........  

**Problems in Pregnancy:**  
- Resuscitation given:  
  1. Multiple Birth  
  2.  

**Congenital Malformations:**  
-  
-  
-  
-  
-  

**Neonatal Problems before Op.:**  
1.  
2.  
3.  
4.  
5.  
6.  
7.  
8.  
9.  
10.  

**Procedures:**  
1.  
2.  
3.  
4.  

**Pre-operative Record up to 12 hrs:**  
**I/V Fluids:**  
1.  
2.  
3.  
4.  
**Duration of Fasting:** ......... hrs  
**No of Transfusions:** ......  

**Fluid Restriction:**  
**I/V Alimentation:**  
**Amount:** ...... ml/kg/day  
**Sample:**  

**Surgical Diagnosis:**  

**Operation required:**  

<table>
<thead>
<tr>
<th>Complications</th>
<th>Anaesthesia Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Blood loss</td>
<td>Estimated blood loss: ...... ml</td>
</tr>
<tr>
<td>2. Blood transfusion</td>
<td>Blood transfusion: ...... ml</td>
</tr>
<tr>
<td>3.</td>
<td>B.P.: ....... H.P.</td>
</tr>
</tbody>
</table>

**Post-operative Anaesthesia:**  
1. TopO2:  
2. TopO2:  
3. TopO2:  
4. TopO2:  
5. TopO2:  

**I/V Fluids:**  
1. Feeds: started at:  
2. Type:  
3. Amount:  
4. Finish:  

**Sample:**  
Post-op:  
6 hrs: ...... ml  
12 hrs: ...... ml  
24 hrs: ...... ml  
48 hrs: ...... ml
ANAESTHESIA RECORD SHEET

Name: .......................... Age: ........... days Sex: M/F Code No: .............
Date of operation ............ Weight ........... grams Premature/Full Term
Anaesthesia Group 1/2 Surgical diagnosis: ..............................................

Basic Technique: (ALL GROUPS)
1. Pre Oxygenation ..........% O₂, for .............. minutes
2. Awake intubation: Ward/Theatre. Tube Size ........... mm
4. Nitrous Oxide and Oxygen (50:50)
   Fresh gas flow: N₂O ..........l/min. O₂ ..........l/min
5. d-Tubocurarine: Initial Dose (0.2-0.4 mg/kg) = ............. mg I/V
   Supplement (0.1-0.2 mg/kg) = ............. mg I/V
   Supplement = ............. mg I/V

6. Additional Drugs: -
   Halothane Group Fentanyl Group
   Induction: (1.2%)= ............. mins Induction: (10-20 g/kg)= ............. mg I/V
   Maintenance: (0.5%)= ............. mins Supplement (3-5 g/kg)= ............. mg IV/IM
   Other drugs 1. ..................mg IV/IM Supplement = ............. mg IV/IM
   2. ..................mg IV/IM Reversal: Naloxone = ............. mg IV/IM

Basic Technique Contd (ALL GROUPS)
7. Reversal: Atropine ............. mg IV/IM
   Neostigmine ............ mg IV/IM
8. Intravenous fluids: (during operation)
   Dextrose-saline .....................mls Blood transfusion ................mls
9. Extubation:
   Theatre/Ward
10. Post-op orders:
11. Complications: and management: -
   1. .
   2. .
   3. .
Operation performed ................. Anaesthetic time ....... mins Op.time.... mins
Comments:
Anaesthetist
Post-operative Data Sheet

Name .................................. GA .... wks  Age ..... days  Code No .......
Sex: M/F  Ward ..........  Birthweight ............ gms

<table>
<thead>
<tr>
<th>Post-operative Record:</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
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<td>IV Fluids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analgesia: Morphine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose</td>
<td></td>
<td></td>
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<tr>
<td>Hours post-op</td>
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<tr>
<td>Other Drugs:</td>
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<td>2.</td>
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<td>3.</td>
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<tr>
<td>Complications:</td>
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Weight: Pre-op          |       |       |       |
Milk Intake (Vol):      |       |       |       |
Urine Output (Vol):     |       |       |       |
**NEONATAL ANAESTHESIA PROJECT**

**NITROGEN BALANCE CHART**

<table>
<thead>
<tr>
<th>TIME</th>
<th>FROM</th>
<th>TO</th>
<th>DATE</th>
<th>FROM</th>
<th>TO</th>
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</thead>
<tbody>
<tr>
<td><strong>INPUT</strong></td>
<td></td>
<td></td>
<td><strong>OUTPUT</strong></td>
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</tr>
<tr>
<td><strong>DAY 1</strong></td>
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<tr>
<td>BLOOD</td>
<td></td>
<td></td>
<td>URINE</td>
<td>Complete 24-hr. collection</td>
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<td>F.P.P.</td>
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<td>F.P.P.</td>
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<td>T.P.N.</td>
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<td>Others</td>
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<td><strong>DAY 2</strong></td>
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<td>BLOOD</td>
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<td>URINE</td>
<td>Complete 24-hr. collection</td>
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<td>Others</td>
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<td><strong>DAY 3</strong></td>
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<tr>
<td>BLOOD</td>
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<td></td>
<td>URINE</td>
<td>Complete 24-hr. collection</td>
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<td>F.P.P.</td>
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<td>T.P.N.</td>
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<td>Others</td>
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</table>
NEONATAL ANAESTHESIA STUDY

1. **URINES**: Please collect all urine passed from _____ to _____ on ___. Please store the urine sample in a fridge after complete collection.

2. **INTRAVENOUS FLUIDS**: (a) Please do not start T.P.N. infusion before _____ on ___.
   (b) Please keep dextrose infusion rate between 4-6 mg/Kg/minute which is provided by:

   - 4% Dextrose = 6-9 ml/Kg/Hour
   - 5% Dextrose = 4.8-7.2 ml/Kg/Hour
   - 10% Dextrose = 2.4-3.6 ml/Kg/Hour

3. **ANALGESIA**: Please do not give analgesia between the following periods:
   and on
   and on
   and on

4. **FEEDING SCHEDULES**: Neonates should not be fed for 6 hours after surgery. Thereafter, feeds may be started, but please do not give any feeds between:
   and on
   and on
   and on

**PLEASE NOTE**: If analgesia or feeding are necessary between the above-mentioned periods, please bleep Dr. Kannwal Anand (Bleep no.364) before giving the drug or the feed.

Thank you very much!