MEASUREMENTS OF CATECHOLAMINES DURING ANAESTHESIA

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SECTION ONE

Introduction

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CHAPTER ONE

1.1 PLASMA CATECHOLAMINES

1.1.1 Biosynthesis of Catecholamines

Noradrenaline, adrenaline and dopamine are collectively known as catecholamines (Hamelberg, 1961; Golberg et al, 1974). Catecholamines are synthesised in the adrenal medulla, brain, sympathetic nerves and sympathetic ganglia (Goldfien, 1963; Cooper et al, 1982) from the amino acid tyrosine to 3,4-dihydroxyphenylalanine (DOPA) catalysed by tyrosine hydroxylase. The enzyme inserts an -OH group on the benzene nucleus at position 3 (Figure 1.1). DOPA is then decarboxylated by DOPA decarboxylase which removes -COOH group at position 8 (Figure 1.1) to give 3,4-dihydroxyphenylethylamine (DOPAMINE), and a beta -OH group is inserted at position 7 (Figure 1.1) by dopamine-beta-hydroxylase to give noradrenaline. In the adrenal medulla noradrenaline is converted to adrenaline by phenylethanolamine-N-methyl transferase (Burgen and Mitchell, 1985), methylating the terminal side chain at position 9 (Figure 1.1).

The concentration of noradrenaline in the nerve terminal acts as a negative feedback control to inhibit the activity of tyrosine hydroxylase that, as the concentration of noradrenaline rises, so the rate of tyrosine conversion to DOPA is reduced. A positive form of feedback control occurs when the activity in the sympathetic fibre increases and

potentiates the synthesis of noradrenaline, probably by reducing tyrosine hydroxylase inhibition because of the increased amount of noradrenaline passing out of the nerve terminal (Cooper et al, 1982).

Measurements of Catecholamines during Anaesthesia

K.J. Achola.

Abstract

Factors affecting catecholamine levels in vitro were studied using the modified high pressure liquid chromatography method with electrochemical detection. Differential centrifugation showed that platelet-rich plasma contained significantly higher catecholamine levels than platelet-poor plasma. Serum samples had significantly higher catecholamine levels than plasma. Plasma or serum samples clotted with glass beads had significantly higher catecholamine levels than those without. Therefore, consistent results can only be obtained when catecholamine samples are spun at the same speed, either plasma or serum can be used, but not both in a single study. Post-dated blood for transfusion was used to study stability of catecholamines, and showed that catecholamines are stable. Hence, the collection of blood samples for catecholamine measurements was modified. Blood samples were collected in Vacutainer tubes containing lithium heparin without anti-oxidants and not pre-cooled, samples were spun at the convenient time. This was welcomed by the clinicians who did not have to interrupt clinical assessment to care for blood samples as with the former method.

The three clinical studies showed no significant differences in catecholamine levels in patients undergoing laryngoscopy with and without tracheal intubation, whether or not the patients were beta blocked or had received topical tracheal analgesia. The mean catecholamine levels were within the normal range. No relationships between baseline catecholamine levels and the baseline blood pressures or heart rate nor between the changes in catecholamine levels from the baseline and the corresponding changes in blood pressures or heart rate.

The Injury Severity Score in minor injured patients had no relationship with plasma catecholamine levels, and no significant rise in noradrenaline levels when the ISS<30 and adrenaline levels when the ISS<17. The studies suggest that catecholamine levels are of no value in assessing the severity of minor injuries, or changes in blood pressures or heart rate during anaesthesia.

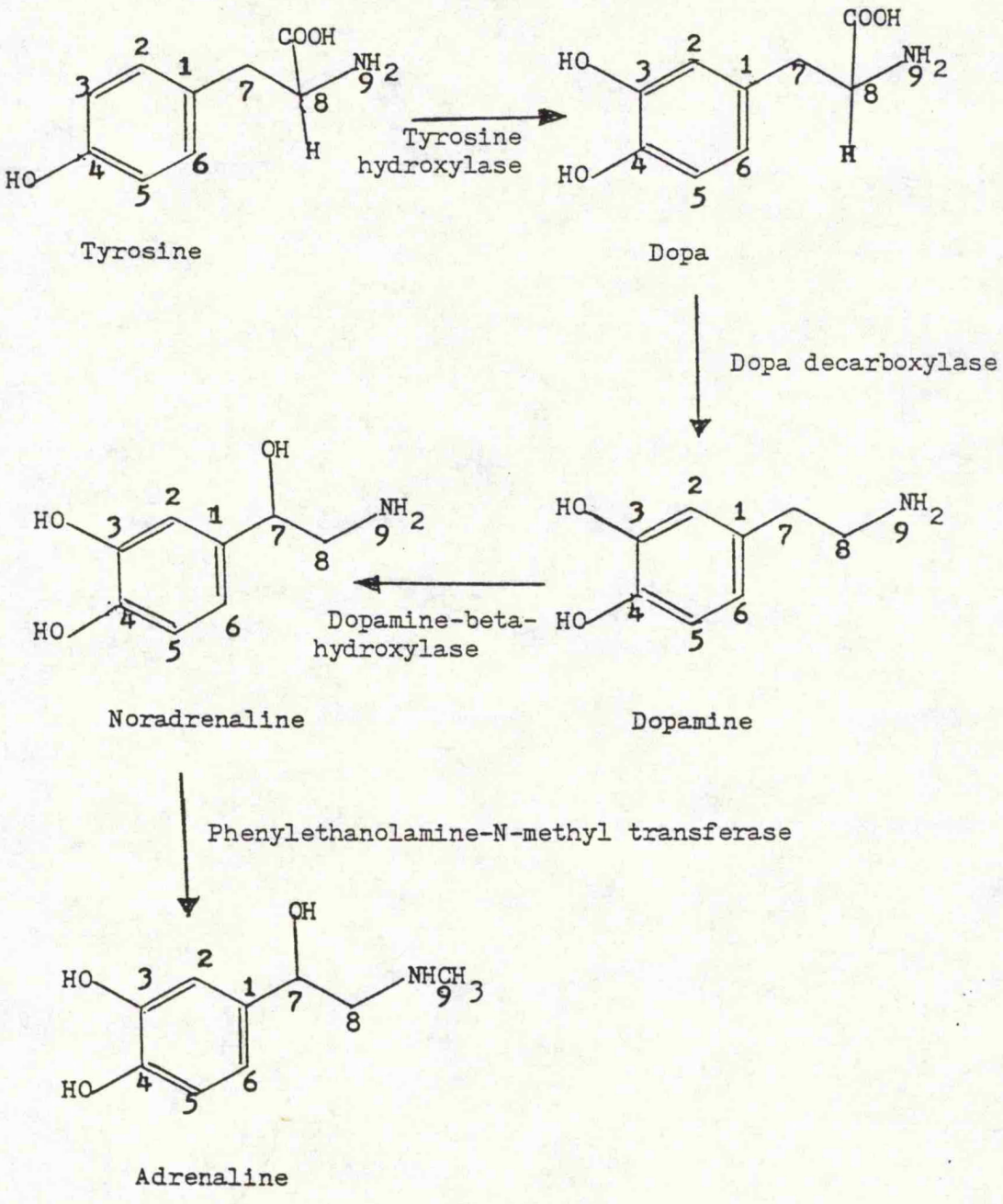


Figure 1.1 Biosynthesis of noradrenaline and adrenaline.

1.1.2 Release of Catecholamine

Noradrenaline is released from the nerve terminals during periods of nerve stimulation and the adrenergic fibres can sustain the output of noradrenaline during prolonged periods of stimulation, provided the synthesis and uptake of noradrenaline is not impaired (Cooper et al, 1982).

The mechanism of catecholamine release from the adrenal medulla is thought to occur when preganglionic fibre is stimulated, acetylcholine is released and thought to combine with the plasma membrane of the chromaffin cells (Cooper et al, 1982; Kruk and Pycock, 1987). The process produces protein conformational change, and alters the permeability of the membrane to calcium ions which then move inward. The influx of calcium ions is believed to be the main stimulus responsible for the secretion of catecholamines (Cooper et al, 1982).

The release of noradrenaline from the storage vesicles is effected by nerve impulse arriving at the nerve terminal and causing noradrenaline to be liberated along with calcium ions, adenosine triphosphate (ATP) and dopamine-beta-hydroxylase by a process of exocytosis (Burgen and Mitchell, 1985; Cooper et al, 1982).

1.1.3 Uptake of Catecholamines

Enzymatic destruction is not the major process that removes

catecholamine liberated in the blood by sympathetic nerve stimulation or after infusion of catecholamines. After their release from the adrenal medulla and postganglionic sympathetic nerve junctions, catecholamines are inactivated by three main mechanisms: The neuronal uptake into the sympathetic postganglionic junctions, known as Uptake-1 (Iversen, 1963; Iversen, 1965) is a major inactivating factor for catecholamine concentrations at physiological levels. It is energy dependent, requiring high energy compound ATP which is broken down by sodium ion-dependent ATPase. The extra-neuronal uptake, designated as Uptake-2 (Iversen, 1965), comes into effect to assist in the clearance of catecholamines when levels rise above the physiological range, such as those concentrations which occur during catecholamine infusions or stimulation of the adrenal medulla (Kruk and Pycock, 1987); and the inactivation by the enzymes catechol-O-methyltransferase, which produces O-methylation, and monoamine oxidase for the deamination of the O-methylated amines (Axelrod, 1957; Axelrod and Tomchick, 1958; Axelrod et al, 1961; Goldfien, 1963; Van Loon, 1983; Snider and Kuchel, 1983).

1.1.4 Storage of Catecholamine

A large amount of noradrenaline is stored within specialised subcellular vesicles in the sympathetic nerve endings and chromaffin cells. The vesicles contain adenosine triphosphate (ATP) in a molar ratio of catecholamine to ATP of about 4 : 1

(Cooper et al, 1982). It is believed that anionic phosphate groups of ATP form an ionic linkage with catecholamines, which exist as a cationic species at physiological pH. The ionic bond serves as a means to bind the amines with the vesicles and protect them from being destroyed by monoamine oxidase (MAO). The vesicles also contain dopamine-beta-hydroxylase which oxidises dopamine to noradrenaline, magnesium ion and calcium ion-dependent ATPase which are utilised during an active process to transport noradrenaline into storage vesicles (Cooper et al, 1982; Burgen and Mitchell, 1985).

1.1.5 Metabolism of Catecholamines

There are two major pathways for the degradation of the free catecholamines. The first is catechol-O-methyl transferase (COMT) which produces 3-O-methylation. COMT is found both inside and outside cells. High concentrations of COMT are found in the liver and lesser concentrations are found in most tissues. COMT is weakly inhibited by pyrogallol and tropolone. The second is monoamine oxidase (MAO) which produces oxidative deamination of catecholamines. MAO is found in all tissues which contain mitochondria, and is bound to the outer membranes. It is found in nerves, muscles, brain, liver and all active tissues (Cooper et al, 1982; Burgen and Mitchell, 1985; Kruk and Pycock, 1987). There are two forms of MAO; type A and B based on substrate specificity and sensitivity to inhibition by selected inhibitors.

Clorgyline is a specific inhibitor of the A-type enzyme, which has a substrate preference for noradrenaline. Deprenyl is a selective inhibitor of the B-type enzyme, and has a substrate preference for dopamine (Cooper et al, 1982).

Noradrenaline is O-methylated to normetadrenaline by COMT, then is degraded by MAO to 3-methoxy-4-hydroxyphenylglycolaldehyde followed by oxidation to 3-methoxy-4-hydroxymandelic acid (VMA) by aldehyde dehydrogenase. Similarly, COMT methylates adrenaline to give metadrenaline then is degraded by MAO to 3-methoxy-4-hydroxyphenylglycolaldehyde, and finally oxidised to 3-methoxy-4-hydroxmandelic acid (VMA) by aldehyde dehydrogenase (Figure 1.2). While dopamine is methylated by COMT to give 3-methoxytyramine (MTA) and is converted to 3-methoxy-4-hydroxyphenylaldehyde by MAO, the aldehyde is oxidised to homovanillic acid (HVA) by aldehyde dehydrogenase followed by sulphate conjugation (Cooper et al, 1982; Kruk and Pycock, 1987)(Figure 1.2)

Other metabolites of catecholamines are catalysed by the same enzymes described above. Thus, MAO degrades noradrenaline to 3,4-dihydroxyphenylglycoladehyde. The glycolaldehyde is oxidised to 3,4-dihydroxymandelic acid (DOMA) by aldehyde dehydrogenase, and finally catalysed by COMT to give 3-methoxy-4-hydroxymandelic acid (VMA) (Figure 1.2), a major metabolite of noradrenaline in urine (Cooper et al, 1982; Katzung, 1987). The 3,4-dihydroxyphenylglycolaldehyde can

also be reduced to 3,4-dihydroxyphenylglycol (DOPEG) by aldehyde reductase, then O-methylated by COMT to 3-methoxy-4-hydroxyphenylglycol (MHPG) followed by sulphate conjugation (Cooper et al, 1982).

Adrenaline is degraded by MAO to 3,4-dihydroxyphenylglycolaldehyde then oxidised to 3,4-dihydroxymandelic acid (DOMA) by aldehyde dehydrogenase, finally O-methylated to 3-methoxy-4-hydroxymandelic acid (VMA) and excreted in urine (Figure 1.2). 3,4-dihydroxyphenylglycolaldehyde can be reduced to DOPEG by aldehyde reductase, then catalysed by COMT to MHPG followed by sulphate conjugation (Cooper et al, 1982).

Dopamine is degraded by MAO to 3,4-dihydroxyphenylacetaldehyde then further degraded by aldehyde dehydrogenase to form 3,4-dihydroxyphenylacetic acid (DOPAC). At this stage DOPAC forms sulphate conjugate, the remainder is methylated to homovanillic acid (HVA) then forms sulphate conjugation.

Inhibition of both COMT and MAO does not markedly potentiate the effects of sympathetic nerve stimulation, although in some tissues it tends to prolong the duration of the response to stimulation. Therefore, neither COMT nor MAO would seem to be the primary mechanism for terminating the action of noradrenaline liberated at the sympathetic nerve terminals. It may be that these enzymes play an important role in terminating transmitter action and regulating catecholamine

function in the central nervous system (Cooper et al, 1982).

In the central nervous system (CNS), the aldehyde derivative of noradrenaline predominates. In the brain, noradrenaline is catalysed to 3,4-dihydroxyphenylglycolaldehyde by MAO, then reduced to 3,4-dihydroxyphenylglycol (DOPEG) by aldehyde reductase. The glycol is O-methylated to 3-methoxy-4-hydroxyphenylglycol (MHPG) by COMT (Cooper et al, 1982). It is thought that VMA is not formed in the brain. The MHPG formed is sulphate conjugated and readily diffuses from the brain into the cerebrospinal fluid (CSF) (Cooper et al, 1982).

The acid derivatives of dopamine rather than aldehyde appear to predominate in the CNS. Thus, dopamine is converted to 3,4-dihydroxyphenylacetaldehyde by MAO then catalysed to 3,4-dihydroxyphenylacetic acid (DOPAC) by aldehyde dehydrogenase. Some of the DOPAC are sulphate conjugated and the rest are metabolised to homovanillic acid (HVA) by COMT then are sulphate conjugated (Cooper et al, 1982). Increased amount of HVA in the brain and CSF is formed during increased activity of dopaminergic neurones in the brain. In human, DOPAC is a minor brain metabolite, while in rat brain DOPAC is a major metabolite of dopamine (Cooper et al, 1982).

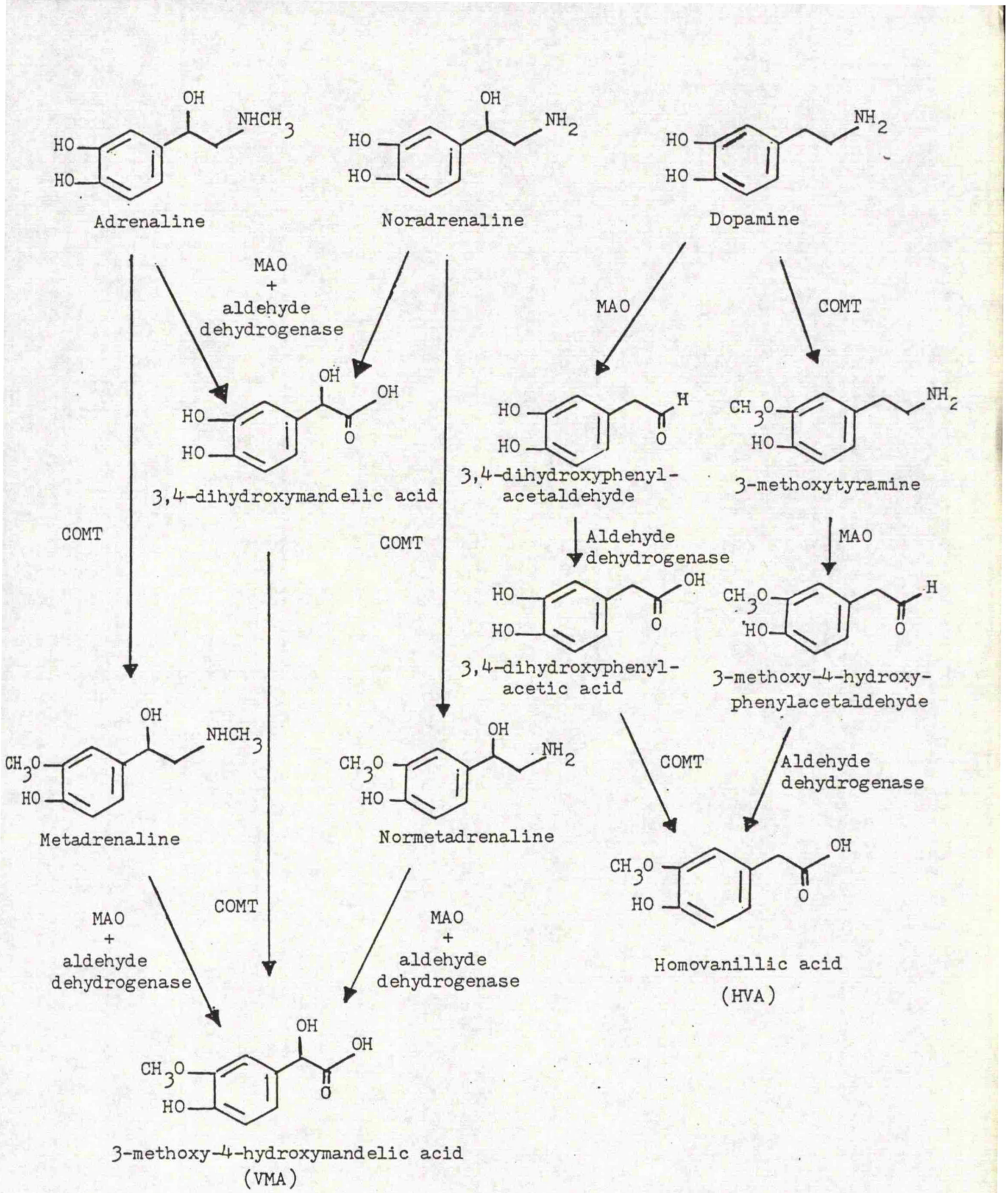


Figure 1.2 Metabolic fate of adrenaline, noradrenaline and dopamine

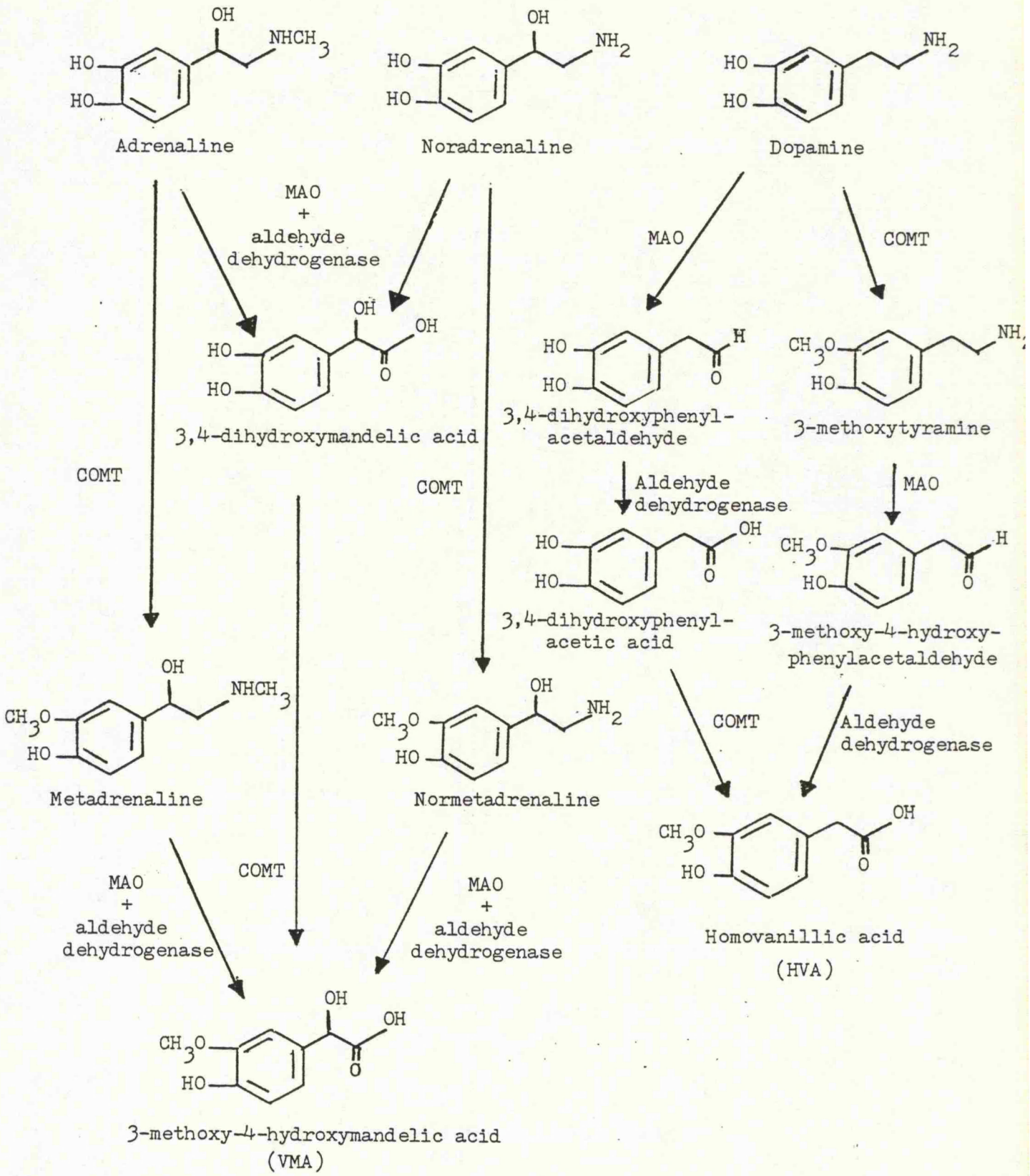


Figure 1.2 Metabolic fate of adrenaline, noradrenaline and dopamine

1.1.6 Stress Response

When sympathetic nerve is stimulated it activates alpha and beta adrenoceptors in various tissues and increases the tone in some smooth muscle and relaxation in others. It increases the rate and force of the heart rate and stimulates the secretion of glands (Gilman et al, 1980; Burgen and Mitchell, 1985). The activation of beta-adrenoceptors lead to increased glycogenolysis in the liver, increased lipolysis in adipose tissues and decreased release of insulin from the pancreas. The increased glycogenolysis leads to elevated blood glucose and decreased glucose uptake because of decreased insulin release. Hence glucose is available for metabolism by the brain. The increased lipolysis provides energy source in the form of fatty acids for skeletal muscle. In situation of stress, the metabolic actions of catecholamines on beta-adrenoceptors serve to ensure a diversion of the energy to the brain and skeletal muscle (Kruk and Pycock, 1987).

On the smooth muscles where alpha-excitation occurs, such as blood vessels, alpha-adrenoceptors stimulation leads to vasoconstriction when the smooth muscle contracts. Sympathetic stimulation causes depolarization of the junction potential which leads to propagating action potential. When catecholamine was applied, similar depolarization of the junction potential occurs (Gilman et al, 1980).

Stimulation of alpha-adrenoceptors usually produces an excitatory response and stimulation of beta-adrenoceptors

produces excitation in the heart but inhibition in most other tissues (Gilman et al, 1980; Katzung, 1987)(Table 1.1).

Table 1.1. Distribution and response of adrenoceptors

Organ	Receptor	Response
Heart	beta-1	increased heart rate and contractility
Coronary arteries	alpha	vasoconstriction
	beta-2	vasodilatation
skeletal arterioles	beta-2	vasodilatation
skin arterioles	alpha	vasoconstriction
Bronchi	beta-2	dilation
Gut	beta-2	relaxation
	alpha	contraction.

Catecholamines, whether blood-borne or released from the sympathetic postganglionic junctions, act on two types of adrenoceptors in the tissues; alpha and beta adrenoceptors (Ahlquist, 1948). Alpha-adrenoceptors are characterised by being most sensitive to the actions of noradrenaline and phenylephrine and least sensitive to the action of isoprenaline (Kruk and Pycock, 1987). Noradrenaline stimulates predominantly alpha-adrenoceptors but possesses a weak beta-adrenoceptor action (Keele et al, 1982).

Alpha-adrenoceptors are of two kinds, alpha-1 and alpha-2. The action of alpha-1 adrenoceptors is associated with

contraction of smooth muscle. They are found at postganglionic nerve terminals of the sympathetic nervous system in most tissues (Table 1.1). Alpha-2 adrenoceptors are located on presynaptic nerve terminals of cholinergic and adrenergic nerves. The actions of alpha-2 adrenoceptors are inhibitory, eg relaxation of intestinal muscle. Noradrenaline is active on alpha-1 adrenoceptors (Meyers et al, 1980; Keele et al, 1982; Katzung, 1987). Phenoxybenzamine is about 100 times more potent in blocking alpha-1 receptors than alpha-2 receptors, while phentolamine is about 3-5 times more potent in blocking alpha-1 than alpha-2 adrenergic receptors (Gilman et al, 1980).

Beta-adrenoceptors are classified by being most sensitive to the actions of isoprenaline and least sensitive to the actions of noradrenaline and phenylephrine (Kruk and Pycock, 1987). There are two kinds, beta-1 and beta-2 adrenoceptors. Both are located on postsynaptic junctions. Adrenoceptors in the heart are called beta-1 adrenoceptors; stimulation increases the force of contraction and the heart rate (Table 1.1). Those beta-adrenoceptors found outside the central nervous system are called beta-2 adrenoceptors (Kruk and Pycock, 1987). These receptors are associated with relaxation of smooth muscle. Beta-2 adrenoceptors are associated with relaxation of smooth muscle of the blood vessels, intestines and bronchioles (Table 1.1) (Gilman et al, 1980; Day, 1979; Keele et al, 1982). Propranolol blocks both beta-1 and beta-2 receptors, while practolol is a selective beta-1 receptor

antagonist (Gilman et al, 1980).

1.1.7 Factors Affecting Catecholamine levels in man

Although major advances in neuroendocrine research have increased the understanding of catecholamine synthesis, release, re-uptake and metabolism, the physiological roles of plasma catecholamines are incompletely understood (Cryer et al, 1974). Infusion of noradrenaline and adrenaline in man caused increased blood pressure, heart rate and increased metabolism of lipids, protein and carbohydrates (Silverberg et al, 1978; Clutter et al, 1980). In addition, high circulating catecholamines are known to occur in phaeochromocytoma (Hamberger et al, 1981) and is associated with an increased blood pressure.

A change in position such as standing up abruptly reduces cardiac output; a sympathetic neuronal reflex is activated by baroreceptors and peripheral vascular resistance is increased to maintain the blood pressure. The activation of the sympathetic neuronal reflex causes an increase in plasma catecholamine levels (Cryer, 1976; Lake et al, 1976; Saar and Gordon, 1979). Also tracheal intubation (Russell et al, 1982), exercise (Christensen and Brandsborg, 1973) and surgery (Halter et al, 1977) all increases plasma catecholamine levels. No account has been offered as to the significance of the inceases of those plasma catecholamine levels.

It has been suggested that plasma catecholamine levels and dopamine-beta-hydroxylase activity (the enzyme that catalyses dopamine to noradrenaline) would reflect sympathetic nervous system activity and medullary function (Benedict and Graham-Smith, 1978). The study of Benedict and Graham-Smith (1978), in patients with septicaemic shock showed that plasma noradrenaline and adrenaline levels were both increased, but there were minimal changes in plasma dopamine-beta-hydroxylase activity. Ziegler et al (1976) reported similar catecholamine levels in normal and dysautonomic subjects during recumbency, whereas there were significant increases in noradrenaline levels in normal subjects after standing for 5 minutes, but no change in dysautonomic subjects. Plasma dopamine-beta-hydroxylase activity was higher in control than in dysautonomic subjects.

Oliver and Schafer (1895) demontrated that the active principle from the adrenal medulla can raise blood pressure; thereafter, increases in plasma catecholamine levels are associated with hypertension. There are reports that suggest the common factors which tend to raise plasma catecholamine levels in vivo are essential hypertension (DeChamplain et al, 1976) and old age (Ziegler et al, 1976). The implication is that the study groups should be carefully selected and matched (according to age, normotensive or hypertensive groups) to avoid variation of plasma catecholamine levels likely to occur due to non-matched groups, other than the factors being investigated.

Attempts to relate abnormal excretion of catecholamine levels and hypertension have not been successful in every attempt. Significant elevation of plasma catecholamine levels have been found in hypertensive patients (Ziegler et al, 1976; DeChamplain et al, 1976), while Skrabal et al (1981) found no significant difference in catecholamine levels between hypertensive and normotensive patients. Lake et al (1977) have suggested that the differences in group mean age could cause differences in plasma noradrenaline levels, because those levels varied with age and because hypertensives were older than the normotensives. In studies which were reported on mean group ages between 20 and 50 years, the mean group noradrenaline levels significantly correlated with the mean age for the normotensives, but not the hypertensives. The hypertensives were older than the normotensives by an average of 3.4 years. In studies in which hypertensive subjects were age matched, there were no significant differences in plasma noradrenaline levels when plasma was taken in either a sitting or a standing position between the normal and the hypertensive groups (Lake et al, 1976).

Bravo and Tarazi (1982) and Miller (1986) have pointed out that measurements of plasma catecholamines have caused some controversy concerning their meaning, and there is little agreement as to whether catecholamine estimations can be used as an index of sympathetic activity, or whether catecholamine levels have diagnostic value in clinical medicine. It is argued that interpretation of noradrenaline levels are

complex because of the complexities of catecholamine metabolism and problems which arise from methodology; also, there is a wide range of normal catecholamine levels. Precision is therefore essential in the methodology and techniques for catecholamine estimations. Adrenal medullary secretion can occur without overall sympathetic stimulation. Therefore, the sampling site for catecholamine estimation is an important factor; forearm venous samples reflect local events while arterial blood represents systemic values (Pflug et al, 1982; Miller, 1986). Noradrenaline secretion reflects an overflow from sympathetic stimulation, and small changes must be interpreted with caution (Miller, 1986).

1.1.8 Catecholamine levels and Anaesthesia

Stimulation of the sympathetic nervous system produces an increase in cardiac output, cardiac work, oxygen consumption and contractility (Eckstein et al, 1950). However, inhalational anaesthetics reduce myocardial oxygen supply by reduction in coronary blood flow, and arterial pressure although myocardial oxygen demand is also decreased. Excessive cardiac depression should be avoided if undue hypotension is to be prevented. If anaesthesia is too light, hypertension and tachycardia occur, but if it is too deep, hypotension results in reduced myocardial perfusion (Miller, 1986).

The response of the sympathetic nerve system to stimulation

by anaesthetic agents has been investigated for many years. It has been suggested that noradrenaline and adrenaline are secreted in response to anaesthetic agents (Price et al, 1959). These authors examined the effects of four anaesthetic agents on plasma catecholamine levels, blood pressure and heart rate in man, using fluorometric techniques. They reported that cyclopropane increased noradrenaline levels with little change in blood pressure, while thiopentone produced no change in catecholamine levels, but marked hypotension; diethylether caused a marked increase in catecholamine levels, together with hypotension and tachycardia, and halothane was reported to produce little change in noradrenaline levels, with a marked hypotension and bradycardia.

During light anaesthesia, anaesthetic agents produce various degrees of central nervous system depression, due to failure of axons to conduct nerve impulses, failure to release transmitter from nerve terminals or failure of the postsynaptic membranes to be excited by the nerve impulse (Brazier, 1961; Paton and Speden; 1965). The body reflexes must, therefore, try to compensate for this abnormal situation. It is assumed that one way the body compensates for this is by increased secretion of noradrenaline and adrenaline levels for their sympathomimetic effects (Hamelberg et al, 1960). Hamelberg et al (1960) also examined the effects of the same anaesthetic agents on catecholamine levels, and found that halothane produced increases in

catecholamine levels while cyclopropane produced little change (in contrast with the study of Price et al, 1959). Hypotension or lack of it in those patients did not appear to relate to catecholamine levels. In addition, hypotensive patients had low catecholamine levels (Hamelberg et al, 1960). This was in contrast to the studies of Rawlinson et al (1978), who found hypotension induced by sodium nitroprusside was accompanied by significant increases in catecholamine levels. Sodium nitroprusside is a potent vasodilator, therefore it reduces peripheral resistance with variable changes in heart rate (Miller, 1986). The reduction of peripheral resistance is usually accompanied by hypotension.

Indeed, during deep cyclopropane anaesthesia greater increases in catecholamine levels were obtained than during light anaesthesia. Ether induced hypotension with a significant rise in catecholamine levels (Hamelberg et al, 1960). These studies suggest that causes of hypotension in anaesthesia are complex and may not be compensated by an increase of the endogenous catecholamine levels. The current studies show that the earlier studies did not take into account that hypotension could have been the major cause of the increase in catecholamine levels; also the results could have been affected by other factors such as age and hypertension and lastly, the time between their baseline samples and the experimental samples were at least 30 to 60 minutes; such timing would produce little difference between the baseline and the experimental results, due to the short

half-lives of catecholamines in vivo.

A more recent study by Joyce et al (1983), using radioenzymatic techniques for catecholamine measurements, examined the effects of thiopentone 3mg per kg on catecholamine responses in three groups of patients, in whom the induction dose was followed by either an intravenous infusion of thiopentone 0.2-0.3mg per kg per minute with 100% oxygen, halothane 1.5% in oxygen, or 70% nitrous oxide in oxygen. The group who received thiopentone infusion showed a decrease in noradrenaline levels (in contrast with the study of Price et al, 1959), 10 and 16 minutes after the start of the infusion; it was implied that the decrease was due to thiopentone infusion. However, there was no change in catecholamine levels in other groups.

Takki et al (1972) studied the effects of laryngoscopy and tracheal intubation on catecholamine levels using fluorometric techniques, in patients who were induced either with thiopentone 5mg per kg followed by suxamethonium 1mg per kg or with propanidid 7mg per kg followed by suxamethonium 0.2mg per kg or with propanidid 10mg per kg alone. No significant changes were found in catecholamine levels, and there were no significant difference in the catecholamine levels in the three groups, when the mean baseline catecholamine levels were compared with the mean catecholamine levels taken 2 minutes after tracheal intubation.

The function of catecholamine levels in maintaining physiological state during anaesthesia is inadequately understood (Hamelberg et al, 1960).

1.1.9 Scope of the Problem to be Investigated

The scope of the problem to be investigated in this thesis arises from the interest in catecholamine measurements over the years and the apparent importance in plasma catecholamine levels reported. Many if not all, report their findings as the change of catecholamine levels from the baseline. The apparent increase of catecholamine levels from the baseline could be due to some factors arising from the blood colletion and during sample preparation before analysis. Those aspects have received little attention over the years. A decrease of catecholamine levels also could be due to body clearance of catecholamines from the circulation. It is known that a half-life of catecholamines is very short, and timing is an important factor in collection of blood for catecholamine estimation. This also has received little attention.

It has been suggested (Russell et al, 1981; Tomori and Widdicomb, 1969) that increases in endogenous catecholamine levels during tracheal intubation may be responsible for the pressor response and increases in heart rate. It would, therefore, be expected that changes in catecholamine levels would correlate with the changes in blood pressures or heart rate. In addition, the endogenous catecholamine levels during

laryngoscopy and tracheal intubation has not been compared with those exogenous catecholamine levels known to cause pressor response.

The aim of this thesis was to examine the factors which affect or interfere with plasma catecholamine levels during sample collections, preparations and measurements, and to modify the published methods if necessary to obtain maximum optimisation for catecholamine estimations. In addition stability of catecholamines was to be investigated in detail. The second part of the work tested the following hypothesis: That measurements of plasma catecholamine levels under controlled conditions during anaesthesia have some biological significance.

Several important questions automatically arose from that hypothesis.

1. Can the catecholamine levels be used to assess changes in blood pressure or heart rate during anaesthesia?
2. Is there a relationship between the baseline catecholamine levels and the baseline blood pressures or heart rate?
3. Do the changes of catecholamine levels from baseline correlate with those changes in blood pressures or heart rate at various stages during induction of anaesthesia and laryngoscopy with or without tracheal intubation?
4. If there is relationship between catecholamine levels and blood pressures or heart rate; is the relationship predictable?

5. What is the range of catecholamine levels obtained during anaesthesia?

The second part of the investigation used patient's blood samples. The author designed the studies on laryngoscopy with and without tracheal intubation, also the study which examined the haemodynamic and catecholamine changes during beta blockade, but not topical tracheal analgesia. In all of those studies the author was involved in sample preparations for the analysis, the analysis of catecholamine levels in those samples and the analysis of statistics, but not sample collections in anaesthetic room.

The third part of the investigations was designed by the author to examine the relationship between catecholamine levels and the Injury Severity Score on minor injured patients. Currently there are reports which have examined the relationship between catecholamine levels and the Injury Severity Score on road crash victims immediately after the injuries, also during surgery. No work has been done on catecholamine levels and minor injuries.

The overall aim of the investigations was to attempt to rationalise and understand the importance, if any, of the endogenous or physiologically secreted catecholamine levels in relation to blood pressure or heart rate.

SECTION TWO

1. Method development and validation of the method

2. Anti-oxidants and stability of catecholamines

3. Factors affecting catecholamine levels

4. Internal quality control system

CHAPTER TWO

2.2 METHOD DEVELOPMENT

2.2.1 The History and Theory of Analytical Development

Analytical method has been defined (Varley et al, 1980) as the procedure, material and equipment used to obtain a reliable result with standard calibration. It has to be sensitive and specific. In the 1950s, the analytical techniques available for measurement of catecholamines were fluorometry and bioassay. Fluorometry was used preferentially to bioassay because it was less tedious. The techniques for catecholamine measurements are summarised.

2.2.2 Fluorometric Technique

The fluorometer is a very sensitive and selective detector for fluorescing solutes because of its ability to measure energy emitted from certain solutes excited by ultraviolet radiation. Fluorescent derivatives of non-fluorescing substances can also be prepared; hence it is a versatile detector.

The earlier fluorescent method for plasma catecholamine measurements, the trihydroxyindole method, was developed by Lund (1950). Lund oxidised noradrenaline and adrenaline with manganese dioxide to noradrenochrome and adrenochrome respectively. Treatment of the products with sodium hydroxide transformed them to noradrenolutine and adrenolutine with an intense yellowish green fluorescence. Adrenolutine decomposed

quickly in an alkaline solution and the fluorescence disappeared in about three to four minutes, while noradrenolutine decomposed slowly in about 20-30 minutes. The addition of an antioxidant such as ascorbic acid prevented further oxidation. In order to measure both adrenaline and noradrenaline, the oxidation with manganese dioxide was performed at a different pH for each substance, adrenaline at pH3 and noradrenaline at pH 6.5.

The ethylenediamine condensation method of Weil-malherbe and Bone (1952) involved the oxidation of catecholamine to adrenochrome and noradrenochrome, which reacted with ethylenediamine to form a fluorescent product. When ethylenediamine condensed with any catechol compound it was found to form a similar fluorescent product. Since there are a number of compounds in biological fluids with catechol structure, this method was less specific than the trihydroxyindole method.

Valk and Price (1956) suggested that the specificity of the trihydroxyindole method was adequate for noradrenaline and adrenaline measurements, but not ethylenediamine method. This indicated that further work on instrumentation was needed to achieve the necessary specificity and sensitivity. McCullough (1968) developed a semi-automated method for the differential measurement of noradrenaline and adrenaline, based on the trihydroxyindole method. Although 5-10ml of plasma was used for each analysis, the levels of catecholamine reported were

similar to the current published levels. Jaattela et al (1975) and Butler et al (1977) also used the trihydroxyindole method and achieved similar catecholamine levels, with 18ml of plasma for each analysis.

The trend to improve the sensitivity of fluorometric procedure continued, and Yamatodani and Wada (1981) reported catecholamine levels in the pmole range with less than 1ml of plasma. They used liquid chromatography with fluorometric detection, the sample loop was 1.0ml with two analytical columns joined together, and less than 1ml of plasma and achieves similar sensitivity as that of high pressure liquid chromatography with electrochemical detection.

2.2.3 Bioassay Technique

The principle of the bioassay technique is based on the response of a strip of tissue, in a water bath, to the presence of catecholamine added to the water bath. Piper et al (1967) showed that the presence of adrenaline caused relaxation of the rat stomach strip, while noradrenaline caused relaxation of the chick rectum strip. The sensitivity and the specificity of the technique were better than for the chemical methods. The bioassay was at least five times more sensitive than chemical methods (Holzbauer and Vogt, 1954). Generally, bioassay is now used in pharmacological procedures (Goldstein et al, 1974) to determine the relationship between the dose (concentration) of a drug and the magnitude of the

biological response which the drug evokes from the tissue. It is used in industry for the preparation of the dose of certain drugs eg heparin, so that each drug contains the same specified pharmacological activity.

2.2.4 Gas Chromatography

Gas chromatography is a useful analytical technique providing separation and quantitation of plasma and tissue catecholamines. However, the technique requires that compounds for analysis must be volatile or are derivatised to become volatile. Halogenated derivatives of catecholamines are employed with this technique because they have a high electron affinity, and are detected with an electron capture detector, providing sensitivity at nanogram range. The trifluoroacetyl or pentafluoropropionyl derivatives of catecholamines (Cooper et al, 1982) are favoured since they are easy to derivatise; in addition the unreacted anhydride is easily separated by volatilisation.

The technique is not accepted widely because derivatives are unstable and the detector is easily contaminated.

2.2.5 Gas Chromatography-Mass Fragmentography

The technique of gas chromatography-mass fragmentography is a combination of gas chromatography, which provides efficient separation of plasma and tissue catecholamines, and mass spectrometry, which provides high structural resolution,

detection and identification of catecholamines. Mass specific recordings are made of preselected ions. The technique uses a few microlitres of plasma and provides a detection range of 10-15 femtomoles. The technique is not favoured because it requires highly skilled personnel to operate it (Cooper et al, 1982; Knox et al, 1977).

2.2.6 Radioenzymatic Assay

The radioenzymatic assay for the measurements of catecholamines provides sensitivity, specificity and precision for both plasma and tissue catecholamines (Engelman et al, 1968).

The general principle of the assay was based on the conversion of noradrenaline and adrenaline in the presence of catechol-O-methyltransferase and S-14C-adenosyl-L-methionine to their respective labelled normetadrenaline and metadrenaline.

The original method (Engelman et al, 1968) was designed to measure total catecholamines. However, the need to measure noradrenaline and adrenaline independently, necessitated the modification of the method, and the introduction of a thin layer chromatography for the separation of the labelled products (Engelman and Portnoy, 1970). The double isotope derivative also enabled internal standard of tracer quantities of the 7-3H-noradrenaline and 7-3H-adrenaline to be added to the samples. This permitted the calculation of

the recovery of catecholamines in each sample when 3H/14C ratios of the samples and those of the standards were compared (Engelman and Portnoy, 1970; Brown and Jenner, 1981).

Further refinement was introduced when Passon and Peuler (1973) demonstrated that the use of S-adenosyl-L-methionine as 3H-methyl donor as opposed to 14C-methyl donor improved the sensitivity of the assay. Cryer et al (1974) confirmed the improved sensitivity of the 3H-methyl donor of S-adenosyl-L-methionine, and also reported that the reproducibility of the assay was better. Hence, there is a choice either to use a single isotope derivative method or a double isotope derivative method for the measurement of plasma or tissue catecholamines.

Radioenzymatic techniques have good sensitivity, though they are time consuming, in that 15 samples take a day to assay (Peuler and Johnson, 1977) whereas high pressure liquid chromatography method with an autosampler in our laboratory can analyse over sixty samples a day. Radioenzymatic methods are difficult to set up and cumbersome to operate, and are very expensive (Holly and Makin, 1983). On a long term basis, high pressure liquid chromatography method is cheaper than the radioenzymatic method (Hjemdahl et al, 1979).

2.2.7 High Pressure Liquid Chromatography

The technology of high pressure liquid chromatography has

reached a level which permits its use in most laboratories at a modest cost. It consists of a short column packed with a stationary phase of small particle size material for the separation of compounds, a pump to drive the mobile phase through the column, an injection valve to introduce the sample on to the column, a detector and an integrator to analyse the peaks.

2.2.8 The Choice of Analytical Technique

Towards the end of 1970s the department of anaesthesia had just started, and we decided to set up analytical technique for plasma catecholamine measurements. The factors which influenced the decision for the choice of the technique were: The simplicity and the reliability of the analytical techniques commonly available; the minimum stages of sample cleanup and the costs of the system as well as the consumables. High pressure liquid chromatography with electrochemical detector was chosen.

2.2.9 Equipments

The equipments used in this thesis are commercially available, and are shown in Appendix One also the addresses of the suppliers.

2.2.10 Oxidation Flowcell and the Detector

The oxidation flowcell used by Keller; Hallman; Hjemdahl was a commercially available TL-3 carbon paste electrode and a TL-5 glassy carbon with a working electrode, an Ag/AgCl as a reference electrode and auxiliary electrode (Bioanalytical Systems). The potential selected on the LC-4A Amperometric Controller was applied between the reference and working electrodes. The auxiliary electrode compensates the current and prevents charge imbalance (Shoup et al, 1980). The repacking and conditioning of the TL-3 is a matter of experience. The TL-3 carbon paste electrode when being repacked, should be cleaned and if there are scratches, Brasso should be used to remove these. After trying this on a badly scratched cell, the result was good and high sensitivity was achieved; subsequently the method was adopted to clean the cells routinely in our laboratory.

The cell is washed with soap and deionised double distilled water and dried with soft tissue, before repacking with fresh carbon paste. Computer card is best for polishing the repacked cell. The polishing is done in a circular motion as outlined in the manual (Shoup et al, 1980), until the cell has a mirror surface. This should not necessarily take more than two minutes.

The TL-5 glassy carbon cell does not need repacking. It only needs polishing with a special cleaning pad and polishing alumina suspension (supplied by the manufacturer).

The reaction on the cell surface occurs when the electrode is maintained at a constant potential (Shoup et al, 1980; Kissinger, 1977), sufficiently positive to force the orthodihydroxy groups of catecholamine to undergo a loss of two electrons and two protons to yield the orthoquinone derivative of catecholamine. Oxidation similar to that of noradrenaline is common to all catechols and catecholamines (Sternson et al, 1973).

2.2.11 Cell Sensitivity

To achieve satisfactory sensitivity with electrochemical detector is still a major problem to some laboratories (Van Loon, 1983). Eriksson and Persson (1982) used 0.025nA or 0.05nA with 0.7V; while Hjemdahl et al (1979) set their potential at 0.72V and 0.1nA sensitivity, and showed that sensitivity and specificity of HPLC with electrochemical detector and those of radioenzymatic technique were comparable. These experiments suggest that sensitivity of the high pressure liquid chromatography system depends on the potential of the cell. Higher potential above the optimum range can be used to increase sensitivity, but at the expense of poor baseline.

2.2.12 High Pressure Liquid Chromatography with Electrochemical detection

The principle of high pressure liquid chromatography with an electrochemical detector is that, the compounds for detection must be electroactive in order that they may be either oxidised or reduced at the carbon electrode surface when a suitable constant potential is applied to the graphite paste or glassy carbon electrode. As compounds are oxidised (eg loss of two electrons and two protons) currents are generated, the change in the current is proportional to the concentration of the compounds which generate the currents (Keller et al, 1976; Shoup et al, 1980). The detector allows pmole levels of the compounds to be measured.

2.2.13 Cation Exchange Chromatography with Electrochemical Detection

In the mid 1970s a reliable and highly sensitive catecholamine assay (Keller et al, 1976) which involved a minimum of sample preparation was described. The method is high pressure liquid chromatogrphy with electrochemical detection.

The principle of ion exhange resin has many applications. The reversibility of the reaction is the most important feature of ion exchange resin. For example, the sulphonic acid exchanger in the hydrogen form reacts with sodium chloride to produce the sodium form of the resin and hydrochloric acid.

The sodium form of the resin can be converted to a hydrogen form by using acid; this is known as regeneration.

Cation exchange chromatography uses the principle of ion exchange process. The separation occurs due to attraction between oppositely charged chemical species on the resin and the compound to be separated. In order to create this attraction, the column is packed with strongly charged exchange groups opposite to those on the compound to be separated. The positively charged compounds are separated on a strong cation exchange resin with negatively charged groups. The nature of the cation exchange resin resembles small beads made of polystyrene, cross-linked to produce an insoluble network (Williams and Wilson, 1976; Varley et al, 1980). One of the groups commonly used on the cation exchanged resin is the sulphonic acid group, the hydrogen ion being capable of exchange with the positively charged ions from the solute.

In general, the physical characteristic of the resin as a whole are unaltered during the exchange process. For more than the past two decades (Moore and Stein, 1951; Hamilton, 1960) this principle has been used to separate several amino acids, on a sulphonated polystyrene resin. More recently, tissue and plasma catecholamine assays have been performed on sulphonated resin (Allenmark and Hedman, 1979); Hjemdahl et al, 1979). Ion exchange chromatography has been used extensively to separate charged chemical species. The advent

of the reverse phase and the reverse phase with ion pair chromatography offers more flexibility and hence better chromatography. It is now probably the method of choice for more polar compounds.

The mechanism of cation exchange chromatography involves an adsorption-desorption process (Williams and Wilson, 1976; Pryde and Gilbert, 1979). At a particular pH, the cation exchange is fully ionised and fully saturated with hydrogen ions from the mobile phase passing through the column. When a solution containing the groups to be exchanged for hydrogen ions eg catecholamines which are injected on to the column, some of the hydrogen ions will be displaced by the protonated catecholamines until a temporary equilibrium is achieved. To elute protonated catecholamines from the column, the hydrogen ion or other cation from the mobile phase must have greater affinity to the resin than the protonated catecholamines. Ionisation is pH dependent. At low pH the protonated catecholamines are fully ionised and are strongly bound to the ion exchange resin. Therefore, to elute them, the pH of the mobile phase must be higher than that of the solution injected on to the column containing catecholamines. The positive charge on the protonated catecholamines will be progressively neutralised by the mobile phase, causing it to be less bound to the resin and finally displaced and eluted from the column; at the same time the column is resaturated with the hydrogen ions for the subsequent ion exchange process.

2.2.14 Mobile Phase for Cation Exchange Chromatography

Keller et al (1976) described the method for tissue catecholamine measurements with electrochemical detector. Hallman et al (1978) and Hjemdahl et al (1979) used the method of Keller et al (1976) for plasma catecholamine measurements, they reported that the water they used was double distilled then finally redistilled from alkaline potassium permanganate. Hjemdahl et al (1979) also reported that an imperfect column may not separate the 3,4-dihydroxybenzylamine hydrobromide (DHBA) peak from the adrenaline peak, and therefore recommended the use of alpha methyldopamine in preference to DHBA as the internal standard.

The author modified the methods of Keller et al, 1976; Hallman et al, 1978; Hjemdahl et al, 1979 to avoid redistillation of water from alkaline potassium permanganate and permitted the use of 3,4-dihydroxybenzylamine hydrobromide (DHBA) as internal standard, even if the column was not perfect. The method was adopted in our laboratory for the measurements of catecholamines in plasma. The chemicals and the suppliers are listed in Appendix Two. The catecholamine levels obtained in our laboratory using the modified methods are similar to those obtained by Hallman and others also with those in the current catecholamine publications.

The mobile phase was acetate citrate buffer pH 4.8 (Appendix

Three). Providing the conditions of chromatography are optimised, sample separations are achieved in less than 15 minutes for noradrenaline, adrenaline and internal standard (DHBA).

2.2.15 Voltammetric Studies

Voltammetric studies are equivalent to scanning a solution in an ultraviolet spectrum in order to find a specific wavelength before using an ultraviolet detector. A low potential of 0.28V was chosen as a starting point for the voltammogram. One hundred microlitres of the working standard solution was injected on to the column. The standard solution was a mixture of noradrenaline, adrenaline, dopamine and 3,4-dihydroxybenzylamine hydrobromide, at a concentration of 1 pmole each (Appendix Six). The detector response was recorded at that potential. The potential was raised, the injection was repeated, and the detector response was recorded. The procedure was repeated until the potential was so high that the repeated injection produced only a small detector response (Table 2.1).

A plot of the peak height versus the applied potential, produced the four curves shown in Figure 2.1. Each point is the mean of two measurements. At the intermediate stage, the peak heights were rising with the increasing applied potential. The potential was controlling the kinetics of the heterogenous electron transfer from the solute to the

electrode surface. But when the plateau was reached, the kinetics were different. The peak heights were rising much more slowly with the increasing applied potential. This indicated that the peak heights were independent of the applied potential. Diffusion to the electrode surface was the rate determining factor; the current was proportional to the rate of the transport of the molecules per unit time and hence, proportional to the actual concentration of the solutes (Shoup et al, 1980).

A working potential was taken just before the plateau region. Such a potential offers maximum sensitivity and a low background current (Kissinger, 1977). Although each compound had its own limiting current, the purpose of the voltammograms was to provide a single potential, which would give the best signals for all the four compounds. In our laboratory, the working potential range was 0.50V to 0.54V, and the optimum was 0.52V.

Figure 2.2 shows clear separations of the chromatograms on aqueous catecholamine standards also, on extracted plasma. Good separation of chromatograms is essential for the calculation of plasma catecholamine levels, reproducibility and reliability of the results. The method for calculating plasma catecholamine levels is discussed in section 2.2.29.

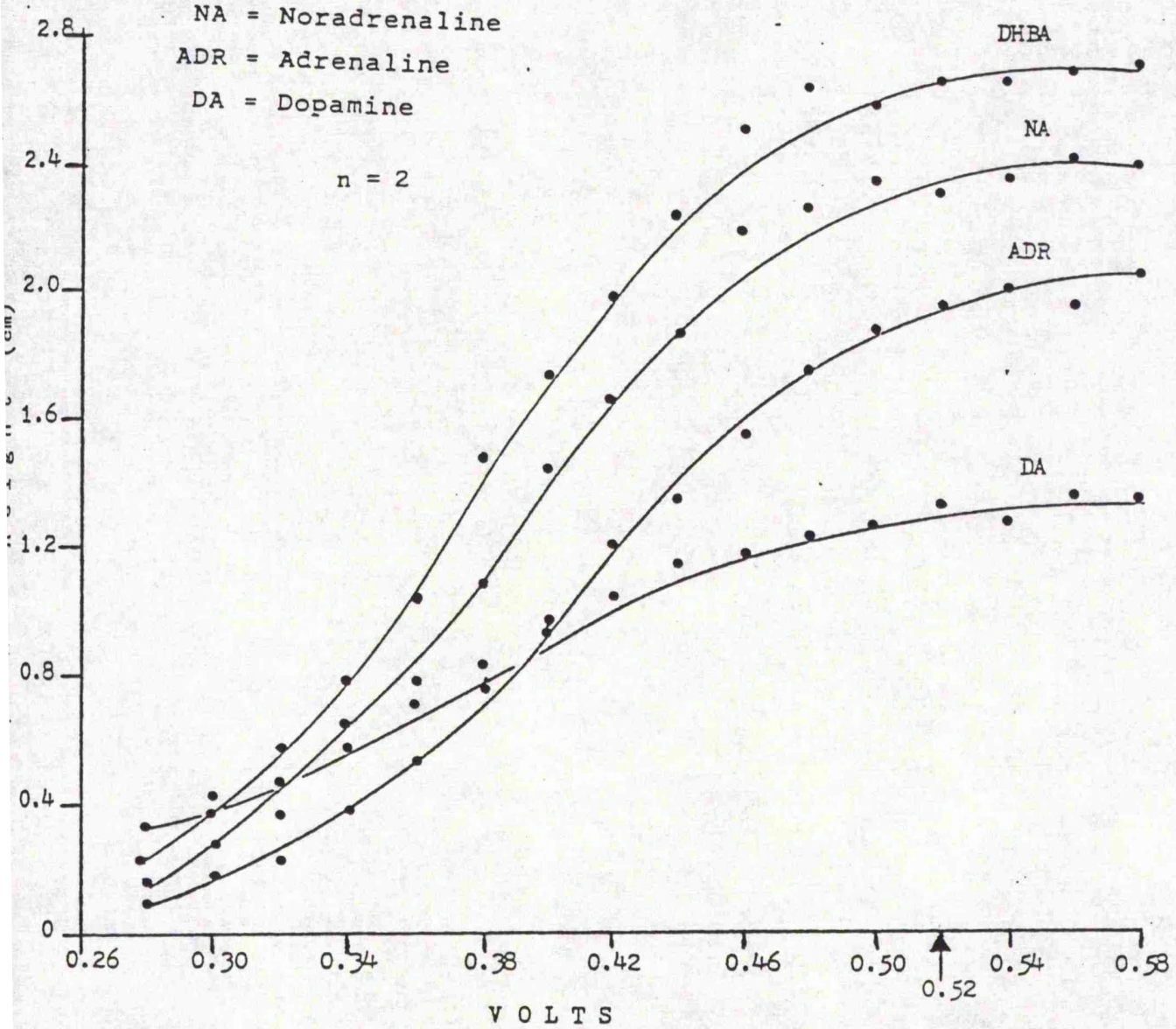


Figure 2.1 Study of voltammograms of aqueous standard containing one pmole of noradrenaline, adrenaline, 3,4-dihydroxybenzylamine hydrobromide and dopamine for selecting optimum voltage on amperometric controller.

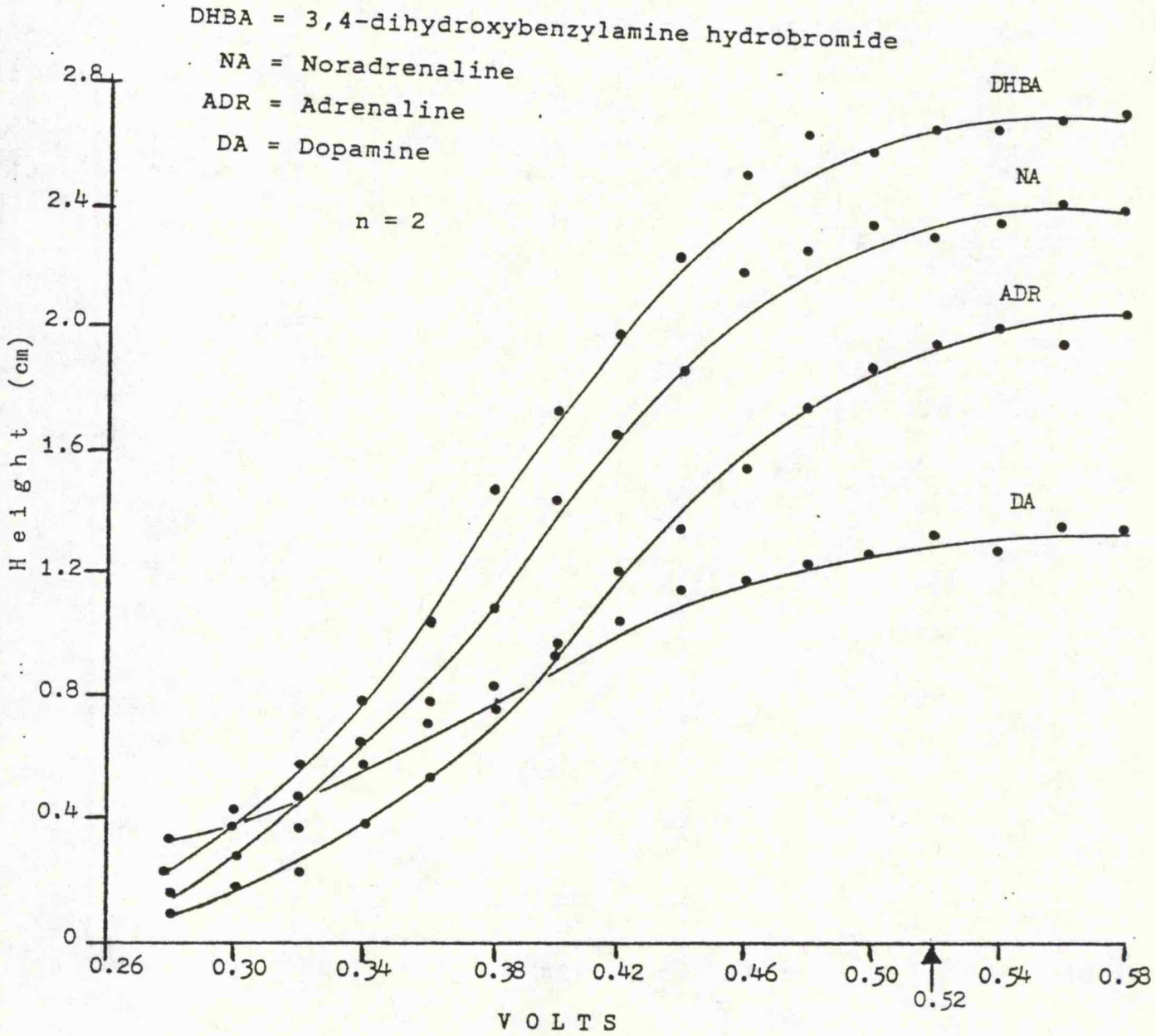


Figure 2.1 Study of voltammograms of aqueous standard containing one pmole of noradrenaline, adrenaline, 3,4-dihydroxybenzylamine hydrobromide and dopamine for selecting optimum voltage on amperometric controller.

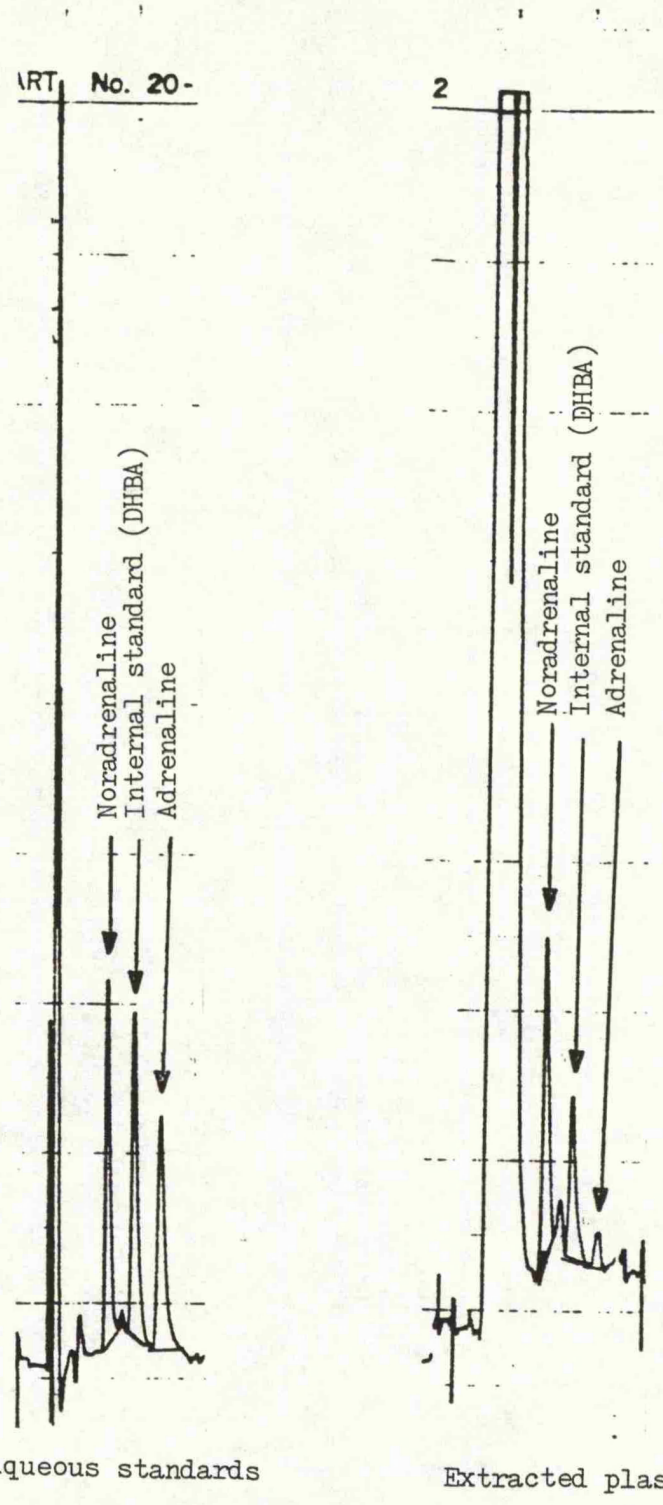


Figure 2.2 Chromatograms of aqueous catecholamine standard and extracted plasma sample on nucleosil 10 SA column, demonstrating good separations of the peaks, which are easy to measure when calculating catecholamine levels on extracted plasma.

2.2.16 Reverse Phase Chromatography with Electrochemical detection

The reverse phase chromatography has been described for plasma catecholamine measurements (Moyer and Jiang, 1978; Hansson et al, 1979; Davis et al, 1981).

The principle of the reverse phase chromatography is that the non-polar mobile phase and the polar stationary phase in the normal phase chromatography are replaced by a polar mobile phase and a non-polar stationary phase. Thus, the more polar compounds are eluted first. The technique is particularly useful for polar compounds (Knox et al, 1977), since the aqueous mobile phase is compatible with biochemical samples.

Another advantage of using an aqueous mobile phase, is to allow the addition of a secondary solute such as ion pairing agents. These agents are used with highly polar ionisable compounds, which tend to elute with, or very close to the solvent front. The addition of an ion pairing agent to the mobile phase delays their elution and moves them away from the solvent front resulting in better separation and thus better chromatography.

Ion pair partition chromatography was developed as an alternative to ion exchange chromatography (Pryde and Gilbert, 1979), for the separation of ionisable compounds. Chromatographic parameters can be determined by using different concentrations of ion pair in the mobile phase

(Moyer and Jiang, 1978; Knox and Jurand, 1976; Knox and Laid, 1976) for selectivity effects. The selectivity has also been shown to depend on the particle size. As the particle size of the packing material reduces from 10 to 5 microns, the peaks become sharper and narrower and, in consequence, the resolution and the sensitivity are improved (Hamilton, 1966).

The exact mechanism by which the retention of an ionised solute is affected by ion pairing materials on a column has yet to be fully resolved (Pryde and Gilbert, 1979).

The mechanism may well be the formatiom of the ion pairs in the mobile phase. The protonated catecholamines which are injected on to the column react with an ion pairing agent, eg 1-heptanesulphonic acid sodium salt in the mobile phase to form ion pairs.

Since there are two phases, it is reasonable to speculate that there would be some interfacial interactions. The interaction would be between the hydrophobic layer of the stationary phase and the solutes from the hydrophilic layer of the mobile phase. The interactions would increase as the amount of ion pairing agent is increased in the mobile phase. These activities would delay the column equilibrium, which in turn would increase the retention time. In practice, the retention time increases as the amount of ion pairing agent in the mobile phase is increased. Also, interfacial interactions would be less when a small amount of ion pairing agent is added in the mobile phase, thereby producing less

reaction time and a shorter time for the column to reach equilibrium, in turn shortening the retention time. In practice, as smaller amounts of ion pairing agent are added in the mobile phase, the shorter is the retention time of the solutes.

2.2.17 Mobile Phase for Microbondapak C18 Column

Moyer and Jiang (1978) recommended the use of 0.07M phosphate buffer pH 5.8 to give the best results; however, when this was tried in our laboratory, unsatisfactory chromatograms were obtained (Figure 2.3) with unidentified peaks interfering with noradrenaline and DHBA. Good chromatograms (Figure 2.4) showing resolved peaks using 0.1M phosphate buffer, the pH adjusted to 5.2 with sodium hydroxide and orthophosphoric acid (Appendix Four).

The mobile phase for Spherisorb 5ODS2 was that which the author adapted for the measurements of plasma catecholamines (Appendix Five); chromatograms are shown in figure 2.5. Good and clear catecholamine chromatograms were achieved.

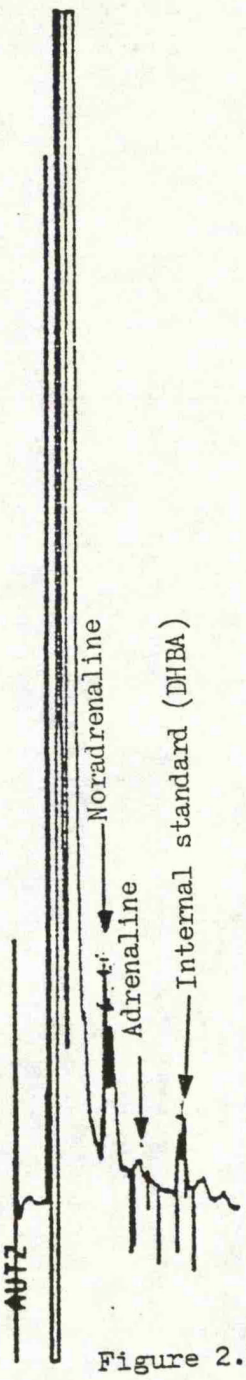


Figure 2.3

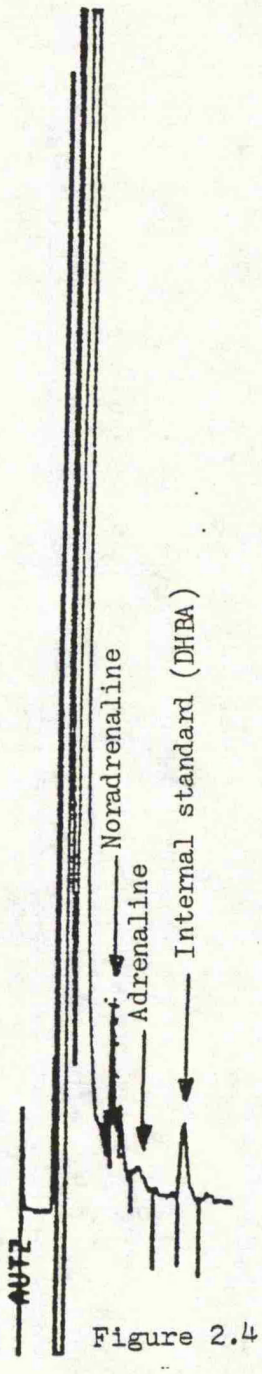


Figure 2.4

Figure 2.3 Chromatograms of catecholamines on extracted human plasma with an internal standard demonstrating unresolved peaks interfering with noradrenaline and internal standard peaks on microbondapak C18 column.

Figure 2.4 Chromatograms of resolved catecholamine peaks on the same plasma sample under optimised chromatographic conditions on microbondapak C18 column.

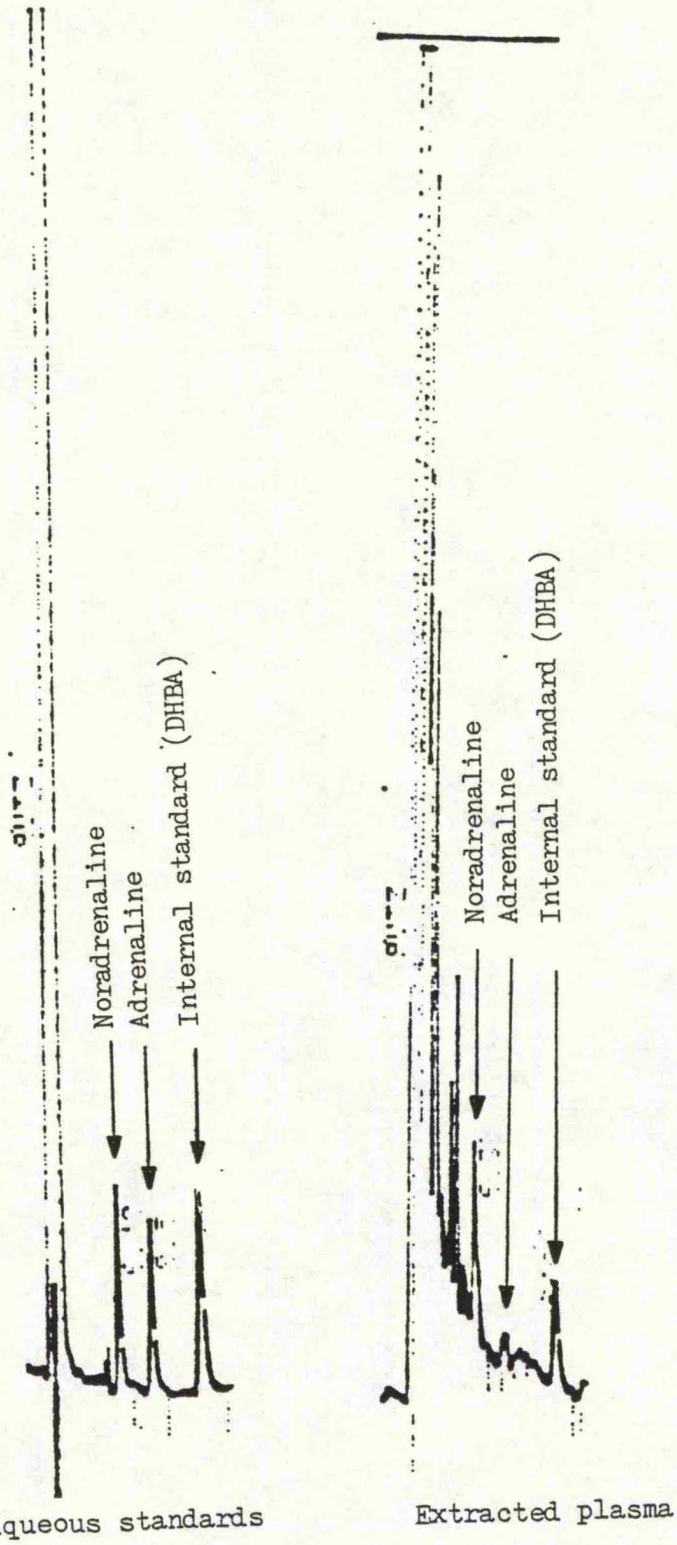


Figure 2.5 Chromatograms of aqueous catecholamine standards and extracted plasma sample on spherisorb 5ODS2 column, demonstrating good separations of the peaks, which are easy to measure when calculating catecholamine levels on extracted plasma.

2.2.18 Assessment of the Technique

The linearity of the method over the analytical range must be investigated. Some methods do not use multipoint standards for calibration curve, but depend on a single point calibration. Although a single point calibration is permissible (Buttner et al, 1976), linearity should always be tested and should never be assumed. The linearity is assessed by analysing serial standard dilutions from a single standard or a mixture of standard solutions. The peak heights are measured and plotted against their corresponding concentrations (Figures 2.6a-2.6c and Table 2.2). Straight lines passing through the origin were obtained. The regression analysis was performed to compare the experimental points and the line of identity.

2.2.19 Results

There were good correlation between the experimental points and the line of identity for the internal standard (DHBA); noradrenaline and adrenaline: ($y= 1.01x + -2.77E-03$; $r= 1.0$; $p<0.001$); ($y= 1.01x + 0.04$; $r=1.0$; $p<0.001$) and ($y= 1.01x + 0.02$; $r=1.0$; $p<0.001$) respectively.

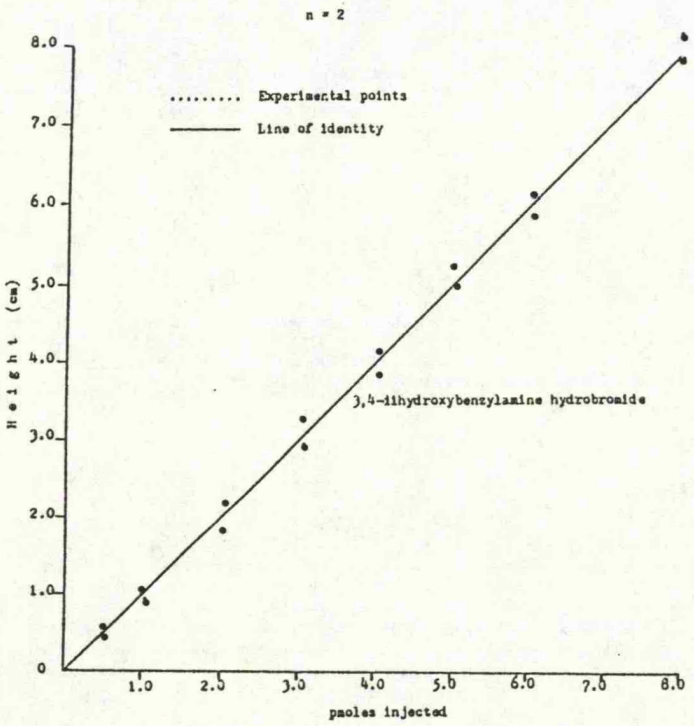


Figure 2.6(a) Aqueous 3,4-dihydroxybenzylamine hydrobromide standard for calibration curve, the X-axis showing concentrations injected and Y-axis the height (cm) measured. The solid line is the line of identity.

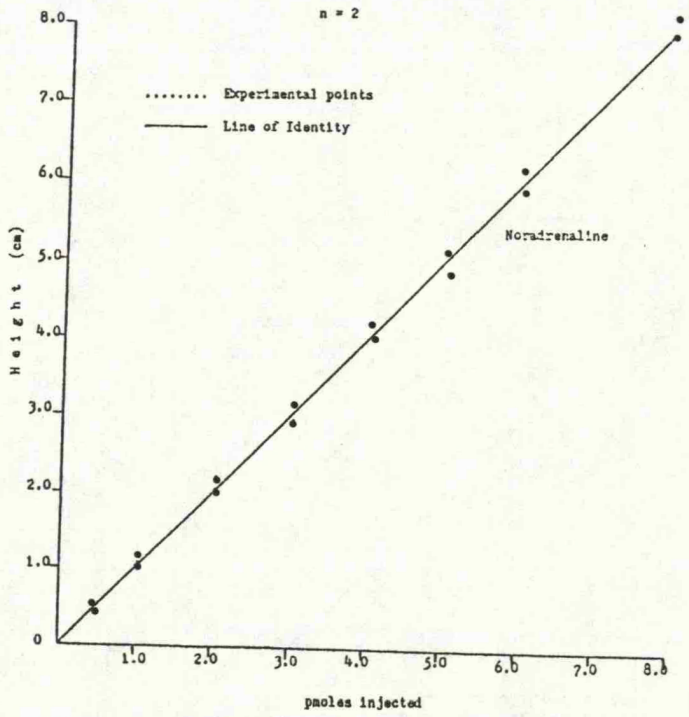


Figure 2.6(b) Aqueous noradrenaline standard for calibration curve, the X-axis showing concentrations injected and Y-axis the height (cm) measured. The solid line is the line of identity.

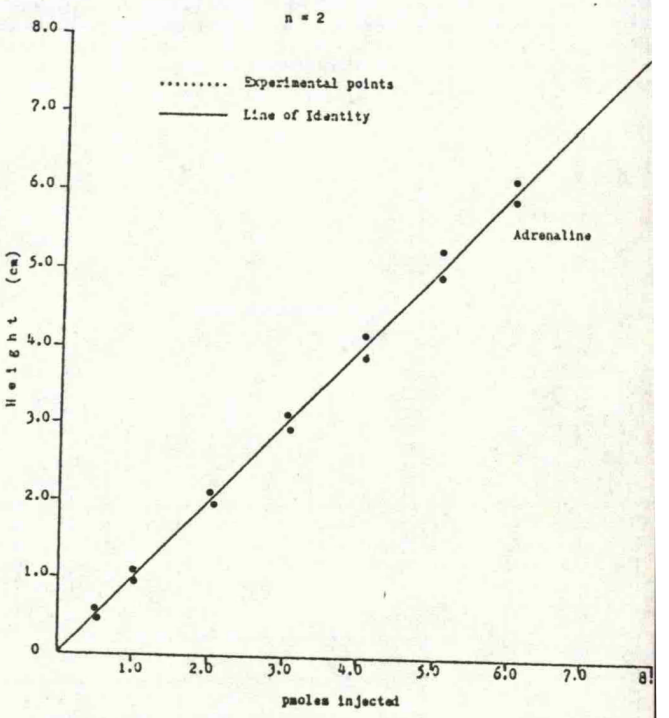


Figure 2.6(c) Aqueous adrenaline standard for calibration curve, the X-axis showing concentrations injected and Y-axis the height (cm) measured. The solid line is the line of identity.

2.2.20 Trizma Buffer

Trizma buffer for adjusting plasma pH during plasma extraction was prepared as described in the Appendix Seven.

2.2.21 Purification of Alumina

Woelm alumina neutral activity grade 1 was prepared following the method of Anton and Sayre (1962) (Appendix Eight).

2.2.22 Validation of the Technique

Assessment of the technique (section 2.2.18) showed that aqueous standards produced linear calibration curves. Extracted plasma must also show linearity over the working range for the method to be used as analytical technique.

The method of plasma extraction was similar to those of Hallman et al, 1978 and Hjemdahl et al, 1979 except that sodium metabisulphite was omitted both during collection of blood samples (Appendix Nine) and during purification of plasma or serum (Appendix Ten). The reason for omitting sodium metabisulphite is discussed in chapter three; sections 3.3.10-3.3.14.

2.2.23 The Recovery of Catecholamines on Cation Exchange Nucleosil 10 SA Column

Method: The object of recovery experiments was to validate a new analytical method, and also to compare the efficacy of

columns composed of different packing materials. Exogenous catecholamines were added to the samples and the samples which also contained internal standard were extracted. The internal standard and the standards whose concentrations were 1.0pmol/ml were used to calculate the endogenous and exogenous catecholamine levels.

2.2.24 Results

The results of this experiment are summarised in Tables 2.3 and 2.4 for plasma noradrenaline and adrenaline respectively. Figure 2.7 shows the total catecholamines recovered from individual samples. Figures 2.8 and 2.9 show the recovery of noradrenaline and adrenaline respectively. The regression analysis was done to compare the experimental points and the line of identity. The results show good correlation between the experimental points and the line of identity for noradrenaline and adrenaline : ($y= 1.01x + 5.99E-03$; $r= 1.0$; $p<0.001$) and ($y= 0.98x + 0.05$; $r= 1.0$; $p<0.001$) respectively. The experiment was repeated and the results were for noradrenaline: ($y= 0.98x + 0.04$; $r=1.0$; $p<0.001$) and for adrenaline: ($y= 0.99x + 5.14E-03$; $r=1.0$; $p<0.001$).

The endogenous catecholamine levels are comparable to those from the intercepts (bracketed). In the first experiment the noradrenaline level was 1.96 (2.04) pmol/ml; and the adrenaline level was 0.24 (0.28) pmol/ml. In the second experiment the noradrenaline level was 2.44 (2.48) pmol/ml;

and the adrenaline level was 0.21 (0.26) pmol/ml.

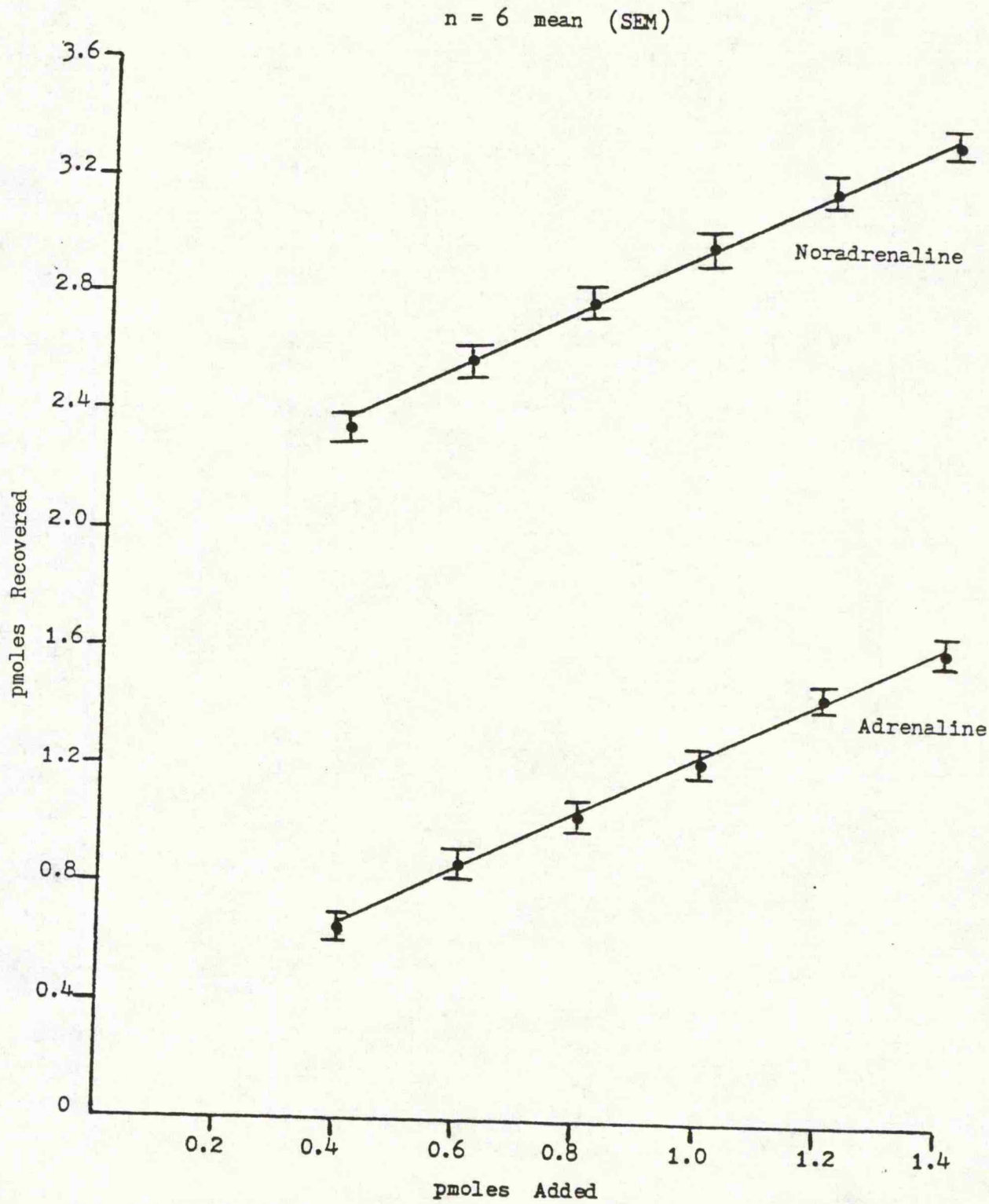


Figure 2.7 Recovery of added catecholamines in human plasma analysed on nucleosil 10 SA column. The X-axis shows the concentrations expected after the addition of catecholamines and Y-axis the amount recovered after correction for incomplete recovery of internal standard D.H.B.A.

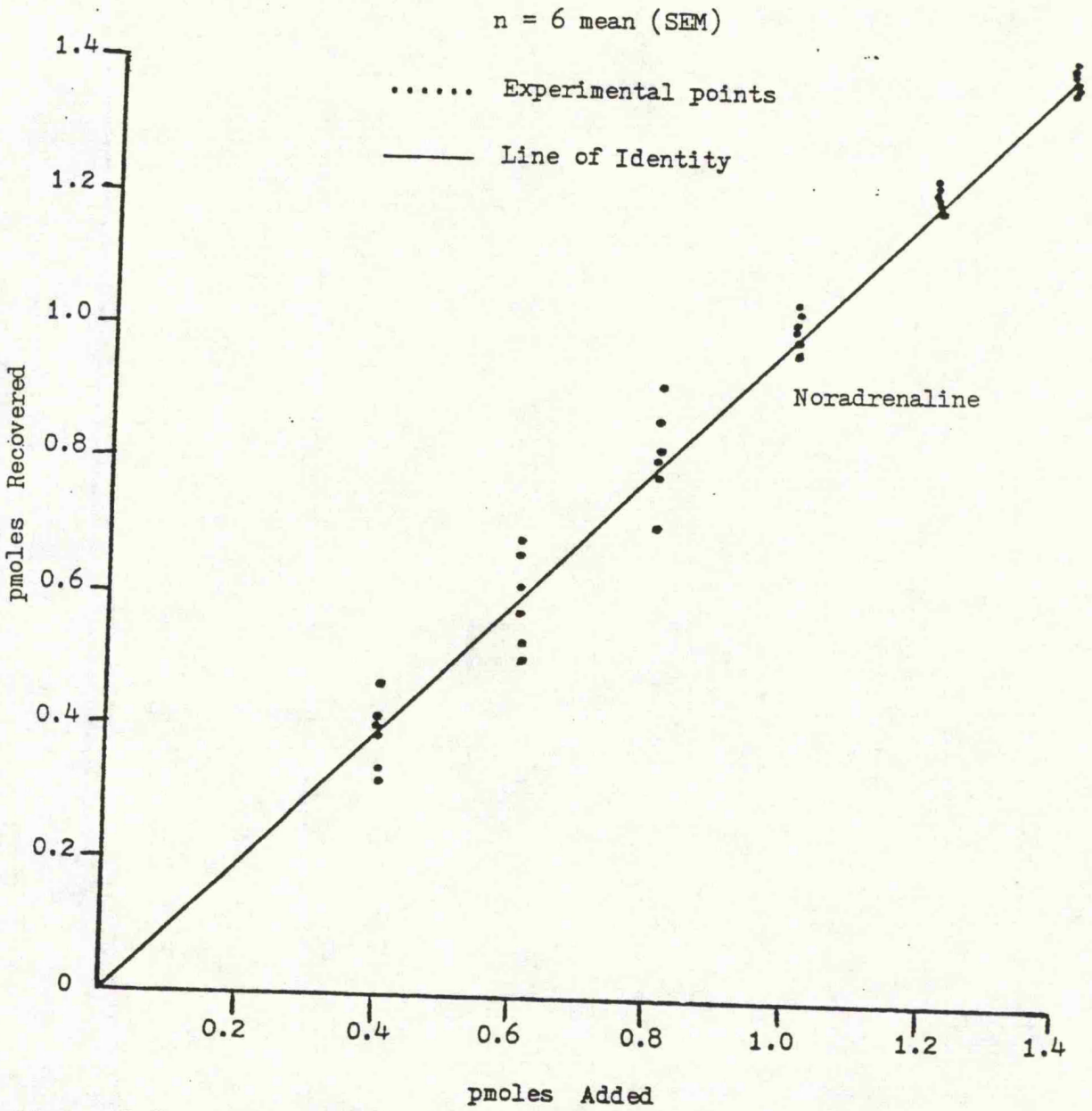


Figure 2.8 Recovery of added noradrenaline in human plasma analysed on nucleosil 10 SA column. X-axis shows the concentrations added and Y-axis the amount recovered after correction for incomplete recovery of internal standard D.H.B.A.

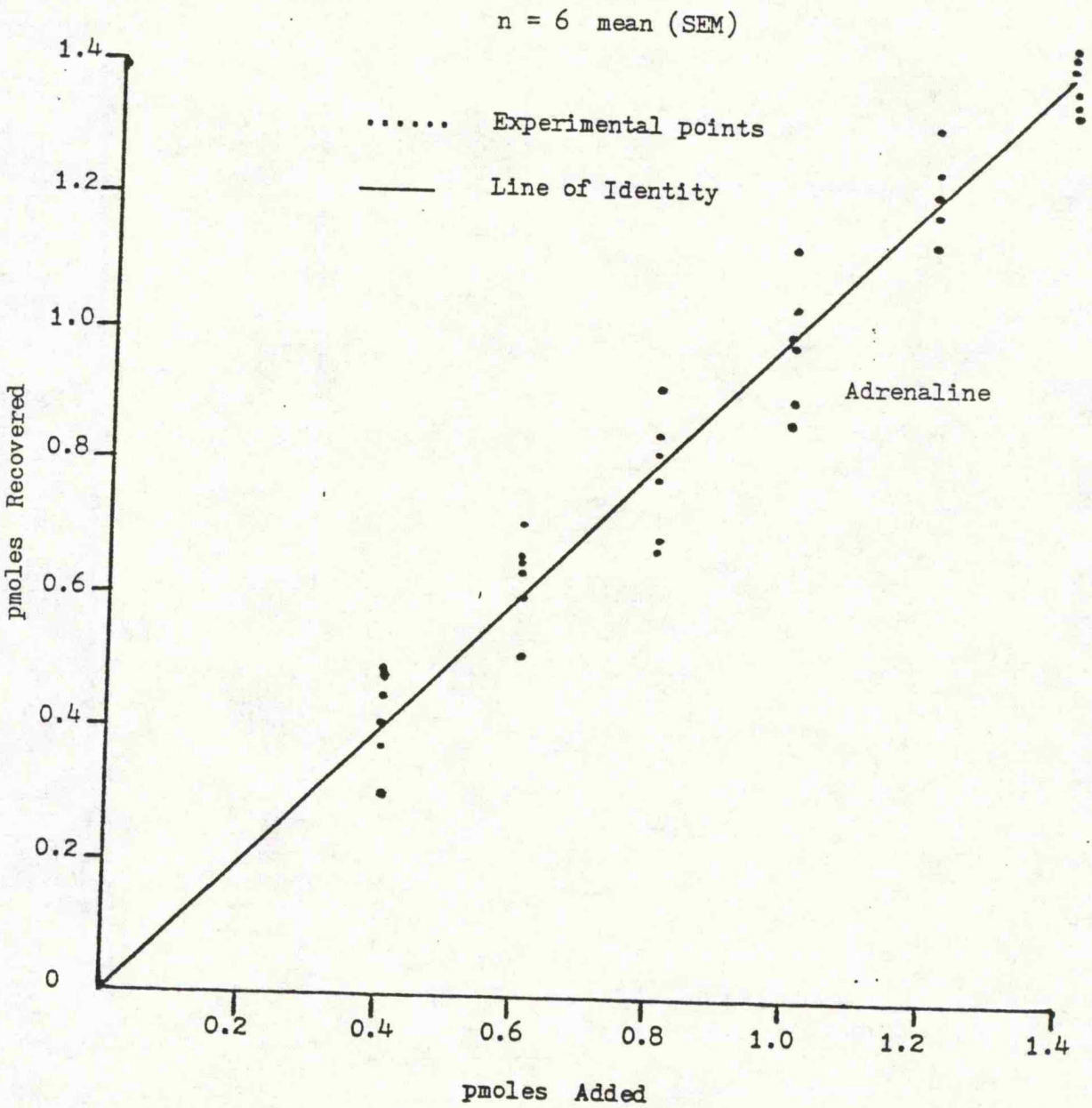


Figure 2.9 Recovery of added adrenaline in human plasma analysed on nucleosil 10 SA column. X-axis shows the concentrations added and Y-axis the amount recovered after correction for incomplete recovery of internal standard D.H.B.A.

2.2.25 The Recovery of Catecholamines on Microbondapak C18 Column

Method: The experimental procedures were similar to those in the previous section, the differences being in the type of column and choice of the mobile phase. In this section, the alkyl carbon chains were chemically bonded to silica to produce a non-polar stationary phase, which can be used with polar mobile phase.

2.2.26 Results

The results in Tables 2.5 and 2.6 are for noradrenaline and adrenaline respectively. Figure 2.10 shows the total plasma catecholamines recovered, whereas Figures 2.11 and 2.12 illustrate noradrenaline and adrenaline recovered. The regression analysis was done to compare the experimental points and the line of identity. The results show good correlation between the experimental points and the line of identity for noradrenaline and adrenaline: ($y= 0.98x + 0.02$; $r=1.0$; $p<0.001$) and ($y= 1.00x + -8.99E-03$; $r=1.0$; $p<0.001$) respectively. The experiment was repeated and the results for noradrenaline were: ($y= 1.00x + 6.00E-03$; $r= 1.0$; $p<0.001$) and for adrenaline were: ($y= 0.97x + 0.02$; $r= 1.0$; $p<0.001$).

The endogenous catecholamine levels are comparable to those from the intercepts (bracketed). In the first experiment the noradrenaline level was 2.52 (2.58) pmol/ml and the adrenaline level was 0.23 (0.20) pmol/ml. In the second

experiment the noradrenaline level was 2.10 (2.18) pmol/ml and the adrenaline level was 0.19 (0.24) pmol/ml.

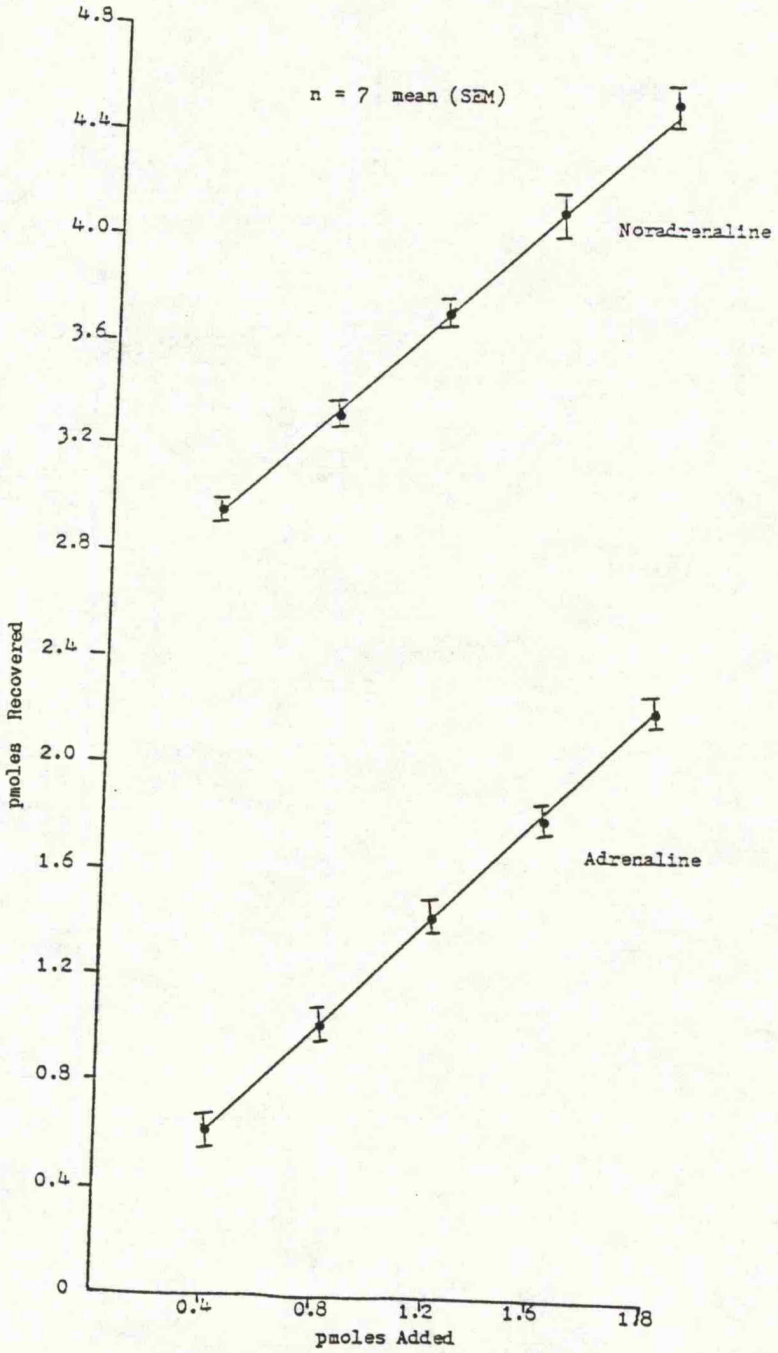


Figure 2.10 Recovery of added catecholamines in human plasma analysed on microbondapak C18 column. X-axis shows the concentrations expected after the addition of catecholamines and Y-axis the amount recovered after correction for incomplete recovery of internal standard D.H.B.A.

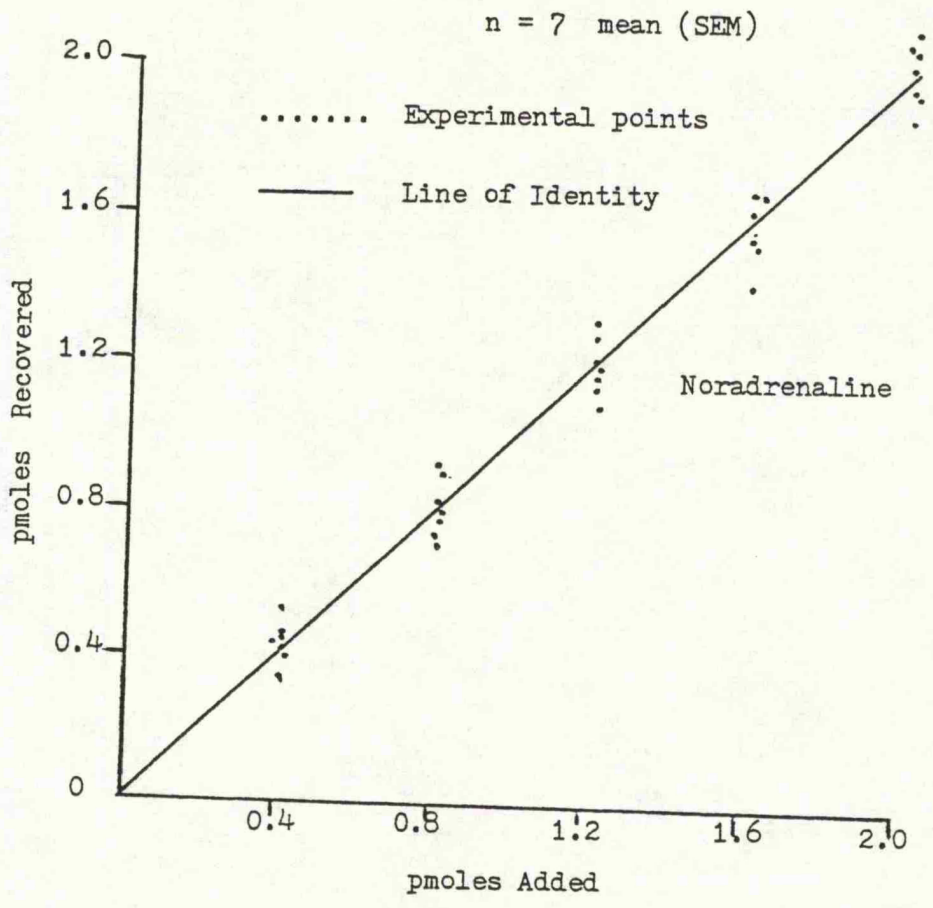


Figure 2.11 Recovery of added noradrenaline in human plasma analysed on microbondapak C18 column. X-axis shows the concentrations added and Y-axis the amount recovered after correction for incomplete recovery of internal standard D.H.B.A.

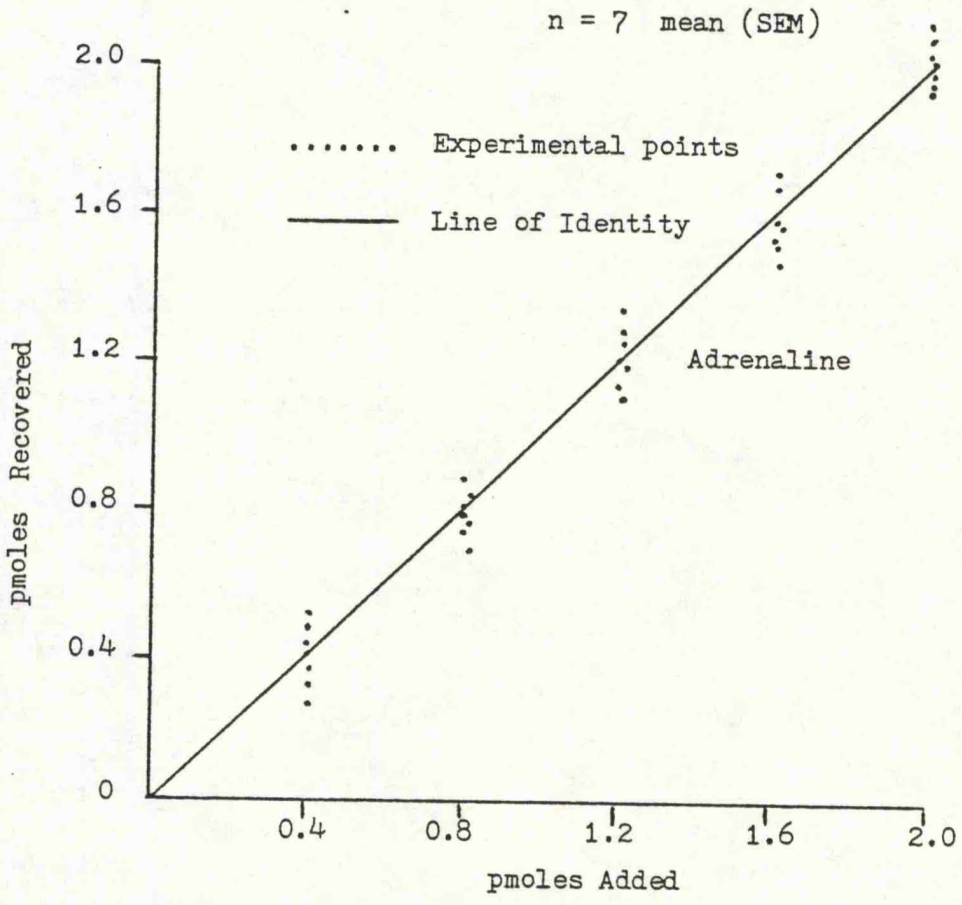


Figure 2.12 Recovery of added adrenaline in human plasma analysed on microbondapak C18 column. X-axis shows the concentrations added and Y-axis the amount recovered after correction for incomplete recovery of internal standard D.H.B.A.

2.2.27 The Recovery of Catecholamines on Spherisorb 5ODS2 Column

Method: The experimental procedures were identical to the previous ones. The column used in this experiment was a reverse phase spherisorb 5ODS2, composed of 12 per cent carbon, suitable to separate polar compounds. The major problems from commercially prepared chemically bonded phases are the residual sinalol groups (Si-OH) arising either from hydrolysis of Si-Cl bonds in the presence of moisture or from non-reacted sinalol groups. These affect the specificity of the column, producing poor separations. In this experiment, a new mobile phase (Appendix Five) adapted by the author was used.

2.2.28 Results

The summary of the results of this experiment is shown in Table 2.7 for plasma noradrenaline and Table 2.8 for plasma adrenaline. Figure 2.13 shows the total plasma catecholamine recovered, whereas Figures 2.14 and 2.15 show the recoveries of exogenous noradrenaline and adrenaline respectively. The regression analysis between the experimental points and the line of identity was performed. The results show good correlations between the experimental points and the line of identity for noradrenaline and adrenaline: ($y= 1.00x + -2.99E-03$; $r= 1.0$; $p<0.001$) and ($y= 0.97x + 0.02$; $r= 1.0$; $p<0.001$) respectively.

The endogenous catecholamine levels are comparable to those from the intercepts (bracketed). In the first experiment the noradrenaline level was 1.94 (2.00) pmol/ml and the adrenaline level was 0.25 (0.22) pmol/ml. In the second experiment the noradrenaline level was 1.83 (1.90) pmol/ml and the adrenaline level was 0.27 (0.30) pmol/ml.

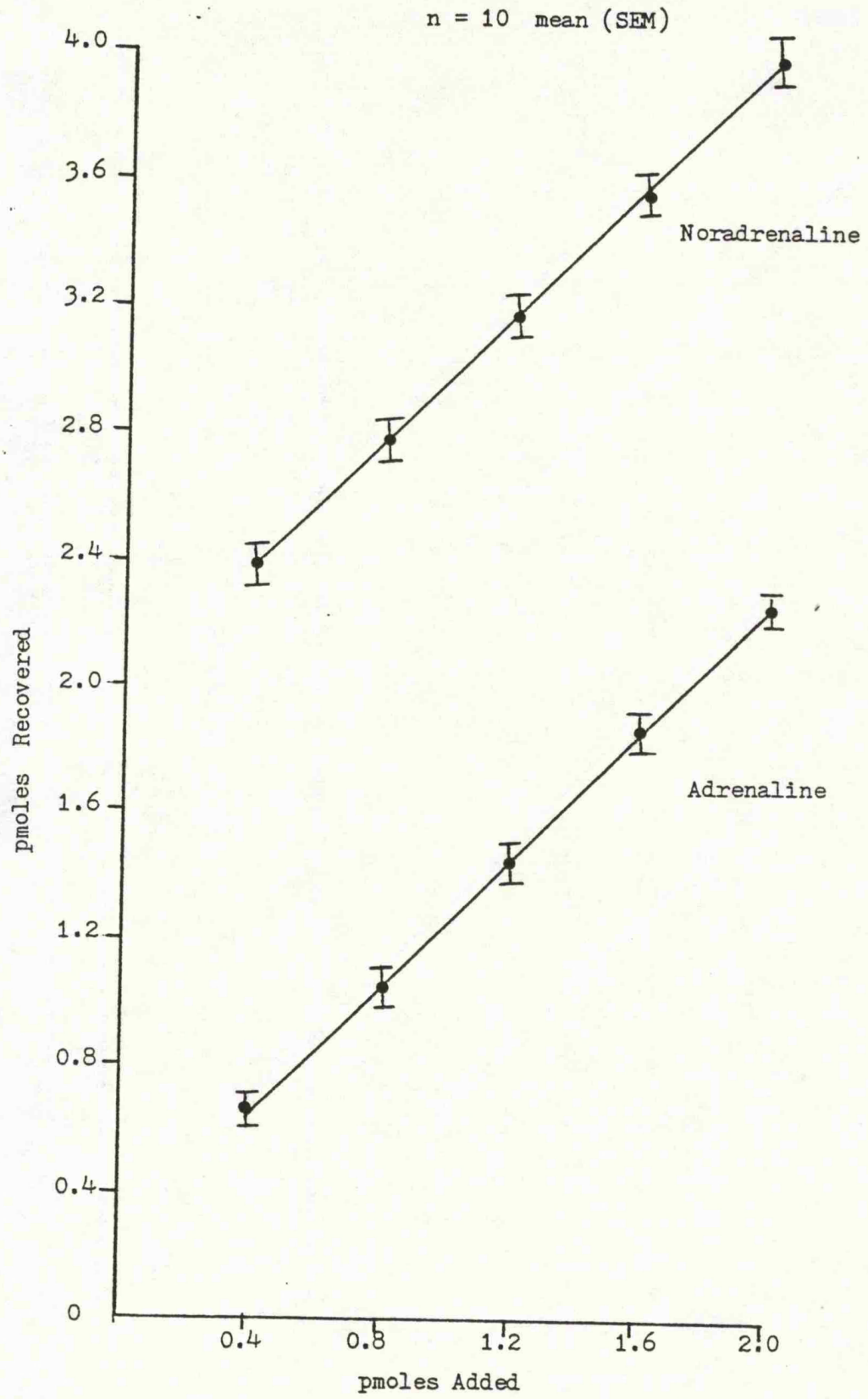


Figure 2.13 Recovery of added catecholamines in human plasma analysed on spherisorb 5ODS2 column. X-axis shows the concentrations expected after the addition of catecholamines and Y-axis the amount recovered after correction for incomplete recovery of internal standard D.H.B.A.

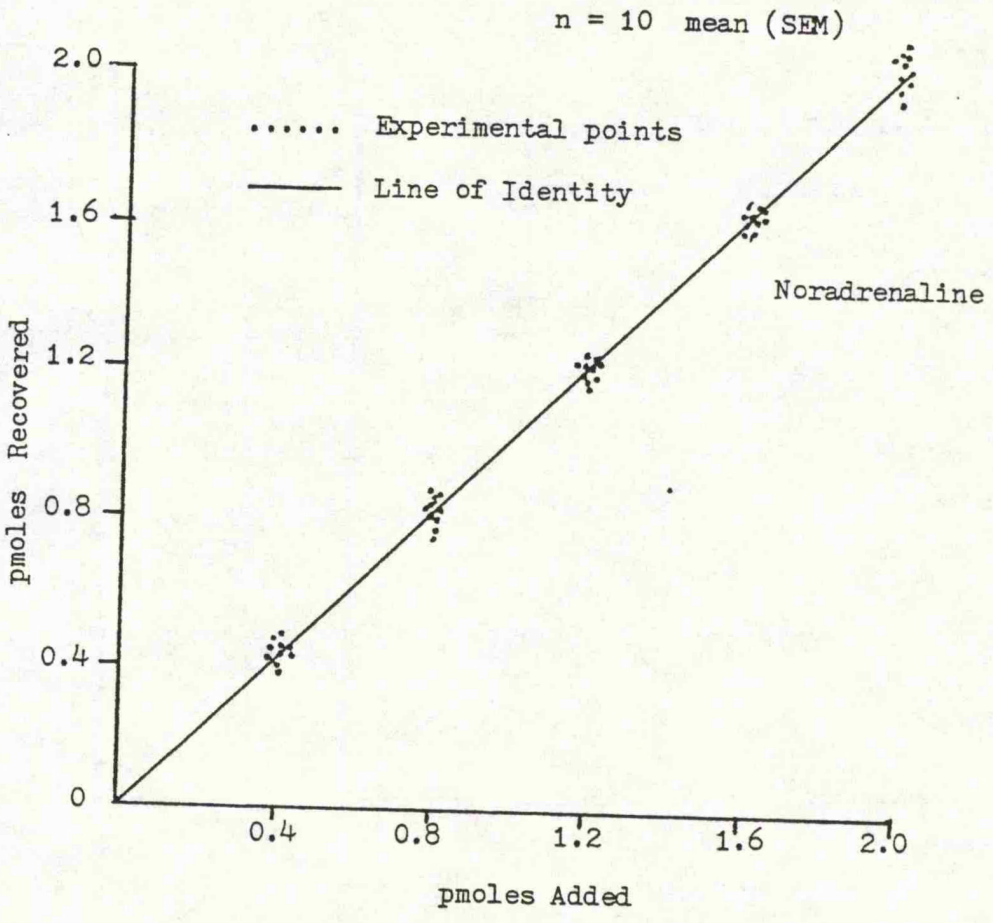


Figure 2.14 Recovery of added noradrenaline in human plasma analysed on spherisorb 5ODS2 column. The X-axis shows the concentrations added and Y-axis the amount recovered after correction for incomplete recovery of internal standard 3,4-dihydroxybenzylamine hydrobromide.

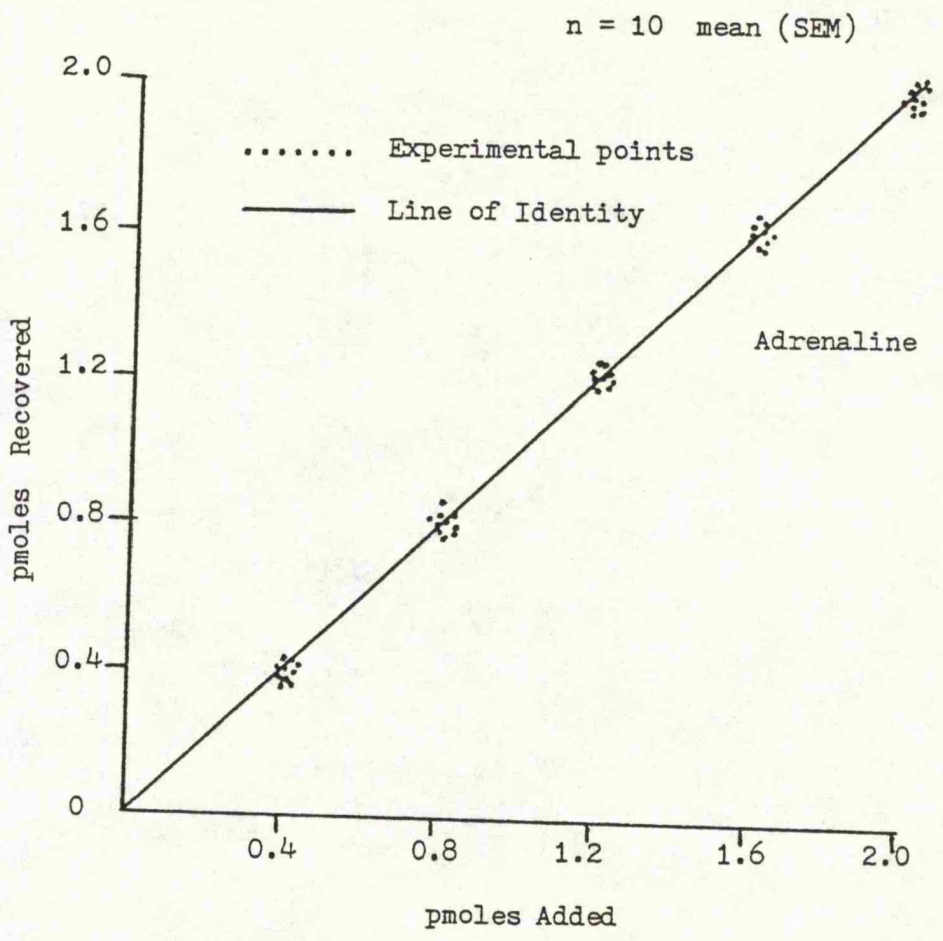


Figure 2.15 Recovery of added adrenaline in human plasma analysed on spherisorb 5ODS2 column. The X-axis shows the concentrations added and Y-axis the amount recovered after correction for incomplete recovery of internal standard 3,4-dihydroxybenzylamine hydrobromide.

2.2.29 Discussion

Voltammetric studies demonstrated that the working potential range for catecholamine measurements was 0.50V-0.54V taken just before the plateau. The optimum was 0.52V and the sensitivity of 1nA or 2nA and the integrator at 4 (302 Laboratory Data Control). Those settings were found to be adequate for catecholamine measurements, produced low background noise and a stable baseline suitable for good chromatography and reproducibility of the chromatograms. Higher potential can be used to increase the sensitivity of the cell, but the baseline noise is also increased, producing poor baseline accompanied with poor chromatograms. Eriksson and Persson (1982) used 0.70V and Hjemdahl et al (1979) used 0.72V both studies showed unsteady baseline. Eriksson and Persson had to use extra damping facility to reduce background noise level.

High pressure liquid chromatography with electrochemical detection is relatively cheaper and simpler to use than the other analytical techniques (sections 2.2.2-2.2.6). The three columns packed with different packing materials had their performance compared for the measurements of plasma catecholamine levels. The result of that investigation showed that there was no significant difference between plasma catecholamine levels obtained in the first and the second experiments on those columns. All produced good clear separations of catecholamine peaks (Figures 2.2; 2.4; 2.5).

Although it is relatively easy to get good chromatograms on aqueous catecholamine standards, it is not easy to achieve good chromatograms of catecholamines on extracted plasma, because their concentrations are low in plasma. Also they are not easily oxidisable at the electrode surface compared with other electroactive compounds in plasma. Hence, much time must be spent to optimise the chromatographic conditions to achieve clear and good chromatograms (Figure 2.4). The importance of this is demonstrated during the calculation of extracted plasma or serum catecholamine levels.

When external standards are used for the calibration (Hallman et al, 1978; Hjemdahl et al, 1979) the percentage recovery of the internal standard; in this case DHBA must be calculated, then it is used for the final calculation. The catecholamine peaks in Figures 2.2 or 2.5 are narrow and clear. The peak height can be measured accurately from the baseline in both aqueous standard and extracted plasma to calculate plasma catecholamine levels. Calculation can either be by hand or by integrator. The height of the catecholamine peaks and the internal standard are measured from the baseline (Figures 2.2 or 2.5). Thus, to calculate the recovery of the internal standard (DHBA), the height of the DHBA in the extracted sample is divided by the height of the DHBA in the aqueous standard, and multiplied by 100 to give the percentage recovery of the DHBA. The concentration of each catecholamine standard and internal standard for calculating plasma catecholamine levels in this thesis is 1.0pmole, and the

volume of plasma for each analysis is 1.0ml, hence, calculation of plasma catecholamine levels is simplified (the factor for standard concentration is 1). Plasma catecholamine levels are calculated in the same way, but the sample values are corrected for the incomplete recovery of the DHBA used as internal standard (IS) as shown below. For example the concentration of plasma noradrenaline (NA) in the extracted sample is calculated as:

$$\text{plasma NA} = \frac{\text{Height of NA in sample}}{\text{Height of NA in std}} \times \frac{100}{\%\text{Recovery(IS)}} = \text{pmol/ml}$$

Plasma adrenaline is also calculated in the same manner as above.

After the column has been used for some time, the efficiency drops. The column can be regenerated. The cation exchange column can be regenerated by washing with acidic mobile phase, the pH being around 3. The reverse phase column (microbondapak C18 or spherisorb 5ODS2) can be regenerated by washing with methanol or acetonitrile. The regeneration procedure can be repeated until the column fails to perform.

2.2.30 Conclusion

High pressure liquid chromatography with electrochemical detector, is simpler to use than other analytical techniques. The system with autosampler can analyse more than sixty samples a day. The optimisation of the method is important to

achieve good and clean chromatograms. Catecholamine levels obtained in our laboratory using the author's methods are similar to the current published levels.

The three columns which were compared, were similar in their performance on plasma catecholamine measurements.

Table 2.1 Study of voltammograms in aqueous standard containing 1pmol of noradrenaline, adrenaline, D.H.B.A. and dopamine n=2

Volt	Noradrenaline Peak Height(cm)	Adrenaline Peak Height(cm)	D.H.B.A. Peak Height(cm)	Dopamine Peak Height(cm)
0.28	0.18	0.13	0.23	0.35
0.30	0.28	0.18	0.43	0.43
0.32	0.38	0.23	0.48	0.48
0.34	0.58	0.35	0.78	0.63
0.36	0.78	0.53	1.03	0.78
0.38	1.08	0.78	1.48	0.83
0.40	1.43	0.98	1.73	0.95
0.42	1.65	1.20	1.98	1.03
0.44	1.85	1.35	2.23	1.15
0.46	2.18	1.55	2.50	1.18
0.48	2.25	1.75	2.63	1.23
0.50	2.35	1.88	2.58	1.25
0.52	2.30	1.95	2.65	1.33
0.54	2.35	2.00	2.65	1.28
0.56	2.40	1.95	2.65	1.35
0.58	2.38	2.03	2.70	1.35

D.H.B.A. = 3,4-dihydroxybenzylamine hydrobromide.

Table 2.2 External standard solution containing D.H.B.A., noradrenaline and adrenaline for calibration curve

n=2

Standard Concentrations pmol/ml	D.H.B.A. Peak Height (cm)	Noradrenaline Peak Height (cm)	Adrenaline Peak Height (cm)
0.50	0.48	0.48	0.50
1.00	0.95	1.13	1.05
2.00	2.05	2.13	2.03
3.00	3.13	3.08	3.05
4.00	4.03	4.13	4.08
5.00	5.13	5.05	5.15
6.00	6.05	6.10	6.08
8.00	8.08	8.13	8.10

Table 2.3 Recovery of added catecholamines in human plasma on nucleosil 10 SA column n=6 mean (SEM)

Noradrenaline

Standard Concentrations Added pmol/ml	Amount Recovered pmol/ml
none	1.96 (0.07)
0.40	2.35 (0.04)
0.60	2.58 (0.04)
0.80	2.79 (0.05)
1.00	2.97 (0.05)
1.20	3.19 (0.05)
1.40	3.36 (0.05)

Table 2.4 Recovery of added catecholamines in human plasma on nucleosil 10 SA column n=6 mean (SEM)

Adrenaline

Standard Concentrations Added pmol/ml	Amount Recovered pmol/ml
none	0.24 (0.03)
0.40	0.65 (0.04)
0.60	0.87 (0.04)
0.80	1.04 (0.05)
1.00	1.23 (0.04)
1.20	1.46 (0.04)
1.40	1.63 (0.04)

Table 2.5 Recovery of added catecholamines in human plasma on microbondapak C18 column n=7 mean (SEM)

Noradrenaline

Standard Concentrations Added pmol/ml	Amount Recovered pmol/ml
none	2.52 (0.04)
0.40	2.95 (0.04)
0.80	3.33 (0.04)
1.20	3.72 (0.04)
1.60	4.10 (0.07)
2.00	4.54 (0.07)

Table 2.6 Recovery of added catecholamines in human plasma on microbondapak C18 column n=7 mean (SEM)

Adrenaline

Standard Concentrations Added pmol/ml	Amount Recovered pmol/ml
none	0.23 (0.05)
0.40	0.62 (0.05)
0.80	1.02 (0.05)
1.20	1.44 (0.05)
1.60	1.81 (0.05)
2.00	2.23 (0.05)

Table 2.7 Recovery of added catecholamines in human plasma on Spherisorb 5ODS2 column n=10 mean (SEM)

Noradrenaline

Standard Concentrations Added pmol/ml	Amount Recovered pmol/ml
none	1.94 (0.04)
0.40	2.37 (0.06)
0.80	2.77 (0.06)
1.20	3.17 (0.06)
1.60	3.56 (0.06)
2.00	3.99 (0.06)

Table 2.8 Recovery of added catecholamines in human plasma on spherisorb 5ODS2 column n=10 mean (SEM)

Adrenaline

Standard Concentrations Added pmol/ml	Amount Recovered pmol/ml
none	0.25 (0.05)
0.40	0.64 (0.05)
0.80	1.04 (0.05)
1.20	1.44 (0.05)
1.60	1.86 (0.06)
2.00	2.24 (0.05)

CHAPTER THREE

3.3 FACTORS AFFECTING CATECHOLAMINE MEASUREMENTS

3.3.1 Blood Collection for Catecholamine Measurements

It has been reported that anti-oxidants should be added to blood during blood collection and the plasma should be separated from red cells as quickly as possible then frozen in order to prevent catecholamine degradation (Carruthers et al, 1970; Peuler and Johnson, 1977). The earlier investigators of plasma catecholamine assays (Anton and Sayre, 1962) also reported that the addition of anti-oxidant eg metabisulphite, promoted catecholamine recovery when aluminium oxide was used for catecholamine extraction. They estimated that plasma catecholamine recovery was better when metabisulphite was used as anti-oxidant than ascorbate, glutathione or thiosulphate.

Powis (1975) reported that there was no evidence of auto-oxidation of catecholamines in vitro when thin layer chromatography was used to analyse catecholamine levels.

There is also contradicting reports on the collection of blood catecholamines. Those which support collection of blood catecholamine with (Peuler and Johnson, 1977; Rawlinson et al, 1978) and without (Hallman et al, 1978; Brown and Jenner, 1981) the addition of anti-oxidants. All agreed that samples should be spun immediately and frozen until analysed. This suggests that before setting up the method for plasma catecholamine measurements, equipment such as an ice-maker,

refrigerated centrifuge, and ultra cold cabinet must be available, yet when the analysis of plasma catecholamines is undertaken, analysis is done at ambient temperature, and a batch of 10 samples would take at least 1 hour before the stage of adding perchloric acid is reached. Some methods of catecholamine measurements do not precipitate protein with acid before storage, but use perchloric acid to elute catecholamines from aluminium oxide when aluminium oxide is used for extraction of catecholamines.

The basis of the established method for the blood collection and for the catecholamine measurements is that, blood is collected in ice-cooled syringe, anti-oxidants are added to the blood sample then is kept cool and spun immediately in a refrigerated centrifuge. Plasma is separated from the blood cells as soon as possible and kept frozen until analysed. The analysis of those samples should be done as soon as possible to avoid catecholamine degradation.

The usual method for catecholamine measurements is aluminium oxide extraction. Catecholamines are adsorbed on aluminium oxide by shaking at alkaline pH with anti-oxidants. The aluminium oxide is washed with distilled water and catecholamines are eluted with acid. The acid containing catecholamines is injected on to the column, the column separates the amines and they are oxidised as they come from the column and detected by electrochemical detector.

3.3.2 Centrifugation of Plasma for Catecholamine Measurements

Weil-Malherbe and Bone (1954) reported that blood platelets are carriers of catecholamines, and that differential centrifugation produced different catecholamine levels in the same sample. They used ethylenediamine condensation method with fluorometric detector for catecholamine assays. The method was reported to be less specific than trihydroxyindole method for catecholamine measurements (Valk and Price, 1956). The present study sought to replicate and confirm the results of Weil-Malherbe and Bone, using high pressure liquid chromatography with electrochemical detector, and in addition, to measure catecholamine levels in thrombocytopenic patients. It was postulated that differential centrifugation would not produce significantly different catecholamine levels in the same samples from thrombocytopenic patients.

A newly developed method requires particular attention and close monitoring of its performance characteristics through the quality control system. The author introduced the use of the internal quality control plasma to be included in all the catecholamine samples estimated in this thesis and those analysed in the department.

3.3.3 Quality Control

The results from a research technique must be dependable, reproducible and accurate. The only way to adhere to these

criteria is to include the quality control samples with all the analyses performed at any time. Quality control procedure is therefore used to monitor the performance of the instruments and the technique. All analytical measurements are subject to some errors arising from a variety of sources and it is essential that these should be kept to a minimum. These errors include poor sample preparation before analysis, errors due to chemicals, errors due to instrumentation, errors due to calculations and errors due to wrong standardisation.

The important factors of quality control (Buttner et al, 1975; Buttner et al, 1976 and Varley et al, 1980) are accuracy, precision, specificity and sensitivity. Accuracy is defined as the agreement between the best estimate and its true value. Precision is the agreement between duplicate measurements, specificity is the ability of an analytical method to determine the compounds it is supposed to measure and sensitivity is the ability of the analytical method to detect small quantities of the compounds. These four factors must be satisfied in plasma catecholamine measurements.

There are two types of quality control systems; the external and the internal quality control systems.

3.3.4 The External Quality Control System

The external quality control system uses the results of the participating laboratories in the scheme. These laboratories

analyse samples of the same specimens in order to use the information in conjunction with those of internal quality control system.

The United Kingdom has two major schemes; The National Quality Control Scheme and The Wellcome Group Control Programme (Varley et al, 1980). They circulate samples to participating laboratories regularly. The overall standard mean and standard deviation between laboratories for the result on the same specimen is calculated, and individual laboratory attempts to get as close to them as possible. The variation between laboratories using different methods is quite large for the same analyte (Varley et al, 1980). Hence, laboratories using the same method are grouped together.

The degree of agreement of the results is related to some measure of satisfactory performance. The performance is found to be better in large laboratories (Varley et al, 1980), probably because of better equipment and the presence of several specially qualified personnel to solve any problem when it arises. Large deviation of the results should prompt an action which may be of clerical or arithmetic errors. If one method gives results different from the majority of other methods, all of which agree with one another, it is necessary to re-examine the calibration standards, pipettes and equipment performances, and to test reagents for evidence of deterioration.

3.3.5 The Internal Quality Control System

In a small research laboratory, it may not be possible to use external quality control facilities; therefore, internal quality control must be rigorously enforced in every run. Quality control plasma or serum can be obtained in different ways. For specialised field of research such as the measurement of plasma catecholamines, the method which was found to be workable (Before post-dated blood for transfusion) was pooled plasma. This can be obtained either from healthy volunteers or by using pooled patients' plasma. Bowers et al, (1975) suggested making a pool of plasma or serum of about 25 litres or more. Such quantity requires a large establishment. In our laboratory, a pool which would last four months was found to be appropriate, and a volume of about 600ml was frequently pooled.

Repetitive analyses on a batch of quality control plasma must be done to calculate the mean of plasma noradrenaline and adrenaline levels. The precision of the results from the quality control are indicators of the quality of the results of the samples in the batch. Statistical manipulation required for quality control is not elaborate; only sample mean, standard deviation and coefficient of variation are required.

The problem to be investigated in this chapter was to study the factors which may affect catecholamine levels, eg the effects of differential centrifugation on plasma samples, the

assessment of stability of catecholamines through the internal quality control system, using pooled plasma and post-dated blood for transfusion. The aim was to modify the collection of blood samples for catecholamine measurements, to allow clinical examinations to proceed without special attention normally given to catecholamine samples.

Therefore, the author decided to examine the following:-

1. The effects of differential centrifugation on plasma samples and samples from thrombocytopenic patients.
2. The advantage of using anti-oxidants in the plasma catecholamines measurements, also the advantage of spinning catecholamine samples immediately in cold conditions, compared with the delay in spinning the samples.
3. The advantage of keeping catecholamine samples in ice, compared with samples left on the bench, and the comparison between serum and plasma catecholamine levels.
4. The addition of the exogenous catecholamines to blood samples and to study the recovery of the endogenous and the exogenous catecholamines with and without anti-oxidants.
5. To study the percentage recovery of the catecholamines in the post-dated blood for transfusion with and without anti-oxidant.
6. The comparison between serum and plasma, and blood samples clotted with glass beads. Glass beads is the quicker method of clotting blood samples with and without anticoagulant.
7. Measurements of plasma catecholamine levels in pooled plasma samples for the study of the stability of

catecholamine in the quality control system.

8. Measurement of plasma catecholamines in post-dated blood for transfusion used in the quality control system.

9. Measurements of plasma catecholamine levels in post-dated plasma for transfusion.

10. Comparison between catecholamine levels using two protein precipitants: 1.2M trichloroacetic acid; 1.2M perchloric acid and those from the usual catecholamine method.

The samples were taken from volunteers by the author while they were seated.

3.3.6 Differential Centrifugation of Plasma for Catecholamine Measurements

Method: Blood was collected from seven healthy volunteers. The volunteers were seated while blood samples were being withdrawn. Four sets of 10ml blood were taken from each. Three 10ml samples contained lithium heparin as an anticoagulant and one 10ml sample (clotted sample) was collected in a plain tube. The tubes with anticoagulant were centrifuged at 170g, 650g and 1500g for 10 minutes. The plasma was separated in polypropylene tubes and stored at -70 degrees C until analysed. The tubes which were spun at 170g were re-spun at 2750g for 10 minutes; plasma was separated and stored as above. The basis of re-spinning the blood samples was to show whether at a high speed centrifugation would produce higher catecholamine levels in extracellular

fluid. The serum samples were spun at 1500g for 10 minutes, serum was separated and stored as above.

3.3.7 Results

Plasma noradrenaline and adrenaline levels spun at different speeds; and serum noradrenaline and adrenaline levels spun at 1500g are shown in Tables 3.1 and 3.2. Tables 3.3 and 3.4 show comparison between those catecholamine levels.

The statistics were either paired or unpaired Student' t test using Hewlett Packard Programmable printing calculator, and the statistical tables from Bailey (1975). The value of $p<0.05$ was taken to be significant.

3.3.8 Differential Centrifugation of Thrombocytopenic Blood

Method: Two sets of 10ml blood samples were collected from each of the seven thrombocytopenic patients when they were seated. One sample was collected in a lithium heparin bottle and one without anticoagulant (clotted). Lithium heparin samples were spun at 170g for 10 minutes (platelet-rich plasma), the plasma was removed into polypropylene tubes. The samples which were spun at 170g were re-spun at 1500g for 10 minutes (platelet-poor plasma); plasma was removed into polypropylene tubes. The serum samples were spun at 1500g for 10 minutes, and the serum was removed into polypropylene tubes. The samples were stored at -70 degrees C until analysed.

3.3.9 Results

There was no significant difference in the mean noradrenaline level spun at different speeds (Table 3.5), the mean adrenaline levels of samples spun at 170g was significantly higher than those spun at 1500g (Table 3.6). Table 3.7 shows the comparison between adrenaline levels spun at different speeds.

3.3.10 The Effect of Anti-oxidants and Delay in Spinning The Samples for Plasma Catecholamine Measurements

Method: Blood was collected in ice cooled heparinized Vacutainer tubes from 10 volunteers. Two sets of samples were obtained: one set contained 200ul of 90g/l reduced glutathione and 60g/l ethyleneglycol bis(beta-aminoethylether)N,N-tetraacetic acid (EGTA) pH adjusted to 6-7 with 6M sodium hydroxide, as described by Yamatodani and Wada (1981); the second set of tubes did not contain anti-oxidants. The first samples collected in both sets were the controls and were spun immediately at zero degrees C for 10 minutes. Plasma was removed into polypropylene tubes and kept at -70 degrees C until analysed. The second set of samples was collected and kept cool in ice for 15 minutes and subsequently spun at zero degrees C. The plasma was removed into polypropylene tubes and kept at -70 degrees C until analysed. The third set of samples was collected and kept in ice for 30 minutes and then spun at zero degrees C. Plasma

was removed into polypropylene tubes and stored at -70 degrees C until analysed using high pressure liquid chromatography with electrochemical detector LC-4A (Bioanalytical Systems).

3.3.11 Results

The samples were analysed in duplicate. There was no statistical difference between the catecholamine levels with and without anti-oxidants and between those which were spun immediately and those which were not (unpaired Student's t test). Tables 3.8 and 3.9 indicate the mean and the individual noradrenaline and adrenaline levels respectively. The mean values are identical, indicating stability of catecholamine levels in vitro with and without anti-oxidants.

3.3.12 The Addition of Exogenous Catecholamines to Blood Samples with and without Anti-oxidants

Method: Blood samples were collected from 4 volunteers with 10ml Vacutainer tubes. The samples were divided into two sets: one set of samples had 200ul of the solution of reduced glutathione and EGTA as described (section 3.3.10), added to heparinized Vacutainer tubes; the second set of samples had no glutathione or EGTA. Four pmoles of exogenous noradrenaline and 4pmoles of exogenous adrenaline were added to each of 10ml of the blood samples except the first ones (zero time) and were spun immediately. The plasma was

separated into polypropylene tubes and was frozen at -70 degrees C until analysed. The rest of the samples were left on the bench and were spun at predetermined times: 30, 60, 120 and 240 minutes. After centrifugation the plasma was removed into polypropylene tubes and frozen until analysed.

3.3.13 Results

The mean and individual noradrenaline and adrenaline levels with anti-oxidants were similar to those without antioxidants (Tables 3.10 and 3.11) respectively. The endogenous and exogenous catecholamine levels were recovered.

3.3.14 The Percentage Recovery of Plasma Catecholamines With and Without Anti-oxidant

Method: The experiment was designed to compare the recovery of plasma catecholamines from samples with and without sodium metabisulphite as anti-oxidant. The experiment was performed on post-dated blood for transfusion. Thirty microlitre solution of 0.1M sodium metabisulphite was added to each of six samples, but was not added to a further six samples. Thereafter, the samples were treated identically.

3.3.15 Results

Table 3.12 indicates the individual and the mean percentage recovery on the samples with and without antioxidant. There were no significant differences between them (unpaired

Student's t test). There were no significant differences between the mean catecholamine levels on the samples with and without anti-oxidant.

The recovery of plasma catecholamine levels (Table 3.12) suggests that the anti-oxidant did not influence the recovery of noradrenaline and adrenaline levels in those samples.

3.3.16 The Comparison Between Serum and Plasma Catecholamine Concentrations

Method: The experiment was set up as a comparative study between serum and plasma catecholamine levels. Blood samples were taken from 10 volunteers. These samples were divided into two sets: one set was collected in Vacutainer tubes without an anticoagulant (clotted samples) and not precooled; the second set of samples was collected in ice-cooled heparin Vacutainer tubes. The heparinised samples were spun immediately at zero degrees C. The plasma was removed into polypropylene tubes. The other set of samples were left on the bench until they were completely coagulated and were then spun at zero degrees C. The serum was removed into polypropylene tubes. All samples from both sets were analysed the same day for catecholamine levels.

3.3.17 Results

The mean serum noradrenaline level was significantly higher than that of plasma noradrenaline, the mean serum adrenaline

level was also significantly higher than plasma adrenaline level. The mean and individual catecholamine levels are shown in Table 3.13, also the statistical analysis.

3.3.18 The Effect of Mechanical Agitation on Blood Catecholamines

Agitation of blood samples with glass beads is a quick method of clotting blood samples with or without anticoagulant. Section 3.3.16 showed that serum contained higher catecholamine levels than plasma. Hence, the investigation in this section was to show whether blood samples agitated with glass beads contain higher catecholamine levels than those without.

Method: There were nine volunteers, and four sets of 10ml blood samples were taken from each volunteer; two 10ml samples were collected into cold lithium heparin Vacutainer tubes and two 10ml blood samples were collected in Vacutainer tubes without an anticoagulant (clotted samples). Ten small glass beads were added immediately to each of two of these samples (one with lithium heparin and one without); the blood samples were mixed by gentle inversion for about 10 minutes to be clotted with glass beads. The samples were then spun at zero degrees C and the serum was separated into polypropylene tubes. The lithium heparin samples were spun immediately and the plasma was separated into polypropylene tubes and all the samples were frozen at -70 degrees C. The samples without

anticoagulant were left on the bench to clot and were then spun at zero degrees and serum separated into polypropylene tubes; these were also frozen at -70 degrees C until analysed.

3.3.19 Results

Each volunteer had four different results for noradrenaline levels (Table 3.14). The first was for plasma noradrenaline and the second for plasma noradrenaline which had beads; the third was for serum noradrenaline which had beads and the fourth was for serum noradrenaline. Similar results were obtained for adrenaline in all the samples. Table 3.15 shows the comparison between those catecholamine levels.

3.3.20 Measurements of plasma Catecholamines in pooled plasma

Method The aim of this experiment was to use the pooled plasma as quality control plasma. Several samples of pooled plasma were included in a batch of samples. Catecholamines were extracted by aluminium oxide, then catecholamines were eluted by perchloric acid.

Plasma samples from patients or volunteers were regularly pooled. The method worked reasonably well. The major source of those samples came from patients.

13.3.21 Results

Some of the pooled samples from patients had many electroactive substances which interfered with catecholamine measurements. However, good results were obtained from some batches. Table 3.16 shows catecholamine levels from pooled plasma. The results show good reproducibility which suggests catecholamines are stable.

3.3.22 Plasma Catecholamines in Post-Dated Blood for Transfusion

There was a need to obtain internal quality control sample from a single source, to eliminate electroactive substances from pooled patients samples. The author investigated the stability of catecholamines in post-dated blood for transfusion.

Method: Post-dated blood for transfusion was used in preference to post-dated plasma for transfusion, so that blood could be spun at the usual speed (1500g) for 10 minutes for plasma catecholamine measurements. It is known that blood for transfusion does not contain anti-oxidants, and it is considered to be post-dated if it is not transfused within 28 days of donation.

Five internal quality control samples (post-dated blood for transfusion) were included in each sample batch analysed daily. Hence stability and reproducibility on post-dated

blood for transfusion were studied for a period of 4 months.

3.3.23 Results

Table 3.17 shows the individual catecholamine levels, the mean and coefficient of variation obtained during week 1, were comparable to those obtained after 4; 8 and 12 weeks (Tables 3.18; 3.19; 3.20). In addition, catecholamine levels on post-dated blood for transfusion were comparable to those on pooled plasma used previously as internal quality control samples. The results indicate that catecholamines are stable. Post-dated blood for transfusion is considered to be post-dated when it is not transfused within 28 days at 4 degrees C.

3.3.24 Catecholamine in Post-Dated Plasma for Transfusion

Method: The experiment in this section was designed to confirm the results in section 3.3.22, that catecholamines are stable. The post-dated plasma for transfusion was obtained in a plastic bag and samples analysed for catecholamine levels. Ten plasma extractions were performed in a single run. The post-dated plasma for transfusion was kept at four degrees C.

3.3.25 Results

The mean and individual plasma noradrenaline and adrenaline levels are shown in Table 3.21. These values are within normal range, and further confirm the stability of plasma catecholamines (compare with catecholamine levels in post-dated blood for transfusion; section 3.3.22).

3.3.26 The Efficacy of the two protein Precipitants For Catecholamine Measurements

Method: Trichloroacetic acid is a known protein precipitant, which keeps in solution the compounds of interest; the concentration normally used is 1.2M (Varley et al, 1980). However Anton and Sayre (1962) reported that a number of other acids including trichloroacetic acid were unsatisfactory compared with perchloric acid for the elution of catecholamines from aluminium oxide, when aluminium oxide is used to extract catecholamines from plasma.

One millilitre of post-dated plasma for transfusion was pipetted into each of twelve glass centrifuge tubes. One millilitre of 1.2M trichloroacetic acid was added to each tube and then the tubes were vortexed for about one minute. Then they were spun at 1500g for 10 minutes, and the supernatants were transferred into clean centrifuge tubes. The pH of the solutions was adjusted to 8.6 with about 3.5ml of Trizma buffer (Appendix Seven). Twenty-five milligrams of aluminium oxide and 100ul of internal standard (DHBA) were

added. The tubes were shaken mechanically for about 10 minutes. The supernatants were discarded, and the aluminium oxide crystals were washed once with double distilled water. 200ul of 0.1M perchloric acid was added to each tube to elute catecholamines from aluminium oxide, the tubes were shaken for 10 minutes, and 100ul of the clear fluid was injected onto the column.

In the second method for protein precipitation, 1.2M trichloroacetic acid was replaced with 1.2M perchloric acid. The methods are described individually in Appendices Eleven and Twelve.

Catecholamine levels from protein extracts using two protein precipitants were compared with each other and those plasma catecholamine levels extracted with the usual method (Appendix Ten).

3.3.27 Results

Three methods for preparing samples for catecholamine measurements were compared. All produced different noradrenaline and adrenaline levels from the same sample (Tables 3.22; 3.23) respectively. Table 3.24 shows the comparison between those catecholamine levels.

3.3.28 Discussion

The differential centrifugations on the same blood sample produced significant different catecholamine levels. The possible mechanism is that centrifugal force on samples during spinning removes catecholamines attached to the cells and proteins into extracellular fluid. Hence, if centrifugation is not standadised, the variations in catecholamine levels may be due to centrifugation effects. It would be appropriate to state the speed of centrifugation with catecholamine reports, so that similar reports would be compared.

The results were in agreement with the study of Weil-Malherbe and Bone (1954). The present study also showed that at a very high centrifugation speed, more catecholamine are liberated into extracellular fluid, those extra catecholamines might have been liberated from their attachment to cells and proteins.

Similar results were obtained with samples taken from thrombocytopenic patients, though, there was no apparent difference between noradrenaline levels spun at different speed.

Some of the design of the experiments in this chapter, was to investigate the evidence available in support of the instability or stability of plasma catecholamines. It is believed that in vitro blood catecholamines or plasma

catecholamines degrade rapidly unless an anti-oxidant is added. Carruthers et al (1970) reported errors in plasma catecholamine measurements occurring during sample collection, storage and analysis, and recommended that samples should be spun immediately and that the plasma should be frozen at -20 degrees C until analysed. They also reported that significant losses occur during prolonged storage and repeated thawing and refreezing.

The catecholamine stability was studied in samples with and without anti-oxidants and with delay in spinning the blood samples. It was assumed that the samples without anti-oxidants and the samples which were not spun immediately would show enhancement of catecholamine degradation. It was further assumed that the degradation of plasma catecholamines would be proportional to the time which had elapsed before the samples were spun. When the studies were done to investigate those assumptions, it was found that there was no evidence of catecholamine degradation in the samples without anti-oxidants and in those which the spinning was delayed (Tables 3.8; 3.9; 3.10; 3.11). This is in agreement with the work of Pattersson et al (1980) with a radioenzymatic assay and with studies employing liquid chromatography with electrochemical detection on rat heart tissue and plasma samples (Eriksson and Persson, 1982; Falconer et al 1982), although the latter authors did not measure plasma adrenaline.

It was assumed that slight catecholamine degradation might have occurred in the previous experiment, but may have not been detected. It was argued that if exogenous catecholamines were added to the samples, degradation may be detected through the recovery process. The samples were left longer on the bench and the first set of tubes (zero time) were spun immediately. The exogenous catecholamines were recovered (Tables 3.10; 3.11) and the differences in all the samples are comparable to the zero time. The present study confirmed the study reported in the above paragraph, that there is no evidence of catecholamine degradation in any of the samples; hence, no advantage was evident with anti-oxidant.

The procedure of collecting blood samples in the cold for catecholamine estimations, and the addition of anti-oxidants are used routinely almost in all laboratories. When such procedures were adopted in our laboratory, we had haemolysis of blood samples, which was due either to the addition of the anti-oxidants or keeping blood in ice before and after blood collection. Haemolysis interfered with catecholamine measurements. The results from the investigations reported earlier compelled us to stop such procedures. Stopping those procedures produced a distinct advantage, in that, there was no more haemolysis of blood samples which used to interfere with catecholamine estimations, and ruined some of the studies. In addition, catecholamine levels are reported in pmole range, that procedures which introduce interferences with the measurements, would introduce large errors in the

catecholamine results, which may either cause misinterpretation of the results or invalidation of the results.

The experiment on serum and plasma catecholamines was designed to investigate the difference between serum and plasma catecholamine levels. Blood serum was collected into the tubes without an anticoagulant and the samples left on the bench to clot, a blood sample may take up to one hour to clot. It was designed so that, if catecholamines were unstable, degradation would occur in the samples without anticoagulants. The mean serum noradrenaline level was significantly higher than the mean plasma noradrenaline level; the mean serum adrenaline level was significantly higher than plasma adrenaline level. Although Hansson et al (1979) and Davis et al (1981) reported that catecholamines can be analysed in either serum or plasma, they made no mention of any differences between them. Here again, there was no evidence of catecholamine degradation. The study of interaction between protein and catecholamines by Danon and Sapira (1972), with tritiated noradrenaline, suggested that about 50% of noradrenaline is bound to plasma protein, Powis (1975) and Markin et al (1966) also reported that catecholamine was bound to protein. In other studies Danon and Sapira (1972) and Roston (1967) reported adsorption of noradrenaline and adrenaline into red cells and erythrocytes. It appears serum would therefore, contain higher catecholamine levels than plasma, because the clot which

forms appears to denature protein and blood cells are ruptured, causing catecholamines attached to protein and blood cells to be released into extracellular fluid, whereas in plasma, blood does not clot, protein and blood cells probably still assume their natural configuration, and hence, less catecholamines are released from them.

The experiment with glass beads produced higher catecholamine levels. This suggests that mechanical agitation denatures protein and ruptures the cells, displacing the catecholamines from cells and proteins into the extracellular fluids as suggested above. There was no haemolysis observed in the samples which had glass beads. Also the acid treated plasma samples showed different catecholamine levels. The samples which were treated with 1.2M perchloric acid showed significantly higher catecholamine levels than those which were not. This is in agreement with the studies discussed above, which reported that catecholamines are attached to proteins and blood cells. Plasma treated with 1.2M trichloroacetic acid produced significantly lower catecholamine levels than those treated with perchloric acid, in addition to those extracted with the usual method. Anton and Sayre (1962) examined the effects of other acids including trichloroacetic acid for the elution of catecholamines from aluminium oxide; they found unsatisfactory results compared with those using perchloric acid. The effects of perchloric and trichloroacetic acids on protein precipitation may be similar, but the difference

between them is that perchloric acid forms ion pairs with catecholamines which makes it a better eluting agent (Persson and Karger, 1974; Knox and Laid, 1976; Knox and Jurand, 1976). The results suggest that only one method can be chosen and used consistently for catecholamine measurements.

The experiment which compared catecholamine recovery on samples with and without anti-oxidant, showed that there was no significant difference between the samples with and without anti-oxidant. The function of the anti-oxidant is questionable. Anton and Sayre (1962) reported that anti-oxidants enhanced the recovery of catecholamines; this was not the case in the present study.

The study which was performed in post-dated blood for transfusion (Tables 3.17; 3.18; 3.19; 3.20) showed that catecholamine levels are normal and comparable to those of pooled plasma (Table 3.16). The present study further confirms the previous studies that plasma catecholamines are stable.

The study on post-dated plasma for transfusion (Table 3.21), showed that catecholamine levels are normal and are similar to those of pooled plasma and those of post-dated blood for transfusion. All these plasma catecholamine levels are within the normal range.

3.3.29 Conclusion

The studies suggest that the speed of centrifugation for plasma catecholamine measurements must be predetermined, and must be standardised for the whole time of the study.

The results from the experiments proved that catecholamines are stable, and that the addition of anti-oxidants either to stabilise or to enhance the recovery of catecholamines, have no effect and are unnecessary. The experiments also proved that strict precautions taken during the collection of blood are unnecessary, and that expensive equipment such as a refrigerated centrifuge, ice maker and ultra cold cabinet only for catecholamine measurements are not essential.

The experiments which used acids to precipitate protein for catecholamine measurements showed that only one method for catecholamine extraction should be used by a laboratory if consistent results are to be obtained.

As a result of the author's experiments on the stability and factors which affect plasma catecholamine levels, the Department's initial attitude towards the collection of blood and the procedures for plasma catecholamine measurements have now changed, no special precautions taken before and after sample collection for catecholamine measurements. Hence, haemolysis of blood samples did not occur thereafter.

Table 3.1 The Effects of Differential Centrifugation on Plasma Catecholamine levels

n=7 mean (SEM) (pmol/ml)

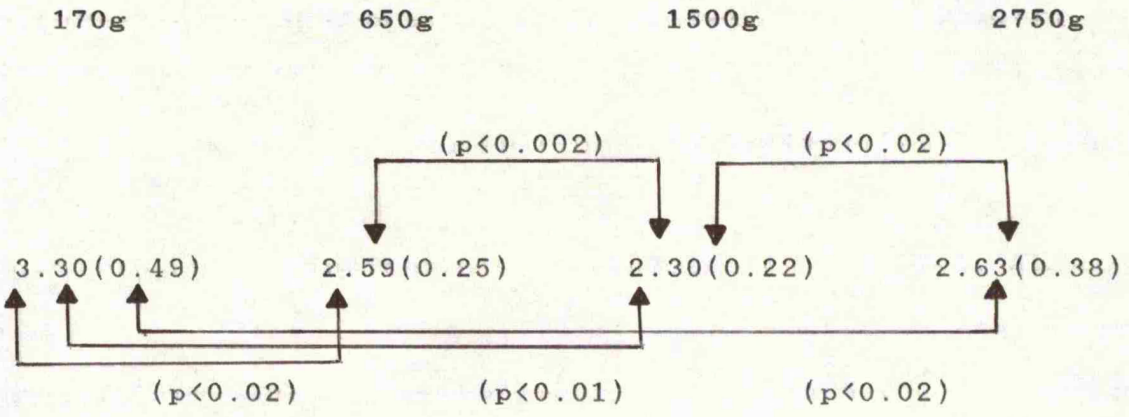
Plasma Noradrenaline				Serum
170g	650g	1500g	2750	1500g
1.99	1.80	1.71	1.78	2.33
2.43	2.25	1.75	1.85	2.75
4.05	3.17	2.75	3.66	4.36
5.38	3.28	2.78	3.44	4.67
2.97	2.39	2.43	2.58	4.99
2.02	1.89	1.74	1.80	2.99
4.26	3.35	3.04	3.29	5.08
3.30(0.49)	2.59(0.25)	2.30(0.22)	2.63(0.32)	3.88(0.43)
Plasma Adrenaline				Serum
0.22	0.20	0.20	0.22	0.22
0.32	0.29	0.25	0.23	0.32
0.38	0.30	0.30	0.31	0.48
0.64	0.64	0.59	0.73	0.79
1.02	0.68	0.64	0.94	1.00
0.14	0.13	0.14	0.19	0.23
0.46	0.45	0.38	0.54	0.61
0.45(0.11)	0.38(0.08)	0.36(0.07)	0.45(0.11)	0.52(0.11)

Table 3.2 The Effects of Differential Centrifugation on Plasma Noradrenaline levels

n=7 mean (SEM) (pmol/ml)

Plasma Noradrenaline levels			
170g	650g	1500g	2750g
1.99	1.80	1.71	1.78
2.43	2.25	1.75	1.85
4.05	3.17	2.75	3.66
5.38	3.28	2.78	3.44
2.97	2.39	2.34	2.58
2.03	1.89	1.74	1.80
4.26	3.35	3.04	3.29
3.30(0.49)	2.59(0.25)	2.30(0.22)	2.63(0.38)

Table 3.3 Statistical Analysis on Plasma and Serum levels Noradrenaline (pmol/ml)



Plasma Noradrenaline Serum Noradrenaline

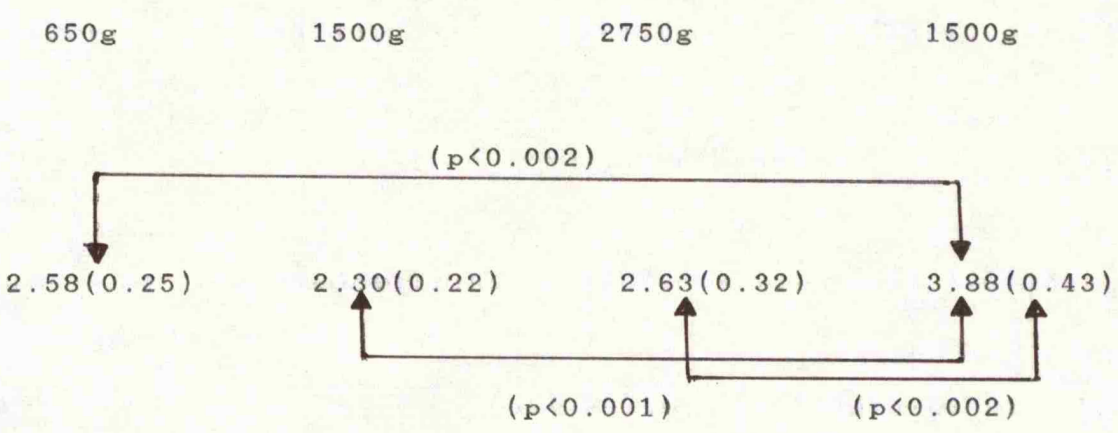


Table 3.4 Statistical Analysis on Plasma and Serum Adrenaline

Adrenaline level (pmol/ml)

Plasma Adrenaline			Serum Adrenaline
650g	1500g	2750g	1500g

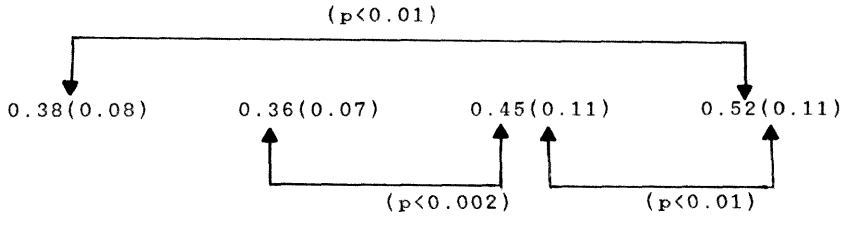


Table 3.5 The Effects of Differential Centrifugation on Plasma Noradrenaline levels from Thrombocytopenic Patients

n=7 mean (SEM) (pmol/ml)

Plasma Noradrenaline		Serum	Platelets
170g	1500g	1500g	(cu.mm)
2.72	2.65	3.36	102,000
3.24	3.18	3.27	103,000
3.85	3.81	3.90	44,000
5.44	5.36	5.91	40,000
3.70	3.61	3.66	69,000
3.26	3.19	3.39	27,000
1.86	1.77	1.82	63,000
3.44(0.42)	**3.37(0.42)**	**3.62(0.46)**	

Table 3.6 The Effects of Differential Centrifugation on Plasma Adrenaline levels from Thrombocytopenic Patients

n=7 mean (SEM) (pmol/ml)

Plasma Adrenaline		Serum	Platelets
170g	1500g	1500g	(cu.mm)
1.14	0.99	1.29	102,000
1.26	1.15	1.24	103,000
1.82	1.65	1.70	44,000
5.05	4.75	5.00	40,000
0.76	0.66	0.75	69,000
0.57	0.46	0.53	27,000
0.57	0.51	0.55	63,000
1.60(0.60	**1.45(0.57)**	**1.58(0.59)**	

Table 3.7 Stastistical Analysis on Plasma and Serum Adrenaline from Thrombocytopenic Patients

Adrenaline (pmol/ml)

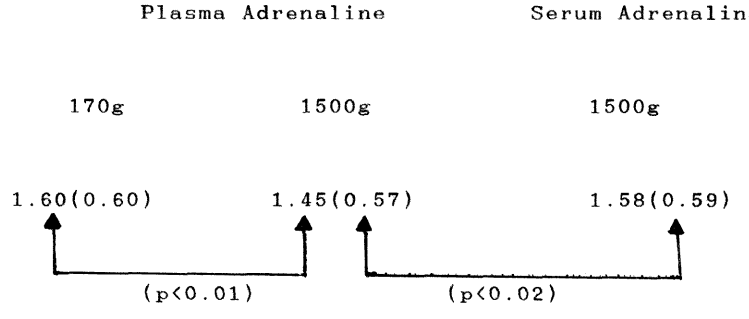


Table 3.8 The Effect of Antioxidants and the Delay in Spining Blood on Catecholamine levels

n=10 mean (SEM)

Noradrenaline (pmol/ml)

Samples without Antioxidants			Samples with Antioxidants		
0 min	15 min	30 min	0 min	15 min	30 min
1.40	1.43	1.38	1.32	1.32	1.30
1.41	1.41	1.39	1.40	1.44	1.44
0.90	0.95	0.92	0.95	0.90	1.02
2.54	2.46	2.47	2.44	2.50	2.45
1.96	1.97	2.12	2.07	2.07	1.97
2.10	1.98	2.05	2.03	1.99	2.06
1.68	1.69	1.66	1.67	1.69	1.68
1.45	1.46	1.49	1.48	1.45	1.50
2.12	2.12	2.09	2.16	1.96	2.09
1.48	1.47	1.50	1.37	1.39	1.36
1.70(0.13)	**1.69(0.13)**	**1.71(0.13)**	**1.69(0.13)**	**1.67(0.13)**	**1.69(0.13)**

Table 3.9 The Effect of Antioxidants and the Delay in Spining Blood on Catecholamine levels

n=10 mean (SEM)

Adrenaline (pmol/ml)

Samples without Antioxidants			Samples with Antioxidants		
0 min	15 min	30 min	0 min	15 min	30 min
0.29	0.32	0.33	0.28	0.33	0.27
0.45	0.42	0.43	0.48	0.50	0.47
0.41	0.42	0.39	0.43	0.39	0.42
0.40	0.43	0.42	0.40	0.36	0.40
0.34	0.32	0.36	0.37	0.35	0.37
0.71	0.65	0.71	0.70	0.69	0.74
0.60	0.56	0.58	0.50	0.46	0.47
0.37	0.36	0.36	0.33	0.35	0.35
0.33	0.32	0.34	0.31	0.35	0.33
0.31	0.28	0.30	0.34	0.33	0.33
0.42(0.04)	0.41(0.04)	0.42(0.04)	0.41(0.04)	0.41(0.04)	0.42(0.04)

Table 3.10 The addition of exogenous Catecholamines to the blood samples with and without Antioxidants

n=4 mean (SEM)

Noradrenaline (pmol/ml)

Samples without Antioxidants

0 min	15 min	60 min	120 min	240 min
1.48	1.94	1.88	1.89	1.89
2.70	3.10	3.08	3.12	3.10
1.94	2.35	2.29	2.35	2.33
1.40	1.82	1.85	1.79	1.82
1.87(0.30)	2.30(0.33)	2.28(0.29)	2.29(0.31)	2.29(0.33)

Samples with Antioxidants

1.51	1.92	1.96	1.96	1.94
2.74	3.23	3.14	3.16	3.20
2.04	2.41	2.39	2.45	2.42
1.30	1.74	1.68	1.67	1.70
1.90(0.32)	2.33(0.33)	2.29(0.32)	2.31(0.33)	2.32(0.33)

Table 3.11 The addition of exogenous Catecholamines to the blood samples with and without Antioxidants

n=4 mean (SEM)

Adrenaline (pmol/ml)

Samples without Antioxidants

0 min	30 min	60 min	120 min	240 min
0.55	0.88	0.97	0.93	0.96
0.69	1.07	1.10	1.14	1.10
0.40	0.79	0.80	0.79	0.81
0.29	0.66	0.68	0.67	0.68
0.48(0.09)	**0.85(0.09)**	**0.89(0.09)**	**0.91(0.12)**	**0.89(0.09)**

Samples with Antioxidants

0.61	0.97	1.03	0.98	1.03
0.79	1.17	1.16	1.15	1.16
0.50	0.92	0.94	0.92	0.94
0.30	0.75	0.76	0.76	0.69
0.55(0.10)	**0.98(0.11)**	**0.97(0.08)**	**0.95(0.08)**	**0.96(0.10)**

Table 3.12 The Percentage Recovery of the Plasma Catecholamines with and without Antioxidant

n=6 mean (SEM) (pmol/ml)

Noradrenaline	Adrenaline	%Recovery
Samples with Antioxidant		
1.89	0.24	71
2.00	0.19	69
1.98	0.30	65
2.00	0.25	74
1.95	0.21	74
2.07	0.29	74
1.98 (0.06)	0.25 (0.02)	71.2 (1.45)
Samples without Antioxidant		
1.90	0.25	75
2.06	0.20	66
1.91	0.26	78
1.98	0.20	81
2.15	0.30	74
1.87	0.22	84
1.98 (0.04)	0.24 (0.02)	76.3 (2.56)

Table 3.13 Comparison between Serum and Plasma Catecholamines

n=10 mean (SEM)

Noradrenaline (pmol/ml)		Adrenaline (pmol/ml)	
Serum	Plasma	Serum	Plasma
3.89	2.60	0.32	0.30
2.58	2.13	0.24	0.20
4.99	2.91	0.32	0.33
2.47	1.71	0.18	0.18
3.82	2.09	0.21	0.16
4.92	3.10	0.32	0.30
3.54	2.65	0.42	0.34
2.24	1.52	0.14	0.05
3.22	2.08	0.18	0.17
2.21	1.53	0.30	0.28
3.39(0.33)	**2.23(0.18)**	**0.26(0.03)**	**0.23(0.03)**

Statistical Analysis on Plasma and Serum Catecholamines (pmol/ml)

Noradrenaline levels		Adrenaline levels	
Serum	Plasma	Serum	Plasma
3.39(0.33)	2.23(0.18)	0.26(0.03)	0.23(0.03)
	(p<0.001)		(p<0.05)

Table 3.14 The Effect of Mechanical Agitation on plasma and serum catecholamine levels with and without glass beads

n=9 mean (SEM)

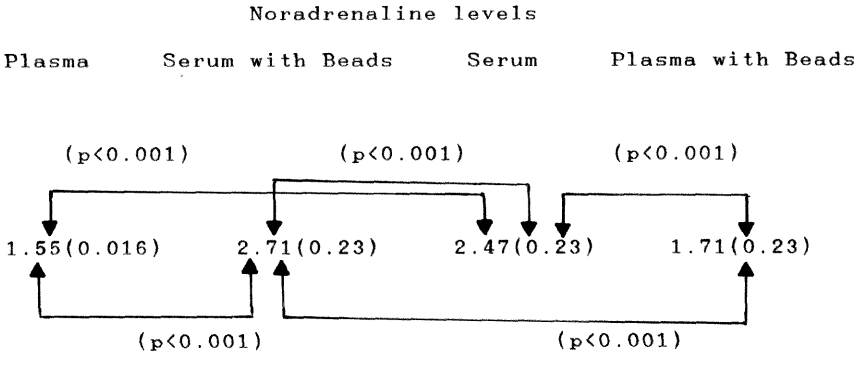
Plasma and Serum Noradrenaline levels (pmol/ml)

Plasma	Plasma with Beads	Serum with Beads	Serum
2.27	2.30	3.86	3.76
2.10	2.24	3.43	3.23
1.03	1.17	1.81	1.60
1.37	1.43	2.26	2.14
2.11	2.15	2.82	2.38
1.17	1.70	2.05	1.91
1.12	1.26	2.21	2.02
1.42	1.50	3.15	2.83
1.39	1.61	2.77	2.32
1.55(0.16)	**1.71(0.14)**	**2.71(0.23)**	**2.47(0.23)**

Plasma and Serum Adrenaline levels (pmol/ml)

0.20	0.22	0.31	0.23
0.27	0.28	0.33	0.30
0.11	0.11	0.15	0.12
0.18	0.21	0.27	0.22
0.32	0.34	0.39	0.34
0.35	0.37	0.38	0.38
0.27	0.27	0.28	0.27
0.19	0.27	0.27	0.25
0.09	0.09	0.13	0.13
0.22(0.03)	**0.24(0.03)**	**0.28(0.03)**	**0.25(0.03)**

Table 3.15 Statistical Analysis on Plasma and Serum Catecholamine levels (pmol/ml) from the Glass Beads Experiment



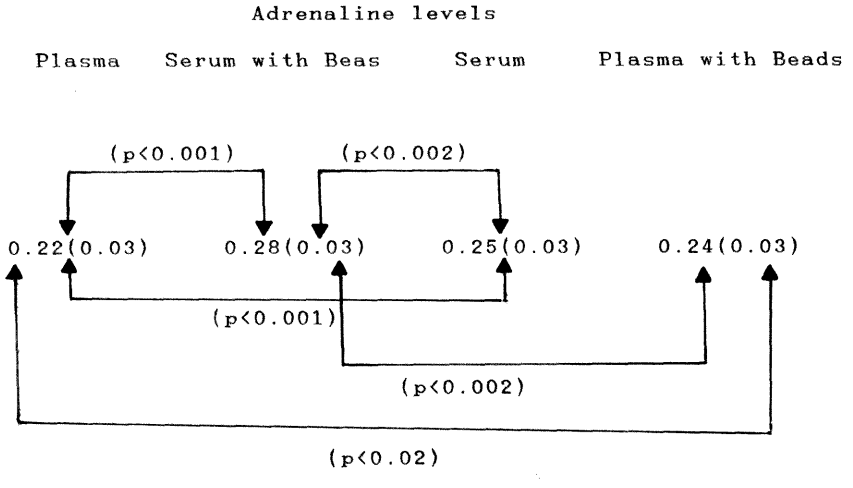


Table 3.16 Catecholamine levels in Pooled Plasma used as internal quality control

n=5 mean (sd)

Noradrenaline levels (pmol/ml)

	Day 1	Day 2	Day 3	Day 4	Day 5
	1.57	1.76	1.84	2.01	1.65
	1.61	1.80	1.92	1.92	1.73
	1.65	1.86	1.93	1.95	1.76
	1.62	1.84	1.90	1.93	1.68
	1.64	1.78	1.87	1.98	1.75
	1.62(0.03)	1.81(0.04)	1.89(0.04)	1.96(0.04)	1.71(0.05)
CV%	1.9	2.2	2.1	2.0	2.9

Adrenaline levels (pmol/ml)

	0.29	0.30	0.32	0.31	0.29
	0.31	0.33	0.30	0.33	0.33
	0.33	0.28	0.31	0.34	0.31
	0.30	0.32	0.30	0.33	0.32
	0.32	0.33	0.33	0.32	0.30
	0.31(0.03)	0.31(0.02)	0.31(0.01)	0.33(0.01)	0.31(0.02)
CV%	6.5	6.5	3.2	3.0	6.5

Table 3.17 Plasma catecholamines on post-dated Blood for transfusion used as internal quality control

n=5 mean (sd)

Noradrenaline levels (pmol/ml)

(A sample from batch one during week 1)

	Day 1	Day 2	Day 3	Day 4	Day 5
	2.25	2.11	2.15	2.32	2.20
	2.00	2.16	1.97	2.24	2.13
	2.15	2.20	2.21	2.28	2.24
	2.31	1.98	2.08	2.33	2.04
	2.26	2.14	2.17	2.29	2.17
	2.19(0.12)	2.12(0.08)	2.12(0.09)	2.29(0.04)	2.16(0.08)
CV%	5.5	3.8	4.2	1.7	3.7

Adrenaline levels (pmol/ml)

	0.31	0.32	0.32	0.26	0.24
	0.28	0.29	0.31	0.27	0.27
	0.30	0.31	0.29	0.24	0.28
	0.28	0.30	0.33	0.28	0.25
	0.30	0.33	0.28	0.25	0.26
	0.29(0.01)	0.31(0.02)	0.31(0.02)	0.26(0.02)	0.26(0.02)
CV%	3.4	6.5	6.5	7.7	7.7

Table 3.18 Plasma catecholamines on post-dated blood for transfusion used as internal quality control

n=5 mean (sd)

Noradrenaline levels (pmol/ml)

(A sample from batch one during week 4)

	Day 1	Day 2	Day 3	Day 4	Day 5
	2.06	2.01	1.98	2.15	2.12
	2.14	1.95	2.18	2.11	1.99
	2.17	1.97	2.05	2.19	2.16
	2.10	2.15	1.95	2.10	2.20
	1.98	2.10	1.97	2.17	1.98
	2.09(0.07)	2.04(0.09)	2.03(0.09)	2.14(0.04)	2.09(0.10)
CV%	3.5	4.2	4.6	1.8	4.8

Adrenaline levels (pmol/ml)

	0.26	0.25	0.26	0.28	0.23
	0.27	0.29	0.31	0.26	0.24
	0.24	0.26	0.29	0.30	0.27
	0.25	0.27	0.27	0.26	0.25
	0.28	0.28	0.25	0.27	0.26
	0.26(0.02)	0.27(0.02)	0.28(0.02)	0.27(0.02)	0.25(0.02)
CV%	6.1	5.9	8.6	6.2	6.3.

Table 3.19 Plasma catecholamines on post-dated blood for transfusion used as internal quality control

n=5 mean (sd)

Noradrenaline levels (pmol/ml)

(A sample from batch One during week 8)

	Day 1	Day 2	Day 3	Day 4	Day 5
	2.10	2.04	1.95	2.20	1.85
	2.00	1.95	2.16	1.99	1.95
	1.97	1.98	2.00	2.15	1.98
	1.92	2.15	1.98	1.93	1.97
	1.85	2.10	2.18	1.96	2.00
	1.97(0.09)	2.04(0.08)	2.05(0.11)	2.05(0.12)	1.95(0.06)
CV%	4.7	4.1	5.2	5.9	3.0

Adrenaline levels (pmol/ml)

	0.29	0.31	0.28	0.30	0.29
	0.27	0.28	0.25	0.33	0.28
	0.25	0.30	0.27	0.26	0.25
	0.30	0.26	0.24	0.29	0.29
	0.28	0.29	0.27	0.30	0.27
	0.28(0.02)	0.29(0.02)	0.26(0.02)	0.30(0.02)	0.28(0.02)
CV%	6.9	6.6	6.3	8.4	6.0

Table 3.20 Plasma catecholamines on post-dated blood for transfusion used as internal quality control

n= mean (sd)

Noradrenaline levels (pmol/ml)

(A sample from batch one during week 12)

	Day 1	Day 2	Day 3	Day 4	Day 5
	2.17	2.09	2.18	2.00	1.91
	2.08	2.14	2.00	1.95	2.17
	2.14	2.10	2.12	2.16	2.04
	2.20	2.27	2.14	2.10	1.98
	2.12	2.15	2.10	2.19	2.03
	2.12(0.05)	2.15(0.07)	2.11(0.07)	2.08(0.10)	2.03(0.03)
CV%	2.4	3.3	3.2	4.9	4.7

Adrenaline levels (pmol/ml)

	0.26	0.25	0.24	0.26	0.29
	0.25	0.28	0.26	0.23	0.25
	0.28	0.24	0.28	0.24	0.26
	0.26	0.23	0.25	0.25	0.31
	0.31	0.24	0.27	0.28	0.28
	0.27(0.02)	0.25(0.02)	0.26(0.02)	0.25(0.02)	0.28(0.02)
CV%	8.8	7.7	6.1	7.7	8.5

Table 3.21 Plasma Catecholamines on Post-dated Plasma for Transfusion

n=10 mean (SEM) (pmol/ml)

Noradrenaline	Adrenaline
2.45	0.26
2.37	0.25
2.39	0.27
2.42	0.23
2.46	0.26
2.39	0.25
2.40	0.24
2.44	0.27
2.39	0.24
2.42	0.26
2.41 (0.01)	0.25 (0.04).

Table 3.22 **Plasma Catecholamine levels with and without acid Extractions**

n=12 mean (SEM)

Noradrenaline levels (pmol/ml)

1.2M Trichloroacetic acid	1.2M Perchloric acid	No Precipitant
1.27	2.54	2.45
1.35	2.48	2.55
1.33	2.57	2.48
1.29	2.51	2.49
1.36	2.58	2.45
1.39	2.53	2.49
1.28	2.55	2.57
1.34	2.52	2.50
1.32	2.50	2.43
1.37	2.55	2.47
1.39	2.49	2.40
1.35	2.52	2.48
1.34(0.01)	2.53(0.01)	2.48(0.01)

Table 3.23 Plasma Catecholamine levels with and without acid Extractions

n= mean (SEM)

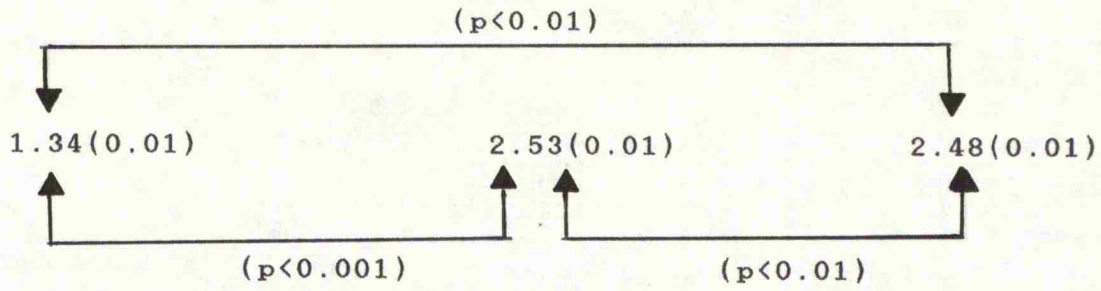
Adrenaline levels (pmol/ml)

1.2M Trichloroacetic acid	1.2M Perchloric acid	No Precipitant
0.39	0.41	0.34
0.33	0.44	0.38
0.36	0.42	0.41
0.35	0.39	0.43
0.32	0.38	0.33
0.29	0.46	0.36
0.33	0.36	0.35
0.36	0.49	0.32
0.29	0.41	0.37
0.31	0.48	0.31
0.37	0.40	0.39
0.34	0.46	0.32
0.34(0.01)	**0.43(0.01)**	**0.36(0.01)**

Table 3.24 Statistical Analysis on Catecholamine levels (pmol/ml) using different Extraction Methods

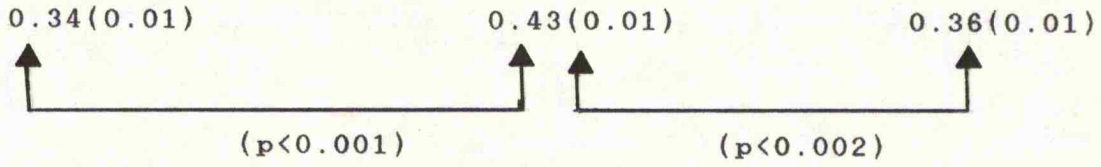
Noradrenaline levels

1.2M Trichloroacetic acid	1.2M Perchloric acid	No Precipitant



Adrenaline levels

1.2M Trichloroacetic acid	1.2M Perchloric acid	No Precipitant



SECTION THREE

Clinical Studies

1. Haemodynamic and catecholamine responses to laryngoscopy with and without tracheal intubation

2. Haemodynamic and catecholamine responses to topical tracheal analgesia

3. Haemodynamic and catecholamine changes during beta blockade

CHAPTER FOUR

4.4 PLASMA CATECHOLAMINE RESPONSES TO LARYNGOSCOPY WITH AND WITHOUT TRACHEAL INTUBATION

4.4.1 Cardiovascular Reflexes To Laryngoscopy and Tracheal Intubation

It has been observed that under light anaesthesia there is always an abrupt and marked hypertension and tachycardia during laryngoscopy and tracheal intubation, due to stimulation by the laryngoscope and endotracheal tube of the sensitive laryngeal and tracheal mucosa. The disturbances disappear after tracheal intubation has been achieved (Reid and Brace 1940). Reid and Brace suggested that the reflexes were "vago-vagal" in nature, i.e. the afferent and efferent pathway of the reflexes were mediated over the vagus nerve. While Burstein et al (1950) suggested that hypertension and tachycardia were due to the stimulation of the cardioaccelerator nerve, which increases cardiac sympathetic tone. Numerous studies regard laryngoscopy and tracheal intubation as a single stimulus, while actually they are not. No study has examined and compared catecholamine responses to laryngoscopy with and without tracheal intubation, or related any significant increases in catecholamine levels to hypertension during laryngoscopy and tracheal intubation.

Russell et al (1981) and Tomori and Widdicomb (1969) suggested that catecholamine released during laryngoscopy and tracheal intubation may cause the hypertension which occurs during such manoeuvres, probably because adrenaline

stimulates beta-1 adrenoceptor in the heart, increasing the rate and the force of the heart and cardiac output. Noradrenaline also stimulates alpha receptors in the periphery causing increased peripheral vasoconstriction which produces increased blood pressure. Russell and others did not suggest the magnitude of catecholamine levels which might cause hypertension. The magnitude of catecholamine levels during such procedures has been generally reported as a significant change of catecholamine levels from the baseline. That does not associate catecholamine levels with hypertension during laryngoscopy and tracheal intubation. If blood samples are taken as soon as possible after the laryngoscopy with or without tracheal intubation, and the catecholamine levels in those samples are compared with those catecholamine levels known to cause a pressor response, then those levels would support or refute the association of catecholamines with hypertension during those procedures. That has not been done.

An excessive pressor response which may occur during laryngoscopy and tracheal intubation may cause cerebral haemorrhage in patients with hypertension or cerebrovascular disease (Forbes and Dally, 1970) and pulmonary oedema on patients with myocardial insufficiency (Fox et al, 1977). There is interest in examining the contribution of catecholamines to the hypertension during such episode.

Inhibition of those hypertensive reflexes by application of

local analgesia prior to laryngoscopy and tracheal intubation has not been uniformly successful during light planes of anaesthesia. Denlinger et al (1974) used lignocaine spray to the trachea and found significant increases in blood pressure following tracheal intubation. Stoelting (1977) used viscous lignocaine gargle and intravenous lignocaine on two groups of patients and observed increases of arterial pressure following laryngoscopy and tracheal intubation also in the control group who received tracheal lignocaine spray. In both of those studies, catecholamine measurements were not done.

The use of beta blockade to inhibit the sympathoadrenal reflex following laryngoscopy and tracheal intubation has been recommended (Prys-Roberts et al, 1973) on those patients who are hypertensive and on antihypertensive treatment. Although there is a general agreement that the patients on a beta blocker should continue the treatment up to the time of surgery, the use of a single dose prior to induction of anaesthesia on patients not taking such treatment is controversial. Werner et al (1980) used practolol and achieved a reduction in systolic pressure during induction of anaesthesia, but hypertensive responses to laryngoscopy and tracheal intubation were not significantly reduced. Coleman and Jordan (1980) used metoprolol and reported a reduction in heart rate during induction of anaesthesia. However, during laryngoscopy and tracheal intubation there were increases of blood pressure and heart rate. Catecholamine estimation was not done on those studies.

Plasma catecholamine measurements have shown no pattern or trend. After induction of anaesthesia, the plasma catecholamine levels may rise above or fall below the baseline levels (Pflug et al, 1982; Mannelli et al, 1982).

The general questions the investigations in this thesis sought to answer have been given in chapter one; section 1.1.9. In addition, the following questions were posed: Does laryngoscopy alone generate the same catecholamine responses as laryngoscopy with tracheal intubation? Does the application of lignocaine with or without laryng-o-jet attenuate catecholamine responses? What are the haemodynamic and catecholamine changes with the a single dose of beta blockade during induction of anaesthesia?

4.4.2 Patients and Methods

The first study was conducted in female gynaecological patients (ASA I or II; Table 4.1) who were about to undergo surgery under anaesthesia and would require tracheal intubation. Patients were asked to take part in the studies, which were approved by the District Ethics Committee. The patients were allocated randomly into groups in whom laryngoscopy alone, or with tracheal intubation, was performed.

The method of premedication, induction of anaesthesia and various stages of laryngoscopy and tracheal intubation were similar in all the studies.

The patients in the present study were premedicated with diazepam 10mg orally 1-2 hours before surgery. In the anaesthetic room a 16-gauge cannula was placed in an antecubital vein under local anaesthesia. A Dinamap cuff was placed on the arm for measurement of blood pressure and electrocardiograph electrodes were attached to the precordium. After a period of stabilisation (usually 5 minutes), baseline readings of the arterial pressure and heart rate were recorded and 10ml of blood was withdrawn from the cannula into a heparinised tube for measurements of plasma noradrenaline and adrenaline levels.

Anaesthesia was induced with intravenous fentanyl 0.1mg and, two minutes later, thiopentone 3-4mg per kg was injected over 60 seconds. Atracurium 0.5mg per kg body weight was administered to provide muscle relaxation for tracheal intubation. An oropharyngeal airway was inserted into the mouth and the patient's lungs were ventilated with 67% nitrous oxide in oxygen using a face mask.

Two minutes after administration of atracurium, the arterial pressure and heart rate were recorded and a second 10ml sample of blood was obtained.

Immediately after blood sampling, the airway was removed and laryngoscopy was performed to provide a clear view of the whole of the vocal cords for 10 seconds. For the patients in the laryngoscopy only group, the laryngoscope was removed, the airway was replaced and ventilation of the lungs

recommenced through the mask. In the patients in the laryngoscopy with tracheal intubation group, the laryngoscopy was performed and the trachea was intubated, the airway was replaced and ventilation of the lungs was recommenced. A 9mm internal diameter cuffed disposable endotracheal tube was used in male patients and an 8mm internal diameter tube in females. In both groups the lungs were ventilated for five minutes and end-tidal carbon dioxide concentration maintained at 5-5.5%.

Further 10ml of blood samples were obtained and arterial pressure and heart rate were recorded at 1, 3 and 5 minutes after laryngoscopy with or without tracheal intubation. At the end of the study, in the laryngoscopy only group the patient's trachea was intubated and anaesthesia continued.

The blood samples were centrifuged at 1500g for 10 minutes. The plasma was separated and stored at -70 degrees C until subsequent analysis for noradrenaline and adrenaline levels using high pressure liquid chromatography with electrochemical detector LC-4A (Bioanalytical Systems).

Paired and unpaired Student's t tests were used to analyse the data.

4.4.3 Results

There were no significant differences between the groups in age and weight (Table 4.2).

The groups had similar baseline catecholamine levels before induction of anaesthesia. Immediately after laryngoscopy the mean catecholamine levels increased above baseline levels in the laryngoscopy alone group (Figure 4.1 and Tables 4.3; 4.4 and statistical analysis), and in the group who had laryngoscopy with tracheal intubation (Figure 4.1 and Tables 4.5; 4.6 and statistical analysis).

There were no significant differences between the groups in blood pressures before induction of anaesthesia (Figure 4.2). Following laryngoscopy with or without tracheal intubation, the groups were significantly different ($p<0.05$), and blood pressures increased above baseline levels (Figure 4.2 and Tables 4.7; 4.8; 4.9; 4.10 and statistical analysis).

Heart rate in both groups were similar before induction of anaesthesia. In the laryngoscopy alone group, there was no change in the mean heart rate after laryngoscopy (Table 4.11), while the mean heart rate increased above baseline levels in the laryngoscopy with tracheal intubation group (Figure 4.3 and Table 4.12 and statistical analysis).

There was no relationship between baseline catecholamine levels and baseline blood pressures or heart rate in either group. There was no relationship between the changes in catecholamine levels from baseline and changes in blood pressures or heart rate during induction of anaesthesia and laryngoscopy with or without tracheal intubation.

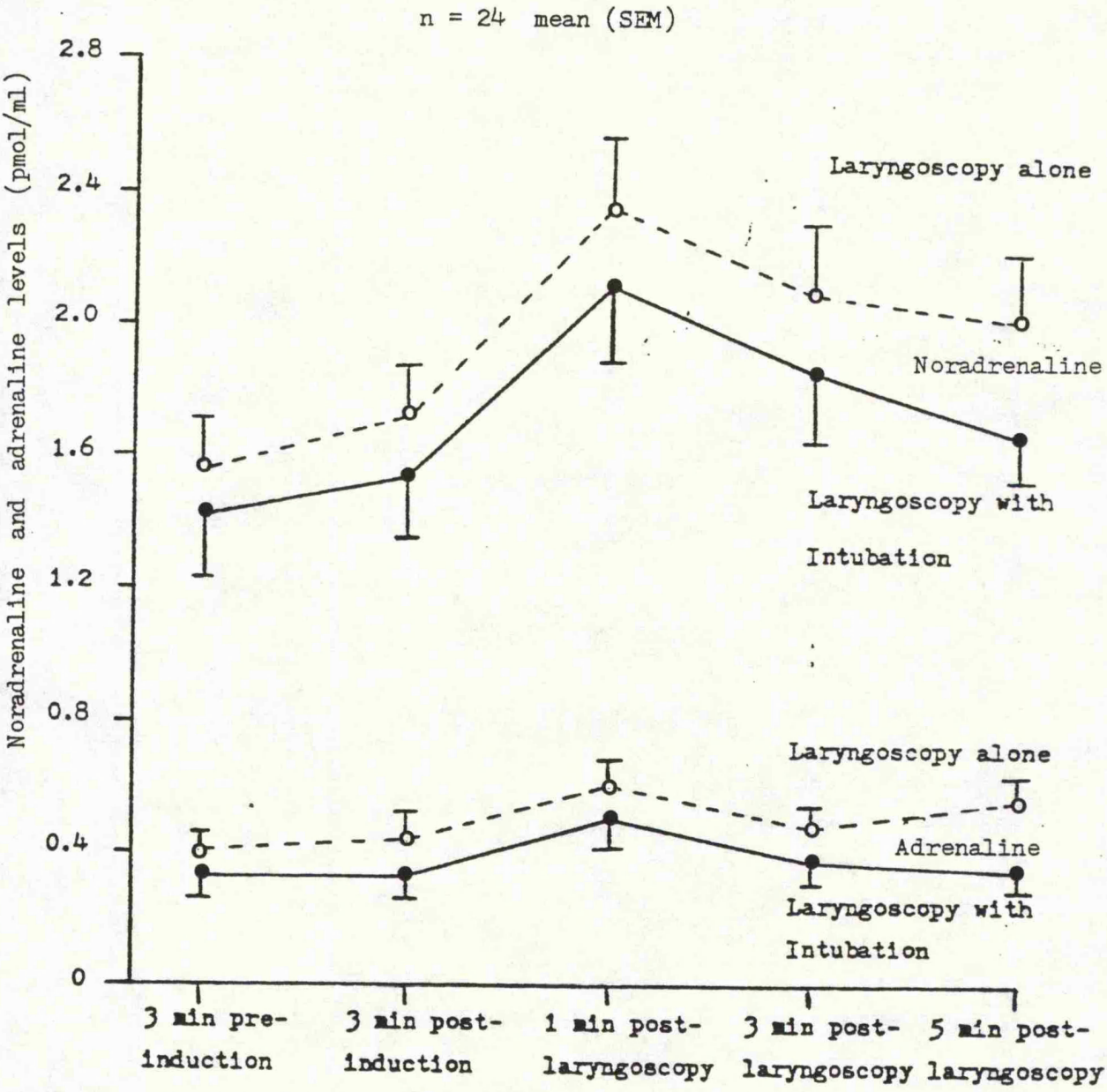


Figure 4.1 Plasma catecholamine levels before, after induction of anaesthesia and following laryngoscopy with and without tracheal intubation.

(see Tables 4.3; 4.4; 4.5 and 4.6 for statistical analysis)

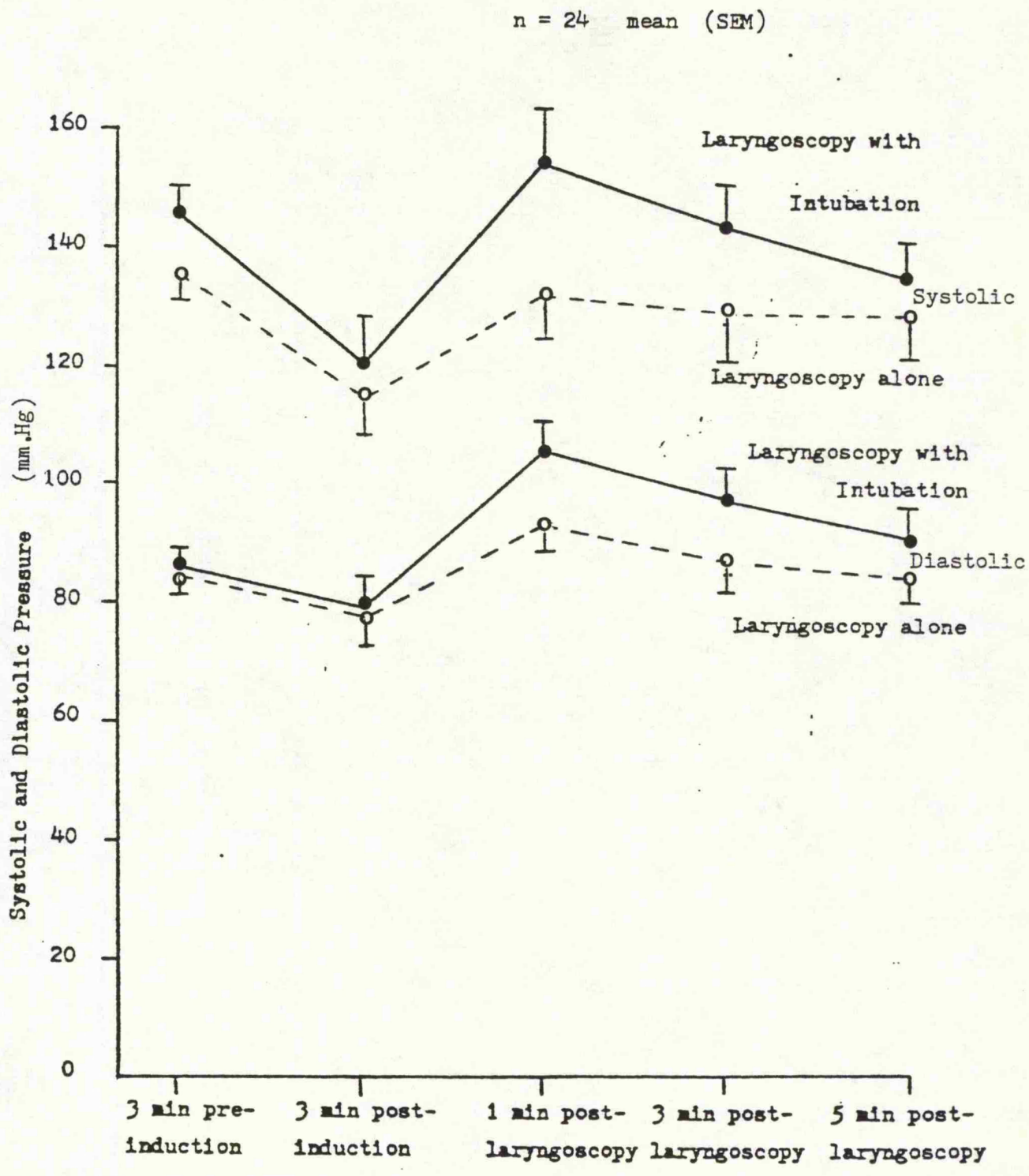


Figure 4.2 Systolic and diastolic pressures before and after induction of anaesthesia and following laryngoscopy with and without tracheal intubation.

(see Tables 4.7; 4.8; 4.9 and 4.10 for statistical analysis)

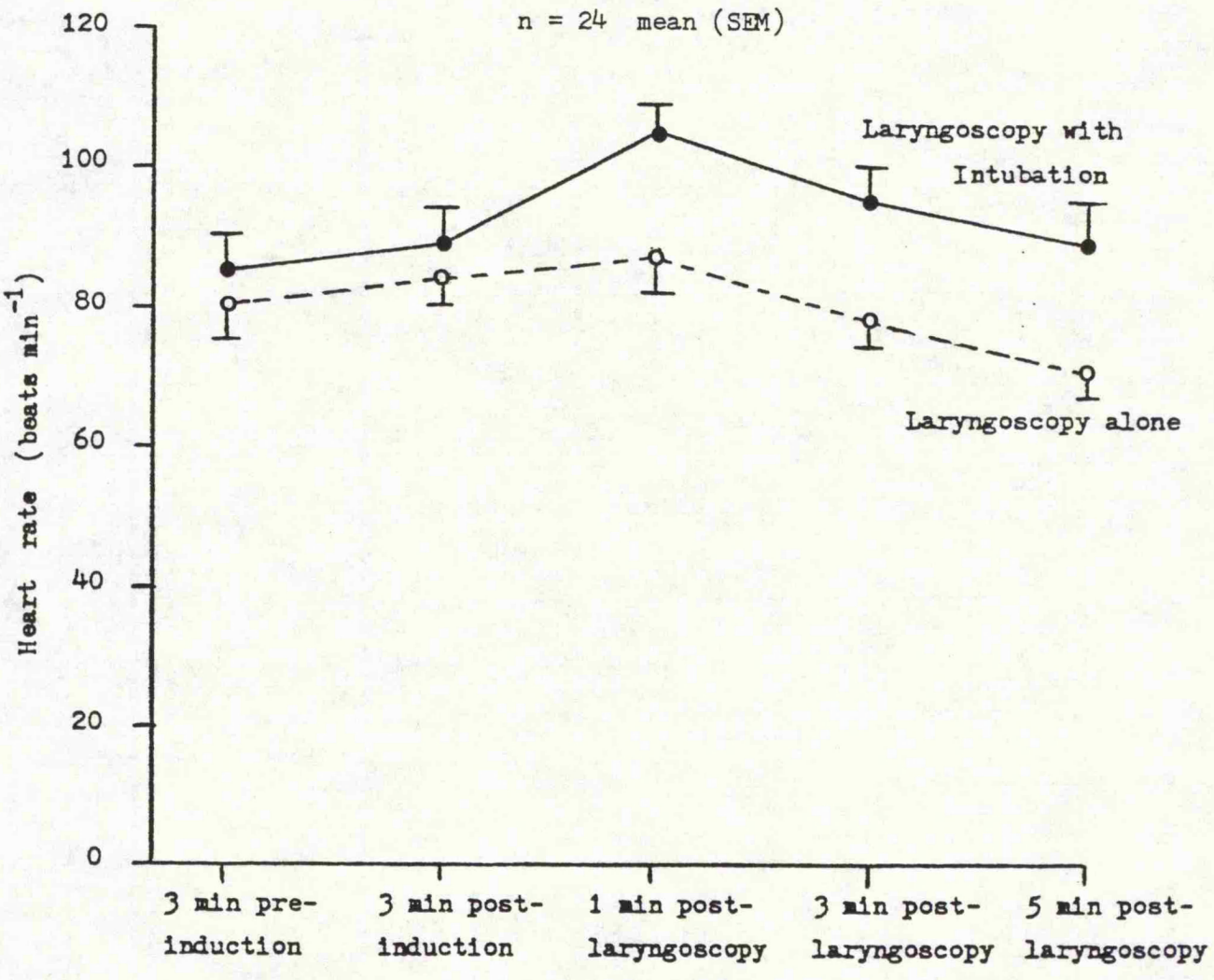


Figure 4.3 Heart rate before and after induction of anaesthesia and following laryngoscopy with and without tracheal intubation.

(see Tables 4.12 for statistical analysis)

4.4.4 Catecholamine Responses to Tracheal Intubation Following Topical Lignocaine Analgesia

The second part of the study in this chapter examined the effects of the application of the topical tracheal lignocaine analgesia using the two devices: The Forrester spray and the laryng-o-jet.

4.4.5 Patients and Methods

In the second study thirty women who were (ASA I or II Table 4.1) about to undergo gynaecological surgery, and who would require tracheal intubation were asked to take part in the study which was approved by the district Ethics committee. The patients were randomised to one of the three groups. Group 1 received topical laryngeal and tracheal lignocaine (160mg as 4%) from a conventional Forrester spray. Group 2 received lignocaine (160mg as 4%) from a commercial device (Laryng-o-jet Tm International Medical Systems Ltd). Group 3 (control) received 4ml of 0.9% sodium chloride for injection to the larynx and trachea delivered from a conventional Forrester spray.

The patients were premedicated with lorazepam 2mg and metoclopramide 10mg administered one and a half hours prior to anaesthesia. In the anaesthetic room, an antecubital vein was cannulated under local analgesia and after stabilisation (usually 5 minutes), 10ml of blood was withdrawn from the cannulated vein into a lithium heparin bottle for

catecholamine measurements. Blood pressures and heart rate were measured using a Dinamap 845. Anaesthesia was induced with papaveretum 10mg, thiopentone 3-5mg per kg and atracurium 0.5-0.6mg per kg to facilate tracheal intubation.

Anaesthesia was maintained with nitrous oxide 66% in oxygen supplemented by 0.2% enflurane administered through a Bain System delivering 70-80ml per kg body weight fresh gas flow. One minute after induction of anaesthesia a further 10ml of blood was withdrawn into a lithium heparin bottle; blood pressures and heart rate were determined at approximately the same time as blood was withdrawn. Laryngoscopy was performed using a Macintosh blade and the spray was applied to the larynx, trachea and laryngopharynx. The laryngoscope was then withdrawn, and 1 minute later, laryngoscopy and tracheal intubation were performed. One and 5 minutes after tracheal intubation, further 10ml samples of blood were withdrawn into lithium heparin bottles, and blood pressures and heart rate were determined.

The blood samples for this study were collected and spun at 4000 rpm then stored at -18 degrees C at Warwick General Hospital before being transported to the laboratory at Leicester Royal Infirmary where they were stored at -70 degrees C prior to analysis using high pressure liquid chromatography with electrochemical detection LC-4A (Bionalytical Systems).

4.4.6 Results

There were no significant differences between the three groups in respect of age and weight (Table 4.13).

The 3 groups had similar mean plasma catecholamine levels. However, immediately after tracheal intubation, the mean catecholamine levels increased above post induction levels in the Forrester and lignocaine group (Figure 4.4 and Tables 4.14; 4.15 and statistical analysis), and in the laryng-o-jet group (Figure 4.4 and Tables 4.16; 4.17 and statistical analysis), similarly, in the control group (Figure 4.4 and Tables 4.18; 4.19 and statistical analysis).

The mean baseline blood pressures were similar in all the groups. Thereafter, the mean blood pressures decreased after induction of anaesthesia in all the groups, and increased immediately above baseline levels after tracheal intubation in the Forrester and lignocaine group (Figure 4.5 and Tables 4.20; 4.21 and statistical analysis); and in the laryng-o-jet group (Figure 4.5 and Tables 4.22; 4.23 and statistical analysis), also in the control group (Figure 4.5 and Tables 4.24; 4.25 and statistical analysis).

The mean baseline heart rate were similar in all the groups. After induction of anaesthesia, the mean heart rate increased above baseline levels in the Forrester and lignocaine group and increased again immediately after tracheal intubation (Figure 4.6 and Table 4.26 and statistical anlysis), and in

the laryng-o-jet group (Figure 4.6 and Table 4.27 and statistical analysis). However, the mean heart rate in the control group increased only following tracheal intubation (Figure 4.6 and Table 4.28 and statistical analysis).

There was no relationship between the baseline catecholamine levels and the baseline blood pressures or heart rate in all the groups. There was also no relationship between the changes in catecholamine levels from the baseline and the changes in blood pressures or heart rate during induction of anaesthesia and laryngoscopy and tracheal intubation.

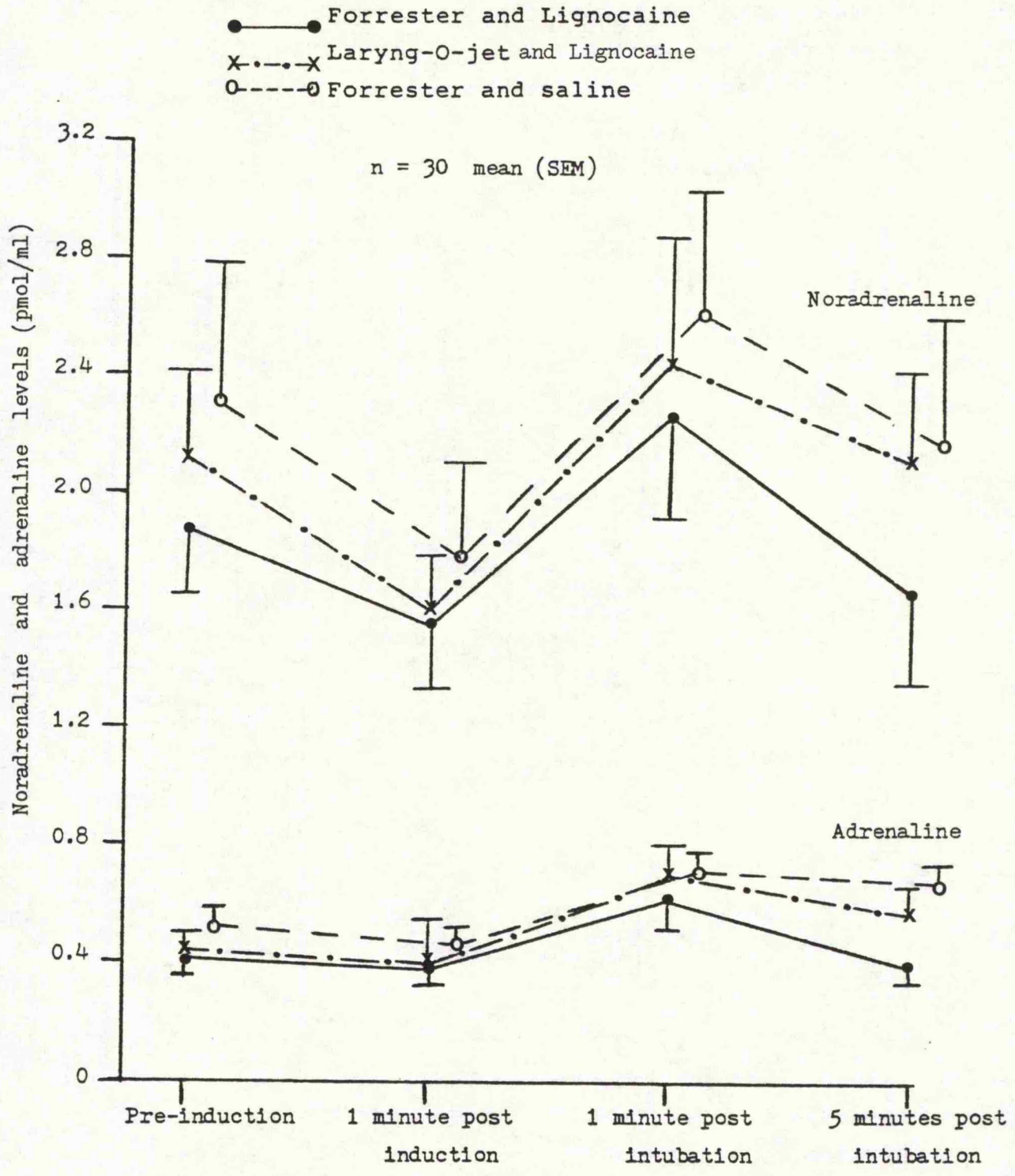


Figure 4.4 Plasma catecholamine levels before and after one minute of the application of topical tracheal analgesia using three different devices.

(see Tables 4.14; 4.15; 4.16; 4.17; 4.18 and 4.19 for statistical analysis)

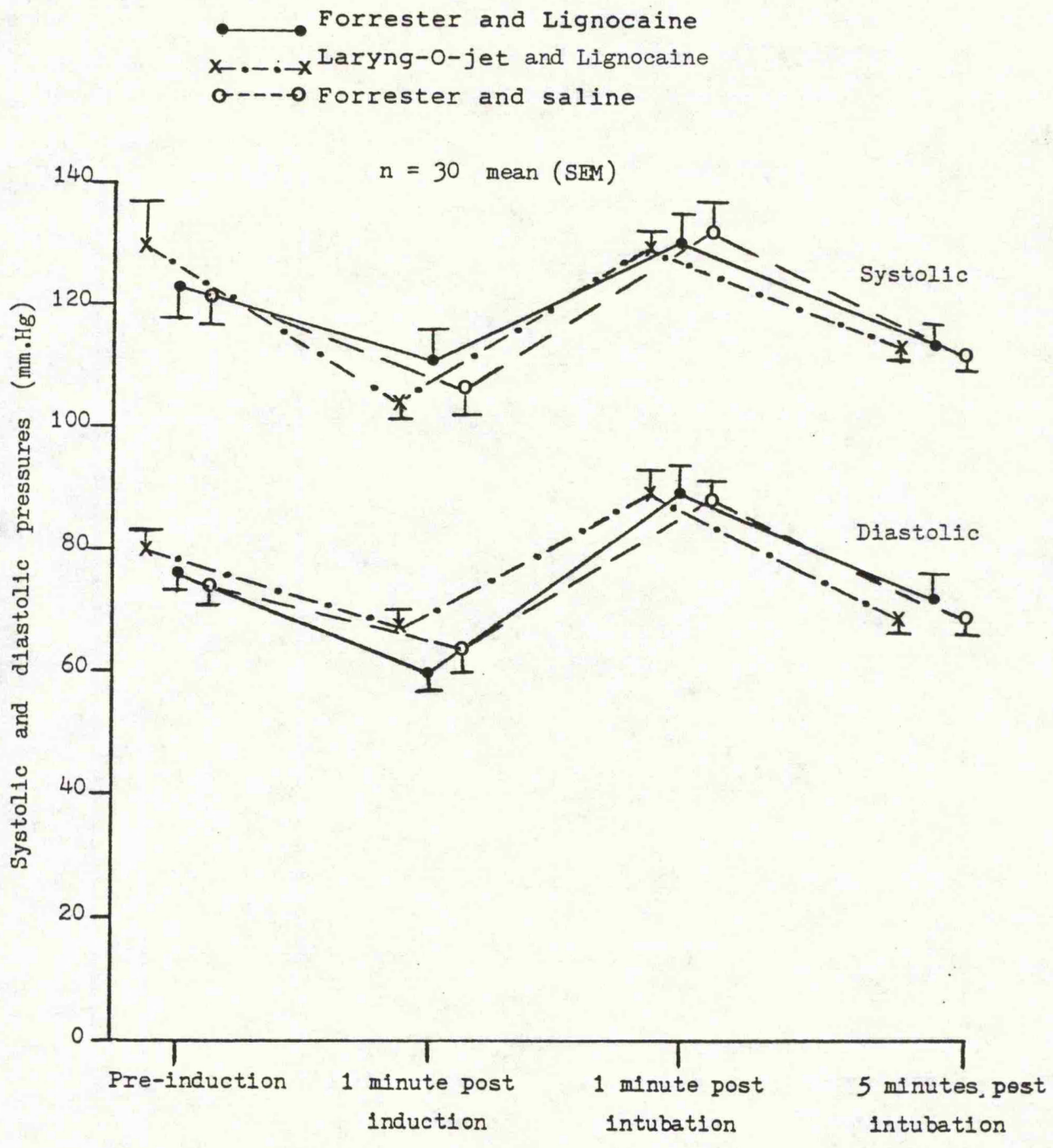


Figure 4.5 Systolic and diastolic pressures before and after one minute following the application of topical tracheal analgesia using three different devices.

(see Tables 4.20; 4.21; 4.22; 4.23; 4.24 and 4.25 for statistical analysis)

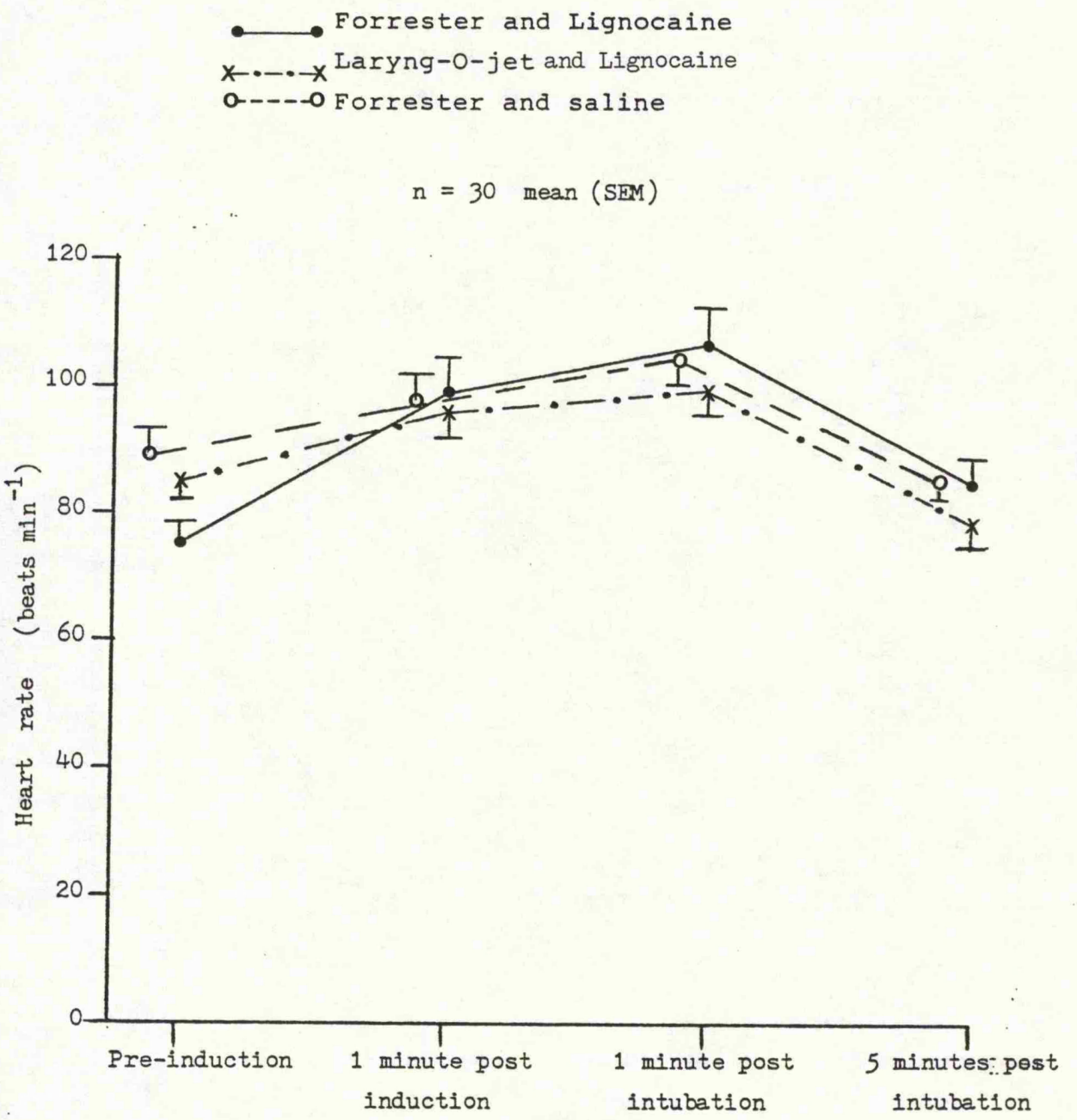


Figure 4.6 Heart rate before and after one minute following the application of topical tracheal analgesia using three different devices.

(see Tables 4.26; 4.27 and 4.28 for statistical analysis)

4.4.7 Haemodynamic and Catecholamine Changes with Beta Blockade during Induction of Anaesthesia

The third part of the study in this chapter looked at the the catecholamine levels and the changes of blood pressures and heart rate, when a single dose of beta blocker (practolol) was administered before induction of anaesthesia.

4.4.8 Patients and Methods

The third study was conducted in twenty adult female gynaecological patients (ASA grade I or II Table 4.1) requiring tracheal intubation for laparoscopic sterilisation. Full informed consent was obtained from all patients for the study, which was approved by the District Ethics Committee.

Patients were randomly allocated into two groups to receive either practolol 10mg or placebo immediately prior to induction of anaesthesia. The premedication was diazepam 10mg orally 90-120 minutes preoperatively. In the anaesthetic room a 16-gauge cannula was placed in a vein in the antecubital fossa after local intradermal injection of 0.5% plain lignocaine. An automatic pressure cuff (Copal digital sphygmomanometer UA-251) was placed on the contralateral arm, and electrocardiograph electrodes attached to record standard limb lead II. After a period of stabilisation (usually 5 minutes), a blood sample (10ml) was withdrawn from the cannula into a lithium heparin bottle for catecholamine measurements, and baseline readings of blood pressures and

heart rate were recorded.

The patients were given either practolol 10mg in a volume of 5ml or an equal volume of saline injected slowly through the cannula over 1 minute period. One minute after the injection a further 10ml blood was withdrawn into a lithium heparin bottle, and blood pressures and heart rate measurements were recorded.

Induction of anaesthesia was achieved with fentanyl 0.1mg, followed by a dose of thiopentone 3-4mg per kg body weight to induce sleep and then atracurium 0.4mg per kg body weight was given to facilitate tracheal intubation. Analgesia was maintained with 67% nitrous oxide in oxygen with artificial ventilation of the lung using a Bain breathing system and a fresh gas flow of approximately 90mls per kg body weight.

Further 10ml blood samples were obtained at 1 minute after induction (assessed by loss of eyelash reflex) and 1, 3 and 5 minutes after tracheal intubation. Blood pressures and heart rate were also recorded at those times. Blood samples for catecholamine measurements were taken with the patients in a supine position.

Blood samples were analysed for plasma noradrenaline and adrenaline levels using high pressure liquid chromatography with electrochemical detector LC-4A (Bioanalytical Systems).

4.4.9 Results

There were no significant differences between the patients who were beta-blocked and those who were not in respect of age and weight (Table 4.29).

The groups were significantly ($p<0.05$) different in the mean baseline noradrenaline levels (Figure 4.7 and Tables 4.30; 4.32). Immediately after tracheal intubation noradrenaline levels in both groups increased above post induction levels (Figure 4.7 and Tables 4.30; 4.32 and statistical analysis). However, the mean baseline adrenaline levels were similar in both groups, but were significantly different ($p<0.05$), one and 3 minutes after tracheal intubation. In both groups, the mean adrenaline levels increased above post induction levels immediately after tracheal intubation (Figure 4.7 and Tables 4.31; 4.33 and statistical analysis).

The mean baseline blood pressures were similar in both groups. However, immediately after tracheal intubation, blood pressures increased above pre-induction levels in both groups (Figure 4.8 and Tables 4.34; 4.35; 4.36; 4.37 and statistical analysis).

The mean baseline heart rates in both groups were similar. But, immediately after the administration of practolol or placebo, and 3 and 5 minutes after tracheal intubation, heart rates were significantly different in both groups (all $p<0.05$). Also the mean heart rate decreased below baseline

level after practolol, but no change occured in the placebo group. Immediately following tracheal intubation, the mean heart rate increased above baseline level in the placebo group, while the increase in the practolol group was not significant (Figure 4.9 and Tables 4.38; 4.39 and statstical analysis).

There was no relationship between the baseline catecholamine levels and the baseline blood pressures or heart rate in either group. There was no relationship between the changes in catecholamine levels from the baseline and the changes in blood pressures or heart rate during induction of anaesthesia and laryngoscopy with tracheal intubation.

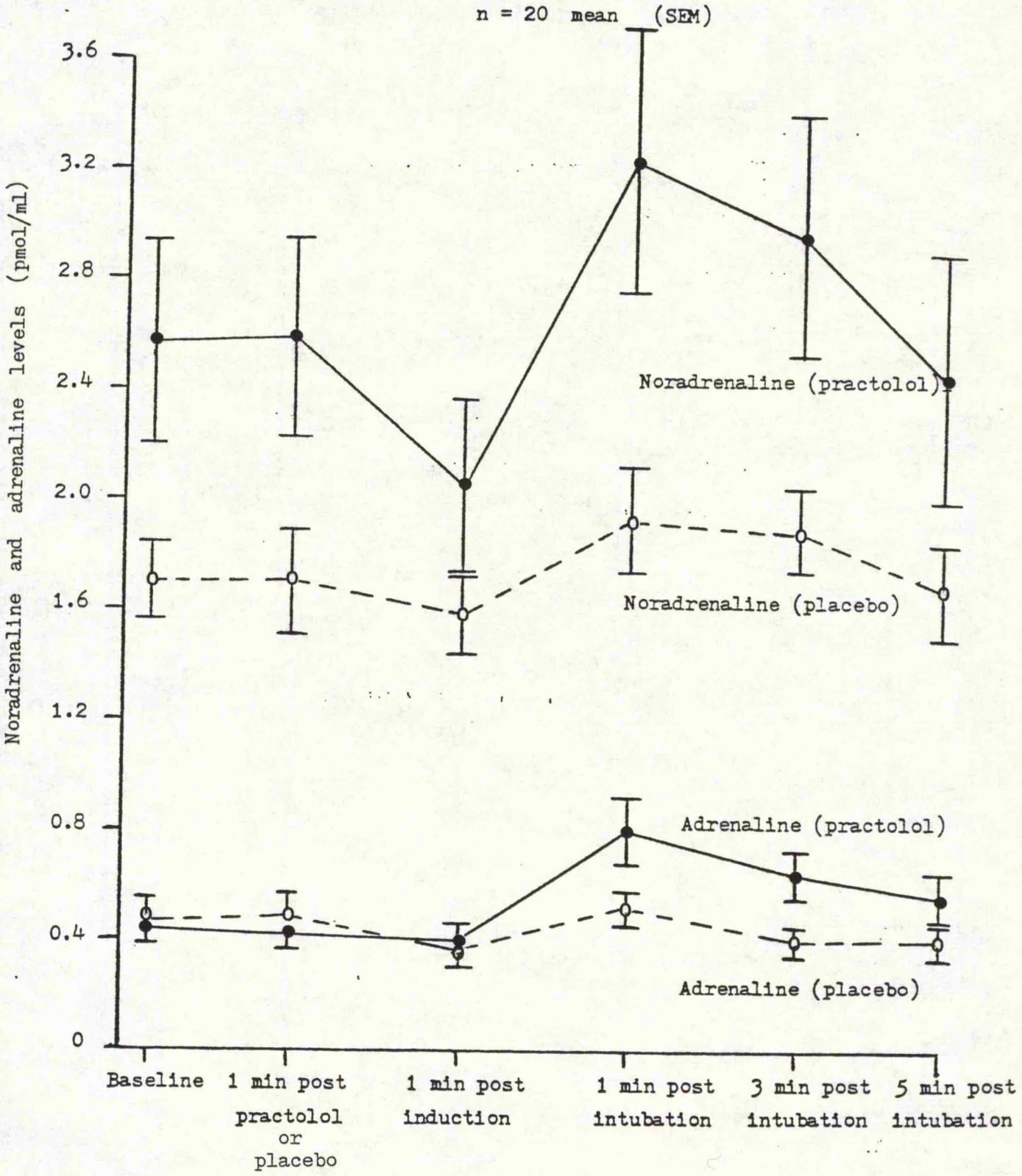


Figure 4.7 Plasma noradrenaline and adrenaline levels before and after intravenous administration of practolol or placebo.

(see Tables 4.30; 4.31; 4.32 and 4.33 for statistical analysis)

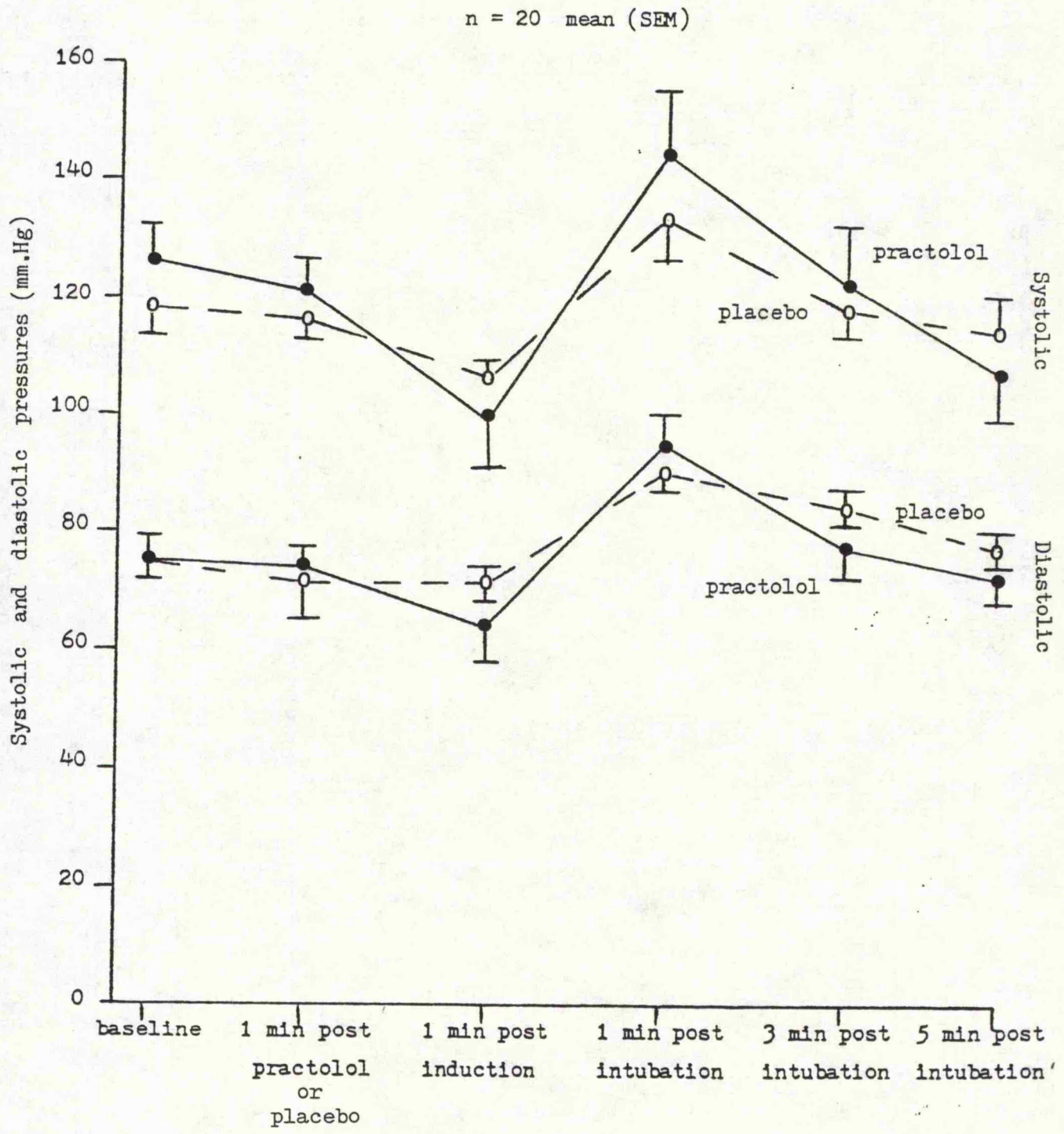


Figure 4.8 Systolic and diastolic pressures before and after intravenous administration of practolol or placebo.

(see Tables 4.34; 4.35; 4.36 and 4.37 for statistical analysis)

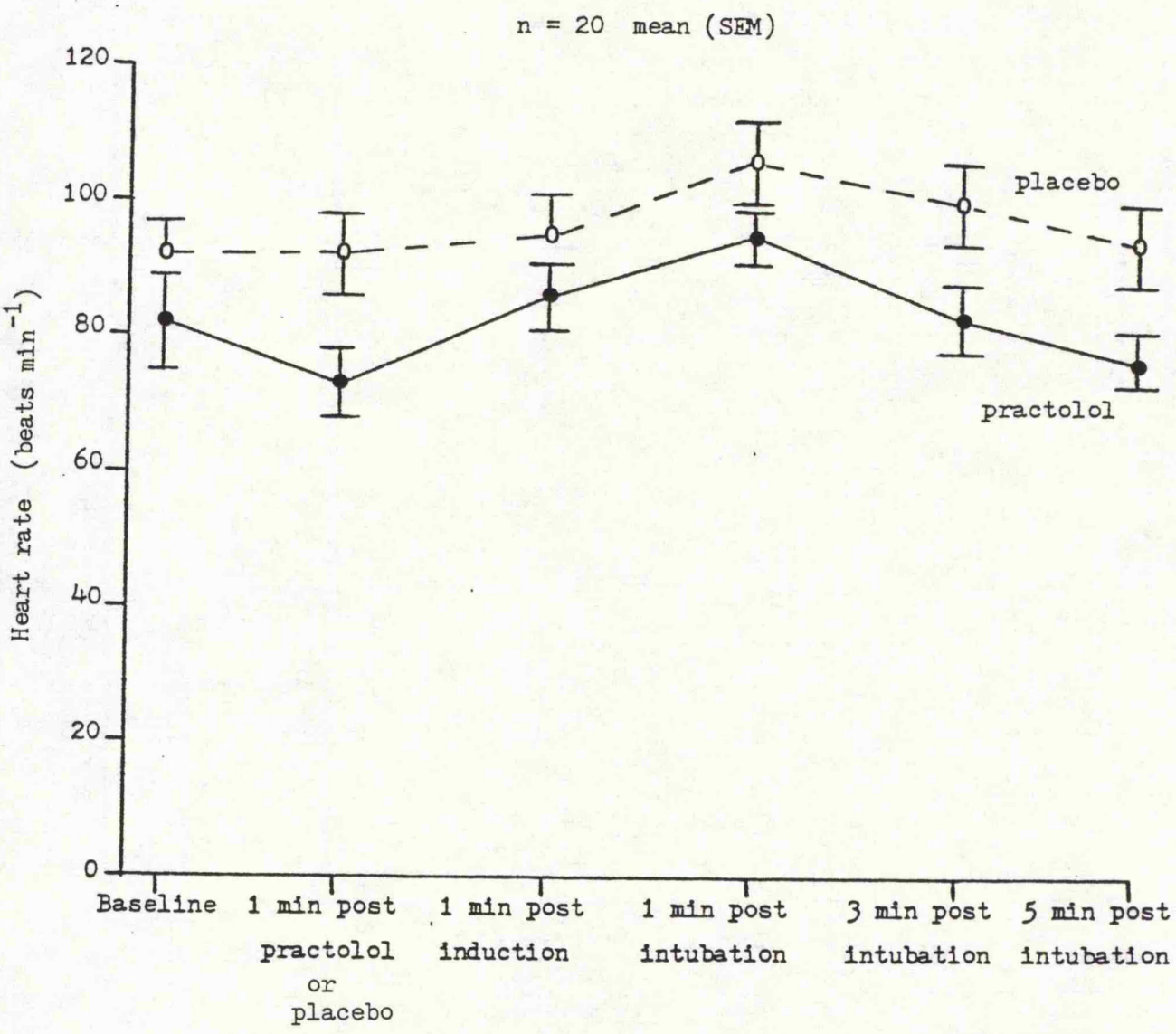


Figure 4.9 Heart rate before and after intravenous administration of practolol or placebo.

(see Tables 4.38 and 4.39 for statistical analysis)

4.4.10 Discussion

In the first study, baseline catecholamine levels (Figure 4.1 and Tables 4.3; 4.4; 4.5; 4.6) indicate that the two groups appeared to be similar.

Systolic and diastolic pressures in the laryngoscopy with and without tracheal intubation (Figure 4.2 and Tables 4.7; 4.8; 4.9; 4.10) also appeared similar, however, after laryngoscopy with and without tracheal intubation, the groups appeared to be different.

Part of apparent differences between laryngoscopy with and without tracheal intubation in respect of catecholamine levels and systolic pressure is related to the fact that baseline values were not comparable (Figures 4.1 and 4.2). In those studies, laryngoscopy with tracheal intubation was accompanied by significant increases in blood pressure and heart rate which were not apparent in the laryngoscopy only group. However, those increases were within physiological range in these ASA group I or II patients (Table 4.1).

The application of lignocaine to the larynx and trachea prior to laryngoscopy and tracheal intubation (study 2) failed to abolish the pressor response to tracheal intubation. Part of the reason for this study was to investigate the efficacy of the laryng-o-jet, which is a convenient commercial disposable device for administration of local analgesia to the larynx and trachea. It is faster than the usual device, hence its

analgesic delivery and effectiveness are assumed. However, the work reported on laryngoscopy with and without tracheal intubation (study 1) demonstrated that the cardiovascular and sympathoadrenal responses to tracheal intubation are largely caused during laryngoscopy. It is therefore not surprising that the application of local anaesthetic had little effect, because to be able to apply analgesia, laryngoscopy must be performed first.

Other investigators have also failed to abolish the pressor response to tracheal intubation. The present study confirmed the sympathoadrenal response to intubation. Similar sympathoadrenal responses with similar anaesthetic technique have been reported (Russell et al, 1981). The catecholamine responses were similar whether active drug or placebo (study 2) was used and there were no significant differences between catecholamine levels in study 2 and those reported on laryngoscopy with and without tracheal intubation (study 1). All are in the normal range and are below the levels of pharmacological action (Silverberg et al, 1978).

Tomori and Widdicombe (1969) observed in cats that there were hypertensive episodes during stimulation of the respiratory tract. They further showed that stimulation at different sites eg nose, epipharynx and laryngopharynx produced different respiratory and hypertensive responses, the reflex from the epipharynx caused stronger hypertensive responses. Tomori and Widdicombe (1969) and Russell et al (1981) assumed

that those responses might be caused by increased catecholamine levels induced by those techniques.

In the third study which examined the effects of beta-blocker on catecholamine levels, showed that there was a significant difference in the baseline noradrenaline levels between the group who received beta-blocker and those who did not. There is no obvious reason to explain that, other than that, the two groups were not matched in terms of age and blood pressures. Ziegler et al (1976) and Lake et al (1977) reported increases in noradrenaline levels with age; this was apparent in the practolol group (Table 4.29). Also DeChamplain et al (1976) observed that noradrenaline levels were higher in hypertensive than in normotensive patients. This effect of hypertension was apparent when the practolol and the placebo groups are compared.

A single intravenous dose of practolol failed to inhibit the pressor response to tracheal intubation. In addition, the rise in catecholamine levels 1 minute following tracheal intubation was greater in magnitude in the practolol group than in the placebo group; thus the two groups were significantly different in respect of noradrenaline levels ($p<0.02$) and adrenaline levels ($p<0.05$).

The present study (study 3) demonstrates that the magnitude of catecholamine levels following tracheal intubation in the placebo group were similar to those obtained in study 1 during laryngoscopy with and without tracheal intubation, and

those of study 2 during topical application of lignocaine prior to tracheal intubation, while catecholamine levels in the practolol group (study 3) were greater in magnitude than in other studies, and heart rate in the group were lower than those in the placebo.

Higher catecholamine levels have been reported with propranolol in hypertensive patients (Esler et al, 1981) and in the stress response (Magnusson and colleagues, 1983). Esler and colleagues suggested that beta-blockade increases plasma noradrenaline levels, due to the decrease in plasma noradrenaline clearance. Diminished pulmonary extraction (Esler et al, 1981) of noradrenaline from a reduced cardiac output is another possible mechanism of a reduced noradrenaline clearance, which may produce disproportionate higher noradrenaline levels in plasma.

Systolic and diastolic pressures were similar in both groups for the whole time of the study, suggesting that a single dose of practolol has little effect on blood pressures. In contrast Magnusson et al (1983) reported that systolic pressure in patients who received metoprolol was significantly lower than in those who did not. However, their patients received metoprolol 200mg in a slow release tablet daily for 4 days in addition to 10mg i.v before induction of anaesthesia.

Heart rates in the practolol group decreased significantly below baseline levels, but no change occurred in heart rate

one minute following administration of placebo. The decrease in the heart rates in the practolol group suggests that practolol blocked beta-1 adrenoceptors in the heart, so that stimulation on the heart by sympathetic nerve impulse had no cardiovascular effects.

The general catecholamine reports take into account their baseline levels, hence, the highest and the lowest baseline catecholamine levels are taken from all the three studies for the discussion in this thesis. Also the changes of those levels immediately after laryngoscopy with or without tracheal intubation are compared (Tables 4.40; 4.41).

There is no apparent association between those catecholamine levels and the corresponding systolic pressures or the age of those patients (Table 4.40; 4.41). It is not surprising that there is no association, because large numbers of subjects are required for such association to be achieved, when the mean of noradrenaline levels and those of systolic pressure and age are compared. This aspect is discussed in chapter 1; section 1.1.7.

The high baseline catecholamine levels would be expected to show corresponding marked increases in catecholamine levels following the stimulation i.e. laryngoscopy with and without tracheal intubation. Some patients with the high baseline catecholamine levels in the studies 1, 2 and 3 had only marginal increases whereas those with the low baseline catecholamine levels had comparatively greater increases in

catecholamine levels after laryngoscopy with or without tracheal intubation. The reason may be that the high baseline catecholamine levels observed may indicate that the physiological catecholamine levels stored in the vesicles and in the adrenal medulla are almost depleted, such that stimulation caused by laryngoscopy with or without tracheal intubation had little or no effects on catecholamine secretions from the vesicles and the adrenal medulla. Thus, 28% of the total patients showed a fall of noradrenaline levels 1 minute after laryngoscopy with or without tracheal intubation, and 16% of the patients showed a fall of adrenaline levels 1 minute after those procedures.

The comparison of the three studies 1 minute after laryngoscopy with or without tracheal intubation shows that in study 1, noradrenaline level decreased in 1 patient and adrenaline levels decreased in 2 patients. In study 2 shows that noradrenaline levels decreased in 11 patients and adrenaline levels decreased in 6 patients, while in study 3 noradrenaline levels decreased in 9 patients; adrenaline levels decreased in 5 patients. There is no obvious reason to explain why study 1 had only 1 and 2 patients with a fall in noradrenaline and adrenaline levels respectively, one minute after laryngoscopy with or without tracheal intubation.

The studies show that the patients with the highest baseline noradrenaline levels do not necessarily have the highest corresponding adrenaline levels, and the patients with the

lowest baseline noradrenaline levels do not appear to show the lowest corresponding adrenaline levels (Tables 4.40; 4.41). That observation appears to be true also with the highest or the lowest adrenaline levels. Hence, the studies appear to suggest that there is no direct relationship between plasma noradrenaline and adrenaline levels. Although the studies show large percentage change of the low catecholamine levels from the baseline, those levels are within the normal or the upper limit of the normal range. The individual patients discussed showed that the patients with the lowest baseline adrenaline levels had greater magnitude of the increase in the adrenaline levels than those of the noradrenaline levels after tracheal inubation (Tables 4.40; 4.41). However, the patients with the lowest or the highest baseline noradrenaline levels, or the highest baseline adrenaline levels do not appear to show consistently high catecholamine levels after tracheal intubation in all the studies (Tables 4.40; 4.41). It is known that the adrenal medulla contains higher adrenaline concentrations than noradrenaline, therefore, it is not surprising that laryngoscopy with or without tracheal intubation produced differential responses to adrenaline and noradrenaline in terms of percentage change from the baseline. The unpredictable pattern of the catecholamine levels in these studies suggests that it is unlikely that catecholamine levels can be used for general clinical assessment.

4.4.11 Conclusion

There were no significant differences between catecholamine levels in the laryngoscopy with and without tracheal intubation, the catecholamine levels in those studies being within normal range for noradrenaline (1.5-3.0pmol/ml) and for adrenaline (0.2-0.5pmol/ml).

The application of local lignocaine analgesia to trachea with or without laryng-o-jet failed to abolish catecholamine responses to laryngoscopy and tracheal intubation. There was no significant difference in catecholamine levels on the groups who had tracheal analgesic treatment and the control group. The catecholamine levels on the three groups were within the normal range.

The studies on the group who were beta blocked, showed that catecholamine levels were greater in magnitude in the group than those who were not beta blocked, though those levels were in the upper limit of the normal range.

There were no relationships between the baseline catecholamine levels and the baseline blood pressures or heart rate nor between the changes in catecholamine levels from the baseline and the corresponding changes in blood pressures or heart rate.

Table 4.1 The American Society of Anesthesiologists' (ASA) Patients Physical Status (Dripps et al., 1977)

Class I

Patients without physiological, biochemical or psychiatric disturbance. Operation is localised, no systemic disturbance eg inguinal hernia, fibroid uterus.

Class II

Patients with mild or moderate systemic disturbance eg mild heart disease, mild diabetes, essential hypertension, anaemia, extreme obesity and chronic bronchitis.

Class III

Patients with severe systemic disturbance eg severe heart disease, severe diabetes with vascular complications, moderate to severe degrees of pulmonary insufficiency, angina pectoris and healed myocardial infarction.

Class IV

Patients with severe systemic disorders not correctable by operation eg patients with heart disease showing marked signs of cardiac insufficiency, persistent anginal syndrome, active myocarditis, advanced degrees of pulmonary, hepatic, renal or endocrine insufficiency.

Class V

The patients in this group have little chance of survival but are operated on in desperation eg ruptured abdominal aneurysm with profound shock, major cerebral trauma with rapidly increasing intracranial pressure, or massive pulmonary embolism.

Table 4.2 Details of the Patients in both groups.

n=24 mean (SEM)

Laryngoscopy Alone		Laryngoscopy and Intubation	
Age (yrs)	Weight (kg)	Age (yrs)	Weight (kg)
63	45	36	75
37	74	30	75
63	80	64	71
25	75	35	85
63	80	61	50
39	54	25	50
	78	40	55
47	57	46	73
33		62	86
23	70	39	65
32	74	51	72
40		55	60
42.3 (4.5)	68.7 (3.9)	45.3 (3.8)	68.1 (3.5)

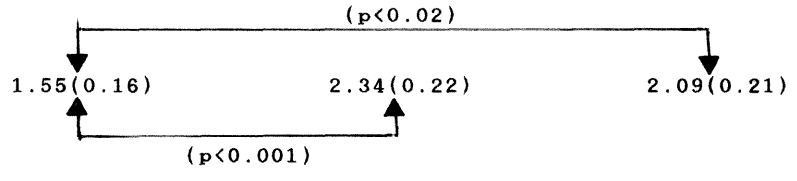
Table 4.3 Plasma Noradrenaline levels before and after laryngoscopy alone.

n=12 mean (SEM) pmol/ml.

3min pre induction	3min post induction	1min post laryngos	3min post laryngos	5min post laryngos
1.19	1.65	2.25	1.79	2.14
1.25	1.26	3.42	2.05	1.16
1.65	1.57	1.79	1.41	1.23
1.38	1.49	2.03	1.47	1.69
0.65	1.16	1.51	1.53	1.69
2.52	2.22	2.71	3.78	2.39
1.41	2.31	3.34	2.94	3.04
1.97	2.89	3.38	2.66	3.53
1.09	1.56	1.98	2.29	2.18
1.43	1.23	1.39	1.46	1.76
1.56	1.04	1.63	1.62	1.49
2.47	2.19	2.66	2.05	1.65
1.55(0.16)	**1.71(0.16)**	**2.34(0.22)**	**2.09(0.21)**	**2.00(0.21)**

Statistical Analysis

Pre-induction 1 min post laryngos 3 min post laryngos



Laryngos=Laryngoscopy

Table 4.4 Plasma Adrenaline levels before and after laryngoscopy alone.

n=12 mean (SEM) pmol/ml.

3min pre induction	3min post induction	1min post laryngos	3min post laryngos	5min post laryngos
0.54	0.67	0.81	0.46	0.55
0.10	0.08	0.37	0.27	0.24
0.17	0.11	0.31	0.12	0.21
0.33	0.45	0.51	0.27	0.20
0.43	0.66	0.74	0.57	0.36
0.13	0.08	0.19	0.22	0.20
0.48	0.73	0.91	0.65	0.70
0.70	0.89	1.21	0.99	1.11
0.75	0.34	0.50	0.85	0.70
0.67	0.43	0.60	0.57	0.35
0.32	0.28	0.31	0.16	0.38
0.35	0.53	0.70	0.40	0.37
0.41(0.06)	**0.44(0.08)**	**0.60(0.08)**	**0.46(0.08)**	**0.45(0.08)**

Statistical Analysis

3 min pre-induction 1 min post laryngos

0.41(0.06) 0.60(0.08)

(p<0.001)

Laryngos=Laryngoscopy

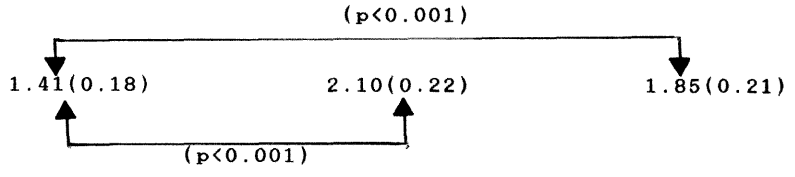
Table 4.5 Plasma Noradrenaline levels before and after laryngoscopy and intubation.

n=12 mean (SEM) pmol/ml.

3min pre induction	3min post induction	1min post laryngos	3min post laryngos	5min post laryngos
1.14	1.31	1.45	1.32	1.54
1.00	1.34	1.45	1.17	1.52
0.98	0.85	1.47	1.42	1.14
0.93	0.71	1.43	1.22	1.27
1.79	1.50	2.10	2.22	1.81
0.62	1.19	2.39	1.35	1.80
2.92	2.92	3.43	3.12	2.34
1.27	1.56	1.97	1.39	1.58
0.99	0.91	1.11	1.19	0.82
1.77	2.09	2.79	2.75	2.18
1.40	1.87	2.53	2.36	2.02
2.05	2.15	3.13	2.73	1.82
1.41(0.18)	**1.53(0.18)**	**2.10(0.22)**	**1.85(0.21)**	**1.65(0.13)**

Statistical Analysis

3min pre-induction 1min post laryngos 3min post laryngos



Laryngos=Laryngoscopy

Table 4.6 Plasma Adrenaline levels before and after laryngoscopy and intubation.

n=12 mean (SEM) pmol/ml.

3min pre induction	3min post induction	1min post laryngos	3min post laryngos	5min post laryngos
0.19	0.17	0.24	0.17	0.44
0.32	0.30	0.54	0.39	0.66
0.23	0.14	0.35	0.27	0.21
0.16	0.12	0.28	0.18	0.07
0.13	0.22	0.30	0.17	0.15
0.20	0.24	0.53	0.26	0.28
0.26	o.26	0.34	0.37	0.21
0.13	0.32	0.22	0.23	0.31
0.69	0.64	0.91	0.92	0.73
0.76	0.81	0.85	0.77	0.41
0.20	0.26	0.28	0.27	0.22
0.42	0.34	1.10	0.41	0.43
0.32(0.06)	**0.32(0.06)**	**0.50(0.09)**	**0.37(0.07)**	**0.34(0.06)**

Statistical Analysis

3 min pre-induction 1 min post laryngos

0.32(0.06) 0.50(0.09)

(p<0.001)

Laryngos=Laryngoscopy

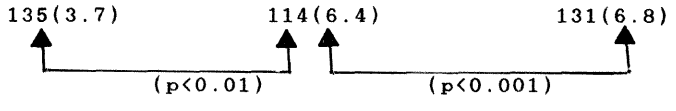
Table 4.7 Systolic arterial pressure before and after laryngoscopy alone.

n=12 mean (SEM) mmHg.

3min pre induction	3min post induction	1min post laryngos	3min post laryngos	5min post laryngos
118	88	102	93	134
145	144	153	147	144
128	93	118	105	112
117	103	134	122	118
150	109	143	132	123
137	117	123	118	114
139	109	134	134	121
161	169	189	196	189
130	119	130	137	128
125	103	107	104	101
134	114	128	137	146
135	103	112	105	99
135(3.7)	**114(6.4)**	**131(6.8)**	**128(7.9)**	**127(7.0)**

Statistical Analysis

3min pre-induction 3min post induction 1min post laryngos



Laryngos=laryngoscopy

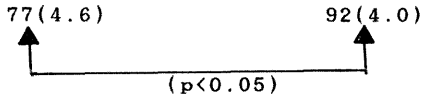
Table 4.8 Diastolic arterial pressure before and after laryngoscopy alone.

n=12 mean (SEM) mmHg.

3min pre induction	3min post induction	1min post laryngos	3min post laryngos	5min post laryngos
66	65	75	58	82
87	105	104	105	91
80	63	76	69	74
74	58	94	77	69
83	70	92	85	79
91	75	101	81	82
93	70	98	88	79
100	110	124	127	115
83	71	98	94	87
81	67	68	66	63
77	84	85	95	96
90	81	87	84	78
84(3.0)	77(4.6)	92(4.0)	86(5.0)	83(4.0)

Statistical Analysis

3 min pre-induction 1 min post laryngos



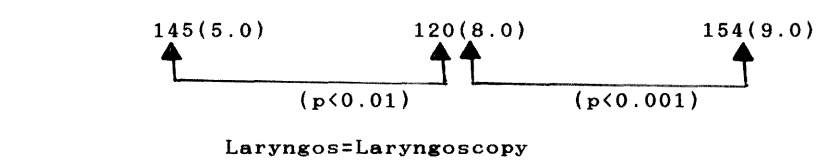
Laryngos=Laryngoscopy

Table 4.9 Systolic arterial pressure before and after laryngoscopy and intubation.

n=12 mean (SEM) mmHg.

3min pre induction	3min post induction	1min post laryngos	3min post laryngos	5min post laryngos
131	139	161	160	170
181	122	145	143	130
170	131	182	161	155
149	120	151	142	135
137	67	96	112	105
125	108	128	123	126
137	94	126	110	104
144	127	152	144	121
154	176	182	168	153
124	129	139	117	121
131	116	182	153	140
155	111	200	188	147
145(5.0)	**120(8.0)**	**154(9.0)**	**143(7.0)**	**134(6.0)**

Statistical Analysis



Laryngos=Laryngoscopy

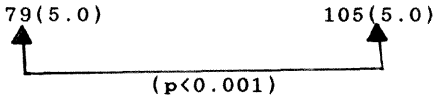
Table 4.10 Diastolic arterial pressure before and after laryngoscopy and intubation.

n=12 mean (SEM) mmHg.

3min pre induction	3min post induction	1min post laryngos	3min post laryngos	5min post laryngos
80	97	107	114	107
103	78	117	108	105
88	83	120	106	109
94	81	114	101	96
78	45	70	74	66
72	55	88	74	76
97	67	93	81	75
82	87	108	92	70
85	107	108	100	93
74	88	93	84	75
93	84	121	109	102
85	78	126	121	101
86(3.0)	**79(5.0)**	**105(5.0)**	**97(5.0)**	**90(5.0)**

Stastistical Analysis

3 min post induction 1 min post laryngos



Laryngos=Laryngoscopy

Table 4.11 Heart rate before and after laryngoscopy alone.

n=12 mean (SEM) beats/min.

3min pre induction	3min post induction	1min post laryngos	3min post laryngos	5min post laryngos
67	74	77	72	78
90	100	85	78	63
55	63	58	51	52
76	85	87	82	75
76	78	95	68	67
108	106	113	104	91
86	95	97	86	73
93	91	98	82	74
68	80	108	96	82
93	70	65	61	60
92	88	85	87	64
60	76	77	66	64
80(4.6)	**84(3.7)**	**84(4.7)**	**78(4.3)**	**70(3.1)**

Laryngos=Laryngoscopy

Table 4.12 Heart rate before and after laryngoscopy and intubation.

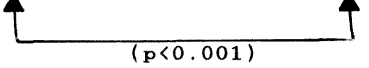
n=12 mean (SEM) beats/min.

3min pre induction	3min post induction	1min post laryngos	3min post laryngos	5min post laryngos
64	66	86	105	125
110	110	119	101	91
84	78	86	73	68
68	79	92	79	75
61	83	108	84	65
84	92	104	88	85
112	120	126	123	121
82	80	100	76	68
85	83	100	91	83
72	77	100	101	86
81	105	107	107	104
115	94	127	111	91
85(5.3)	**89(4.6)**	**105(4.0)**	**95(4.8)**	**89(5.7)**

Stasistical Analysis

3 min pre-induction 1 min post laryngos

85(5.3) 105(4.0)



Laryngos=Laryngoscopy

Table 4.13 Details of patients

mean (SEM)

Forrester&lignocaine		Laryng-o-jet		Forrester&saline	
Age(yrs)	Wt(kg)	Age(yrs)	Wt(kg)	Age(yrs)	Wt(kg)
32	73	48	75	39	47
43	71	31	52	40	60
30	68	46	65	36	54
24	50	20	57	53	71
41	58	36	67	51	60
39	55	27	53	29	69
62	68	38	53	28	58
31	60	22	57	43	53
22	69	42	58	20	62
30	63	41	62	37	59
35(4)	63(2)	35(3)	60(2)	38(3)	59(2)

Table 4.14 Changes in plasma noradrenaline levels in patients who received topical lignocaine spray before tracheal intubation from Forrester device.

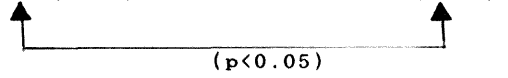
n=10 mean (SEM) pmol/ml.

Baseline	1'post induc	1'post intub	5'post intub
1.64	2.67	2.47	2.58
1.26	0.95	1.46	1.10
3.07	2.22	2.58	2.56
2.04	1.49	1.19	0.92
2.12	1.62	1.95	0.85
0.72	0.61	0.69	0.52
2.19	1.44	4.63	3.50
1.24	1.43	3.05	2.15
1.95	0.88	2.10	0.76
2.61	2.15	2.54	1.77
1.88(0.22)	1.55(0.21)	2.27(0.35)	1.67(0.31)

Statistical Analysis

1' post induction 1' post intubation

1.55(0.21) 2.27(0.35)



' = minute

induc = induction

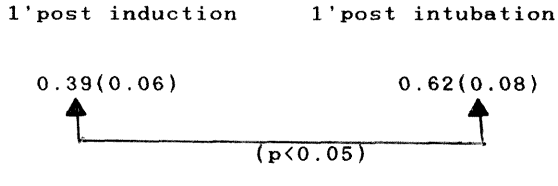
intub = intubation

Table 4.15 Changes in plasma adrenaline levels in patients who received topical lignocaine spray before tracheal intubation from Forrester device.

n=10 mean (SEM) pmol/ml.

Baseline	1'post induc	1'post intub	5'post intub
0.69	0.56	0.64	0.56
0.46	0.46	0.74	0.38
0.58	0.82	0.89	0.64
0.27	0.53	0.52	0.43
0.36	0.33	0.39	0.30
0.58	0.30	0.45	0.16
0.32	0.21	0.91	0.71
0.48	0.25	0.94	0.52
0.30	0.22	0.25	0.24
0.19	0.22	0.47	0.10
0.42(0.05)	0.39(0.06)	0.62(0.08)	0.40(0.06)

Statistical Analysis



' = minute

induc = induction

intub = intubation

Table 4.16 Changes in plasma noradrenaline levels in patients who received topical lignocaine spray before tracheal intubation from Laryng-o-jet device.

n=10 mean (SEM) pmol/ml

Baseline	1'post induc	1'post intub	5'post intub
2.41	2.45	5.23	3.94
1.72	1.35	2.18	2.47
2.65	2.04	2.83	1.98
1.83	1.46	1.91	2.30
4.16	1.74	1.82	1.27
1.24	1.21	1.33	0.89
1.56	1.08	2.17	2.46
1.20	1.29	1.08	1.67
1.59	0.73	1.29	1.13
2.79	2.60	4.55	3.04
2.12(0.29)	1.60(0.19)	2.44(0.44)	2.12(0.30)

Statistical Analysis

1' post induction 1' post intubation

1.60(0.19) 2.44(0.44)

(p<0.02)

' = minute

induc = induction

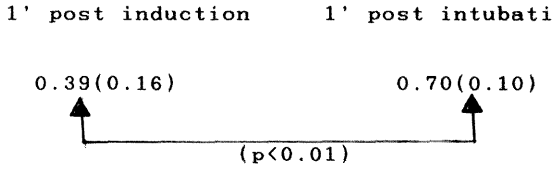
intub = intubation

Table 4.17 Changes in plasma adrenaline levels in patients who received topical lignocaine spray before tracheal intubation from Laryng-o-jet device.

n=10 mean (SEM) pmol/ml

Baseline	1'post induc	1'post intub	5'post intub
0.62	0.54	0.88	0.44
0.60	0.42	0.60	0.40
0.32	0.23	0.87	1.01
0.49	0.42	0.64	0.44
0.36	0.44	0.77	0.55
0.19	0.35	0.86	0.56
0.86	0.68	1.19	1.40
0.24	0.20	0.20	0.13
0.21	0.17	0.22	0.25
0.40	0.49	0.76	0.52
0.43(0.07)	**0.39(0.16)**	**0.70(0.10)**	**0.57(0.12)**

Statistical Analysis



' = minute

induc = induction

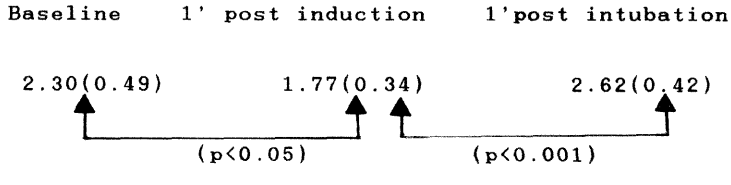
intub = intubation

Table 4.18 Changes in plasma noradrenaline levels in patients who received topical saline spray before tracheal intubation from Forrester device.

n=10 mean (SEM) pmol/ml.

Baseline	1'post induc	1'post intub	5'post intub
2.39	1.88	2.92	2.05
4.49	3.48	4.53	3.05
1.81	1.56	2.44	2.48
1.94	1.21	1.75	1.60
1.20	1.21	2.21	1.70
1.21	0.70	1.34	1.14
1.77	1.09	1.54	2.45
1.19	0.76	2.01	1.21
1.33	1.89	2.03	1.40
5.65	3.87	5.38	4.65
2.30(0.49)	**1.77(0.34)**	**2.62(0.42)**	**2.17(0.34)**

Statistical Analysis



' = minute

induc = induction

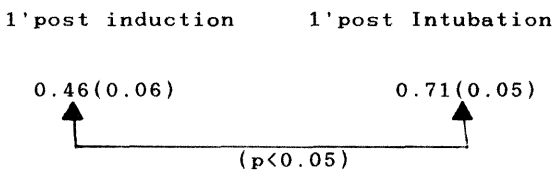
intub = intubation

Table 4.19 Changes in plasma adrenaline levels in patients who received topical saline spray before tracheal intubation from Forrester device.

n=10 mean (SEM) pmol/ml

baseline	1'post induc	1'post intub	5'post intub
0.42	0.47	0.95	0.89
0.98	0.72	0.70	0.41
0.61	0.51	0.93	0.73
0.48	0.70	0.79	0.68
0.27	0.40	0.55	0.50
0.22	0.12	0.49	0.77
0.29	0.35	0.86	0.84
0.49	0.32	0.54	0.73
0.69	0.67	0.54	0.57
0.67	0.36	0.71	0.66
0.51(0.07)	0.46(0.06)	0.71(0.05)	0.68(0.05)

Statistical Analysis



' = minute

induc = induction

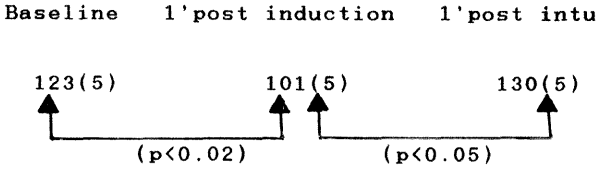
intub = intubation

Table 4.20 Changes in systolic arterial pressure in patients who received topical lignocaine spray before tracheal intubation from Forrester device.

n=10 mean (SEM) mmHg

Baseline	1'post induc	1'post intub	5'post intub
120	125	127	125
134	104	144	110
108	87	115	117
137	101	121	108
146	87	131	126
127	119	124	116
124	73	153	125
116	101	135	103
127	121	145	112
95	95	107	97
123(5)	**101(5)**	**130(5)**	**114(3)**

Statistical Analysis



' = minute

induc = induction

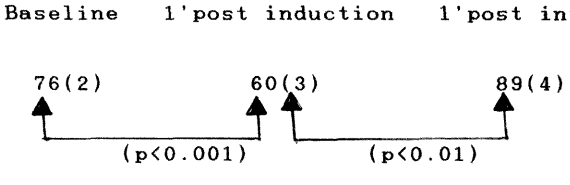
intub = intubation

Table 4.21 Changes in diastolic arterial pressure in patients who received topical lignocaine spray before tracheal intubation from Forrester device.

n=10 mean (SEM) mmHg

Baseline	1'post induc	1'post intub	5'post intub
81	61	94	80
79	75	105	69
74	59	100	87
78	54	75	60
78	49	90	84
87	74	88	74
77	54	85	65
69	57	91	83
72	62	94	66
60	58	63	53
76(2)	**60(3)**	**89(4)**	**72(4)**

Statistical Analysis



' = minute

induc = induction

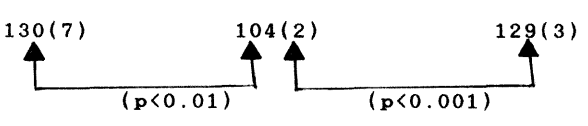
intub = intubation

Table 4.22 Changes in systolic arterial pressure in patients who received topical lignocaine spray before tracheal intubation from Laryng-o-jet device.

n=10 mean (SEM) mmHg

Baseline	1'post induc	1'post intub	5'post intub
171	104	131	120
98	93	122	117
144	103	131	109
139	103	128	117
138	119	126	125
96	98	104	102
124	104	131	118
121	109	130	111
141	106	144	124
126	104	141	100
130(7)	**104(2)**	**129(3)**	**114(2)**

Statistical Analysis



' = minute

induc = induction

intub = intubation

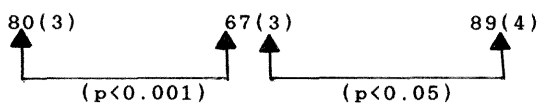
Table 4.23 Changes in diastolic arterial pressure in patients who received topical lignocaine spray before tracheal intubation from Laryng-o-jet.

n=10 mean (SEM) mmHg

Baseline	1'post induc	1'post intub	5'post intub
94	80	103	76
66	63	85	74
88	65	83	71
78	61	76	71
87	78	97	76
69	52	65	56
82	70	101	74
78	64	84	69
85	75	106	61
76	66	88	59
80(3)	**67(3)**	**89(4)**	**69(2)**

Statistical Analysis

Baseline 1'post induction 1'post intubation



' = minute

induc = induction

intub = intubation

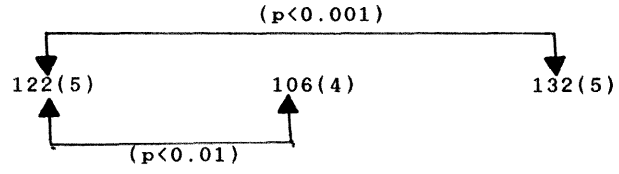
Table 4.24 Changes in systolic arterial pressure in patients who received topical saline spray before tracheal intubation from Forrester device.

n=10 mean (SEM) mmHg

Baseline	1'post induc	1'post intub	5'post intub
140	116	145	120
150	109	129	108
119	90	127	109
130	121	133	123
108	122	144	111
101	92	127	109
102	99	119	107
122	96	153	110
117	100	101	108
131	114	138	118
122(5)	**106(4)**	**132(5)**	**112(2)**

Statistical Analysis

Baseline 1'post induction 1'post intubation



' = minute

induc = induction

intub = intubation

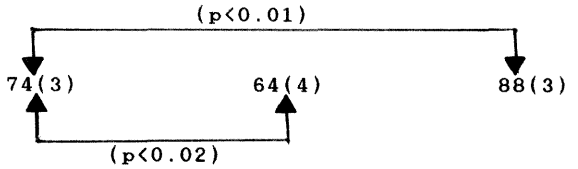
Table 4.25 Changes in diastolic arterial pressure in patients who received topical saline spray before tracheal intubation from Forrester device.

n=10 mean (SEM) mmHg

Baseline	1'post induc	1'post intub	5'post intub
84	69	91	65
86	60	88	64
63	42	77	70
79	68	89	72
67	84	100	67
69	60	90	70
71	63	89	75
68	58	87	67
68	52	67	66
86	79	98	77
74(3)	**64(4)**	**88(3)**	**69(2)**

Statistical Analysis

Baseline 1'post induction 1'post intubation



' = minute

induc = induction

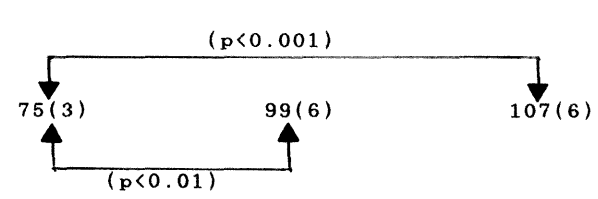
intub = intubation

Table 4.26 Changes in heart rate in patients who received topical lignocaine spray before tracheal intubation from Forrester device.

n=10 mean (SEM) beats/min.

Baseline	1'post induc	1'post intub	5'post intub
81	92	97	84
92	97	125	88
76	115	115	89
83	119	105	84
83	131	141	104
77	84	93	86
88	94	101	87
81	113	101	103
68	73	117	71
64	71	75	57
75(3)	99(6)	107(6)	85(4)

Statistical Analysis



' = minute

induc = induction

intub = intubation

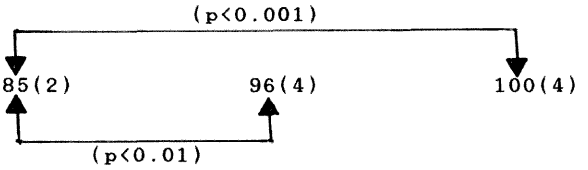
Table 4.27 Changes in heart rate in patients who received topical lignocaine spray before tracheal intubation from Laryng-o-jet device.

n=10 mean (SEM) beats/min.

Baseline	1'post induc	1'post intub	5'post intub
98	111	108	96
77	74	92	87
93	104	113	83
82	102	101	84
92	94	90	81
82	104	88	61
85	107	111	84
89	93	122	88
78	78	92	63
76	92	84	68
85(2)	**96(4)**	**100(4)**	**79(4)**

Statistical Analysis

Baseline 1'post induction 1'post intubation



' = minute

induc = induction

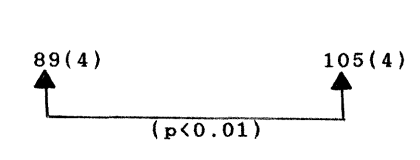
intub = intubation

Table 4.28 Changes in heart rate in patients who received topical saline spray before tracheal intubation from Forrester device.

n=10 mean (SEM) beats/min.

Baseline	1'post induc	1'post intub	5'post intub
100	93	110	81
106	97	108	89
90	94	107	84
101	86	85	72
79	93	105	83
80	108	113	96
84	102	111	94
61	68	83	65
84	101	100	91
104	125	129	101
89(4)	**97(5)**	**105(4)**	**86(3)**

Statistical Analysis



' = minute

induc = induction

intub = intubation

Table 4.29 Patients data in both groups

mean (SEM) n=20

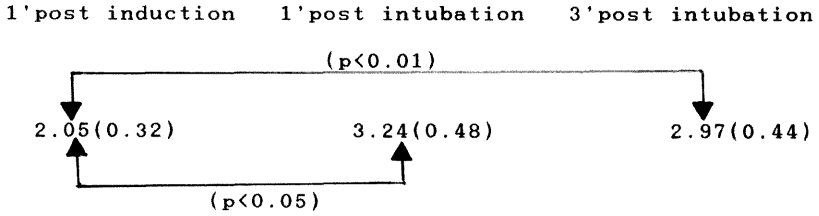
Practolol		Placebo	
Age (years)	Wt (kg)	Age (years)	Wt (kg)
30	61	30	63
41	71	34	55
43	82	26	55
32	61	26	64
28	64	41	61
	58	27	59
31	43	26	64
37	60	26	65
34	65	30	67
27	52	27	67
34(2.0)	**62(3.0)**	**29(2.0)**	**62(1.0)**

Table 4.30 Plasma noradrenaline levels in the group of patients who received intravenous injection of practolol before induction of anaesthesia.

n=10 mean (SEM) pmol/ml

Baseline	1'post pract	1'post induc	1'post intub	3'post intub	5'post intub
1.75	1.97	1.66	3.36	1.88	1.70
1.20	1.57	2.80	3.30	2.26	1.90
5.08	5.08	4.56	6.49	4.55	5.26
1.27	1.19	1.78	3.67	5.05	2.32
3.32	2.79	2.31	4.79	4.93	4.95
2.42	2.04	1.54	1.74	3.39	1.65
2.82	2.46	1.31	1.55	1.46	1.33
3.10	3.71	1.54	1.97	1.56	1.72
3.06	2.83	1.90	2.44	2.22	1.93
1.67	2.23	1.05	3.11	2.43	1.69
2.57(0.37)	2.59(0.36)	2.05(0.32)	3.24(0.48)	2.97(0.44)	2.45(0.45)

Statistical Analysis



' = minute

pract = practolol

induc = induction

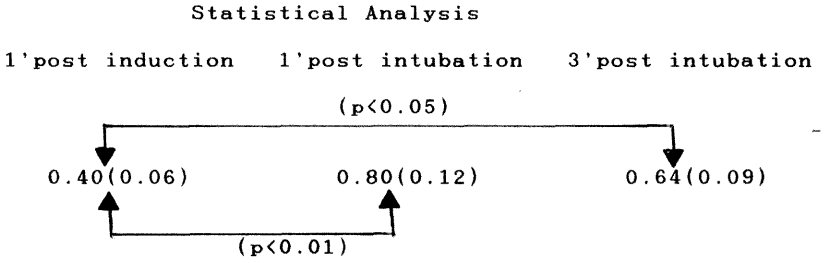
intub = intubation

Table 4.31 Plasma adrenaline levels in the group of patients who received intravenous injection of practolol before induction of anaesthesia.

n=10 mean (SEM) pmol/ml

Baseline	1'post pract	1'post induc	1'post intub	3'post intub	5'post intub
0.35	0.31	0.40	0.70	0.35	0.43
0.42	0.35	0.35	0.35	0.37	0.27
0.42	0.38	0.39	1.40	0.71	0.78
0.78	0.66	0.73	1.35	1.23	0.93
0.36	0.25	0.21	0.78	0.77	1.06
0.34	0.25	0.27	0.35	0.32	0.26
0.20	0.22	0.19	0.83	0.81	0.70
0.61	0.61	0.65	1.01	0.61	0.43
0.45	0.57	0.44	0.55	0.50	0.33
0.51	0.57	0.40	0.72	0.74	0.39
0.44(0.05)	0.42(0.05)	0.40(0.06)	0.80(0.12)	0.64(0.09)	0.56(0.09)

Statistical Analysis



' = minute

pract = practolol

induc = induction

intub = intubation

Table 4.32 Plasma noradrenaline levels in the group of patients who received intravenous injection of normal saline before induction of anaesthesia.

n=10 mean (SEM) pmol/ml

Baseline	1'post place	1'post induc	1'post intub	3'post intub	5'post intub
2.32	1.95	2.46	2.89	1.99	2.65
2.00	1.72	1.96	1.55	1.87	1.41
1.66		1.10	1.47	1.04	0.87
1.36	1.38	1.39	2.38	1.85	1.45
1.07	1.10	1.61	1.83	2.49	2.27
2.13	1.75	1.35	1.60	1.80	1.46
1.80	1.66	1.43	1.47	2.19	1.51
0.97	0.97	1.10	1.51	1.20	1.20
1.72	2.90	1.32	1.58	1.93	1.90
2.01	1.87	2.03	3.00	2.51	1.93
1.70(0.14)	1.70(0.19)	1.58(0.14)	1.93(0.19)	1.89(0.15)	1.67(0.17)

Statistical Analysis

1'post induction	1'post intubation
1.58(0.14)	0.52(0.06)

($p<0.05$)

' = minute

place = placebo

induc = induction

intub = intubation

Table 4.33 Plasma adrenaline levels in the group of patients who received intravenous injection of normal saline before induction of anaesthesia.

n=10 mean (SEM) pmol/ml

Baseline	1'post place	1'post induc	1'post intub	3'post intub	5'post intub
0.75	0.59	0.69	0.51	0.41	0.51
0.56	0.52	0.50	0.49	0.43	0.49
0.25		0.20	0.40	0.25	0.28
0.20	0.26	0.22	0.93	0.20	0.11
0.64	0.80	0.40	0.48	0.68	0.73
0.35	0.25	0.26	0.43	0.28	0.26
0.88	0.80	0.51	0.41	0.56	0.48
0.37	0.40	0.37	0.42	0.30	0.27
0.24	0.32	0.15	0.58	0.50	0.50
0.45	0.47	0.35	0.50	0.37	0.34
0.47(0.08)	0.49(0.08)	0.37(0.06)	0.52(0.06)	0.40(0.05)	0.40(0.06)

Statistical Analysis

1'post induction 1'post intubation

0.37(0.06) 0.52(0.06)

($p<0.05$)

' = minute

place = placebo

induc = induction

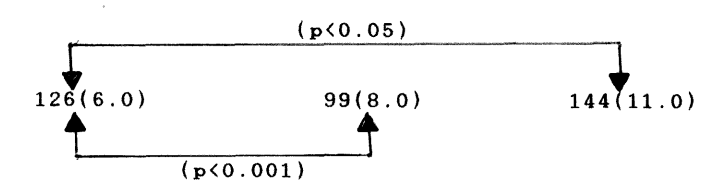
intub = intubation

Table 4.34 Systolic arterial pressure in the group of patients who received intravenous injection of practolol before induction of anaesthesia.

n=10 mean (SEM) mmHg

Baseline	1'post pract	1'post induc	1'post intub	3'post intub	5'post intub
141	122	119	181	135	122
134	136		150	116	97
144	133	107	155	133	115
134	136		198	190	156
132	126	101	136	123	117
118	113	101	161	117	109
90	94		90	77	76
99	100	72	114	81	82
147	142	122	146	125	116
117	112	72	105	122	83
126(6.0)	121(5.0)	99(8.0)	144(11)	122(10.0)	107(8.0)

Statistical Analysis



' = minute

pract = practolol

induc = induction

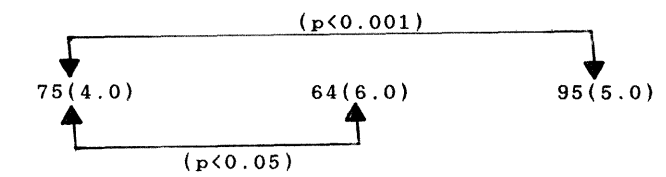
intub = intubation

Table 4.35 Diastolic arterial pressure in the group of patients who received intravenous injection of practolol before induction of anaesthesia.

n=10 mean (SEM) mmHg

Baseline	1'post pract	1'post induc	1'post intub	3'post intub	5'post intub
64	64	65	101	81	77
87	82		104	83	66
86	79	73	113	89	70
79	82		117	116	93
71	72	53	89	78	76
70	70	67	102	75	68
71	66		80	67	64
65	68		79	65	74
95	87	82	100	65	83
61	69	42	64	55	52
75(4.0)	74(3.0)	64(6.0)	95(5.0)	77(5.0)	72(4.0)

Statistical Analysis



' = minute

pract = practolol

induc = induction

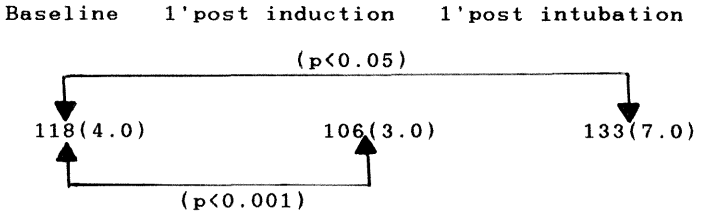
intub = intubation

Table 4.36 Systolic arterial pressure in the group of patients who received intravenous injection of normal saline before induction of anaesthesia.

n=10 mean (SEM) mmHg

Baseline	1'post place	1'post induc	1'post intub	3'post intub	5'post intub
107	109	96	119	112	108
113	113	105	122	109	105
96		81	108	93	91
110	106	101	123	105	100
128	127	119	182	158	160
140	127	110	130	127	115
122	114	109	123	114	116
128	119	116	127	125	119
121	122	109	142	122	110
113	110	110	147	117	114
118(4.0)	116(3.0)	106(3.0)	133(7.0)	118(5.0)	114(6.0)

Statistical Analysis



' = minutes

place = placebo

induc = induction

intub = intubation

Table 4.37 Diastolic arterial pressure in the group of patients who received intravenous injection of normal saline before induction of anaesthesia.

n=10 mean (SEM) mmHg

Baseline	1'post place	1'post induc	1'post intub	3'post intub	5'post intub
63	34	61	82	72	64
66	69	70	88	76	70
67		52	75	74	68
70	63	76	90	76	74
92	90	76	110	102	102
80	80	72	90	91	76
69	64	64	80	76	77
94	89	82	101	92	85
71	72	71	94	86	79
79	74	82	91	92	79
75(3.0)	71(6.0)	71(3.0)	90(3.0)	84(3.0)	77(3.0)

Statistical Analysis

Baseline 1'post intubation

75(3.0) 90(3.0)

(p<0.001)

' = minute

place = placebo

induc = induction

intub = intubation

Table 4.38 Heart rate in the group of patients who received intravenous injection of practolol before induction of anaesthesia.

n=10 mean (SEM) beats/min.

Baseline	1'post pract	1'post induc	1'post intub	3'post intub	5'post intub
91	71	88	82	96	84
68	63		101	82	71
74	73	78	87	74	79
77	55		113	114	93
108	97	96	102	85	77
68	63	83	84	67	55
81	71		91	80	78
54	59	64	77	60	56
130	106	92	104	90	86
68	75	103	113	80	89
82(7.0)	73(5.0)	86(5.0)	95(4.0)	83(5.0)	77(4.0)

Statistical Analysis

Baseline 1'post practical

82(7.0) 73(5.0)

(p<0.05)

' = minute

pract = practolol

induc = induction

intub = intubation

Table 4.39 Heart rate in the group of patients who received intravenous injection of normal saline before induction of anaesthesia.

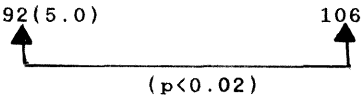
n=10 mean (SEM) beats/min.

Baseline	1'post place	1'post induc	1'post intub	3'post intub	5'post intub
76	81	102	113	96	79
107	93	97	100	99	94
84		83	85	76	71
81	94	63	96	89	83
99	101	97	104	106	113
98	123	105	113	117	97
88	67	104	109	103	108
75	83	82	111	101	93
86	75	83	84	80	72
123	110	132	147	136	132
92(5.0)	92(6.0)	95(6.0)	106(6.0)	100(6.0)	94(6.0)

Stastistical Analysis

Baseline 1'post intubation

92(5.0) 106(6.0)



(p<0.02)

' = minute

place = placebo

induc = induction

intub = intubation

Table 4.40 The highest noradrenaline and adrenaline levels (pmol/ml) from patients in each study, with the corresponding percentage change of catecholamine levels from the baseline after tracheal intubation are compared, the systolic pressure (mm.Hg) and age in years.

Study 1	noradrenaline	%change	Adrenaline	%change	SP	Age
Laryngoscopy	2.52	8	0.13	46	137	39
Laryngoscopy/int	2.92	17	0.26	31	137	40
Study 2						
Forrester/lignocaine	3.07	-16	0.58	53	108	37
Laryng-o-jet	4.16	-56	0.36	114	138	30
Control	5.65	-5	0.67	6	131	36
Study 3						
Practolol	5.08	28	0.42	233	144	43
Placebo	2.32	25	0.75	32	107	30
Study 1	**Adrenaline**	**%change**	**Noradrenaline**	**%change**	**SP**	**Age**
Laryngoscopy	0.75	-33	1.09	82	130	33
Laryngoscopy/int	0.76	12	1.77	58	124	39
Study 2						
Forrester/lignocaune	0.69	-7	1.64	51	120	32
Laryng-o-jet	0.86	38	1.56	39	124	38
Control	0.98	-29	4.49	1	150	40
Study 3						
Practolol	0.78	73	1.27	189	134	32
Placebo	0.88	-53	1.80	-18	122	26

SP = systolic pressure

int = intubation

Table 4.41 The lowest noradrenaline and adrenaline levels (pmol/ml) from patients in each study, with the corresponding percentage change of catecholamine levels from the baseline after tracheal intubation are compared, the systolic pressure (mm.Hg) and age in years.

Study 1	Noradrenaline	%change	Adrenaline	%change	SP	Age
Laryngoscopy	0.65	132	0.43	72	150	63
Laryngoscopy/int	0.62	282	0.20	165	125	25
Study 2						
Forrester/Lignocaine	0.72	-4	0.58	-22	127	39
Laryng-o-jet	1.20	-10	0.24	-17	121	22
Control	1.19	69	0.49	10	122	22
Study 3						
Practolol	1.20	175	0.42	-17	134	41
Placebo	0.97	56	0.37	14	128	26
Study 1	**Adrenaline**	**%change**	**Noradrenaline**	**%change**	**SP**	**Age**
Laryngoscopy	0.10	270	1.25	174	145	37
Laryngoscopy/int	0.13	131	1.79	17	137	63
Study 2						
Forrester/lignocaine	0.19	147	2.61	-3	95	30
Laryng-o-jet	0.19	353	1.24	7	96	37
Control	0.22	123	1.21	11	101	29
Study 3						
Practolol	0.20	315	2.82	45	90	31
Placebo	0.20	365	1.36	75	110	26

SP = systolic pressure

int = intubation

SECTION FOUR

1. The development of the Injury Severity Score

2. Catecholamines and the Injury Severity Score in minor injured patients

CHAPTER FIVE

5.5 PLASMA CATECHOLAMINE AND THE INJURY SEVERITY SCORE

5.5.1 Development of the Injury Severity Scores

Investigations on the road crash accidents have shown that there are differences between the nature and severity of injuries between groups of the patients. Although the descriptions of the injuries were based on numerical scores, their assignments were subjective and analysis of the data showed no relationship with the deaths between the groups. These observations led to the recent developments of the two basic research approaches. The first approach was the development of The Abbreviated Injury Scale AIS (1971), and the second was The Comprehensive Research Injury Scale CRIS (1972).

The Abbreviated Injury Scale (1971) categorised injuries according to the body area, and the numerical scores represented the severity ratings as minor (1); moderate (2); severe, not life threatening (3); severe, life threatening (4); critical, survival is uncertain (5); fatal within 24h were assigned (6-9). The assignments of these scores were subjective, and showed a weak relationship with mortality.

The Comprehensive Research Injury Scale (1972) was devised as an extension of The Abbreviated Injury Scale. It was to introduce objective ratings to describe the injuries. Thus, engineers consider the energy dissipation as the force required to produce an injury, while physicians think of an

injury in terms of threat to life; permanent impairment; treatment period and incident which caused the injury in evaluating the severity of an injury. Numerical scores (1-5) were assigned to each heading.

Under energy dissipation, the rating of the energy required to produce an injury was little (1); minor (2); moderate (3); major (4) or maximum (5).

The rating of the threat to life had an inherent subjectivity (The Comprehensive Injury Score, 1972), except cases where the injury was so severe that death was an obvious outcome. The rating was none (1); minor (2); moderate (3); severe (4); and maximum (5).

Permanent impairment is defined as functional abnormality after maximum medical rehabilitation has been achieved, and disability is defined as a reduction of the ability to do useful activity (The Comprehensive Injury Score, 1972). Hence, impairment is directly related to the injury which caused it, while disability covers factors such as occupation, attitude and education. For example, an injury to a finger may represent total disability to a pianist or a violinist, but has no threat to life. On the other hand injuries to brain or a major organ may cause impairment to the whole body. The rating of the impairment was 20% or less (1); 21-40% (2); 41-60% (3); 61-90% (4) and 91-100% (5).

The length of the treatment period reflects the severity of

the injury. The Comprehensive Research Injury Scale (1972), reported that energy dissipation required to amputate a toe was 2, and that required to amputate above the knee was 5. However, both injuries required the same treatment period 3. Therefore the severity of both injuries is the same. Length of the treatment was rated as 2 weeks or less (1); 2-8 weeks (2); 8-26 weeks (3); 26-52 weeks (4); and 52 weeks or more (5).

Incident is related to the factors which cause the injuries, and whether they can be prevented to eliminate other injuries. Chest injuries in crash accidents led to the installation of energy absorbing steering columns. Incident was rated as unusual (1); occasional (2); common (3); very common (4); and most frequent (5).

Because of the non-linearity between numerical scores and mortality rate, a third scale was introduced, that described injuries to more than one part of the body. The scale which described multiple injuries to the patient was defined as the Injury Severity Score ISS (Baker et al, 1974). Chest injuries were classified according to the AIS rating as chest wall stiffness (1); simple rib fracture (2); multiple rib fractures (3); flail chest (4); and aortic laceration (5). For the ISS to be used effectively (Baker et al, 1974), the AIS grades 6-9 were omitted. The second modification of the AIS was to separate facial injuries from cranial and neck injuries, since facial injuries are common to crashes which

might overshadow other head injuries.

Baker et al (1974) observed that death increased disproportionately with the AIS rating of the most severe injury, and the death rate on the patients with two injuries of grade 4 and 3 was not comparable to that on the patients with two injuries of grade 5 and 2, (sum = 7 in both). Such observations led them to investigate the possibility of squaring the AIS grades from the most severe injury in each body area, then sum them. They found that by squaring the AIS grades in each of the two most severely injured areas, and adding the two results together, the death rates were similar for comparable totals. Thus, the patients whose two most severely injured areas were graded 5 and 0 and for those graded 4 and 3 (sum of squares = 25 in both), the death rates were 22% and 24% respectively.

They further showed that if the AIS grades for each of the three most severely injured areas were squared and the results were added together, comparable totals were associated with similar mortality rates; also, the correlations between total injury severity and mortality were improved. The inclusion of the grade of the fourth most severely injured area had no appreciable effect. Hence, the Injury Severity Score was defined as the sum of the squares of the highest AIS grade in each of the three most severely injured areas. For instance, the method for calculating the ISS is as follows; a patient with laceration of the aorta AIS

= 5, multiple closed long bone fractures AIS = 4, and retroperitoneal haemorrhage AIS = 3, the total ISS = 50 (25+16+9).

Scores in the ISS were designed in such a way that equal scores in different groups of patients indicate similar injury severity, irrespective of its nature, making it convenient to study a mixture of casualties. This has been confirmed (Stoner et al, 1977) in a study which showed a positive relationship between cortisol levels and the ISS in different groups of patients.

The relationship between the Injury Severity Score and catecholamine levels on road crash victims has been studied (Davies et al, 1984; Frayn et al, 1985). The results showed that there were correlations between catecholamine levels and the Injury Severity Score on those patients who were critically injured.

The aims of this study are to compare the changes in plasma catecholamine levels and the Injury Severity Scores on a mixture of less severely injured patients, to determine whether there is a correlation between catecholamine levels and the ISS, and to asssess the range of the Injury Severity Scores likely to produce significantly raised catecholamine levels in plasma.

5.5.2 Patients and Methods

Twenty one patients were studied. The patients came from the scene of their injuries to the accident and emergency department at the Royal Infirmary, Leicester. Transport from the scene of injury normally takes about thirty minutes. A further thirty minutes approximately was spent by the medical officer in charge in examining, the patient, making a diagnosis and calculating the injury severity score. The method of calculating the ISS is outlined in section 5.5.1. By the time a blood sample was taken from the antecubital vein, the total time since the injury was at least one hour.

It was not possible for the author to take the blood samples and calculate the ISS on those patients; both procedures were fully discussed with the medical officer in charge who examined the patients.

The blood sample (10ml) was taken into a lithium heparin bottle for catecholamine measurements. The samples were spun at 1500g for 10 minutes; plasma was then separated into polypropylene tubes and stored at -70 degrees C before analysis. Samples were analysed for noradrenaline (Norad) and adrenaline (Adr) levels using high pressure liquid chromatography with electrochemical detector LC-4A (Bioanalytical Systems).

5.5.3 Results

Table 5.1 gives details of the individual patient, age; gender; diagnoses; injury severity scores and catecholamine levels.

One patient had disproportionately high noradrenaline and adrenaline levels, and his data were excluded from the statistical analysis (Table 5.1). The patients with injuries (ISS of 1-22; n=20) (Figure 5.1 and Table 5.2), showed no correlation between noradrenaline and the ISS. There was significant correlation between adrenaline levels and the ISS (Figure 5.2 and Table 5.3; n=20) because of the patient with the high adrenaline level of 4.04pmol/ml. Similarly, Figure 5.3 and Table 5.4; n=19) showed correlation between adrenaline levels and the ISS, again another patient with high adrenaline level of 2.35pmol/ml. When the data on those two patients were excluded (Figure 5.4 and Table 5.5; n=18), there was no correlation between the ISS and adrenaline levels.

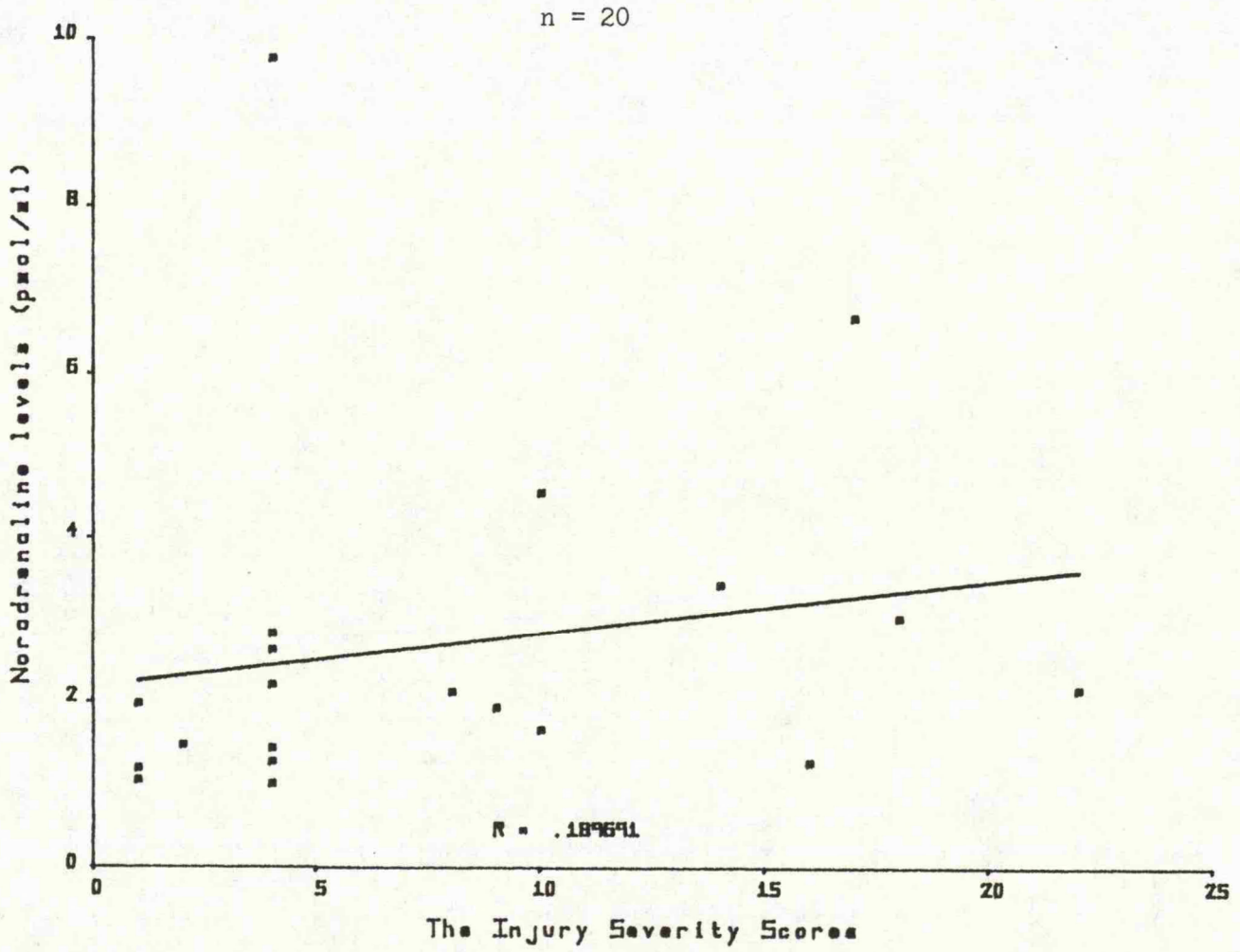


Figure 5.1 Relationship between plasma noradrenaline levels and the Injury Severity Score in patients with minor injuries. Each point represents the results from each patient. Linear correlation coefficient ($r = 0.19$; $p > 0.05$)

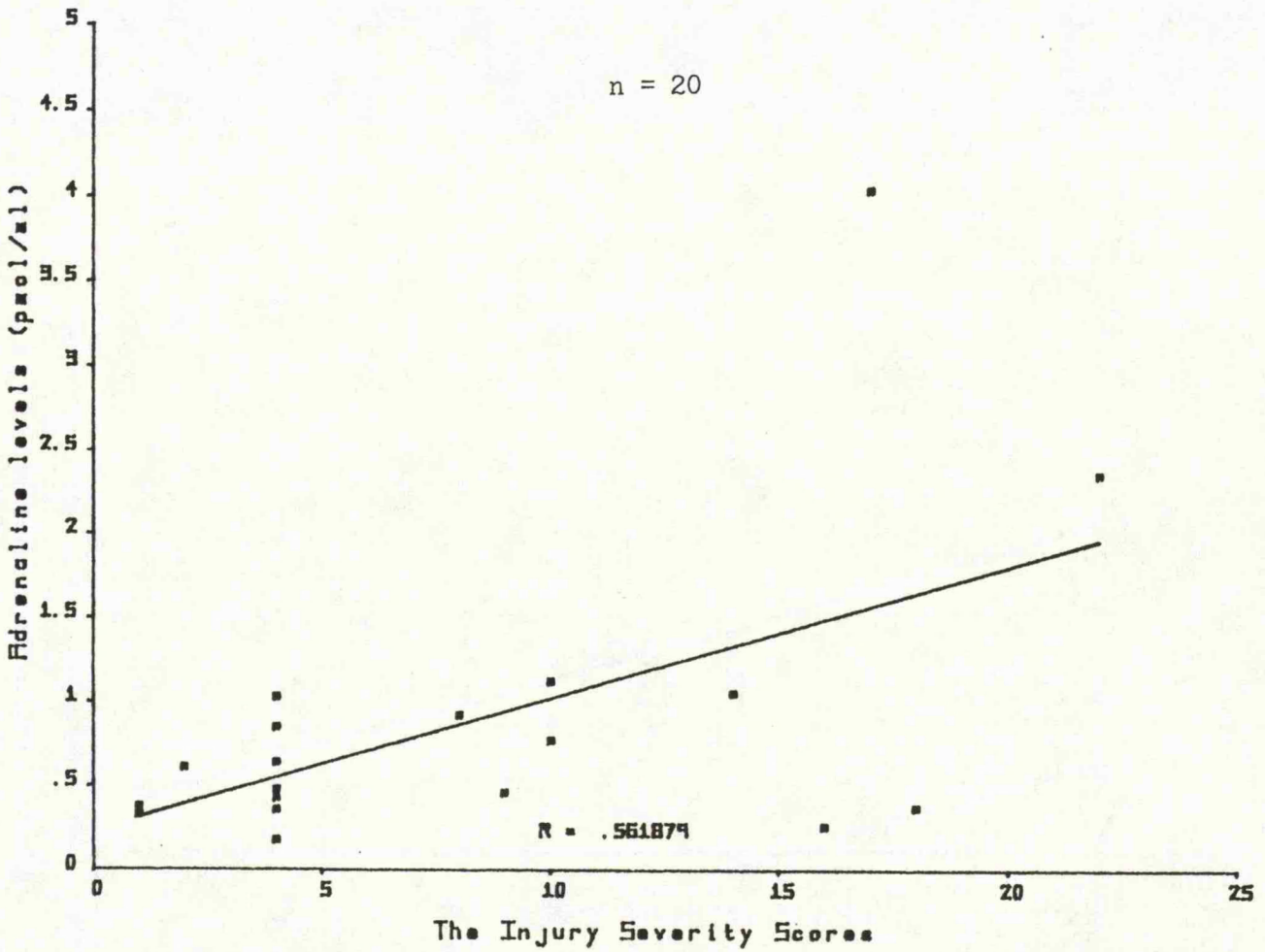


Figure 5.2 Relationship between plasma adrenaline levels and the Injury Severity Score in patients with minor injuries. Each point represents the results from each patient. Linear correlation coefficient ($r = 0.56$; $p < 0.05$)

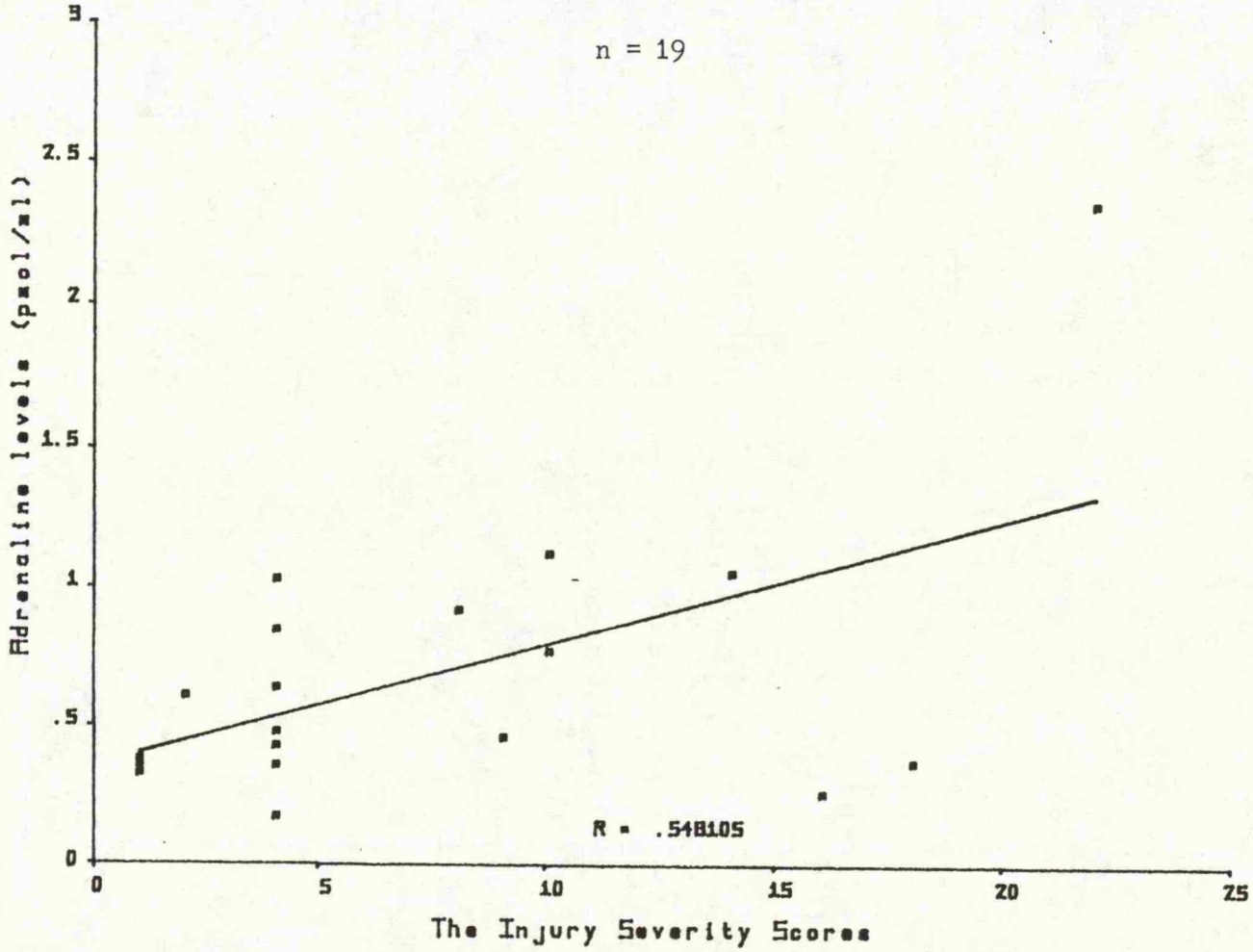


Figure 5.3 Relationship between plasma adrenaline levels and the Injury Severity Score in patients with minor injuries. Each point represents the result from each patient. Linear correlation coefficient ($r = 0.55$; $p < 0.05$)

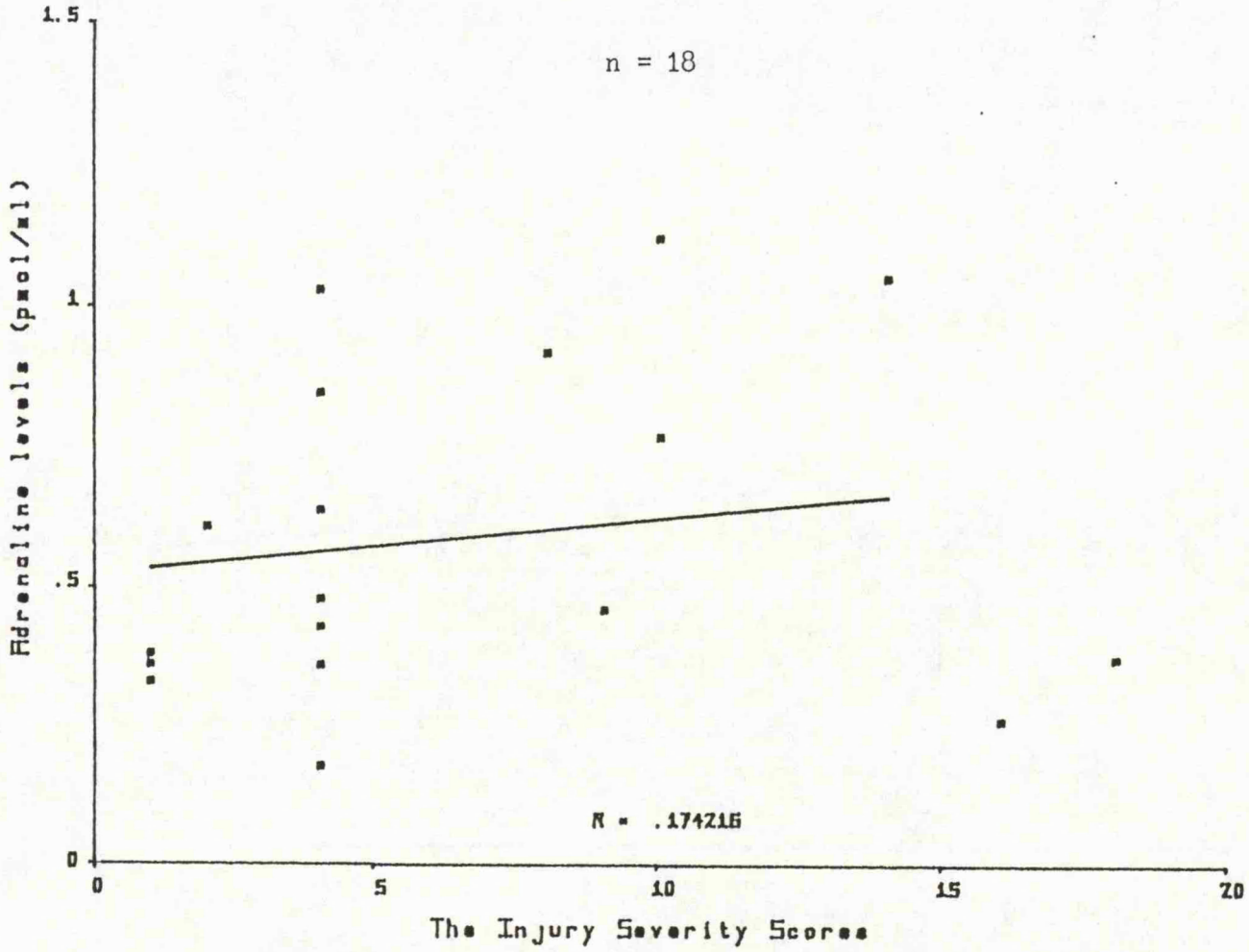


Figure 5.4 Relationship between plasma adrenaline levels and the Injury Severity Score in patients with minor injuries. Each point represents the results from each patient. Linear correlation coefficient ($r = 0.17$; $p > 0.05$)

5.5.4 Discussion

A wide range and increases in noradrenaline and adrenaline levels were obtained in the present study. The results support those of Davies et al (1984) and Frayn et al (1985). They reported increases in catecholamine levels in critically injured patients; levels were highest immediately after injury, partly due to hypovolaemia (Frayn et al, 1985). One patient had multiple injuries with an ISS of 34, a noradrenaline level of 37.47pmol/ml; and an adrenaline level of 15.40pmol/ml; influenced the correlation between ISS with catecholamine levels, hence, his data were excluded in the statistical analysis. The patient later died through his injuries. Deaths associated with high catecholamine levels have been found (Benedict and Grahame-Smith, 1978; Davies et al, 1984); those patients who died either from injuries or other causes had persistently high catecholamine levels.

There was no relationship between noradrenaline levels and the ISS n=20 (Figure 5.1 and Table 5.2). The relationship between the ISS and adrenaline levels (Figure 5.2 and Table 5.3; n=20 and Figure 5.3 and Table 5.4; n=19) showed that adrenaline levels on two patients influenced the significant correlation between the ISS and adrenaline levels. When the data on those patients were excluded the regression analysis showed no correlation between the ISS and adrenaline levels (Figure 5.4 and Table 5.5; n=18). The patient with an ISS of 17 had almost three times as much noradrenaline and twice as

much adrenaline levels in plasma as the patient with an ISS of 22 (Table 5.1). Such anomaly highlights the difficulties in the interpretation of catecholamine results.

Lack of a correlation between injury severity scores and catecholamine levels in patients with minor injuries may be due to the short half-lives of the secreted noradrenaline and adrenaline in blood (Ferreira and Vane, 1967; Ginn and Vane, 1968; Vane, 1969). In the present study there was a mixture of casualties; the majority of the patients had minor injuries, and the blood samples were taken at least one hour after the injuries. It appears that an ISS of less than 30 would not produce a correlation with noradrenaline levels; and the ISS of less than 17 would not produce a correlation with adrenaline levels.

Davies et al (1984) suggested that catecholamine responses to the injury occur in moderate and severe injuries (ISS>9). The present study shows that, the injury which produces high noradrenaline levels occur with ISS>30, and is associated with multiple injuries. Hence the injury associated with the low noradrenaline levels in minor injury occurs with ISS<30. While the injury associated with low adrenaline levels occurs with ISS<17.

5.5.5 Conclusion

In conclusion, minor injuries did not produce significantly high catecholamine levels in blood, or catecholamines had

been cleared from the blood at the time the blood samples were taken, resulting in no correlation between catecholamine levels and the ISS. Half-lives of catecholamines in blood are known to be very short. Hence, it is unlikely that plasma catecholamine levels can be used as indices of injury on the patients with minor injuries. The noradrenaline level is determined not only by the rate of nerve firing rate, but also by the rate at which it is removed from the circulation.

Table 5.1 Details of the Patients, the Injury Severity Score and Catecholamine levels (pmol/ml)

Age	Sex	Diagnosis	ISS	Norad	Adr
75	F	F'ture of Tibia&Fibula	4	2.21	0.85
16	M	F'ture of Tibia&Fibula	4	2.63	0.64
44	F	Compound Fracture	4	2.83	0.43
20	M	Fracture of Pelvis	8	2.12	0.92
60	M	F'ture of Tibia&Fibula	4	1.44	0.48
62	M	Bruised Ribs	1	1.98	0.33
37	M	Compound Fracture	10	4.55	1.12
80	F	Fracture of Pelvis	17	6.66	4.04
41	M	Bruised Legs	2	1.48	0.61
32	M	Bruised Ribs	1	1.06	0.36
82	F	Shoulder Dislocation	4	9.78	1.03
20	M	Bruised Ribs	1	1.20	0.38
67	F	Colles's Fracture	4	1.28	0.36
90	F	Fracture of Femur	9	1.93	0.46
33	F	Head Injuries	18	3.01	0.37
13	F	F'ture of Radius&Ulna	4	1.02	0.18
28	M	Crashed Pelvis	16	1.26	0.26
9	M	Stab to the back	10	1.66	0.77
60	M	Head Injuries	14	3.42	1.05
59	M	Multiple Injuries	22	2.14	2.35
21	M	Multiple Injuries	34	37.47	15.40

F'ture = Fracture

Table 5.2 Details of the Patients, the Injury Severity Score and Noradrenaline levels (pmol/ml)

Diagnosis	ISS	Norad
F'ture of Tibia&Fibula	4	2.21
F'ture of Tibia&Fibula	4	2.63
Compound Fracture	4	2.83
Fracture of Pelvis	8	2.12
F'ture of Tibia&Fibula	4	1.44
Bruised Ribs	1	1.98
Compound Fracture	10	4.55
Fructure of Pelvis	17	6.66
Bruised legs	2	1.48
Bruised Ribs	1	1.06
Shoulder Dislocation	4	9.78
Bruised Ribs	1	1.20
Colles's Fracture	4	1.28
Fracture of Femur	9	1.93
Head Injuries	18	3.01
F'ture of Radius&Ulna	4	1.02
Crashed Pelvis	16	1.26
Stab to the back	10	1.66
Head Injuries	14	3.42
Multiple Injuries	22	2.14

F'ture = Fracture

Table 5.3 Details of the Patients, the Injury Severity Score and Adrenaline levels (pmol/ml)

Diagnosis	ISS	Adr
F'ture of Tibia&Fibula	4	0.85
F'ture of Tibia&Fibula	4	0.64
Compound Fracture	4	0.43
Fracture of Pelvis	8	0.92
F'ture of Tibia&Fibula	4	0.48
Bruised Ribs	1	0.33
Compound Fracture	10	1.12
Fracture of Pelvis	17	4.04
Bruised Legs	2	0.61
Bruised Ribs	1	0.36
Shoulder Dislocation	4	1.03
Bruised Ribs	1	0.38
Colles's Fracture	4	0.36
Fracture of Femur	9	0.46
Head Injuries	18	0.37
F'ture of Radius&Ulna	4	0.18
Crashed Pelvis	16	0.26
Stab to the back	10	0.77
Head Injuries	14	1.05
Multiple Injuries	22	2.35

F'ture = Fracture

Table 5.4 Details of the Patients, the Injury Severity Score and Adrenaline levels (pmol/ml)

Diagnosis	ISS	Adr
F'ture of Tibia&Fibula	4	0.85
F'ture of Tibia&Fibula	4	0.64
Compound Fracture	4	0.43
Fracture of Pelvis	8	0.92
F'ture of Tibia&Fibula	4	0.48
Bruised Ribs	1	0.33
Compound Fracture	10	1.12
Bruised Legs	2	0.61
Bruised Legs	1	0.36
Shoulder Dislocation	4	1.03
Bruised Ribs	1	0.38
Colles's Fracture	4	0.36
Fracture of Femur	9	0.46
Head Injuries	18	0.37
F'ture of Radius&Ulna	4	0.18
Crashed Pelvis	16	0.26
Stab to the back	10	0.77
Head Injuries	14	1.05
Multiple Injuries	22	2.35

F'ture = Fracture

Table 5.5 Details of the Patients, the Injury Severity Score and Adrenaline Levels (pmol/ml)

Diagnosis	ISS	Adr
F'ture of Tibia&Fibula	4	0.85
F'ture of Tibia&Fibula	4	0.64
Compound Fracture	4	0.43
Fracture of Pelvis	8	0.92
F'ture of Tibia&Fibula	4	0.48
Bruised Ribs	1	0.33
Compound Fracture	10	1.12
Bruised Legs	2	0.61
Bruised Ribs	1	0.36
Shoulder Dislocation	4	1.03
Bruised Ribs	1	0.38
Colles's Fracture	4	0.36
Fracture of Femur	9	0.46
Head Injuries	18	0.37
F'ture of Radius&Ulna	4	0.18
Crashed Pelvis	16	0.26
Stab to the back	10	0.77
Head Injuries	14	1.05

F'ture = Fracture

SECTION FIVE

1. Final Discussion

2. Concluding remark

3. Future research

CHAPTER SIX

6.6 FINAL DISCUSSION AND CONCLUSION

6.6.1 Measurements of Catecholamine

During the estimations of plasma or serum catecholamine levels, it was found that plasma samples from the patients had some electroactive substances other than catecholamines, possibly from drugs administered or their metabolites. Some of those electroactive substances interfered with catecholamine peaks. Hence, the need to optimise conditions for catecholamine measurements. The developments and the validations of the methods for catecholamine estimations are discussed in chapter 2. The catecholamine levels obtained with those methods are comparable with the catecholamine levels reported in the past and the current publications.

Attempts were made to exclude all factors which the author found to interfere with catecholamine levels during sample preparations for catecholamine measurements, as discussed in chapter 3. Hence, changes in catecholamine levels obtained in the groups of patients studied were either due to the stimulation induced by laryngoscopy with or without tracheal intubation or trauma.

6.6.2 Differential Centrifugation of Plasma Samples

Platelet-rich plasma had significantly higher catecholamine levels than the platelet-poor plasma. Serum samples had significantly higher catecholamine levels than plasma

samples. In addition, acids used to precipitate protein for catecholamine measurements produced different catecholamine levels. These observations showed that a high proportion of catecholamines come from blood cells and protein. Those investigations suggest that the speed of centrifugation must be pre-determined, plasma or serum can be used but not both during a single study, and acid chosen to precipitate protein must be used consistently in order to obtain consistent results.

6.6.3 Anti-oxidants and the Stability of Catecholamines

One of the significant finding in this thesis is that catecholamines are stable compounds. Therefore, the collection of blood samples was modified. The blood samples were collected in Vacutainer tubes containing lithium heparin. The tubes were not pre-cooled, the collected samples were not ice-cooled and anti-oxidants were excluded in the samples collection and during sample analysis, the samples were spun when it was convenient. The modification of the samples collection and centrifugation were welcomed by the clinicians, who did not have to interrupt clinical assessment after blood collection to care for those samples as with the former method.

The introduction of the internal quality control system as an integral part of the catecholamine measurements in this thesis and in all the catecholamine analysis in the

department served two purposes. Firstly, to monitor the quality of the results and the system and secondly, to study the stability of catecholamines over a period of time. The stability of catecholamines on post-dated blood for transfusion was examined in a batch for four months. The catecholamine levels in post-dated blood for transfusion showed that all the results in four months were similar. The author is not aware of similar investigations on catecholamine levels in post-dated blood or plasma for transfusion.

6.6.4 Catecholamine levels during Anaesthesia

Another significant finding of this thesis is that the mean plasma catecholamine levels during anaesthesia were within the normal range in all the groups of patients studied, whether they were beta blocked or not, and whether they had topical tracheal analgesia or not. The normal catecholamine levels found in all the studies showed that the increases in catecholamine levels associated with laryngoscopy and tracheal intubation are lower than the levels previously associated with pharmacological effects (Silverberg et al, 1978). Although Black et al (1962) conceived the idea of beta blockade to reduce adrenergic drive after myocardial infarction, which might have been caused by high catecholamine levels in blood, since adrenaline stimulates beta-1 adrenoceptors in the heart and produces increased force and heart rate and cardiac output; these effects cause

increased blood pressure and increased oxygen consumption. The catecholamine levels during beta-blockade in this study were below pharmacological effects.

The three clinical studies demonstrate that 1 minute after laryngoscopy with and without tracheal intubation, the patients with the lowest baseline catecholamine levels, had greater increases in catecholamine levels than those with higher baseline catecholamine levels. Those increases were disproportionately greater on adrenaline levels than on noradrenaline levels when the percentage change of the catecholamine levels from the baseline and 1 minute after laryngoscopy with or without tracheal intubation are compared. The patients with high baseline catecholamine levels had modest increases or decreases in catecholamine levels 1 minute after laryngoscopy with or without tracheal intubation. In addition, the patients with high baseline noradrenaline levels do not necessarily have corresponding high baseline adrenaline levels. Hence, there is no relationship between noradrenaline and adrenaline levels before and after laryngoscopy with or without tracheal intubation. Therefore, the change of catecholamine levels is unpredictable. Hence, catecholamine levels cannot be used to assess changes in the blood pressures or heart rate during anaesthesia.

The studies suggest that catecholamine levels within the physiological range may be responding to stimuli which differ

from those that cause changes in blood pressures or heart rate, and this may be one of the reason that could explain the lack of the relationships between catecholamine levels and blood pressures or heart rate in these studies.

6.6.5 Catecholamine levels and the Injury Severity Score

The Injury Severity Score in patients with minor injuries failed to correlate with plasma catecholamine levels, probably due to short half-lives of catecholamines following their secretion in the blood. The studies also suggest that catecholamine levels are of no value in assessing the severity of minor injuries. Noradrenaline levels in blood depend on the nerve firing rate and the rate of its clearance from blood. Thus, if the clearance is reduced, plasma noradrenaline would be disproportionately high, otherwise, the catecholamine control mechanisms which maintain catecholamine levels at the physiological range predominate.

6.6.6 Catecholamine Infusion in man

Infusion of noradrenaline into man usually produces increased metabolism, blood pressure and heart rate (Silverberg et al, 1978); the threshold for noradrenaline levels which produce those changes is above 1800pg/ml (10.65pmol/ml). The reported value is much higher than the normal noradrenaline levels, although occasionally it can be reached during provocative manoeuvres in phaeochromocytoma (Hamberger et al, 1981; Vater

et al, 1983), in critically ill patients (Russell et al, 1982) and patients with severe injuries (Davies et al, 1984). Clutter et al (1980) reported thresholds for adrenaline levels which produce increases in blood pressure and heart rate to be within normal range for noradrenaline (1.5-3.0pmol/ml) and for adrenaline (0.2-0.5pmol/ml).

6.6.7 Catecholamines, Blood Pressures and Heart Rate

The relationships between catecholamine levels and blood pressures or heart rate are complex, probably due to the different systems which control blood pressure or heart rate and catecholamine levels in blood. The changes in catecholamine levels in the circulation are controlled by their re-uptake into sympathetic nerve junctions for those levels at the physiological range, and into tissues for higher catecholamine levels such as those from transfusion of catecholamines or from stimulation of the adrenal medulla. In addition, O-methylation enzymatically inactivates catecholamines followed by deamination of the O-methylated amines by monoamine oxidase. Changes in blood pressures and heart rate are controlled by feedback mechanisms through the sympathetic nervous system and the baroreceptor reflexes. The different control mechanisms may be another factor which could account for the lack of relationships between blood pressures or heart rate and catecholamine levels.

6.6.8 Concluding Remark

Although Russell et al (1981) reported a significant correlation between catecholamine levels and the mean arterial pressure, and was confirmed by Derbyshire et al (1983) in our department, the subsequent studies in this department have consistently failed to reproduce those reports. The studies of Turner et al (1986) and those of Shribman et al (1987); Derbyshire et al (1987) and Achola et al (1988) all failed to find correlation between catecholamine levels and mean arterial pressures.

It appears that there are many factors both in vivo and in vitro, which would make interpretation of catecholamine results difficult, even under the stringent conditions of analysis. Therefore, it is not surprising that catecholamine levels are reported as a change from the baseline and the importance of those changes in terms of clinical assessment have not been clearly demonstrated. This thesis failed to show the significance of those changes.

The studies in this thesis suggest that plasma or serum catecholamine measurements have limited application in general clinical assessment.

Finally, in an attempt to reproduce the studies of Russell et al (1981) with the author's method, the department of anaesthesia used similar anaesthetic techniques as those of Russell and others. The results showed that pre-induction

plasma catecholamine levels in the patients about to undergo anaesthesia and surgery were almost identical to those reported by Russell et al (1981), who showed that mean (SEM) plasma noradrenaline was 1.82 (0.24) and adrenaline was 0.61 (0.19). Derbyshire et al (1983) found that plasma noradrenaline was 1.99 (0.34) and adrenaline was 0.54 (0.10) pmol/ml; providing further evidence in the accuracy of the author's method.

6.6.9 Future Research

It would be of interest to investigate whether there are higher peak levels of catecholamines soon after laryngoscopy with or without tracheal intubation than those observed in this thesis. Therefore the relevant questions for future research are:

1. Are the maximum peaks of catecholamine levels missed by the one minute delay between the end of the laryngoscopy with or without tracheal intubation and the time of blood collection? This may be difficult research to undertake, and is probably approached best by in vivo voltammetry. The technique is sensitive but lacks specificity. Recent advances in this area indicate that the pretreatment of carbon fibre electrodes for in vivo studies allows for greater selectivity of the molecules of biological interest.

The groups of patients to be investigated would be hypertensives, and the controls would be normotensives. The

results would be interesting because hypertensives are sensitive to stimuli. Probably they would be significantly sensitive to laryngoscopy with or without tracheal intubation than the normotensive patients. In view of the short half-lives of catecholamines, those catecholamine levels would be of great interest, and would demonstrate whether those levels approach their pharmacological action.

2. Why do catecholamine levels during induction of anaesthesia decrease below the baseline levels or increase above the baseline levels or show no change, while blood pressure always decreases below the baseline level and heart rate always rises above the baseline level?

APPENDIX ONE

Equipments and The Suppliers

Beckman-RIIC Ltd, High Wycombe, England: A double piston high pressure liquid chromatography pump.

Bioanalytical Systems, West Lafayette, USA: LC-4A amperometric detector; TL-3; TL-5 and polishing alumina.

Fisons Scientific Apparatus, Loughborough, England: Fisons Cliffco ultracold cabinet; Fistreem distillator.

Hewlett-Packard Ltd, Berks, England: Hewlett packard 97 programmable printing calculator.

HPLC Technology Ltd, Cheshire, England: Nucleosil 10SA stainless steel column with pre-column.

Laboratory Data Control Ltd, Stone, England: 302 Computing integrator, Sekonics printer.

Luckham Ltd, Sussex, England: Shaker and carrying plates.

Magnus Scientific Instrumentation Ltd, Bucks, England: Jun-Air compressor.

Millipore (UK) Ltd, Harrow, England: Microbondapak C18 stainless steel column.

Talbot Scientific Ltd, Cheshire, England: Talbot AS-3 Autosampler.

Technical Ltd, Cheshire, England: Spherisorb 5ODS2 stainless steel column.

V.A. Howe & Company Ltd, London, England: Heraeus Christ Minifuge 2.

APPENDIX TWO

Chemical and Suppliers

Aldrich Co Ltd, Dorset, England: 3,4-dihydroxybenzylamine hydrobromide.

BDH Chemicals Ltd, Dorset, England: Adrenaline and noradrenaline.

Fisons Scientific Apparatus, Loughborough, England: Citric acid monohydrate, diaminoethanetetraacetic acid disodium salt, glacial acetic acid, hydrochloric acid, perchloric acid, phosphoric acid, sodium acetate trihydrate, sodium dihydrogen orthosphosphate, sodium hydoxide pellets, sodium metabisulphite and trichloroacetic acid. All are analytical grade chemicals. High pressure liquid chromatography grade chemicals were:- Acetonitrile, 1-heptane sulphonic acid sodium salt and methanol.

Koch-light Laboratories Ltd, Slough, England: Woelm aluminium neutral activity grade 1.

Sigma Chemical Company Ltd, Dorset, England: 3,4-dihydroxyphenylalanine hydrochloride, ethyleneglycol-bis-(beta-aminoethylether) N,N-tetraacetic acid, reduced glutathione, Tris(hydroxymethyl)aminomethane.

APPENDIX THREE

Mobile Phase for Cation Exchange Chromatography for Nucleosil 10 SA column

Acetate-Citrate Buffer pH 4.8

1. Citric acid monohydrate	7.98g
2. Sodium acetate trihydrate	9.44g
3. Sodium hydroxide	2.4g
4. Diaminoethanetetraacetic acid disodium salt	50mg

These were dissolved in one litre of deionised double distilled water. The pH was adjusted to 4.8 with glacial acetic acid. The mobile phase was filtered through sartorius acetate filter pore size 0.45um and was degassed with helium gas for about two minutes to remove dissolved gases, then was ready to be used at a flow rate of 1.4ml per minute.

The function of EDTA is well established as a chelating agent. Waraszkrewicz et al (1981) used EDTA specially to remove iron(II) leached from the stainless steel of the chromatographic system. The unwanted metallic ions are oxidised by the cell to give a false response to the detector.

APPENDIX FOUR

Mobile Phase for Ion Pair Chromatography for uBondapak C18 Column

The Mobile Phase pH 5.2

1. Sodium dihydrogen orthophosphate 15.6g

2. 1-Heptane sulphonic acid sodium salt 1.0g

3. Diaminoethanetetraacetic acid disodium salt 50mg

These were dissolved in one litre of deionised double distilled water. Then the working mobile phase was made as follows:

900 ml of buffered solution and 120ml of methanol HPLC grade.

The pH was adjusted to 5.2 with sodium hydroxide and orthophosphoric acid. The mobile phase was filtered and degassed with helium gas for about 2 minutes. The mobile phase was ready to be used at a flow rate of 1.0ml per minute.

APPENDIX FIVE

Mobile Phase for Ion Pair Chromatography for Spherisorb 5ODS2 Column

The Composition of the Mobile Phase:

1. Sodium dihydrogen orthophosphate	15.6g
2. Diaminoethanetetraacetic acid sodium salt	50mg
3. 1-Heptane sulphonic acid sodium salt	1.0g

These chemicals were dissolved in one litre of deionised double distilled water. The working mobile phase was a mixture of:

900 ml of buffered solution and 120ml of methanol HPLC grade.

The pH was adjusted to 5.2 with sodium hydroxide and orthophosphoric acid then filtered and degassed. It was then ready to be used at a flow rate of 1.0ml per minute.

APPENDIX SIX

Preparation of Catecholamine Standard Solutions

The stock standard solutions were made in 0.1M perchloric acid. Then the working standard solutions were made from stock solutions, then aliquoted and kept frozen at -70 degrees Celsius. Fresh aliquots were used each day for the standardisation.

The Stock Solutions

Accurately weighed amounts of 0.169g noradrenaline, 0.183g adrenaline, 0.234g 3,4-dihydroxyphenylamine hydrochloride and 0.344g 3,4-dihydroxybenzylamine hydrobromide were dissolved in a litre of 0.1M of perchloric acid made in deionised double distilled water. The concentration of each substance in this solution is 1,000,000 nmol/l.

The Working Standard Solutions

Dilution of the stock solution 1 in 1000 gives 1,000 pmol/ml of each compound. A further 1 in 100 dilution of the 1,000 pmol/ml gives 10 pmol/ml of each compound. This was aliquoted in polypropylene tubes and kept frozen at -70 degrees C.

The preparation of internal standard solution (3,4-dihydroxybenzylamine hyrobromide) is made in the same way as described above. The aliquots from 10 pmole per ml solution were kept frozen as above.

APPENDIX SEVEN

Preparation of 1.0M Trizma Buffer pH 9.1

1. Tris(Hydroxymethyl)aminomethane (Trizma Base) 12.11g

2. Diaminoethanetetraacetic acid disodium salt 2.0g

The chemicals were dissolved in 100ml volummetric flask with deionised double distilled water. This was titrated with 2.0M hydrochloric acid AR grade to pH 9.1.

APPENDIX EIGHT

Purification of Aluminium Oxide

1. 50g Woelm neutral activity grade 1 was added to 500ml of 2M hydrochloric acid AR grade in a large beaker covered with a watch glass. The beaker was heated to about 100 degrees C in a fume cupboard for about 45 minutes on a hot plate equipped with magnetic stirrer.

2. The beaker is removed to allow heavier aluminium oxide to settle. The yellowish supernatant solution and finer particles of aluminium oxide are discarded.

3. The precipitate is washed twice with fresh 200ml portions of 2M hydrochloric acid, and heated for about 10 minutes with a fresh portion of 200ml of the acid to about 70 degrees C while being stirred.

4. The supernatant solution and the finer particles of aluminium oxide are discarded.

5. The precipitate is washed twice with 200ml portions of 2M hydrochloric acid, then heated to about 70 degrees C with a fresh 200 ml of the acid for about 10 minutes while being stirred.

6. The supernatant solution and finer particles are discarded.

7. The acid washed aluminium oxide is washed repeatedly with

deionised double distilled water until the pH of the water is 3.4. The finer particles of aluminium oxide are discarded each time.

8. Aluminium oxide is transferred to an evaporating dish, heated at 120 degrees C in the oven for six hours.

9. The temperature of the oven is reduced to about 40 degrees C and aluminium oxide is kept at that temperature to keep it dry.

10. The aluminium oxide treated this way will always give satisfactory results, when used for plasma extraction.

APPENDIX NINE

Collection of Blood for Catecholamine Measurements

The procedure adopted in our Laboratory for collection of blood for catecholamine measurements is: 10ml of blood withdrawn into lithium heparin Vacutainer tube without anti-oxidants and not pre-cooled, the blood is mixed gently by inversion. The blood sample is centrifuged for 10 minutes at 1500g when it is convenient. Plasma is separated into polypropylene tubes and stored at -70 degrees C until analysed.

APPENDIX TEN

Purification of Plasma for Catecholamine Measurements

1. 25mg of acid washed aluminium oxide was placed in a stoppered glass tube.

2. 100ul of 10pmol/ml of 3,4-dihydroxybenzylamine hydrobromide (IS) was added.

3. 1.0ml of plasma was added.

4. The pH of the mixture was titrated to 8.6 with 100ul of Trizma buffer.

5. The tube was mechanically shaken for 10 minutes.

6. The fluid was aspirated off.

7. Aluminium oxide was washed two times with cold deionised double distilled water. After the final wash, the fluid was removed as much as possible.

8. Catecholamines were eluted from aluminium oxide by mechanically shaking the tube with 200ul of 0.1M perchloric acid.

9. 100ul of clear fluid was injected onto the column, through 100ul sample loop.

APPENDIX ELEVEN

Protein Precipitation by Trichloroacetic Acid

1. 1ml plasma
2. 1ml of 1.2M trichloroacetic acid
3. Vortex for about one minute
4. Spin tubes at 1500g for ten minutes
5. Remove the clear supernatant fluid into stoppered centrifuge tubes
6. Add 3.5ml of Trizma buffer pH 9.1 to titrate the pH to 8.6
7. Add 100ul of internal standard (BHBA)
8. Add 25mg of aluminium oxide
9. Shake the tubes mechanically for 10 minutes
10. Discard the supernatant fluid
11. Wash aluminium oxide once with deionised double distilled water
12. desorb catecholamine with 200ul of 0.1M perchloric acid
13. Shake tubes mechanically for 10 minutes
14. Inject the clear supernatant fluid onto the column.

APPENDIX TWELVE

Protein Precipitation by Perchloric Acid

1. 1ml plasma
2. 1ml of 1.2M perchloric acid
3. Vortex the tubes for about one minute
4. Spin the tubes at 1500g for 10 minutes
5. Remove the clear supernatant fluid into stoppered centrifuge tube
6. Add 3.5ml of Trizma buffer pH 9.1 to titrate the pH to 8.6
7. Add 100ul of internal standard
8. Add 25mg of aluminium oxide
9. Shake the tubes mechanically for 10 minutes
10. Discard the supernatant fluid
11. Wash aluminium oxide once with deionised double distilled water
12. Desorb catecholamine with 200ul of 0.1M perchloric acid
13. Shake tubes mechanically for 10 minutes
14. Inject the clear supernatant fluid onto the column.

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