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AN EVALUATION OF A EUTECTIC TOPICAL ANAESTHETIC

(EMLA cream 5%)

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A thesis submitted in fulfilment of the requirements

for the degree of Master of Dental Science

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Department of Oral Surgery

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ABSTRACT

Eutectic Mixture of Local Anaethetics cream 5% (EMLA) is a 1:1 oil/water emulsion of a eutectic mixture of lignocaine 2.5% and prilocaine 2.5%. It is a new topical anaesthetic approved for medical purposes. Its medical indications include reducing the pain experienced during venous cannulation, the harvesting of skin grafts and the removal of genital warts.

A series of studies was carried out to investigate the efficacy and safety of EMLA for dental applications. Study 1 compared EMLA, NUM and Xylocaine 5% topical anaesthetics with placebo, for their efficacy in reducing the pain experienced during needle insertion in the oral mucosa. Results from study 1 showed that EMLA was the most effective agent when compared with placebo. Study 2 compared EMLA with Xylocaine 5% but no significant difference was found. Study 3 examined the depth of anaesthesia from longer application times of EMLA, Xylocaine 10% and placebo by electrical pulp testing. Results showed that EMLA was effective in blocking the response to the maximum stimulus of the pulp tester (300 volts) in 92% of subjects tested. Study 4 evaluated the plasma concentrations of prilocaine and lignocaine from 8 mL of EMLA applied to the oral mucosa. Analysis of venous blood samples by high pressure liquid chromatography showed the maximum plasma concentration for prilocaine to be 223 ng/mL and for lignocaine to be 418 ng/mL. Both concentrations were well below known toxic levels for the drugs.

Dedicated to Georgina Hall

Friend, Teacher and Healer.

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INTRODUCTION AND AIMS OF THE STUDIES

The application of a topical anaesthetic agent to the oral mucosa is often the first clinical step performed in the dental treatment of a patient. It is, therefore, somewhat surprising that there have been very few studies carried out to investigate the efficacy of these preparations. The main indication for the use of these agents in dentistry is to reduce the pain experienced during the administration of local anaesthetic injections. Other indications for the use of topical anaesthetics include the drainage of intra-oral abscesses and the removal of loose deciduous teeth.

Topical anaesthetic agents commonly used in recent years in Australia include Xylocaine 5% Ointment™[1] , Xylocaine 10% Special Adhesive™[1] and NUM Topical Anaesthetic Ointment™[1]. A new topical anaesthetic, EMLA™ cream 5%[1], which has undergone clinical trials since 1980, is currently being used to achieve anaesthesia for medical purposes.

Considering that relatively few studies have been carried out to evaluate topical anaesthetic agents, the objectives of this study were as follows :-

1. To conduct clinical trials of topical anaesthetic agents, and in particular

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[1]Astra Pharmaceuticals Pty. Ltd. (Australia)

EMLA, for their efficacy in reducing the pain experienced during needle insertion in the oral mucosa.

2. To establish the degree of pulpal anaesthesia that could be achieved by prolonged administration of topical anaesthetic agents and to assess their potential for further clinical applications in dentistry.

3. To measure the plasma concentrations of lignocaine and prilocaine, the active components of EMLA, when it is applied to the oral mucosa.

4. To observe any adverse local or systemic effects from the application of EMLA.

CHAPTER 1

HISTORY AND METHODS OF TOPICAL ANAESTHESIA

1.1 HISTORY OF TOPICAL ANAESTHETICS

The early history of local anaesthetics is to be found in the daily activities of the inhabitants of Bolivia and Peru. The South American natives chewed the leaves of the plant Erythroxylum coca in Bolivia and Erythroxylum truxillense in Peru. These leaves provided a powerful nerve stimulant which prevented fatigue and often enabled the people to perform great feats of endurance in the rarefied atmosphere of the high mountain regions. The plants were also used by the natives to act as a general nerve tonic and as an aphrodisiac. The plants are also cultivated in Java and Formosa, with Java Coca (Eryth. trux.) exported in the form of a coarse powder which is then refined to produce cocaine (Wren, 1975).

The first scientific investigation into substances capable of producing local anaesthesia was carried out by Gaedicke, a French chemist, in 1855. He produced a local anaesthetic agent in the form of a crude extract of cocaine from the South American plant Erythroxylum coca. Wohler continued to refine the extract and in 1860 isolated pure cocaine. The first reported

application to mucous membrane of cocaine was by Vasilius von Anrep in 1880. In 1884, William Halsted from New York utilised the drug for dental application to anaesthetise the mandibular nerve by the injection of cocaine 4 per cent intra-orally. Köller, also in 1884 began using cocaine for ophthalmic operations after being introduced to it by Sigmund Freud. Soon afterwards the danger of cocaine from its addictive properties became apparent. Following this a German chemist, Alfred Einhorn, in 1904 synthesised procaine which became the commonly used local analgesic for a great many years (Roberts and Sowray, 1970,p. 2).

In 1943, Nils Löfgren, a Swedish chemist synthesised lignocaine which became available for general use five years later. Löfgren and Tegener in 1953 synthesised prilocaine and reported its clinical use in 1960. Subsequently, prilocaine underwent extensive clinical trials in the period 1960-65.

A later development of local anaesthetic agents was their incorporation into surface delivery systems to provide for topical anaesthesia of the dermis and oral mucosa. Few studies have been done on the efficacy of topical anaesthetics on the oral mucosa. An early study by Adriani et al.(1963) investigated various agents and factors affecting their efficacy. The authors in their conclusion suggested first, that there was a concentration for each drug, above which no further enhancement of anaesthesia would occur. Second, drug combinations such as benzocaine with amethocaine were potentially useful due to the different latencies of action thus prolonging the

duration of anaesthesia. The duration of anaesthesia was found to be longest on the lip, followed by the palate and then the tongue. For medical applications, Crawford (1965) compared topical lignocaine with prilocaine in conscious patients undergoing bronchograms. Results showed that lignocaine 2 per cent and prilocaine 3 per cent were comparable in reducing the cough reflex and in obtaining adequate anaesthesia. Bergman, Siegel and Ciancio (1968) measured the plasma concentrations of carbon-14 labelled lignocaine in rabbits for comparison following intravenous and topical administrations. The authors found that concentrations were similar after ten minutes although intravenous injection had, as expected, a higher initial peak level. An extensive investigation by Adriani and Dalili (1971) compared thirty preparations of topical anaesthetic agents available on the American market and concluded that cutaneous barriers were more easily traversed by the bases of local anaesthetic drugs than by their acid-salts. Roller and Ship (1975) tested lignocaine in topical film strip form and although the technique appeared promising at first, they did not find it effective enough for surgical biopsy of the oral mucosa. Gangarosa (1981) studied the efficacy of topical anaesthetic preparations delivered by iontophoresis. He reported from earlier trials that deciduous teeth could be extracted after iontophoretic application of lignocaine 2 per cent with adrenaline 1:25,000. However, the method required a rather complex system of electrodes to obtain positively charged anaesthetic agent and negatively charged mucosa. Pashley and Parsons (1987) reported a case where topical application of lignocaine 5 per cent ointment resulted in sharp, severe pain in a patient with dentinal hypersensitivity. The

authors concluded that due to the high concentration of the polyethylene glycol vehicle, the ointment was very hypertonic and caused a large osmotic pressure difference at the dentine surface which resulted in pain. Harbet (1989), in a clinical report, suggested that topical ice may reduce the pain experience from dental needle insertion in the palate. He recommended that a five minute application time was necessary to achieve a sufficient degree of anaesthesia.

1.2 METHODS OF OBTAINING TOPICAL ANAESTHESIA

Roberts et al. (1970,p. 50-52) and Somers and Mouser (1991) have described a number of methods whereby topical anaesthesia of the oral mucosa can be attained. The majority of these methods employ a local anaesthetic agent. Currently in Australia, there are many proprietary topical anaesthetic agents available for use on the oral mucosa. However, the method of obtaining topical anaesthesia and the number of anaesthetic substances used are in fact, quite limited.

1. Solutions

Solutions that are available for oral use contain either lignocaine hydrochloride (4 per cent), amethocaine hydrochloride (0.5 - 2 per cent) or benzocaine base (2.5 per cent). The anaesthetic agents are combined with sterile water to form solutions. The solutions can be used as a mouthrinse or with a gargling action. The indications for the use of solutions include

patients with a gagging reflex of the soft palate during the taking of impressions and patients with painful oral ulceration who need relief when taking meals. No studies have been carried out to assess the efficacy of solutions for topical use in the oral cavity.

2. Sprays

Topical anaesthetic sprays use lignocaine base (10 per cent) or benzocaine base (1.5 per cent) combined with propellants, to provide surface anaesthesia of areas with difficult access, such as the oropharynx and trachea. Sprays are useful in reducing the reflex activity during endotracheal intubation, and instrumentation of laryngoscopic and bronchoscopic procedures.

3. Jellies

Jellies contain lignocaine hydrochloride (2 per cent) combined with methylcellulose base in order to achieve topical anaesthesia of accessible mucous membranes. A jelly also containing chlorhexidine gluconate (0.05%) is available for catheterisation of the urethra to reduce the risk of infection. For the application of a topical anaesthetic agent to proximal parts of the digestive tract, a more viscous form of jelly is available. This form contains lignocaine hydrochloride (2 per cent) in the aqueous solution adjusted to suitable consistency with carboxymethylcellulose.

4. Ointments and Creams

This method of topical anaesthesia is perhaps, the most common used in dentistry. Ointments have bases (such as petroleum jelly, polyethylene glycol or lanolin) that are not water soluble. Due to the lipophilic property of ointments, the anaesthetic agents within the ointment are lignocaine base, benzocaine base, amethocaine base or a combination of the anaesthetic bases. Ointments are easy to apply and localise and adhere well to the oral mucosa. The available generic preparations of ointments are Xylocaine 5% Ointment, Xylocaine 10% Special Adhesive and NUM. EMLA cream 5% contains an emulsion of oil and water. Details of the above agents, and in particular EMLA, are presented in the following section (1.3) of the thesis.

5. Lozenges

Benzocaine (7.5 mg) lozenges are sucked to produce surface analgesia of the tongue, palate and mucosa. No studies investigating the efficacy of lozenges for use in the oral cavity have been reported.

6. Refrigeration

The freezing of tissues usually employs a volatile material such as ethyl chloride (15 per cent) combined with dichlorotetrafluoroethane (65 per cent). As the refrigerant evaporates there is heat loss from the tissues which may provide a degree of analgesia. No comprehensive studies of these agents have been carried out to assess their efficacy.

1.3 TOPICAL ANAESTHETIC OINTMENTS AND CREAMS

A primary aim of this thesis was to investigate the efficacy of topical anaesthetics, and in particular topical ointments and creams, for their applications in the oral cavity. The topical anaesthetic ointments that are currently approved for oral mucosal use in Australia are Xylocaine 5% Ointment, Xylocaine 10% Special Adhesive and NUM Topical Anaesthetic Ointment.

Xylocaine 5% Ointment has as its active agent lignocaine 5 per cent in the base form. The drug is carried in a vehicle of polyethylene glycol. Xylocaine 10% Special Adhesive has a 10 per cent concentration of lignocaine, again in the base form. The agent is also carried in a polyethylene glycol vehicle. NUM Topical Anaesthetic Oinment is a combination of two anaesthetic agents. The agents are benzocaine base (15 per cent) and amethocaine base (1.7 per cent). The carrier medium again is polyethylene glycol. A small concentration of hibitane is also present in NUM to provide for disinfection of the tissue when applied to the oral mucosa.

In recent years a new topical anaesthetic preparation has been developed, EMLA cream 5% (Eutectic Mixture of Local Anaesthetics), which is eutectic in composition. A eutectic is a mixture of two solids and has an important physical property whereby the melting point of the mixture is lowered. This enables EMLA to overcome a major barrier, namely the high

melting points of anaesthetic bases, which results in a more rapid rate of absorption through tissues.

1.3.1 EMLA cream 5%

EMLA is a new topical anaesthetic that is currently approved for medical applications in Australia. The first published study evaluating EMLA for dermal use was by Juhlin, Evers and Broberg (1980). Since then clinical trials have been carried out to evaluate other possible uses for EMLA. These have included the reduction of pain from venous cannulation and otological procedures. These studies are reported more extensively in chapter 2.

EMLA is a mixture of lignocaine and prilocaine in their base forms Evers,1988). Lignocaine and prilocaine bases have melting points of 69°C and 37°C respectively. However, when combined in eutectic form the melting point of the mixture is lowered to 17°C. This property allows the anaesthetic agents to form an oil at mouth temperature (37°C) and thus allows increased absorption of the drugs. This oil is combined with castor oil and other additives (information regarding anaesthetic oil/ castor oil / additive ratios not released by manufacturer) and then emulsified in water to achieve a high water content (50 per cent) of the cream. The oil droplets contain a high concentration of the local anaesthetic agents in the base form (80 per cent). In a conventional lignocaine-in-oil mixture, the concentration of local anaesthetic in the droplets is only 20 per cent, because in the conventional preparation

the crystalline form of the anaesthetic has to be dissolved in castor oil before an emulsion can be produced. The eutectic mixture, because of the high concentration of bases present, facilitates more rapid diffusion of the two anaesthetic bases than would be achieved if the bases were used alone in a conventional formulation of similar concentration.

Illustration 1. Topical Anaesthetic Agents

	2
1	3
	4

1. NUM
2. Xylocaine 10% Special Adhesive
3. Xylocaine 5% Ointment
4. EMLA cream 5%

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 PHARMACOLOGY OF LOCAL ANAESTHETIC AGENTS

2.1.1 Lignocaine

Lignocaine (N-diethylaminoacetyl-2,6-xylidine) (Fig. 1a) was the first amide-type local anaesthetic to be synthesised. It has a molecular weight of 234.3 and its empirical formula is $C_{14}H_{22}N_2O$. It is the most widely used local anaesthetic in dentistry and is the standard with which all other local anaesthetics are compared (Malamed, 1986,p. 45). Lignocaine base is the active form of the drug which blocks the conduction of the nerve impulses. The base is a white crystalline powder and has a melting point of 66 - 69°C. The base form is used in topical ointments such as Xylocaine 5% Ointment and Xylocaine 10% Special Adhesive. The base is very insoluble in water but soluble in ethanol, ether and chloroform. Lignocaine hydrochloride is the acid-salt of the base. It is a white crystalline powder with a higher melting point of 76 -79°C. The acid-salt is highly soluble in water and it is in this form that the drug is used for administration by injection or by topical application in the form of solutions and jellies.

The drug has a relatively short half-life of ninety minutes following administration by injection in the oral cavity. Its primary site of metabolism is the liver where 90 per cent of the drug undergoes biotransformation into inactive metabolites. In the liver microsomal fixed-function oxidases convert lignocaine to monoethylglyciene and xylidide (which has local anaesthetic properties). Excretion is via the kidneys with less than 10 per cent in the form lignocaine and more than 80 per cent as its various metabolites. Lignocaine has a rapid onset of action of two to three minutes following injection to block conduction of nerve impulses. The drug has an dissociation constant (pK_a) of 7.85 which creates a favourable rate of ionization to produce more available free base for blocking.

A 2 per cent concentration of lignocaine hydrochloride is considered as the optimum concentration for blocking nerve conduction and to minimise toxic effects when used in the injected form. Lignocaine has a vasodilating effect and is often used with a vasoconstrictor such as adrenaline in various concentrations to prolong the duration of anaesthesia and to reduce potential adverse reactions due to its rapid absorption.

Lignocaine has a high potential for crossing the blood-brain barrier and a high concentration in blood can cause depression of the central nervous system. Lignocaine in sub-toxic levels (0.5 - 4.0 mg/L) possesses anticonvulsant properties and the drug has been used intravenously to treat both grand mal and petit mal seizures. This effect is achieved by decreasing

the excitability of the central nervous system neurones, thus raising the seizure threshold. In higher plasma concentrations (4.5 - 7.0 mg/L), however, lignocaine can cause preconvulsive signs and symptoms. These initial clinical signs and symptoms are excitatory. Signs include slurred speech, muscle twitching and tremor in the facial muscles. Symptoms include numbness of the tongue and circumoral region, flushed sensation of the skin, vertigo and tinnitus, and drowsiness and disorientation. Higher plasma concentrations (> 7.5 gm/L) can lead to tonic-clonic seizure. Other actions of the local anaesthetic on the central nervous system is an increase in the pain reaction threshold and its ability to attain a degree of anaesthesia.

Lignocaine also has a direct effect on the myocardium. The drug can decrease electrical excitability of the myocardium, conduction rate and the force of contraction. Lignocaine is known to have antiarrhythmic actions in animals and humans. Blood levels for antiarrhythmic activity range from 1.8 - 5.0 mg/L. The vasodilatory effects of lignocaine are caused by relaxation of smooth muscle in the walls of blood vessels which can result in hypotension. Malamed (1986,p. 44) has suggested that the maximum dose of lignocaine without causing toxic effects is 4.4 mg/kg of body weight.

2.1.2 Prilocaine

Prilocaine (α-Propylaminopropio-o-toluidide) (Fig. 1b) is also an amide-type local anaesthetic agent. The empirical formula of the base is $C_{13}H_{21}ClN_2O$

and it has a molecular weight of 220.3. The base is a white crystalline powder with a melting point of 36 - 38°C. The base is freely soluble in ethanol, ether and chloroform. The acid-salt of prilocaine is prilocaine hydrochloride and has a melting point of 167 - 168°C. It is the acid-salt form that is used for administration by injection.

The drug undergoes metabolism mainly in the liver, with some metabolism also occurring in the lungs. An important metabolite of prilocaine is orthotoluidine. This metabolite can induce the formation of methaemoglobin if a large dose of prilocaine is administered. A dose in excess of 400 mg of prilocaine is required to produce significant levels of methaemoglobin in the blood (1 per cent). Methaemoglobin levels greater than 20 per cent of total haemoglobin can produce greyish or slate-blue cyanosis of the lips, mucous membranes and nail beds. At these levels respiratory and circulatory distress is possible. Prilocaine is metabolised more rapidly than lignocaine and thus has a shorter half-life. Prilocaine and its metabolites are excreted primarily through the kidneys. The anaesthetic has an onset of action of two to four minutes and the ideal dental concentration is 4 per cent for injection. Prilocaine has a dissociation constant of 7.9. The drug can also cause vasodilation but to a lesser degree than lignocaine. Malamed (1986,p. 49) has recommended that the maximum dosage of prilocaine without causing toxic effects is 6 mg/kg of body weight.

2.1.3 Benzocaine

Benzocaine is a white crystalline powder and has a melting point of 88 - 91°C. The formula for benzocaine is $C_9H_{11}NO_2$ and it has a molecular weight of 165.2. The drug is metabolised by hydrolysis to p-aminobenzoic acid. Benzocaine (Ethyl p-aminobenzoate) (Fig. 1c) is an ester of aminobenzoic acid and is considered a simple ester in that it contains no basic nitrogen group. Due to the absence of the nitrogen group it is poorly soluble in water and the drug is unable to form an acid-salt. The low water solubility and consequently slow absorption from topical application prolongs the anaesthetic effect of benzocaine and also reduces its toxicity.

Although the drug has low toxicity, Kleniewska and Rudzki (1976) reported a high level of skin sensitivity of 5 per cent in a group of nearly nine hundred patients. A case report by Klein and Feinberg (1990) mentioned acute toxic methaemoglobinaemia caused by benzocaine topical ointment (20 per cent) when applied for five minutes to remove archbars on a small child. The patient complained of nausea and dizziness, and physical examination showed cyanosis and agitation. Blood levels of methaemoglobin in the child was found to be 13.8 per cent of total haemoglobin. Potter and Hillman (1979) estimated that blood levels of 15 - 25 mg/kg body weight of the drug can cause significant methaemoglobin formation.

2.1.4 Amethocaine

Amethocaine (2-Dimethylaminoethyl p-butylaminobenzoate) (Fig. 1d) is also an ester-type anaesthetic and was developed by Eisleb in 1931. Amethocaine base has a molecular weight of 264.4 and its formula is $C_{15}H_{24}N_2O_2$. It is a white, waxy solid with a melting point of 41 - 46°C and is only very slightly soluble in water. Amethocaine hydrochloride is the acid-salt and is a white hygroscopic powder with a melting point of 147 - 150°C. The hydrochloride salt is a white odourless crystalline powder (m.w. 300) and possesses a bitter taste. The salt is very soluble in water.

The drug has frequently been used to obtain spinal anaesthesia with a rapid onset of action following intrathecal injection. Detoxification takes place at a very slow rate and it is hydrolysed mainly in the liver. A metabolite is p-aminobenzoic acid which inhibits the action of some antibiotics such as sulphonamides. Woolfson, McCafferty and Boston (1990) used a percutaneous application of amethocaine to assess its efficacy for cutaneous anaesthesia. The investigators found that a thirty minute application time of 4 per cent ointment resulted in profound anaesthesia of the dermis to pinprick challenge.

Fig. 1a Lignocaine

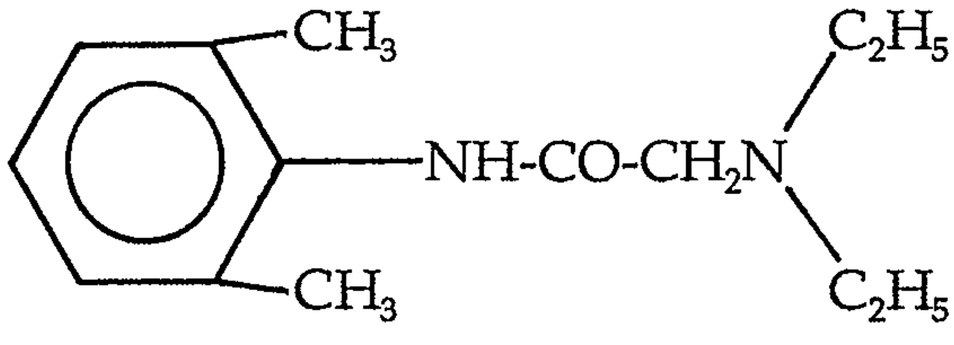


Fig. 1b Prilocaine

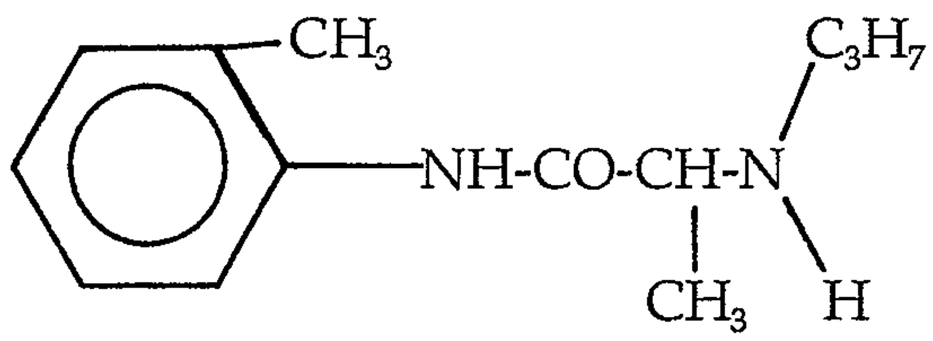


Fig. 1c Benzocaine

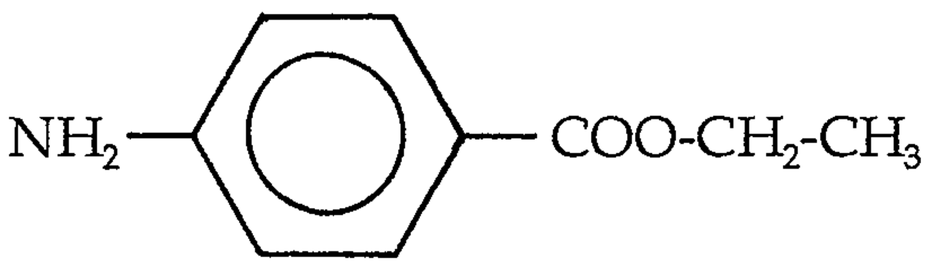
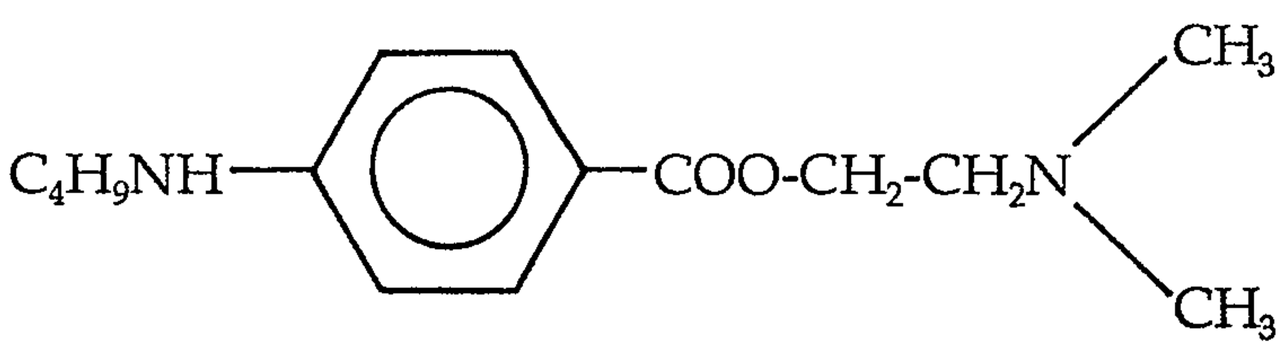


Fig. 1d Amethocaine



2.2 PHYSICAL PROPERTIES OF TOPICAL ANAESTHETICS

2.2.1 Oil/water distribution ratio

The oil/water distribution ratio (or partition coefficient) is the extent to which a substance will dissolve in water. The ratio is important in determining the rate of transfer of non-electrolytes across the oral mucosa. Walton and Lacey (Meyer, Squier and Gerson,1984,p.99) studied the oil/water distribution ratios of several alkaloidal drugs. They compared the ratio of the sublingual to subcutaneous dose needed to produce similar pharmacological effects. Drugs with high oil/water distribution ratios required far lower sublingual doses than drugs with lower ratios to produce similar pharmacological effects to those obtained by subcutaneous injection. The buccal absorption test of Beckett and Triggs (Meyer et al.,1984,p.97) confirmed this work. They demonstrated that the percentage disappearance of a solute is directly related to its oil/water distribution ratio. The buccal absorption test consists of introducing a standard volume of a drug solution of known concentration into the oral cavity of a subject, who swirls it around the mouth for a measured period of time and then expels it. The returned liquid is measured and analysed for solute concentration. From a knowledge of the volume and concentration of the solution taken into the oral cavity, and of the solution expelled, the amount which has been absorbed can be calculated.

An oil/water distribution ratio range from 1:40 - 1:2,000 of water is

required for a drug to be useful in the oral cavity. A ratio of less than 1:40 makes the drug too water-soluble and absorption through the lipid membrane is reduced. However, a ratio greater than 1:2,000 makes it difficult to achieve adequate concentrations of the drug in an aqueous solution.

2.2.2 Diffusion coefficient

The diffusion coefficient (or permeability coefficient) of a solute is defined as the number of moles of solute which can passively diffuse across unit area of membrane in unit time when the concentration gradient is one. The diffusion coefficient is mathematically expressed in Fick's Law, and this equation can be used to calculate the rate of diffusion of a drug :-

$$\text{Rate of diffusion} = P \times A \times (C_a - C_b)$$

where P = diffusion coefficient; A = area of membrane barrier between compartments a and b; C_a and C_b are the concentrations of the solute in compartments a and b.

The coefficient can vary with the temperature, the viscosity of the solution and the molecular weight of the solute. The mouth temperature is considered constant at 37°C and therefore does not affect the diffusion coefficient. Viscosity changes are also considered to have minimal effect on the coefficient when the drug under investigation is present in low concentrations. The size of a penetrating drug molecule can also affect its permeability but to a lesser extent than the oil/water distribution ratio.

2.2.3 Concentration gradient

The concentration gradient is defined by the difference in concentrations of the solutes on both sides of a membrane. Siegel (Meyer et al.,1984,p.100) has suggested that there should be a linear relation between the concentration gradient and the amount transferred across a membrane. An in vitro study by Bergman et al.(Meyer et al.,1984,p. 100) of the rates of transfer of the anaesthetic agents lignocaine and prilocaine, showed that when there was a ten-fold increase in concentration at the test site (lingual frenum), there was a ten-fold increase in the rate of transfer. Siegel (Meyer et al.,1984,p.101), conducting in vivo experiments with ethanol and glycerol, also confirmed the linearity of buccal absorption in relation to the concentration gradient, and felt that this mechanism was consistent with passive (simple) diffusion as the mechanism of transportation of drugs across the epithelium.

2.2.4 Degree of ionization

Rabinowitz and Myerson (1971) have stated that in general, the ionized form of a drug does not penetrate the lipid membrane, whereas the lipid soluble non-ionized form can diffuse through passively. The degree of ionization is described by the Henderson-Hasselbach equation:

for a weak acid, $pH = pK_a + \log$ (ionized form/non-ionized form)

for a weak base, $pH = pK_a + \log$ (non-ionized form/ionized form)

For weak acids and bases, the non-ionized form of the molecule has a much higher oil/water distribution ratio than the ionized form. Thus, the rate of transfer of a weak acid or base is dependent on the concentration gradient of the non-ionized form rather than the total concentration.

Bergman et al.(Meyer et al.,1984,p. 101) used the Henderson-Hasselbach equation in studies of prilocaine and lignocaine in vitro and in vivo. They measured the rate of penetration when altering the pH of the solution containing the drugs. They found that an increase in pH of the weak basic anaesthetic solution to a value greater than the pK_a resulted in a higher rate of penetration, and a decrease in pH to a value lower than the pK_a caused a decreased rate of penetration. The degree of non-ionization present before absorption takes place is significant. The mean pH of saliva is 6.0 and in general drugs are considered to be adequately absorbed by passive diffusion through the oral mucosa if the pK_a of an acid is greater than 2, or for a base less than 10.

2.3 MUCOSAL ABSORPTION OF DRUGS

The first investigator to describe the ability of substances to be absorbed through the oral mucosa was Field in 1858, when he reported treating patients for angina pectoris by placing nitroglycerine on the tongue. Brunton, in the Gaulstonian Lectures delivered before the Royal College Of Physicians in 1877, also showed that sublingual application of glyceryl trinitrate could bring about dramatic relief from the symptoms of angina pectoris. More recently, work carried out by Siegel (Meyer et al.,1984,p.95) suggested that the epithelium and basal lamina of the oral mucosa constitute the major barriers to mucosal absorption, while the lamina propria offers little resistance to penetration.

Some drugs are absorbed rapidly by oral mucous membranes because of their thin, smooth surface and rich blood supply. Drug administration via the oral mucosa has a number of advantages as suggested by Rabinowitz et al.(1971) which are as follows :-

1. There is no pain or discomfort compared with parenteral or rectal methods.
2. The use of aseptic techniques is less critical.
3. Self-administration is possible.
4. The drug is not subject to alteration or breakdown by gastro-intestinal secretions.
5. Rapid onset of action is possible and high blood levels can be attained.
6. Excess drug can be quickly and easily removed, thus reducing the risks

involved by accidental overdosing in parenteral administration.

2.3.1. Methods of diffusion

Substances are thought to cross the oral mucosal barrier by either facilitated (carrier-mediated) diffusion or passive (simple) diffusion.

1. Transport by facilitated diffusion (active transport)

The energy source for facilitated diffusion is the concentration gradient. It is thought that the solute molecule combines with a carrier receptor of the membrane to form a solute-carrier complex which diffuses through the membrane along the concentration gradient. Once through the membrane the solute molecule is released and the carrier molecule is then free to return to its original site to combine with another solute molecule.

2. Transport by passive diffusion

Siegel (Meyer et al.,1984,p. 98) has described the rate of passive diffusion of a substance across the membrane in a complex mathematical equation. He states the factors involved in passive diffusion are :-

1. oil/water distribution coefficient
2. diffusion coefficient
3. area of the membrane
4. thickness of membrane
5. concentration gradient

2.3.2 Morphological barriers to diffusion

1. Solute Binding

The equation for passive diffusion (Fick's Law) states that a linear relationship should exist between the concentration gradient and the rate of diffusion. However, Beckett and Pickup (Meyer et al., 1984,p.102) have reported a non-linear relationship using the buccal absorption test. A model proposed by Dearden and Tomlinson (Meyer et al.,1984,p.102) to explain this non-linearity was that protein binding occurred on or in the oral epithelium. Kates (Meyer et al.,1984,p. 102) used the buccal absorption test and measured blood levels for sublingual administration of propranolol. Results substantiated Dearden's model by showing that the time in which 50 per cent of the drug appeared (half-time) in the blood was about three times longer than half-time disappearance from the administered solution. A study by Odomosu and Wilson (Meyer et al.,1984,p. 98) also verified the model following their results for oral administration of ascorbic acid. The location for this binding mechanism is thought to be a filamentous material on the epithelium derived in part, from components of saliva. An alternative explanation for this non-linear relationship is that the concentration of a drug on one side of a membrane (i.e. C_a) may decrease in a linear manner relative to time but the concentration of the drug on the other side of the membrane (C_b) may not rise in parallel, because blood flow may remove drug from the membrane area. Thus, C_b remains relatively low and the concentration gradient will no longer be directly related to time but will be related linearly

to the rate of diffusion (Mylecharane,1991).

2. Basal Lamina

The basal lamina of the oral mucosa acts as a barrier with selective properties for the diffusion of substances. The lamina is the intervening layer between the epithelium and the underlying connective tissue. Under electron microscopy the lamina appears as a continuous layer 50 - 100 nm thick which contains a network of fine filaments (3 - 4 nm) in an amorphous matrix. Chemical analysis of the basal lamina indicates that its primary components are collagen and proteoglycans. The role of the basal lamina in the absorption of drugs is not well understood (Ross, Reith and Romrell,1989,p.54). For example some large molecular weight compounds such as dextran 70 (molecular weight 70,000) and small molecular weight compounds e.g. inulin, a plant polysaccharide (m.w. 5,000) have slow diffusion rates and yet dextran 20 (m.w. 20,000) has a relatively rapid rate of diffusion.

3. Zonula occludens

The zonula occludens, also referred to as a tight junction, forms part of the junctional complex that is responsible for the adherence of epithelial cells to each other (Ross et al.,1989,p.59). The zonula occludens is located at the most apical part of the lateral surface of the cell and represents a ring of plasma membrane union between neighbouring cells. The greater the number of membrane contacts formed by the zonula, the more impervious the epithelium will be to drug absorption.

4. The keratin layer

The oral mucosa contains areas of both keratinized and non-keratinized epithelium. Areas of keratinized epithelium are located in the expanse of the hard palate and the labial and lingual gingiva adjacent to teeth. During the maturation of a keratinocyte, there is a gradual loss of cytoplasm while tonofibrils are converted to keratin. This is followed by breakdown of the cell nucleus and other organelles and the thickening of the cell membrane. At the same time as keratin is produced, a glycolipid is formed as membrane-bound lamellar bodies (Ross et al.,1989,p.353). The glycolipid spreads to fill the intercellular space forming a barrier to water. The lipid membrane is thus important in allowing through drugs according to its oil/water distribution ratio.

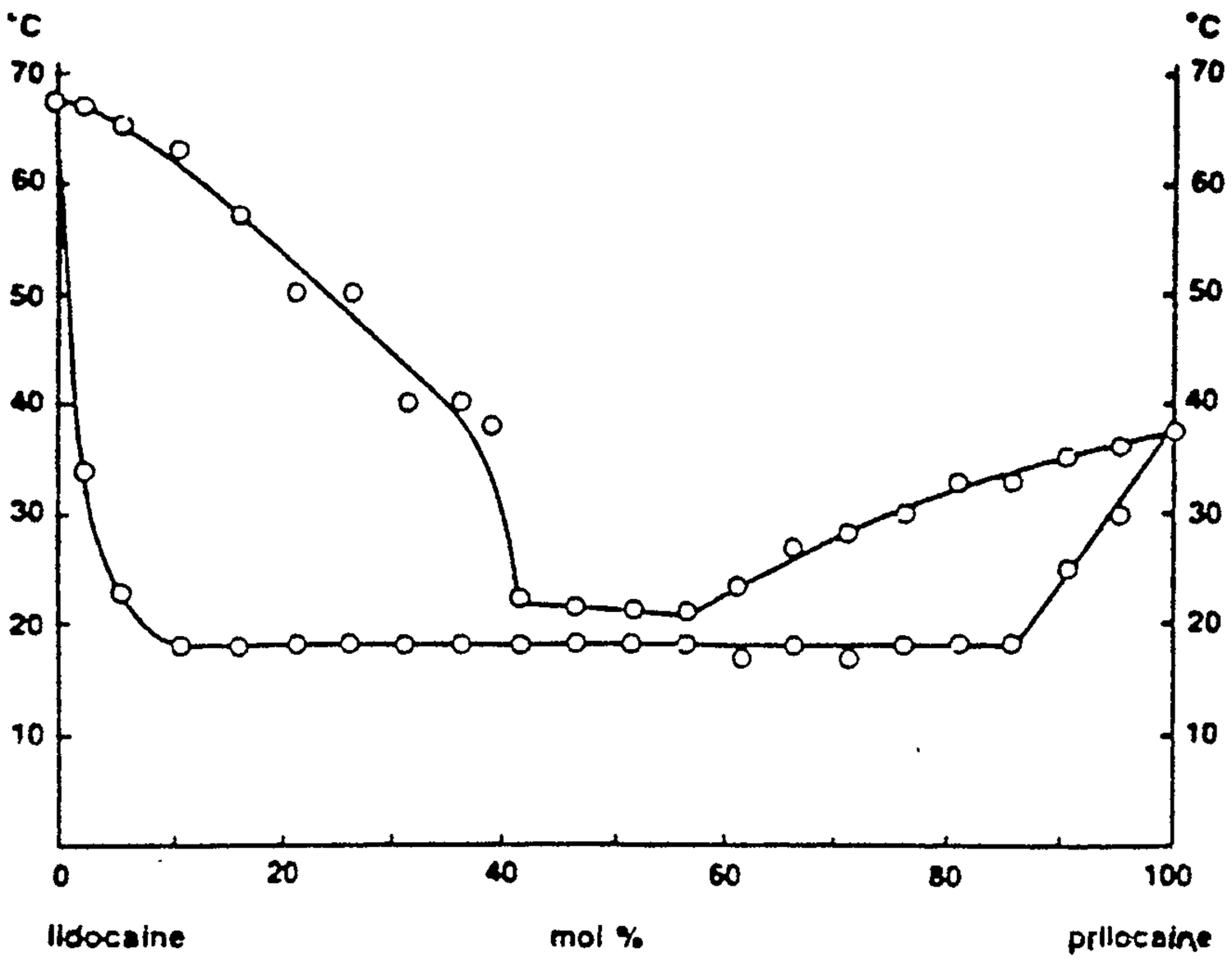
2.4 LIGNOCAINE - PRILOCAINE EUTECTIC SYSTEM

Both lignocaine base and prilocaine base are poorly soluble. The bioavailability of poorly soluble drugs can be enhanced by their fusion to water soluble carriers. Brodin et al. (1984) studied the eutectic lignocaine-prilocaine system of EMLA to investigate its physical characteristics and aqueous solubility. The investigators prepared a mixture of lignocaine (49.6 per cent by weight) and prilocaine (50.4 per cent by weight) which was heated gently until liquefaction occurred. Different methods were employed to observe and measure the changes that occurred:-

1. Hot stage microscopy

Lignocaine and prilocaine in various ratios were mixed and heated. The solidified mixtures were then examined under a binocular microscope with a hot stage. Phase transitions of the drug mixtures were recorded both visually and by photoelectric sensor. The phase diagram (Fig. 2) showed that in higher concentrations of lignocaine or prilocaine, only solid solutions existed. However, when lignocaine and prilocaine were mixed in a ratio of approximately 1 : 1 an oil was clearly visible at 19°C. Results indicated that a 1 : 1 ratio of lignocaine to prilocaine was necessary for the eutectic mixture in EMLA cream 5% (lignocaine 2.5 per cent : prilocaine 2.5 per cent) to form an oil at mouth temperature.

Fig. 2 Phase diagram of the nonequilibrated lignocaine-prilocaine system determined by hot stage microscopy (Brodin et al.,1984).



2. Xray diffraction

Xray diffraction was carried out on the 0-15 per cent concentrations of lignocaine and prilocaine by an Xray powder diffraction camera. This confirmed the existence of the solid solutions when compared with hot stage microscopy.

3. Infra-red spectrophotometry

The infra-red spectrum of crystalline EMLA was compared to those of the single components (lignocaine and prilocaine). Results showed that no chemical interaction takes place between lignocaine and prilocaine in the eutectic form.

4. Solubility determination

Both lignocaine base and prilocaine base are known to decrease the surface tension of water only slightly and thus do not form micelles (minute droplets). The investigators measured the temperature dependence (ΔH) of lignocaine and prilocaine when EMLA was in aqueous solution. Results showed that lignocaine and prilocaine decreased the solubility of each other; however, the total solubility of the mixture in water was decreased only marginally. The eutectic mixture in EMLA cream 5% has the major advantage of being an oil at room temperature. Thus, it is possible to emulsify the eutectic mixture of anaesthetic bases directly in water.

2.5 STUDIES ON THE APPLICATION OF EMLA

2.5.1 Oral Mucosal

There have been only two published studies evaluating the efficacy of EMLA on the oral mucosa. The first was undertaken by Holst and Evers (1985). In this study EMLA cream 5%, Xylocaine 5% Ointment and placebo were compared for efficacy to reduce the pain experience from dental needle insertion. This double blind study employed two groups. Group one consisted of ten females undergoing twelve paired applications with a two minute and a five minute application time. A 30 gauge needle was inserted to a depth of 2 mm and the pain experience was assessed on a ten centimetre visual analogue scale. Results showed that EMLA was significantly better than Xylocaine and placebo at both two and five minute time intervals. Group 2 consisted of twenty subjects and each had a paper disc with impregnated EMLA or placebo left on the lower buccal sulcus for two minutes, followed by the insertion of a 27 gauge needle into the mucosa. Results showed EMLA to be significant in reducing the pain experience when compared with placebo. The authors concluded that although effective, EMLA was difficult to localise at the application site because of its fluid consistency. Some volunteers reported a slight burning feeling for the first twenty seconds with the application of dry discs. Two critical comments of this study were, first, the investigators did not report the period between each test sample. If the period between each paired application was too short, the spread of the

agent may have resulted in a lower pain score for subsequent test samples. Second, the method of utilising twelve paired samples in each subject can result in lower pain scores during the course of the study because patient anxiety is lessened .

The second study for oral mucosal application was by Haasio et al.(1990). A random study using ten volunteers compared EMLA with Xylocaine 10% Spray for pain threshold response from electrical stimulation. Four grams of EMLA (100 mg of lignocaine and 100 mg of prilocaine) was applied for four minutes to the upper gingiva by an electric toothbrush, or Xylocaine spray applied to each quadrant (50 mg lignocaine per quadrant). Results showed no statistical difference between the two preparations. The maximum analgesic effect for EMLA was reached at thirteen (S.D. ± 8) minutes, and for Xylocaine fourteen (S.D.± 5) minutes. Lignocaine and prilocaine plasma concentrations were measured by gas chromatography and the range for EMLA was 0.08 to 0.10 µgm/mL for prilocaine and 0.14 to 0.20 µgm/mL for lignocaine. Xylocaine 10% spray had a range of 0.12 to 0.35 µgm/mL for lignocaine. The plasma concentrations showed EMLA to be more readily absorbed than Xylocaine. A five minute application of EMLA and Xylocaine produced plasma concentrations of 0.20 µgm/mL for EMLA and 0.12 µgm/mL for Xylocaine although twice the amount of lignocaine was used for the spray. Throughout the thirty minutes the prilocaine plasma concentration varied by only a small amount as mentioned. However, the concentration of lignocaine from the EMLA peaked at the five minute interval

and reduced to 0.14 µgm/mL at thirty minutes. Lignocaine from Xylocaine had a low five minute concentration but was still rising at the thirty minute interval (0.35 µgm/mL). A conclusion from this study was that EMLA should have maximum analgesic efficacy within five minutes because peak concentration of lignocaine was measured at the five minute interval. However, the authors reported that maximum analgesic efficacy was found at thirteen minutes. The disparity between the time of peak concentration and maximum analgesic efficacy could be explained by the degree of standard errors in the results, although this was not suggested by the investigators.

2.5.2 Dermal

1. Assessment of the pain experienced from needles and cannulas

The majority of the published reports of investigations of EMLA have been the evaluation of the material in reducing the pain during intravenous cannulation through the skin. Investigators have used large numbers of patients utilising different pain scoring methods over a range of application times. Ehrenström Reiz and Reiz (1982) carried out a study involving sixty children with an age range of six to fifteen years. They examined the pain experience from venous cannulation comparing EMLA with placebo. They found that EMLA was significant ($p<0.001$) in reducing pain compared with placebo. A follow-up study by Ehrenström Reiz, Reiz and Stockman (1983) investigated the minimum application time for EMLA to be effective when applied to the skin. The study was a double-blind trial in which fifty three

female and sixty six male patients were subjected to intravenous cannulation. Results showed that the minimum effective time to obtain dermal anaesthesia was forty five minutes in adults.

Hállen and Uppfeldt (1982) carried out a study to evaluate EMLA with a number of objectives. They investigated the value of EMLA for diminishing pain from an injection of premedication and during insertion of intravenous catheters. The investigators also looked for any adverse reactions of EMLA from application to the skin. For patients receiving premedication, fifty eight subjects had 1 mL of cream applied one hour prior to needle insertion in a thick layer over the puncture site on the thigh and held in place by an occlusive dressing. Another group of fifty three patients who were to undergo cannulation prior to surgery had 1 mL of cream occluded on the dorsum of the hand, cubital fossa or forearm for at least one hour. When the dermis was examined for adverse reactions, the investigators found "paleness" occurred frequently in both active and placebo groups. "Redness" was observed under the occlusive tape but not where the cream had been applied. All reactions were transient and not detected an hour after the procedure.

Hállen, Carlsson and Uppfeldt (1985) examined the efficacy of EMLA in reducing the pain experienced during venepuncture. The study was a random, double-blind, cross-over trial using thirty one adult subjects. Pain was registered on a ten centimetre visual analogue scale. Twenty eight subjects indicated lower pain scores for EMLA when compared with placebo.

The remaining three subjects had similar scores for EMLA and placebo for pain experience. The authors also reported transient skin reactions (blanching, erythema and oedema) from both EMLA and placebo. However, these reactions were not found to be intensified by repeated applications.

Dohlwitz and Uppfeldt (1985) carried out a study on children to evaluate the degree of pain relief from venepuncture with shortened application times of EMLA due to urgent administration of a general anaesthetic agent. Application times of at least twenty minutes were used. Pain scores from the application of EMLA were significantly lower when compared with placebo treatment ($p<0.001$) irrespective of the application time. Local adverse reactions included transient colour changes, which the authors called "paleness" or "redness" of the skin. They reported these colour changes as not constituting any clinical problem.

Similar findings for the efficacy of EMLA in reducing the pain experienced from needle cannulation were reported by several other investigators:- Möller (1985); Kurien, Kollberg and Uppfeldt (1985); Maunuksela and Korpela (1986); Manner et al. (1987); Cooper et al.(1987); Hopkins, Buckley and Bush (1988); Halperin et al.(1989); Hellgren et al.(1989); Joyce and Skjonsby (1990).

Evers et al.(1985) assessed the efficacy of EMLA 2.5% and EMLA 5%. Their results showed that pain produced by the insertion of an intravenous

cannula was successfully abolished in the cubital area when the agents were left in place for sixty minutes. Temporary blanching of the skin areas was frequently observed on removal of the occlusive tape bandages but was not prolonged. Repeated application of EMLA 5% did not produce local skin reactions. Tests for delayed hypersensitivity reactions were negative.

The skin reactions from these studies suggest that EMLA may cause local irritation or sensitivity. However, Hállen, Olsson and Uppfeldt (1984) and Hállen et al.(1985) showed that placebo held in place by an occlusive dressing produced similar skin reactions. Therefore, it appears more likely that the occlusive dressing is responsible for dermal reactions, especially if firmly adhered to the skin.

Two studies have been carried out on patients requiring venous cannulation for haemodialysis. Watson, Szymkiw and Morgan (1988) compared EMLA with lignocaine injection and placebo for venous cannulation prior to haemodialysis. Visual analogue and verbal rating scales from twenty six patients showed EMLA to be highly effective ($p<0.001$). EMLA was also shown to provide more pain relief and improve the ease of venepuncture compared with lignocaine injections, with patients expressing a strong preference for EMLA. Wehle et al.(1989) investigated thirty one patients requiring treatment over a period of twelve to eighteen months. Seventeen out of thirty-one patients completed the study in a double-blind cross-over design. EMLA provided considerable pain relief and was

significantly better than placebo for all except one patient. There was a low incidence of local skin reactions which did not correlate to the number of EMLA applications.

2. Harvesting of skin grafts

Ohlsén , Englesson and Evers (1985) evaluated the efficacy of EMLA for the harvesting of split-skin grafts. The cream was applied to the donor sites of one hundred and forty six patients with a minimum application time of ninety minutes. One hundred and twenty three patients (84.3 per cent) reported adequate analgesia when the dermatome was used. Operations were completed in another twenty patients (13.7 per cent) without supplementary local or general anaesthesia but subjects did indicate a moderate level of pain. Three patients (2.0 per cent) required additional anaesthesia. Transient irritation following application was claimed by six patients. Local dermal reactions to the cream included erythema in forty two patients, pallor in sixty two patients and oedema in fourteen patients. Blood levels for lignocaine and prilocaine were monitored in one hundred and six patients with the highest level for any patient being 1,100 ng/mL for lignocaine, and 200 ng/mL for prilocaine. No systemic side effects were observed.

Two other studies have been carried out that have investigated EMLA for the harvesting of skin grafts. Lähteenmäki et al.(1988) compared the efficacy of a single application with two successive applications of EMLA for

skin grafts. Either 30 g or 60 g (2×30 g doses) were applied to the donor site two to five hours before operation. No difference in pain experience was established between the two application methods. In each group, 92 per cent of subjects rated the pain from harvesting as either none or slight, and 8 per cent rated it as either moderate or severe. Goodacre et al.(1988) compared EMLA to infiltration anaesthesia for harvesting grafts. Pain experience was measured by visual analogue scales, first for pain on application and second for pain on harvesting. EMLA produced no discomfort on application, while infiltration anaesthesia produced varying degrees of pain. EMLA and infiltration anaesthesia were found to be equally effective in reducing the pain. The authors' final comment was that EMLA should be considered the treatment of choice for the harvesting of skin grafts.

3. Curettage and removal of skin lesions

Hállen, Ljunghall and Wallin (1987) examined the efficacy of EMLA for the removal of genital warts by electrocautery. Fifty seven male patients and fifty one female patients were tested. Each lesion had 1 mL of cream applied under plastic film for twenty to one hundred and five minutes prior to surgery. Analgesia was sufficient in 96 per cent of males and 40 per cent of females. Supplementary local injection was carried out on 60 per cent of the females, but the investigators reported the additional injection(s) to be not as painful as without topical analgesia. Dermal effects observed in patients included local pallor (30 per cent), "redness" (53 per cent) and oedema (15 per

cent).

Ljunghall and Lillieborg (1989) applied EMLA prior to cautery of condylomata acuminata on the vulval mucosa. The agent was applied to the lesions for ten, fifteen or twenty minutes. Cautery of these genital warts was successful in 92 per cent of patients who reported experiencing either no pain or only slight pain. Rylander et al.(1990) performed a similar study on eighty females with condylomata of the genital mucosa. Results of the study showed that significantly less pain was experienced by patients with EMLA. The most effective degree of anaesthesia was achieved after an application time of five to fifteen minutes. A previous study by Wagner and Mensing (1989) found EMLA to be totally effective in patients treated for removal of mollusca contagiosa and condylomata acuminata and for biopsies of the glans penis, but for the removal of verrucae plantaris, anaesthesia was incomplete but acceptable.

Rosdahl et al.(1988) used EMLA in children for the curettage of molluscum contagiosum on all parts of the body and found that from a one hour application time 93 per cent of the children indicated no pain to slight pain for the procedure on a ten centimetre visual analogue scale (median score 3 mm).

2.5.3 Plasma concentrations

There have been only five studies carried out to measure the plasma concentrations of lignocaine and prilocaine from the application of EMLA. Haasio et al.(1990) measured plasma concentrations of the drugs when applied to the oral mucosa as previously discussed in chapter 2.5.1. The other four studies measured levels of lignocaine and prilocaine when EMLA was applied to the skin. Engberg et al. (1987) measured the plasma concentrations of lignocaine, prilocaine and methaemoglobin (met-Hb) after the application of EMLA to the skin of infants. Twenty two infants aged three to twelve months had 2 mL of EMLA placed for four hours over a 16 cm^2 area of dermis. Blood was taken prior to application and at two, four and eight hours after application. Their findings showed that plasma concentrations of lignocaine and prilocaine were below toxic levels in all infants and only minor increases were measured of met-Hb in a few subjects.

Nilsson et al.(1990) measured met-Hb levels in infants under three months from EMLA. Two grams of agent was applied for four hours over 16 cm^2 and blood samples taken at four, eight and twelve hours after application. Maximum values were obtained at eight hours and were significantly higher ($p<0.001$) than levels before the application. Plasma concentrations of the anaesthetic agents were low, the highest value for prilocaine being 78 ng/mL and for lignocaine 412 ng/mL. The authors concluded that although met-Hb levels were low, the enzyme capacity of

erythrocyte met-Hb reductase could be overloaded when EMLA is administered at the same time as other met-Hb inducing agents, and the use of EMLA should be restricted in that age group.

Enander Malmros, Nilsen and Lillieborg (1990), measured the plasma concentrations of lignocaine and prilocaine in eight subjects requiring cleansing of leg ulcers. EMLA cream 2% (8-10 g) was applied for sixty minutes to ulcers measuring 31-80 cm^2. Their findings showed that the maximum individual plasma concentrations were 205 ng/mL for lignocaine and 79 ng/mL for prilocaine. They reported that these levels were twenty times lower than those associated with toxicity.

Haugstvedt, Friman and Danielson (1990), in a comparative study, measured lignocaine and prilocaine levels when EMLA was applied to the skin for the removal of mollusca (skin lesions) in children. Twenty children, with an age range of two to eight years, had 10-16 g of EMLA occluded over an area of 100-160 cm^2 with an application time of two hours. Blood samples were taken prior to application and at two, three, four and five hours. The investigators found that the highest concentration of lignocaine and prilocaine for any subject was 315 ng/mL for lignocaine and 215 ng/mL for prilocaine.

The above studies show that plasma concentrations for lignocaine and prilocaine were well below known toxic levels as suggested by Malamed (1986) when large quantities of EMLA was used. The applications extended

over large areas of skin and in some studies for many hours. It is clear that EMLA when used for dermal purposes, does not cause toxic effects in adults and children. However, care is necessary when using large quantities of EMLA in infants due to the potential risk of high levels of met-Hb being formed.

2.5.4 Other studies

Several studies relating to otological use of EMLA have been published. Whittet, Williams and Wright (1988) compared EMLA with cocaine 5 per cent in controlling pain arising from myringotomy and found EMLA to be significantly better than cocaine. Timms, O'Malley and Keith (1988) conducted a double-blind trial of EMLA in fifteen patients undergoing electrocochleography. Their results showed EMLA to be both effective and safe. Roberts and Carlin (1989) compared EMLA with prilocaine injection to provide anaesthesia of the tympanic membrane of adults. Their findings showed that EMLA and prilocaine injection were equally effective in anaesthetising the eardrum. Anniko and Schmidt (1988) conducted a pilot study where EMLA was applied on the organ of Corti of the guinea pig . They found morphological damage was evident at the site of application and that EMLA had ototoxic potential. In a later study, Anniko et al.(1989) studied the effects of a number of compounds when applied to the inner ear of rats. The authors demonstrated that EMLA, when applied to the site, caused both morphological and functional change. On the morphological level, under the

light microscope, there was a graded morphological damage to the organ of Corti. Functional alterations were observed by changes in auditory recordings.

Pipkorn and Andersson (1987) examined what effect EMLA had on the allergic response in a skin prick test. A random double-blind study recruiting twenty one subjects who suffered from seasonal allergic rhinitis was undertaken. A one hour application time of EMLA was followed by a preloaded injection of a standardised test needle containing pollen allergen, and observation of the weal and flare reaction. The investigators found that topical dermal anaesthesia reduced the reaction of the flare response to histamine by 49 per cent ($p<0.01$) and to allergen by 21 per cent ($p<0.05$). No reduction of the histamine and allergen induced weal response was observed. The authors concluded that flare response is partly mediated by neural reflex activity, being ameliorated by EMLA. Shuttleworth et al.(1988) evaluated the efficacy of EMLA in relieving artificially induced pruritus in twenty healthy volunteers. All subjects showed a marked reduction in sensitivity to histamine after the treatment with EMLA. The agent was also found to be effective in alleviating pruritus induced by cowage and papain. The authors suggested that EMLA could be useful in some conditions where persistent itch is a distressing symptom.

Arendt-Nielsen and Bjerring (1988) studied three parameters of pain using EMLA and a local anaesthetic injection of lignocaine. The pain response was produced by laser stimulation to the skin. Lignocaine produced total

sensory block almost immediately after injection. When EMLA was applied for fifteen minutes both pain and sensory thresholds increased. Total sensory block was reached twenty minutes after the application of EMLA and lasted for eighty minutes. In a follow-up study by Bjerring, Andersen and Arendt-Nielsen (1989), investigations were carried out to measure the vascular responses after cutaneous application of EMLA by skin reflectance spectroscopy and laser Doppler blood flowmetry. In healthy subjects, EMLA produced a biphasic vascular response with initial vasoconstriction after a ninety minute application time. After three hours of application, vasodilation occurred and the authors suggested that this was most likely due to the effect of smooth muscle relaxation from the analgesics. Support for this finding was shown in a study by Harper, Beck and Spence (1989) where EMLA was applied to skin and the histamine flare was measured by Doppler velocimetry. After an injection of histamine into tissue treated with EMLA, subjects showed no difference in hyperaemic responses at the injection site and at one centimetre but a marked reduction was found at two centimetres ($p<0.05$). At all three sites the decay of the histamine-induced hyperaemia was faster following EMLA treatment than with the placebo ($p<0.05$). The experiments showed that the indirect effect of histamine on the cutaneous microvasculature in the peripheral flare around the injection site was greatly diminished by the prior application of EMLA.

Honnens de Lichtenberg et al.(1989) compared EMLA with the infiltration of carbocaine (1 per cent) in thirteen males undergoing

vasectomies. Twelve patients indicated their preference for infiltration anaesthesia. EMLA was only effective on the skin, and had to be supplemented by a local anaesthetic agent when the incision reached subcutaneous tissue. However, el-Kholy (1989) administered a combination of EMLA and cocaine in twelve patients to successfully manipulate the fractured nasal bones without discomfort.

Stow, Glynn and Minor (1989) evaluated EMLA for the treatment of post herpetic neuralgia. Five female and six male patients, with an age range of fifty to eighty five years, had EMLA applied for twenty four hours. Pain intensity was significantly reduced six hours after the commencement of application. In a subgroup of patients with facial post herpetic neuralgia, there were significant improvements in pain intensity from six to ten hours after application.

Lowrie, Jones and Eastley (1989) studied the effects of EMLA on tourniquet pain on ten male patients. Tourniquet inflation time was tolerated significantly longer with EMLA when compared with placebo. Pain scores increased in both groups but was significantly less at forty minutes with EMLA.

In reviewing the use of EMLA, a number of conclusions can be drawn from the studies carried out. First, EMLA is very effective for dermal applications such as the harvesting of split-skin grafts and for the reduction

of the pain experienced during venous cannulation. However, the depth of analgesia from the application of EMLA appears to be limited to the dermis. When underlying connective tissue has been contacted by instruments, the success rate for pain-free procedures is reduced. Second, EMLA when applied for dermal use, adheres well to the skin and can be occluded easily. More recent animal studies of EMLA suggest the preparation may be effective in reducing the inflammation process and this area deserves further investigation in human clinical trials.

The majority of studies carried out have investigated the clinical applications of EMLA when applied to the skin. However, EMLA may well have more potential when applied to the oral mucosa because of the increased rate in drug absorption of the oral mucosa compared to the skin and the physical property of EMLA to be an oil at mouth temperature would further facilitate the absorption of the drug.

CHAPTER 3

EXPERIMENTAL STUDIES

3.1 " THE EFFICACY OF THREE TOPICAL ANAESTHETIC AGENTS COMPARED WITH PLACEBO "

3.1.1 Introduction

Topical anaesthetic agents have been used in dentistry for a number of years, with a view mainly to reduce the pain experienced during administration of local anaesthetic injections. Other indications for the use of topical anaesthetic agents include drainage of intra-oral abscesses and the removal of loose deciduous teeth. Such applications in dentistry have led to a decrease in the level of pain experienced by patients resulting in better acceptance of dental procedures.

Topical anaesthetic agents which have been commonly used in recent years include Xylocaine 5% Ointment (lignocaine 5 per cent) and NUM (benzocaine 15 per cent, amethocaine 1.7 per cent).

There has been only one previous study (Holst et al., 1985) which

evaluated the efficacy of EMLA in the oral cavity for the reduction in pain experience from needle insertion. As no information on the relative efficacy of topical anaesthetic agents which are currently approved for use in Australia is available, it was felt that a study to assess their efficacy should be carried out. Considering only few clinical studies have evaluated the efficacy of EMLA it was decided to include EMLA in this investigation with two other commonly used topical anaesthetic agents for comparison with placebo for reducing the pain experience from dental needle insertion in the oral mucosa.

3.1.2 Aims

The aims of this study were to evaluate the efficacy of EMLA cream 5%, NUM and Xylocaine 5% Ointment compared with placebo for reduction in pain experience from needle insertion in the oral mucosa and also to examine for any local effects to the oral mucosa from the application of EMLA.

3.1.3 Patients and Methods

Materials used in this study were EMLA cream 5%, Xylocaine 5% and NUM. The investigation was a randomised, double-blind study. A sample of sixty subjects, ages ranging from twenty to thirty years, was divided into three groups each comprising twenty volunteers. Subjects with a history of

known allergy to local anaesthetic agents and those with any evidence of inflammation or pathosis of the oral mucosa were excluded. Approval for this study was granted by the Ethical Review Committee (University of Sydney).

The buccal sulcus adjacent to the upper first premolar, on both sides was selected as the site of application and the side for topical anaesthetic or placebo testing was randomly chosen. 0.2 mL of the test sample was dispensed by a 1 mL plastic syringe onto a cotton applicator stick by an assistant. The cheek was retracted and the test site was wiped free of excess saliva with cotton gauze. The applicator stick was applied for two minutes (Illustration 3) and the cheek allowed to return to its normal position. The test sample was then removed with fresh gauze by the assistant. The investigator then inserted a standard 27 gauge infiltration-type (Monoject)®[2] needle (Illustration 2) through the mucosa to a depth of 5 mm with an endodontic stopper on the needle shank to standardise the depth (Illustration 4). No local anaesthetic was injected and a new needle was used for each test site. Pain experience was assessed by two methods (Appendix A1):-

1) Analogue scale

Each subject indicated the degree of pain experience on a plain, horizontal 100 mm visual analogue pain scale with "no pain" at 0mm and "severe pain" at 100mm.

2) Descriptive table

Subjects were asked to indicate the appropriate response from one of three

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[2] Sherwood Medical, St Loius, U.S.A.

categories, namely, i) total anaesthesia (no pain), ii) partial anaesthesia (mild to moderate pain), or iii) no anaesthesia (severe pain).

Pain experience was recorded by the subject after the withdrawal of the needle. The paired test sample was applied after a period of ten minutes on the contra-lateral side and pain assessed in the same manner.

Subjects also completed a questionnaire (Appendix A2) in which they were asked to list any adverse effects that occurred during the following twenty four hours, and to give comments regarding the taste of the test agent.

Statistical analysis was performed using Mann-Whitney U test for the horizontal analogue scores, and Chi-squared test for the descriptive table response. The results were considered significant at $p<0.05$ for both pain response methods.

3.1.4 Results

The male/female ratio was comparable between the groups (Fig. 3).

Table 1 shows the visual analogue pain scores for each group. All three topical anaesthetic agents were significantly better than placebo in reducing the pain experience ($p<0.05$). Group 1 compared EMLA cream 5% (Fig. 4a) with placebo (Fig. 4b). EMLA 5% produced a range of scores from 0 to 26

with placebo (Fig. 4b). EMLA 5% produced a range of scores from 0 to 26 and fourteen subjects indicated "no pain" experience. Placebo had a range from 0 to 48 with three subjects indicating no pain. Statistical analysis showed a significant reduction in pain intensity with EMLA 5% compared with placebo ($p = 0.002$). Group 2 compared Xylocaine 5% (Fig. 5a) with placebo (Fig. 5b). Xylocaine 5% produced the lowest range of scores (0-12) for any of the agents. The paired placebo had a wide range of pain scores (0-70). For Xylocaine 5%, ten subjects, and for placebo, six subjects reported no pain experience. Xylocaine 5% was found to be significantly better when compared with placebo ($p = 0.047$) in the reduction of pain intensity experienced. Group 3 compared NUM (Fig. 6a) with placebo (Fig. 6b). Both NUM (0-49) and placebo (1-51) produced a wide range of scores. Three subjects reported no pain following the application of NUM, while none of the subjects receiving placebo reported no pain. The level of pain reduction was significant ($p = 0.005$) compared with placebo.

Results for the descriptive table responses are shown in Tables 2,3 and 4. Statistical analysis showed EMLA and Xylocaine to be significant in reducing pain compared with placebo ($p<0.05$), however, NUM was not significant ($p>0.05$). In Group 1 (Table 2) eighteen subjects recorded total anaesthesia (no pain) for EMLA and the remaining two subjects indicated partial anaesthesia. For the paired placebo response twelve subjects indicated no pain and eight subjects indicated mild to moderate pain. Statistical analysis with the Chi-squared test gave a result of $p = 0.028$. In Group 2

(Xylocaine-placebo) seventeen subjects reported no pain and three subjects reported mild to moderate pain when Xylocaine was used. The paired placebo response had ten subjects with no pain and ten subjects with mild to moderate pain. Statistical analysis gave a value of $p = 0.018$. Group 3 compared NUM to placebo. Ten subjects indicated no pain and ten subjects indicated mild to moderate pain for NUM. The placebo pairing had only five subjects reporting no pain from needle insertion and fifteen subjects with mild-moderate pain. Statistical analysis gave a value of $p = 0.102$.

3.1.5 Discussion

EMLA and Xylocaine were shown to be effective in reducing the pain experience in both pain-scoring methods. Analysis of the horizontal analogue scales showed all three topical anaesthetic preparations to produce a significant reduction in pain during needle insertion when compared with placebo, but in terms of efficacy, EMLA was the superior agent. The average pain scores between the groups showed EMLA (mean = 4) and Xylocaine (mean = 3) to be lower when compared with NUM (mean = 10). EMLA appeared to be very effective in producing profound anaesthesia of the mucosa to the depth of the needle penetration (5 mm) in a majority of subjects.

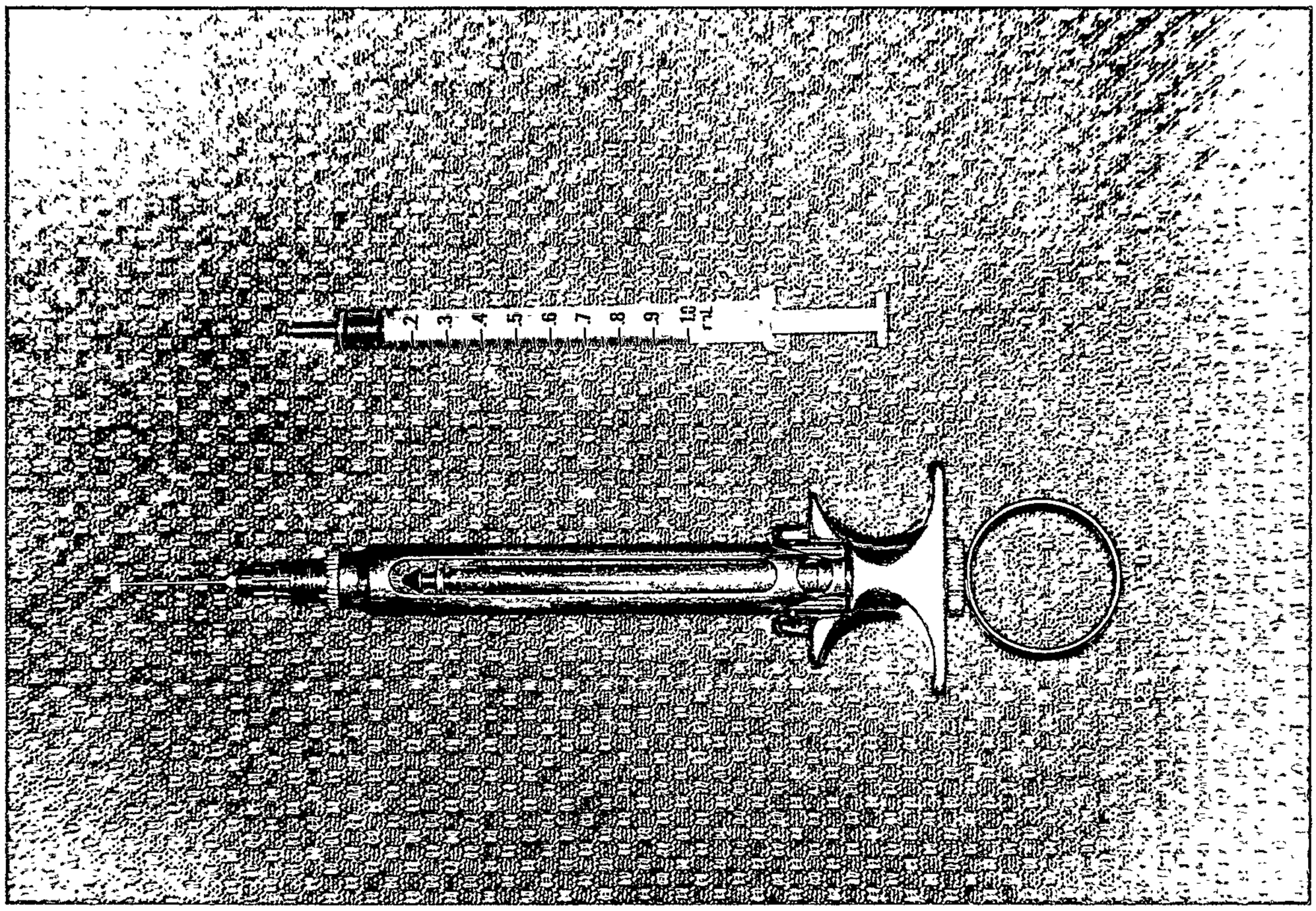
During the study, the subjects found the visual analogue scales simple to complete. However, some subjects when indicating the response in the descriptive table, had difficulty in choosing the response between no pain and

mild to moderate pain. They found it hard to differentiate the pressure sensation from needle insertion, from actual pain experience. Error was quite possible because some subjects indicated mild to moderate pain and yet later stated that no pain was felt.

Most subjects tested with EMLA found the material to have a bland taste and it was generally well accepted. EMLA was easily applied and adhered well to the mucosa after the removal of saliva. However, EMLA, with its creamy consistency, spread to the surrounding area when not carefully isolated. No adverse local effects were observed from two minute applications of EMLA cream 5%.

Illustration 2.

Equipment, Experiment 3.1



1
2

1. 1 mL syringe for standard application of 0.2 mL of agent
2. 27 gauge dental needle fitted with endodontic rubber stop

Illustration 3. 0.2 mL of test agent applied to buccal sulcus

Illustration 4. Insertion of 27 gauge needle to 5 mm.

Fig. 3 Male / female ratio, Experiment 3.1

Fig. 4a. EMLA 5% pain response

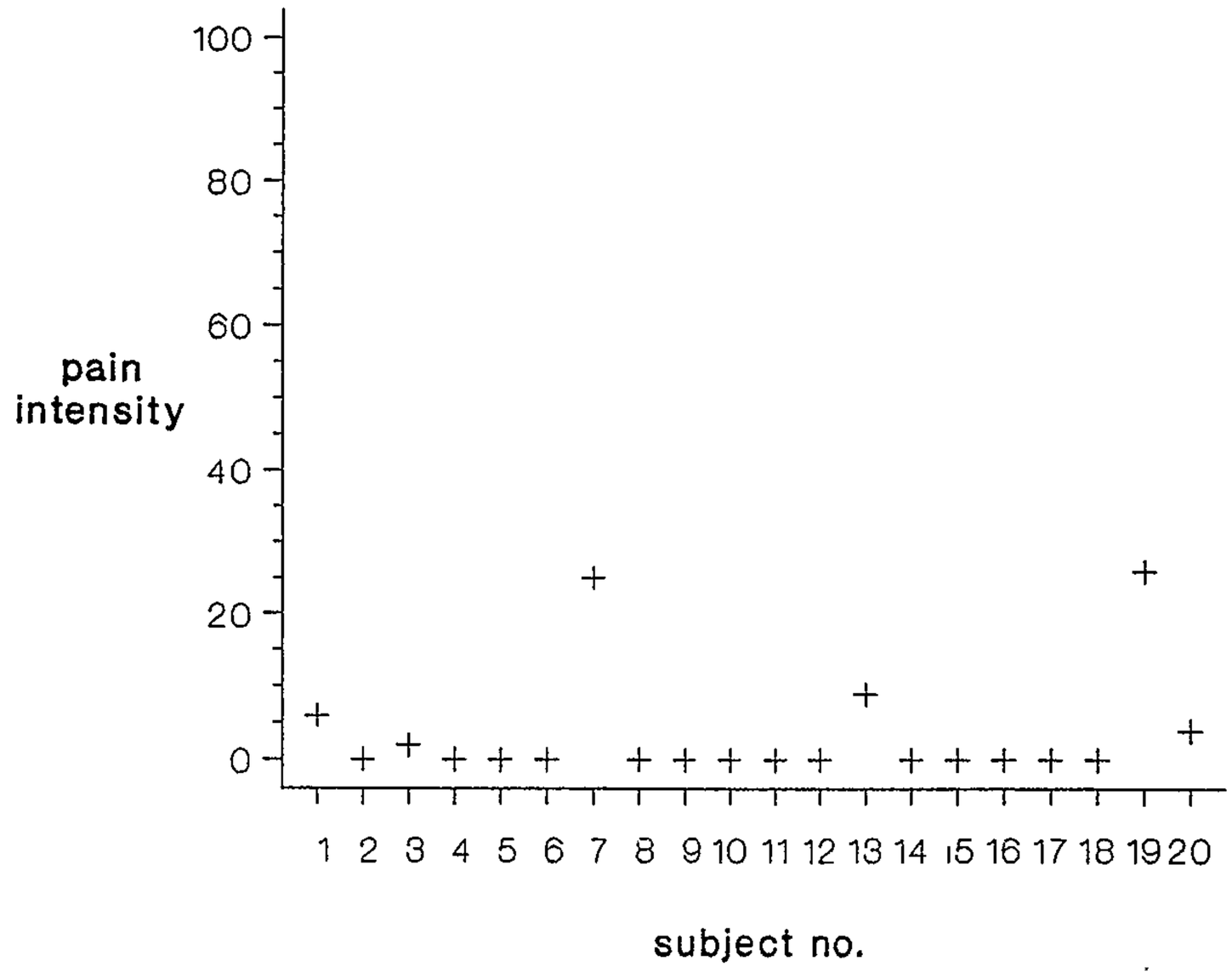


Fig. 4b. Placebo pain response

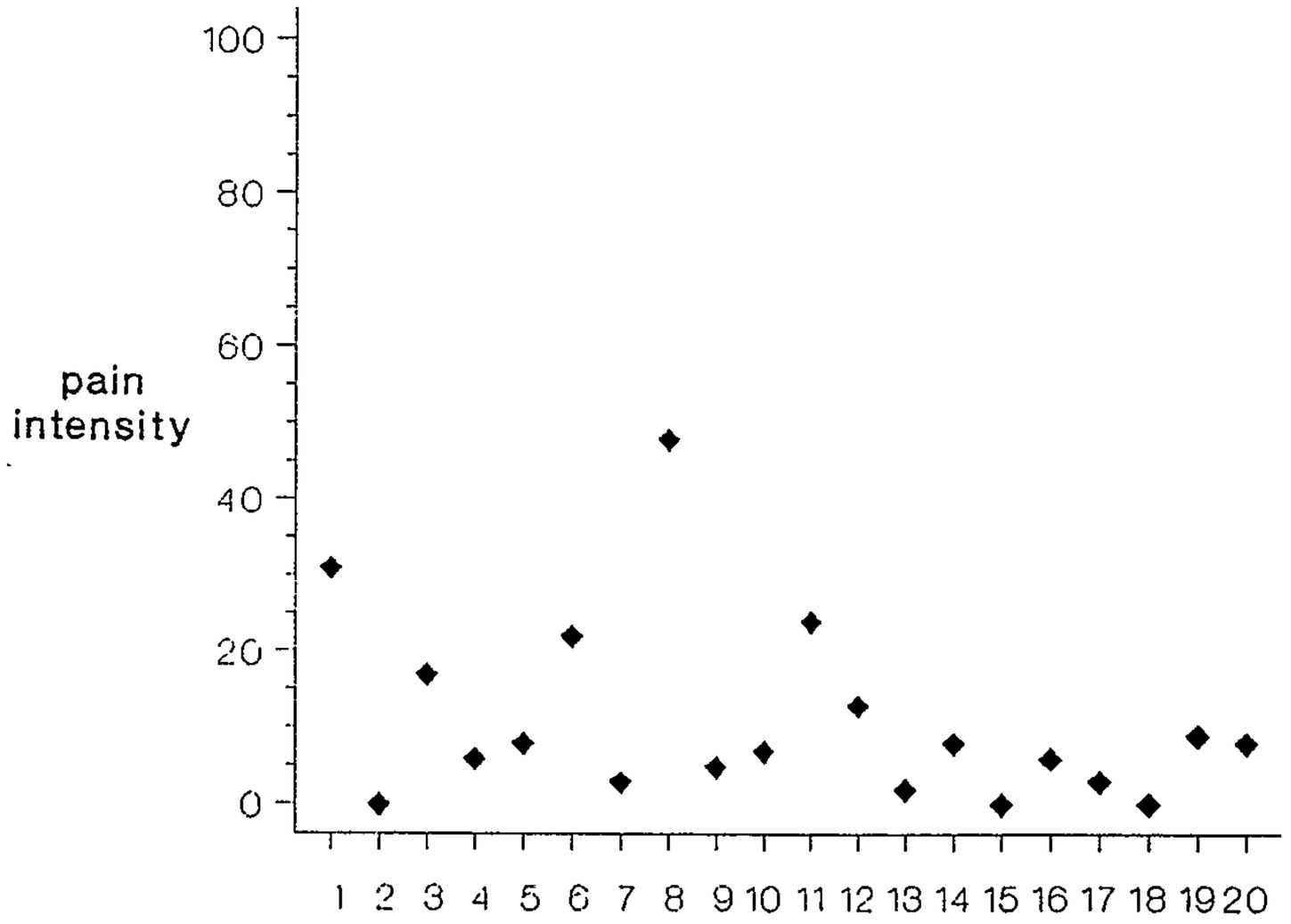


Fig. 5a. Xylocaine 5% pain response

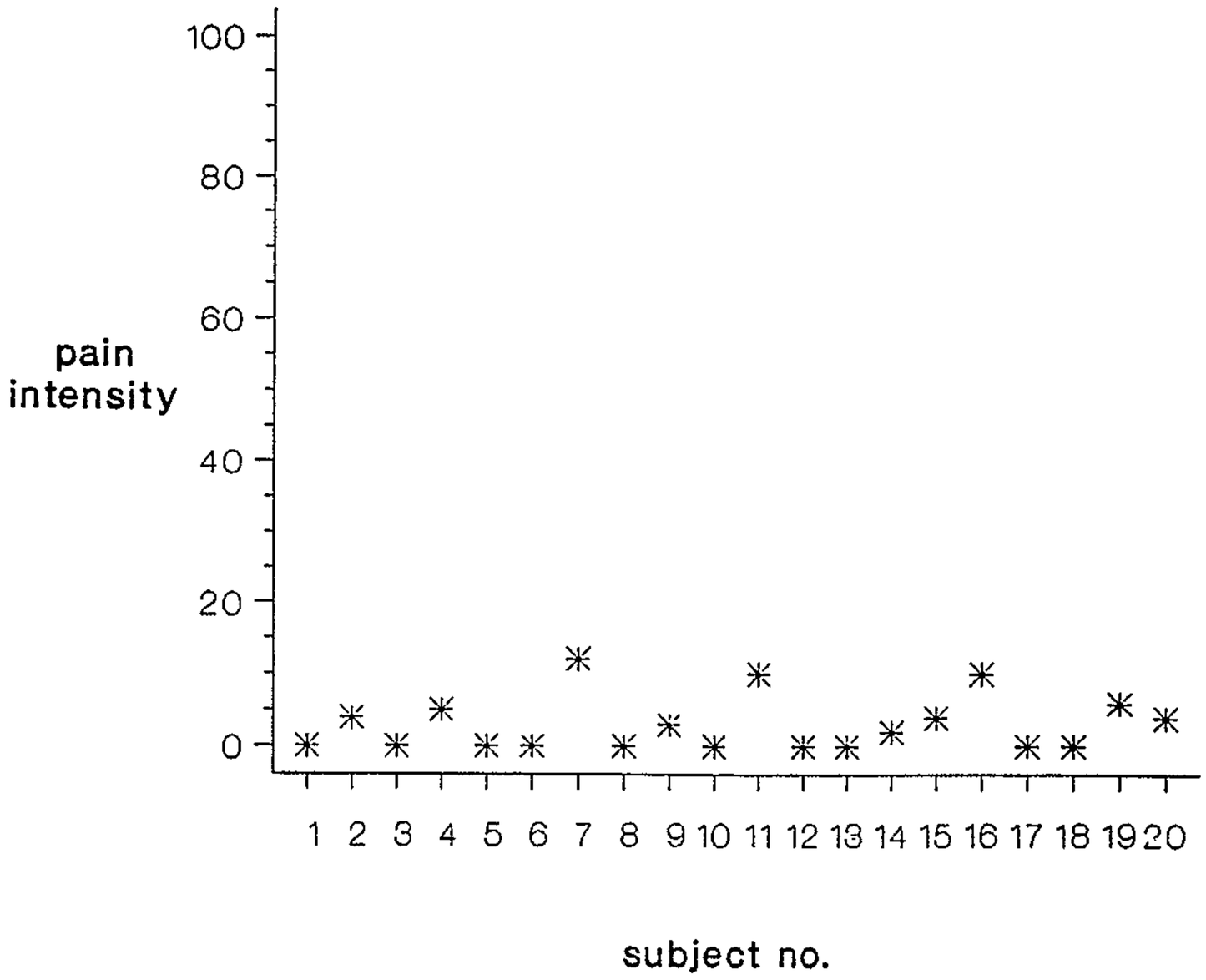


Fig. 5b. Placebo pain response

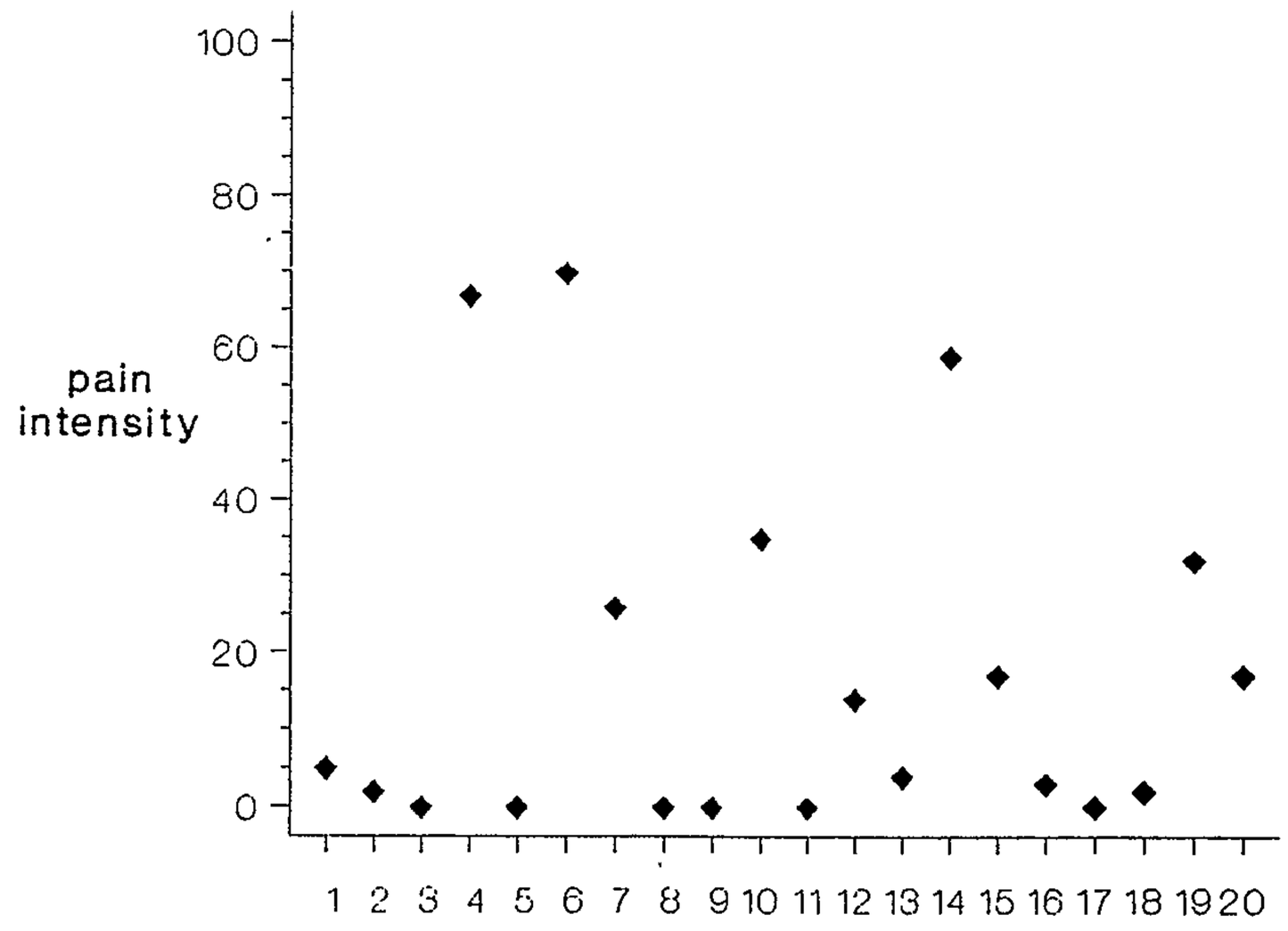


Fig. 6a. NUM pain response

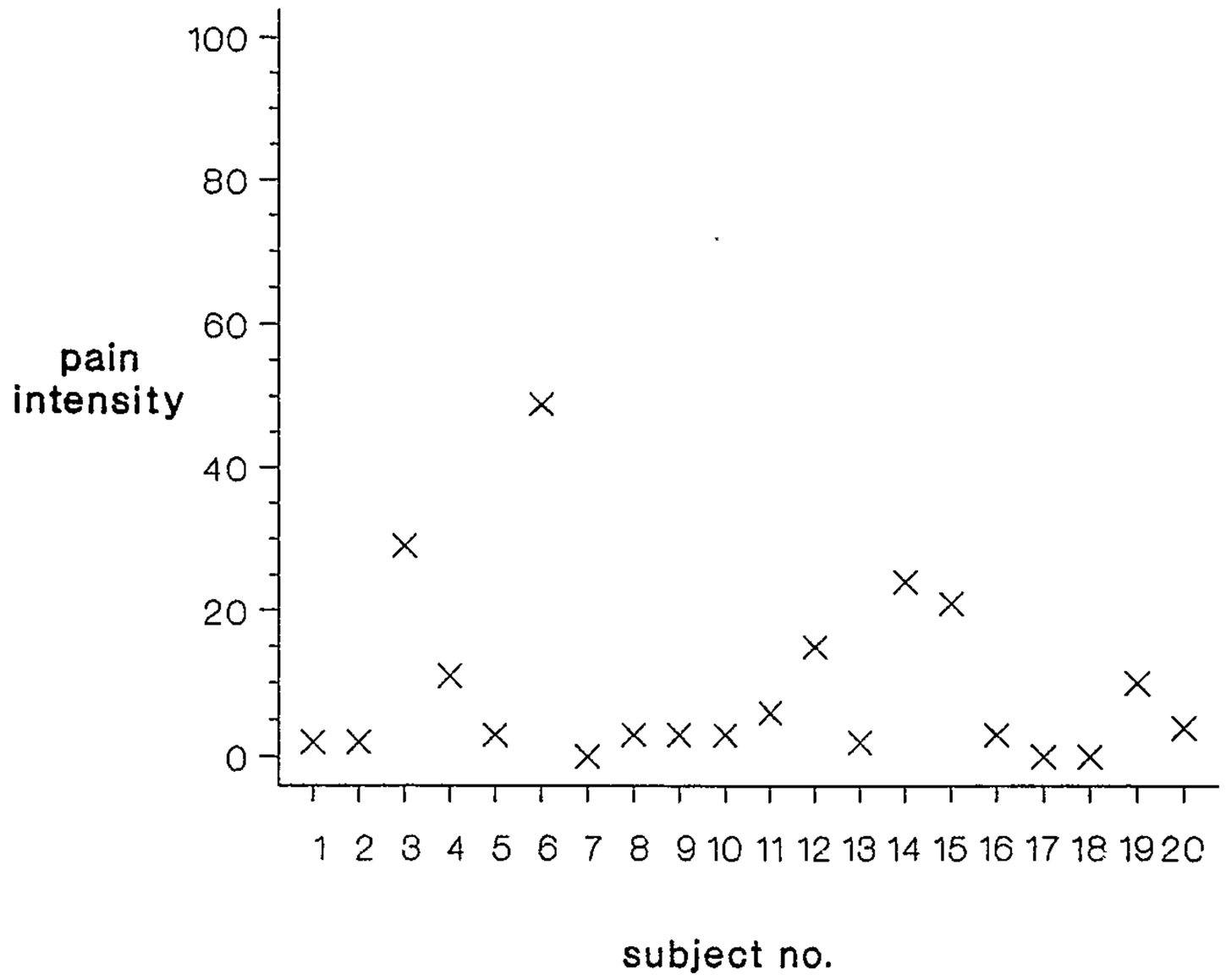


Fig. 6b. Placebo pain reponse

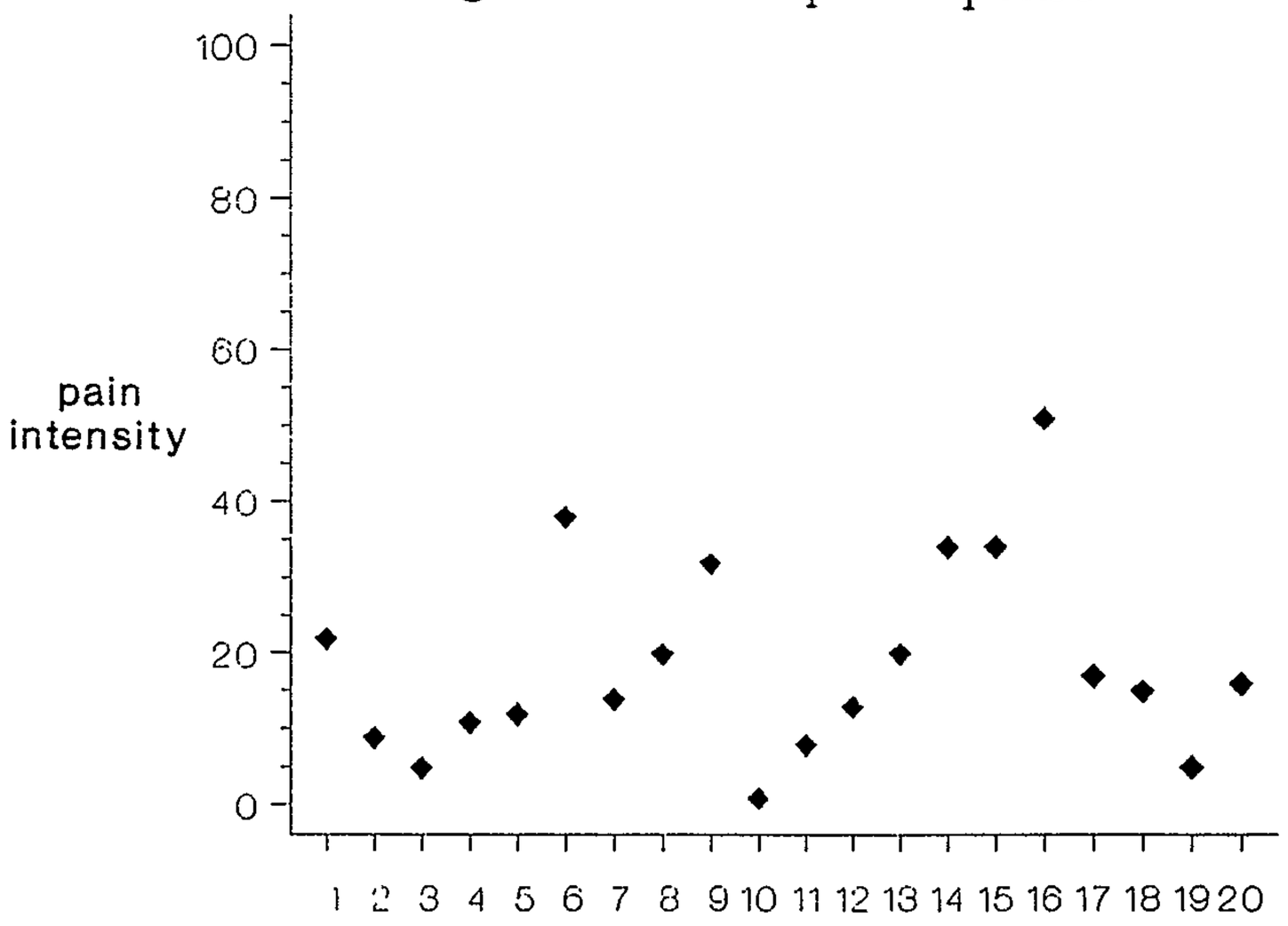


Table 1

Visual analogue scores, Experiment 3.1.

	Group 1		Group 2		Group 3	
subject	EMLA	placebo	Xylocaine	placebo	NUM	placebo
1	6	31	0	5	2	22
2	0	0	4	2	2	9
3	2	17	0	0	29	5
4	0	6	5	67	11	11
5	0	8	0	0	3	12
6	0	22	0	70	49	38
7	25	3	12	26	0	14
8	0	48	0	0	3	20
9	0	5	3	0	3	32
10	0	7	0	35	3	1
11	0	24	10	0	6	8
12	0	13	0	14	15	13
13	9	2	0	4	2	20
14	0	8	2	59	24	34
15	0	0	4	17	21	34
16	0	6	10	3	3	51
17	0	3	0	0	0	17
18	0	0	0	2	0	15
19	26	9	6	32	10	5
20	4	8	4	17	4	16
Mean	4	11	3	18	10	19
S.D.	7.88	12.15	3.88	23.39	12.58	12.89

Table 2

Descriptive table response, Group 1, Experiment 3.1.

EMLA cream 5% versus placebo

Subject	Sex	No pain	Mild to moderate pain	Severe pain
1	male		●□	
2	female	●□		
3	male	●	□	
4	female	●	□	
5	female	●□		
6	male	●	□	
7	male	□	●	
8	male	●	□	
9	female	●□		
10	male	●	□	
11	male	●	□	
12	male	●	□	
13	male	●□		
14	male	●□		
15	female	●□		
16	female	●□		
17	male	●□		
18	female	●□		
19	male	●□		
20	male	●□		

EMLA cream 5% (●)

placebo (□)

Table 3

Descriptive table response, Group 2, Experiment 3.1.

Xylocaine 5% versus placebo

Subject	Sex	No pain	Mild to moderate pain	Severe pain
1	female	●	□	
2	female	●□		
3	female	●□		
4	male		●□	
5	female	●	□	
6	male	●	□	
7	female		●□	
8	male	●□		
9	female	●	□	
10	female	●□		
11	male	□	●	
12	male	●	□	
13	male	●□		
14	male	●	□	
15	male	●	□	
16	male	●□		
17	male	●□		
18	male	●□		
19	male	●	□	
20	male	●□		

Xylocaine 5% ●

placebo □

Table 4

Descriptive table response, Group 3, Experiment 3.1.

NUM versus placebo

Subject	Sex	No pain	Mild to moderate pain	Severe pain
1	male	●	□	
2	male	●	□	
3	female	□	●	
4	female		●□	
5	male	●□		
6	male		●□	
7	female	●	□	
8	male		●□	
9	female		●□	
10	male	●□		
11	male	●□		
12	male		●□	
13	male	●	□	
14	male		●□	
15	male		●□	
16	male	●	□	
17	male	●	□	
18	female	●	□	
19	female	□	●	
20	male		●□	

NUM ●

placebo □

3.2 " A COMPARISON OF THE EFFICACY OF EMLA CREAM 5% TO XYLOCAINE 5% OINTMENT "

3.2.1 Introduction

Analysis of the results of experiment 3.1 showed EMLA and Xylocaine 5% to be the most effective of the topical anaesthetics. It was felt that a direct comparison of these two agents should be made to assess which of the two agents was more effective in reducing the pain experience under similar double-blind conditions.

3.2.2 Aims

The aim of this experiment was to compare the effectiveness of EMLA cream 5% with Xylocaine 5% Ointment in reducing the pain experience of dental needle insertion in the oral mucosa.

3.2.3 Patients and Methods

Twenty two subjects (fifteen males and seven females) were compared in a random, double-blind trial using identical methods as in study 3.1. Sites of application, quantities of agents and application times were identical to those in study 3.1. The pain scoring methods and statistical analyses employed were the same as in study 3.1. Approval for this study was granted

by the Ethical Review Committee (University of Sydney).

3.2.4 Results

Table 5 shows the pain scores for the group from the analogue scales. EMLA had the lower range of scores (0-12) compared to Xylocaine (0-48). However, more subjects recorded a 0 response to Xylocaine (13 subjects) compared with EMLA (11 subjects). EMLA had a lower average score for the group (mean = 2) compared with Xylocaine (mean = 6). Eight subjects had lower pain experience with EMLA, four subjects had lower pain experience with Xylocaine and the remaining eight subjects indicated identical pain experience with EMLA and Xylocaine. Statistical analysis gave a value of 0.89 ($p>0.05$) and therefore no statistical significance was established between EMLA and Xylocaine in reducing pain experience.

The descriptive table response had seventeen subjects indicating no pain from EMLA, with five subjects marking mild to moderate pain. Xylocaine had fifteen subjects reporting no pain and seven subjects with mild to moderate pain. Seven subjects had EMLA producing less pain than Xylocaine, five subjects indicated Xylocaine was more effective than EMLA and the remaining ten subjects indicated no pain for both EMLA and Xylocaine. Statistical analysis with the Chi squared test gave a value of 0.49 ($p>0.05$).

3.2.5 Discussion

Results from both pain scoring methods showed there was no statistically significant difference in effectiveness between EMLA and Xylocaine in achieving reduction in pain from needle insertion through the oral mucosa. The pain scoring methods were subject to a degree of error as in study 3.1. The author suggests that the horizontal analogue scales offers the most accurate way of measuring pain experience when comparing two different agents. The analogue scale offered the subject a greater range from which to choose (0-100) compared to the three category table response. This descriptive table lacked the range necessary to provide for small degrees of difference in pain experience. Subjects could only choose from one of three available responses :- total anaesthesia, partial anaesthesia or no anaesthesia. This was apparent in two subjects where they indicated total anaesthesia from the table response and yet visual analogue scores were different (6,0 and 1,3).

In conclusion, no statistically significant difference was found between EMLA cream 5% and Xylocaine 5% Ointment in achieving a reduction in pain experience from needle insertion after a two minute application on the oral mucosa.

Fig. 7a. EMLA 5% pain response

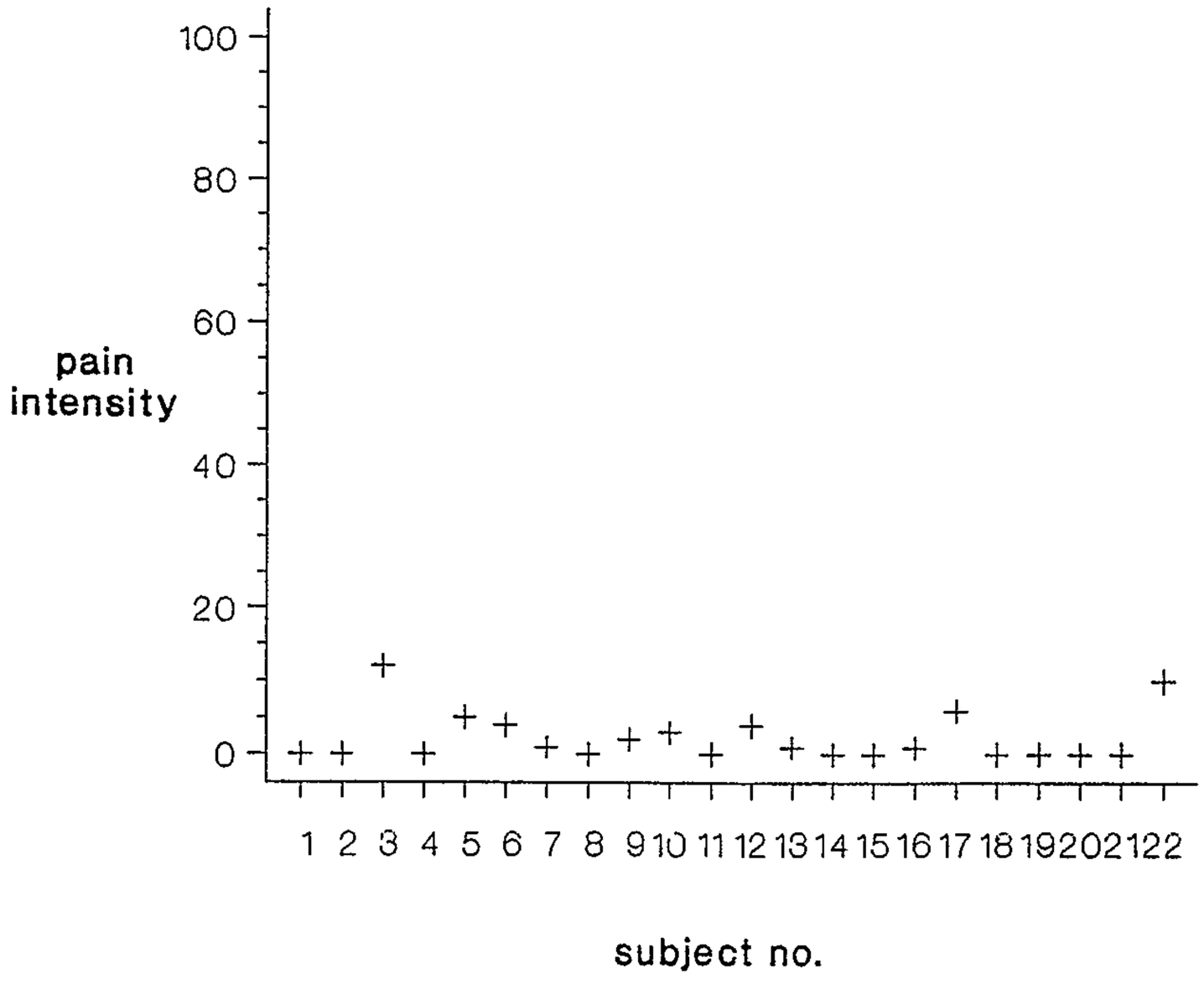


Fig. 7b. Xylocaine 5% pain response

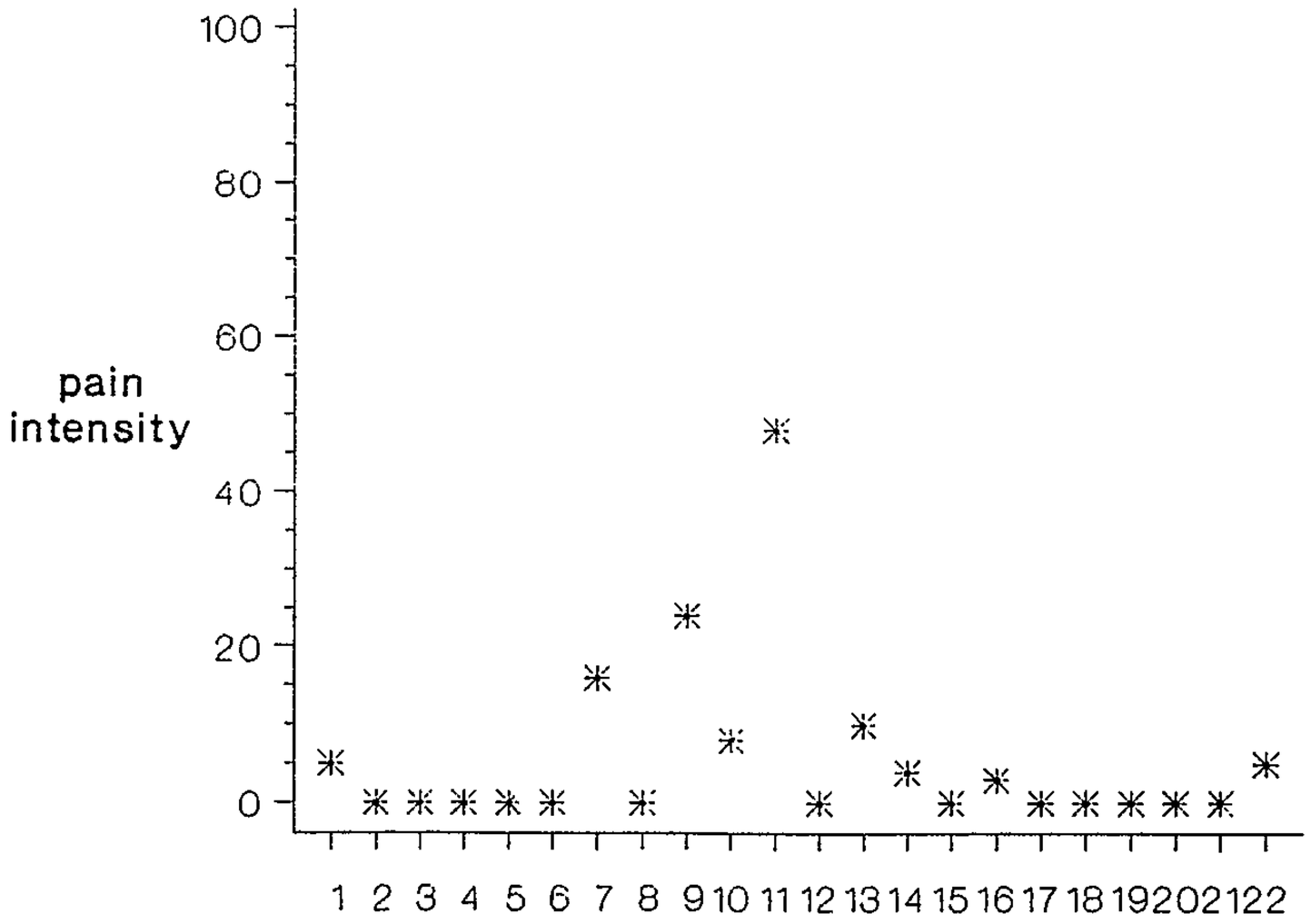


Table 5

Visual analogue scores, Experiment 3.2.

EMLA cream 5% versus Xylocaine 5%

subject	EMLA 5%	Xylocaine 5%
1	0	5
2	0	0
3	12	0
4	0	0
5	5	0
6	4	0
7	1	16
8	0	0
9	2	24
10	3	8
11	0	48
12	4	0
13	1	10
14	0	4
15	0	0
16	1	3
17	6	0
18	0	0
19	0	0
20	0	0
21	0	0
22	10	5
mean	2	6
S.D.	3.49	11.31

Table 6

Descriptive table response, Experiment 3.2.

EMLA 5% versus Xylocaine 5%

Subject	Sex	No pain	Mild to moderate pain	Severe pain
1	male	□	●	
2	female	●□		
3	male	●	□	
4	male	●□		
5	male	●	□	
6	male	●	□	
7	female	□	●	
8	male	●□		
9	female	□	●	
10	female	□	●	
11	female	□	●	
12	male	●	□	
13	male	□	●	
14	male	□	●	
15	male	●□		
16	male	●□		
17	male	●□		
18	female	●□		
19	female	●□		
20	male	●□		
21	male	●□		
22	male	●	□	

EMLA 5% □

Xylocaine 5% ●

3.3 " THE EFFICACY OF TOPICAL ANAESTHETICS TO PRODUCE PULPAL ANAESTHESIA "

3.3.1 Introduction

The results of study 3.1 showed that EMLA produced profound mucosal anaesthesia to the depth of needle insertion (5mm) after a two minute application time. It was felt that a study should be carried out to assess if EMLA could produce a degree of anaesthesia of the pulp of a tooth if the agent were applied for a longer period. It was also thought that Xylocaine 10% Special Adhesive should be included in the study, to evaluate whether a higher concentration of lignocaine would be more effective.

3.3.2 Aims

The aims of study 3.3 were twofold. First, to assess the level of pulpal anaesthesia, if any, of a tooth produced by longer application times of EMLA cream 5% and Xylocaine 10% Special Adhesive. Second, to examine what effects EMLA 5% and Xylocaine 10% had on the oral mucosa from these longer application times.

Materials used in this experiment were :-

1. EMLA cream 5%

2. Xylocaine 10% Special Adhesive (lignocaine 10 per cent).

3.3.3 Patients and methods

This experiment was a random double-blind study in its design with the subject unaware of the test agent and an electrical pulp tester providing a stimulus which was free of operator influence and error. Volunteer subjects recruited for study 3.3 were in good health and not allergic to local anaesthetics. Subjects with pacemakers, hearing aids or other attached electronic devices were excluded. Teeth to be tested had to be vital, caries free, have good periodontal condition and be free of metallic restorations. Approval for this study was granted by the Ethical Review Committee (University of Sydney). Initially, only two groups of subjects were tested (groups 1 and 2). However, based on the results of group 1 it was decided to expand the study for EMLA (group 3) and to include a placebo group (group 4). The four groups were :-

Group 1

Thirteen subjects received EMLA, pulp testing was carried out prior to application, at fifteen minutes and thirty minutes after application.

Group 2

Twelve subjects received Xylocaine 10%. Pulp testing was carried out at the same intervals as in group 1.

Group 3

Twelve subjects received EMLA, testing was carried out prior to application, at ten minutes and forty minutes after application.

Group 4

Ten subjects received placebo (petroleum jelly) as a control group. Pulpal testing was carried out at the same intervals as for group 3.

The instrument used for measuring pulpal response was an electrical pulp tester[3] (Illustration 5). The display count reads from 0 (15 volts) to 80 (300 volts). The pulp tester has a rate control dial which was set at 1. This provided an electrical stimulus to increase from 0-80 (15-300 volts) over a period of forty-eight seconds.

The upper right central incisor was selected as the tooth to be used for assessing each preparation. The pulp test site was standardised as the centre of the incisal third of the buccal surface. The tooth was pulp tested with the electric pulp tester prior to the placement of the topical anaesthetic agent (0 minute) to record a baseline response. The subject indicated to the investigator when a painful stimulus was first felt. The probe was then withdrawn from the tooth surface and the digital reading recorded.

The area was then wiped free of saliva with fresh cotton gauze and 0.5 mL of the test agent was applied to the buccal sulcus adjacent to the tooth (Illustration 6). Isolation of the agent was achieved by covering the test agent with Stomahesive bandage®[4].

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[3]Analytic Technology, U.S.A.

[4]ConvaTec, Squibb and Sons Pty. Ltd., Victoria.

The tooth was again pulp tested at the fifteen and thirty minute intervals or the ten and forty minute intervals according to the group number (Illustration 7). At the completion of the last testing interval the bandage was removed and the mucosa wiped gently with fresh cotton gauze and examined for any adverse local effects.

3.3.4 Results

Tables 7, 8, 9 and 10 gives the responses for each subject in study 3.3.

Fig. 8a shows the baseline response of group 1 (EMLA). The majority of subjects responded between 20-40. This was within the manufacturer's specified response range for a healthy, vital upper central incisor tooth. Fig. 8b shows the fifteen minute response to electrical testing with the application of EMLA. Eight subjects indicated no pain response to the maximum level of the tester (300 volts) and four subjects required a higher voltage than their baseline reading to elicit the pain response. The remaining one subject indicated the pain response at the same voltage level as the baseline reading. Fig. 8c shows the thirty minute response with the application of EMLA. Ten subjects had indicated no pain response up to the maximum level. Three subjects responded at thirty minutes, who had previously indicated no response at fifteen minute interval. Only one subject felt a stimulus at both the fifteen and thirty minute interval. The average scores for the group 1 was 27 for the baseline reading, 70 at fifteen minutes and 72 at thirty minutes.

Fig. 9a shows the group 2 (Xylocaine 10%) baseline response. This was comparable to group 1 (EMLA). Fig. 9b shows the fifteen minute response to Xylocaine. Three subjects indicated no response to the maximum setting. Eight other subjects required an increased stimulus to elicit a response. One subject reported the stimulus at a lower level than the baseline response. The thirty minute group response is shown in Fig. 9c. Three different subjects indicated no response to 300 volts at this interval. However, the three subjects with no response to 300 volts at fifteen minutes, indicated a response at the thirty minute interval. Seven subjects in group 2 required an increased voltage at thirty minutes to elicit a response, while the remaining six subjects peaked at the fifteen minute interval for pulpal anaesthesia. The average scores for the group was 23, 53 and 58 for the baseline reading, fifteen and thirty minute interval respectively.

Fig. 10a shows the group 3 results. The baseline scores are comparable to groups 1 and 2. At the ten minute interval (Fig. 10b) two subjects indicated no response to the maximum voltage. Seven subjects indicated a response at a higher level, and three subjects indicated a response at a lower level, compared to the baseline reading. At the forty minute interval (Fig. 10c) only two subjects indicated no response to the maximum stimulus. The average scores for each interval was 18 for baseline, 42 for ten minutes and 62 for the forty minute interval.

Group 4 was the placebo group. The baseline response (Fig. 11a) was again comparable to the baseline responses of the other groups. The fifteen minute response (Fig. 11b) had two subjects indicating no response to the maximum level of the pulp tester. Seven other subjects required a higher voltage for response when compared to the baseline scores. Fig. 11c shows the forty minute scores for group 4. Four subjects reported no response to the maximum stimulus. Five other subjects indicated higher response scores compared to the ten minute scores. The average scores for the intervals were 19 for baseline, 43 at the ten minute interval and 57 for the forty minute interval.

3.3.5 Discussion

EMLA was shown to produce a high degree of anaesthesia of the pulp when testing was carried out by a high voltage electrical stimulus. In the fifteen to thirty minute interval, twelve out of thirteen subjects tested in group 1 showed profound pulpal anaesthesia to electrical testing. The average scores from groups 1 and 3 indicated that pulpal anaesthesia from EMLA was peaking in the fifteen to thirty minute period, with a reduced level of anaesthesia in the forty minute interval (Fig. 12). It was clinically evident that when the occlusive bandage was removed at the forty minute interval, there was no drug or small quantities of the drug observed in the majority of subjects suggesting that a high level of absorption or dispersal of EMLA had occurred.

Illustration 6. 0.5 mL of EMLA ap

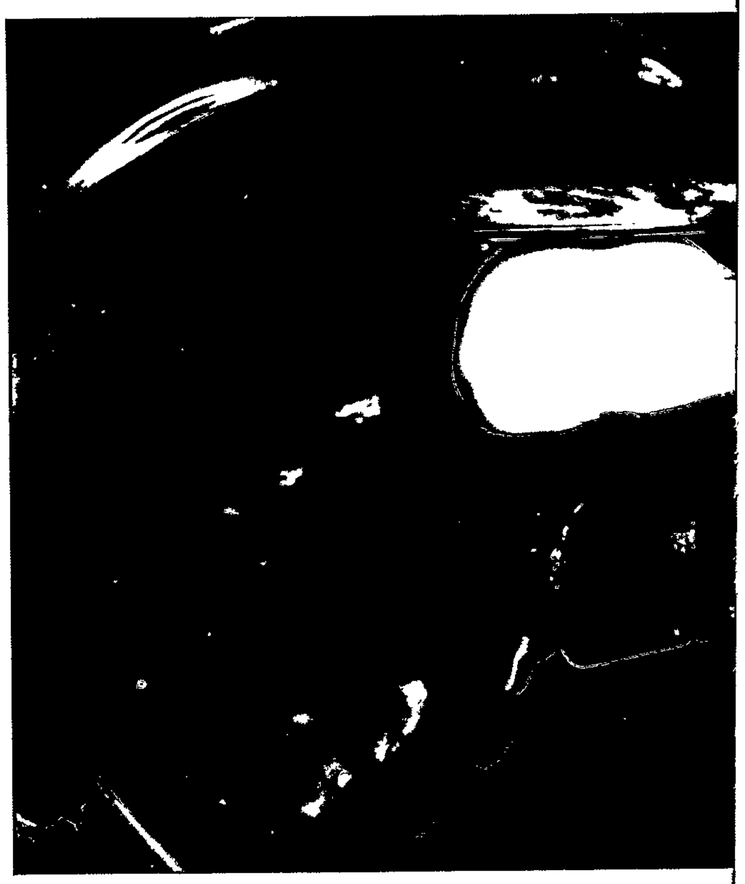

Illustration 7. Pulp test probe cont

Xylocaine 10% showed little or no degree of pulpal anaesthesia when the mean of group 2 was compared with placebo (group 4). At the fifteen minute interval Xylocaine produced a mean score of 53 compared with the placebo average of 43 at the ten minute interval and at the thirty minute interval the mean score for Xylocaine was 58 compared with a mean of 57 for the placebo group at forty minutes.

Interestingly, the placebo group showed a degree of pulpal anaesthesia at both the ten minute and forty minute interval. The effect of lowering the pain threshold for the dental pulp is difficult to explain. A suggestion is that the electrical device may have caused a degree of pulpal accommodation to occur. However, pain fibres in the dental pulp are unmyelinated C fibres that do not exhibit adaptation as do other sensory fibres. The lower pain threshold, more likely, is due to the release of endorphins, enkephalins or other centrally released pain mediating substances. It is widely known that a constant electrical source has this effect which can be readily observed in the therapeutic use of transcutaneous electrical nerve stimulators (TENS) for the treatment of chronic pain. The electrical pulp tester in the study may have acted in the same way as TENS.

The results were not subjected to statistical analysis because the sample size in the groups was too small. As there was a clear difference in pulpal response among the groups it was felt that increasing the sample size for statistical reasons was not justified on ethical grounds.

Fig. 8a. EMLA 5%, 0 minute response

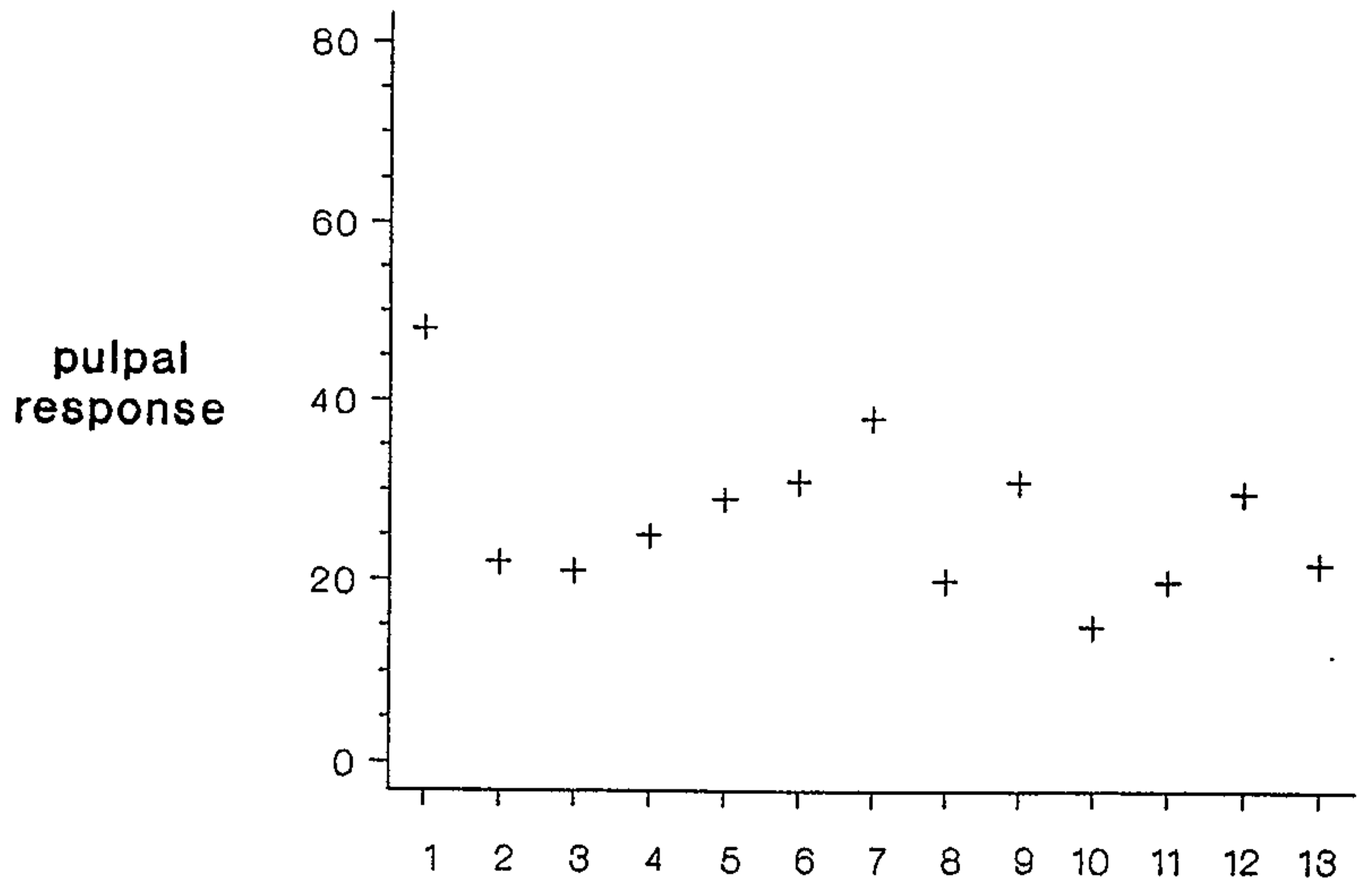

Fig. 8b. EMLA 5%, 15 minute response

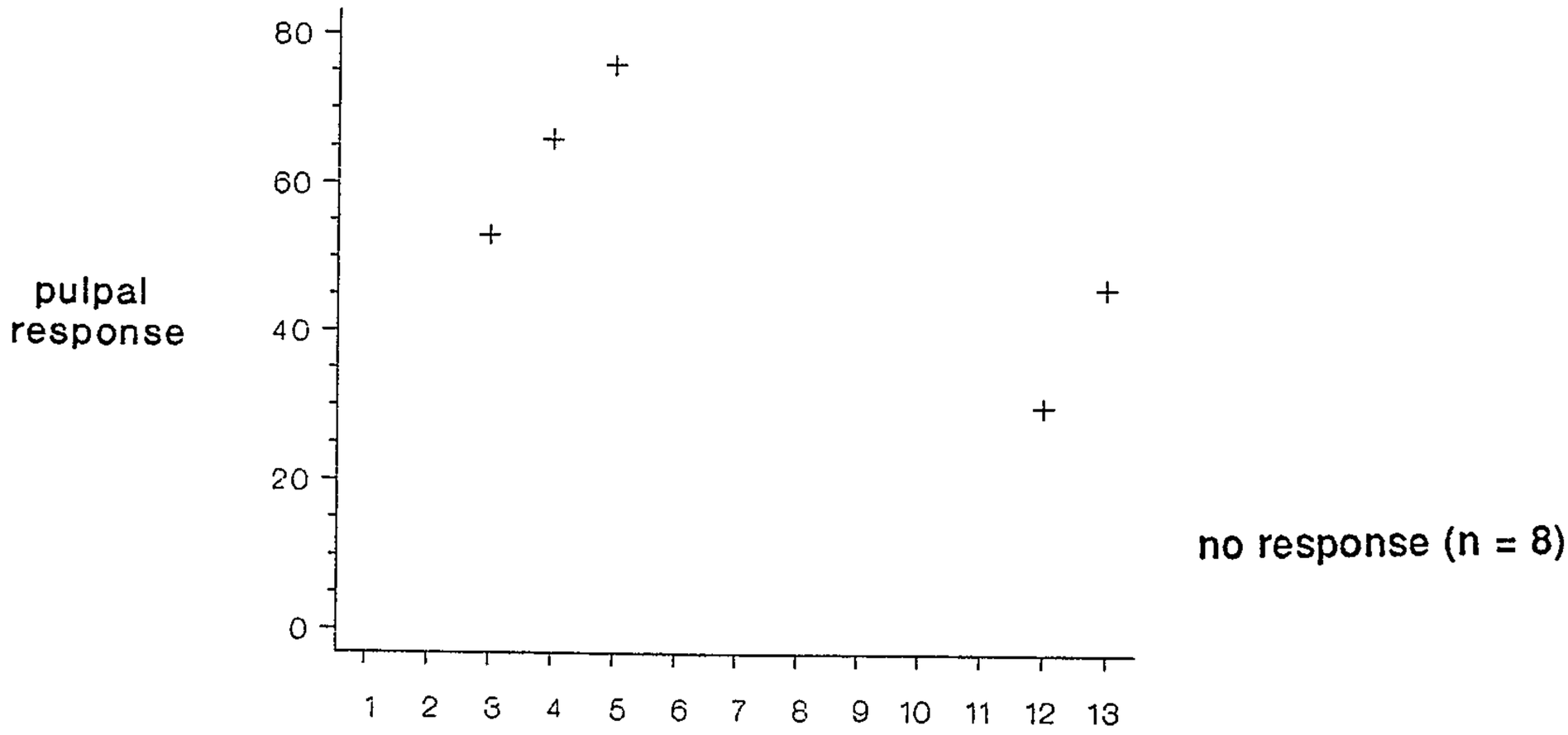

Fig. 8c. EMLA 5%, 30 minute response

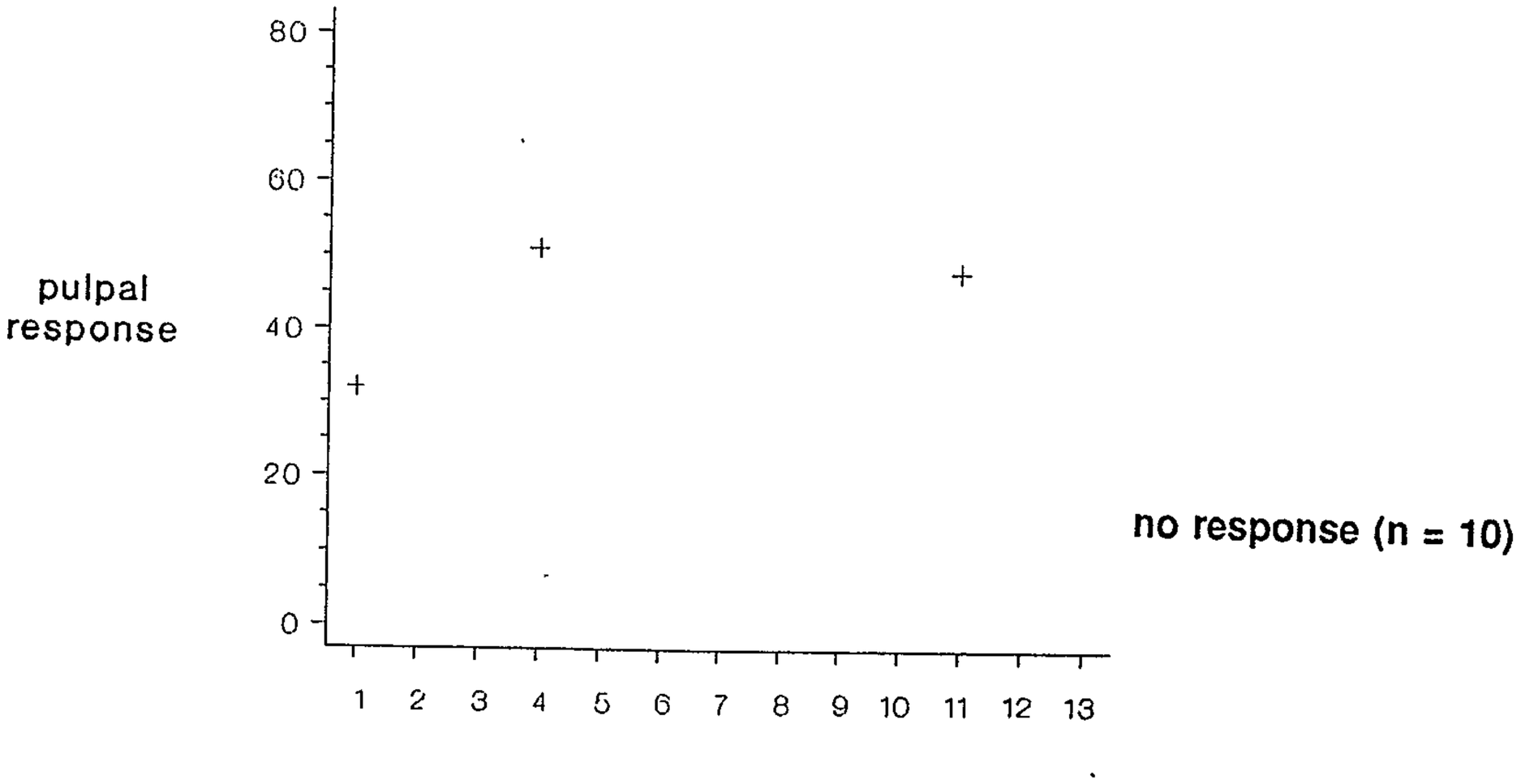

Fig. 9a. Xylocaine 10%, 0 minute response

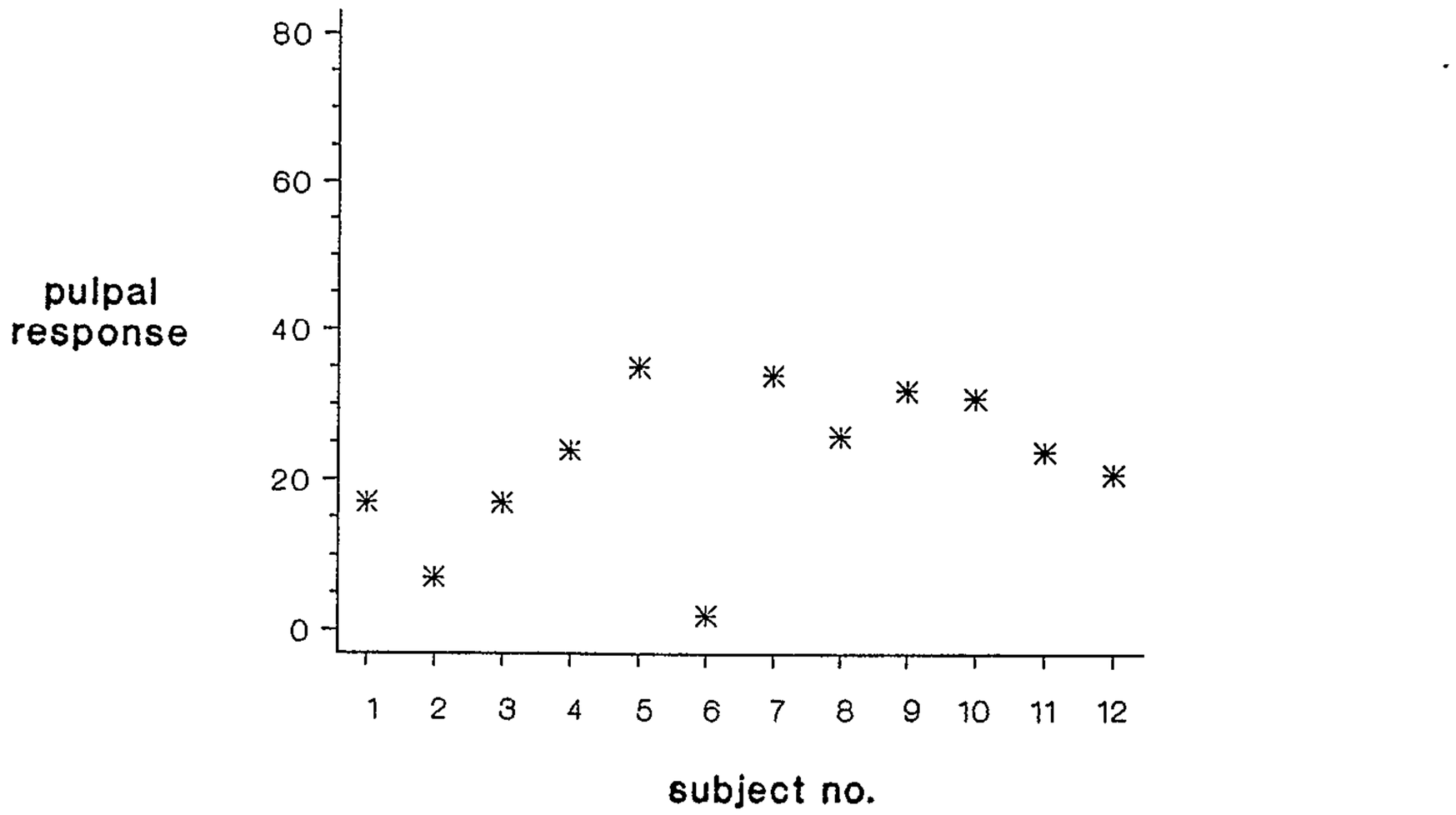

Fig. 9b. Xylocaine 10%, 15 minute response

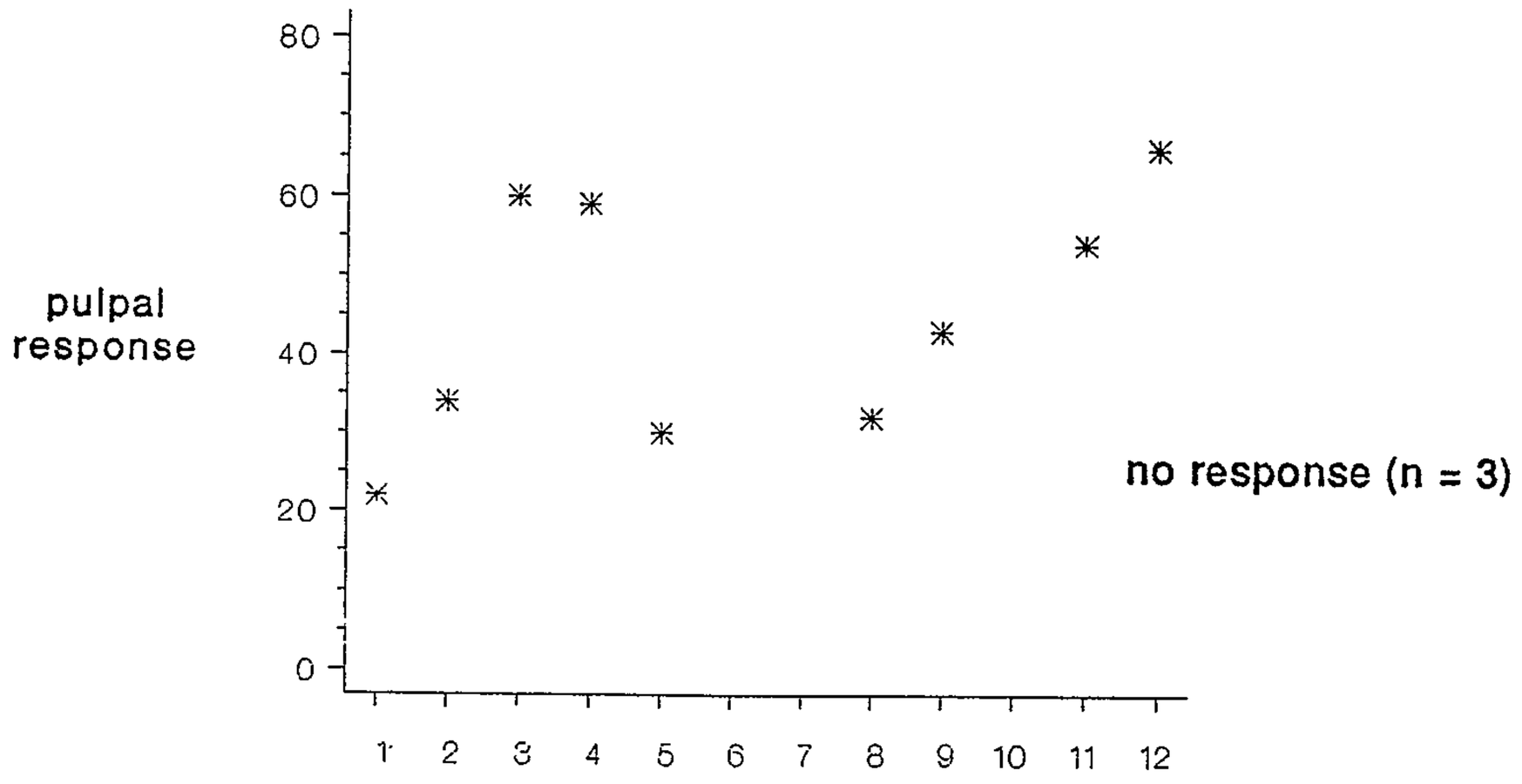

Fig. 9c. Xylocaine 10%, 30 minute response

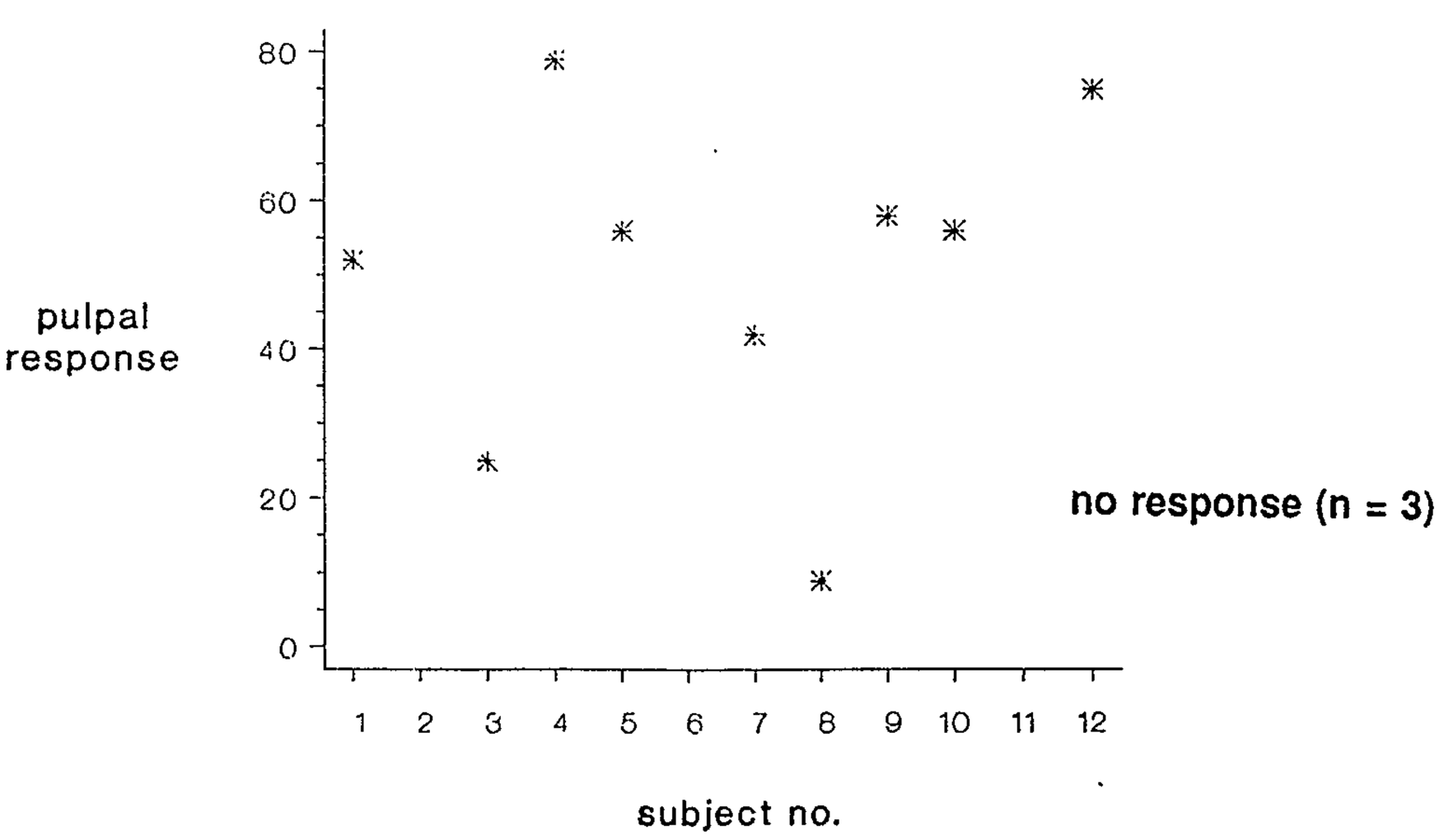

Fig. 10a. EMLA 5%, 0 minute response

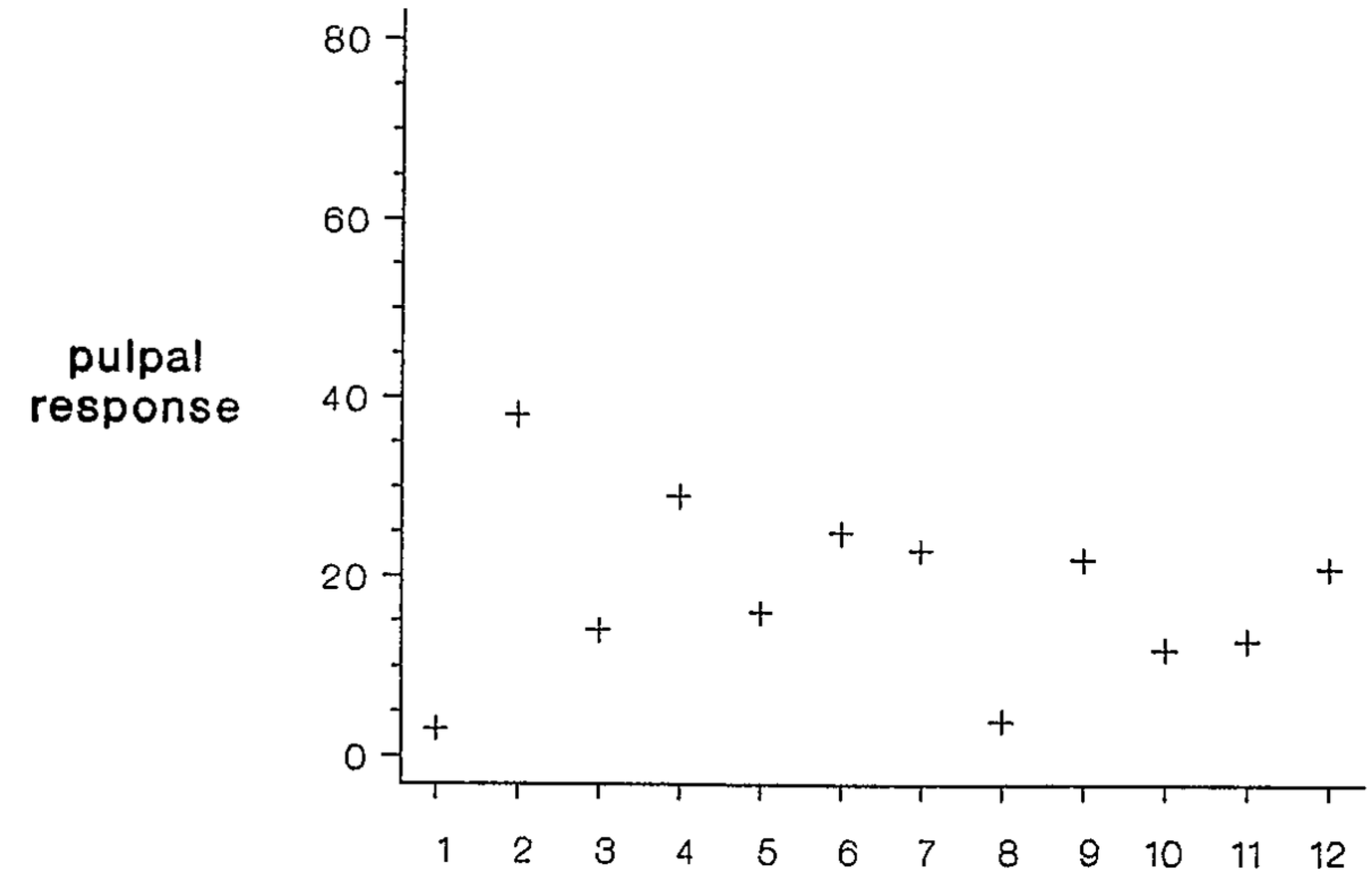

Fig. 10b. EMLA 5%, 10 minute response

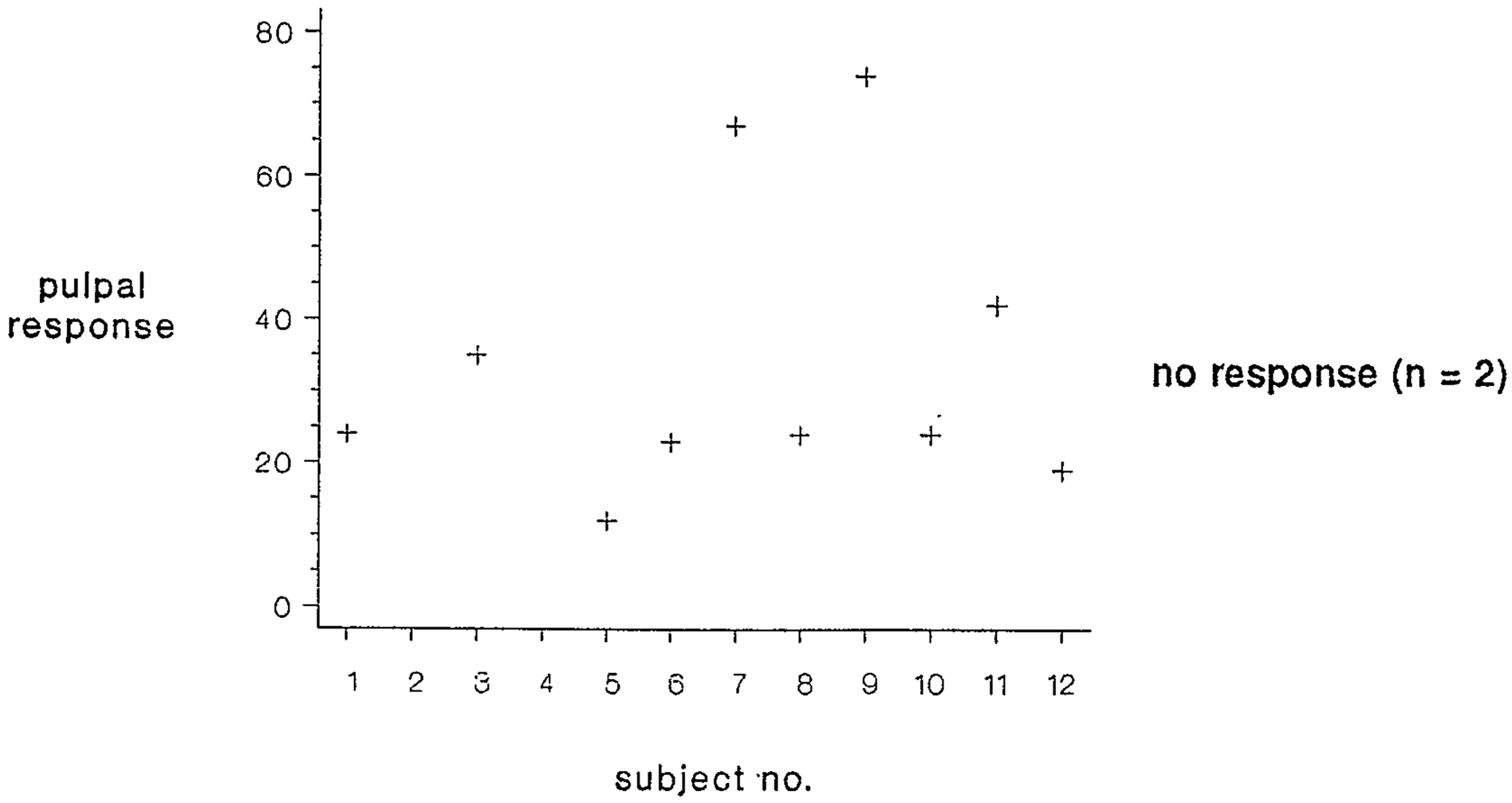

Fig. 10c. EMLA 5%, 40 minute response

Fig. 11a. Placebo, 0 minute response

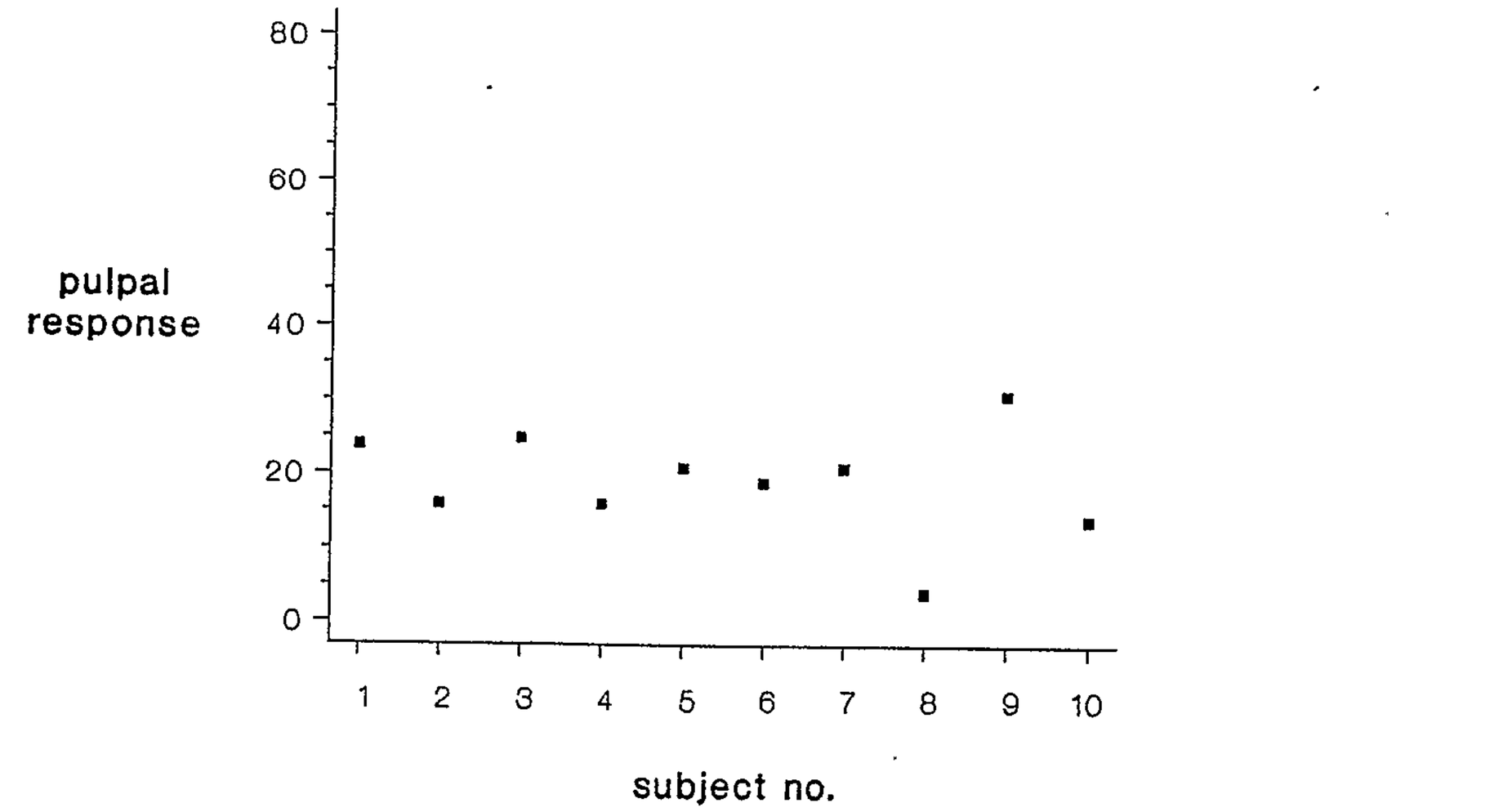

Fig. 11b. Placebo, 10 minute response

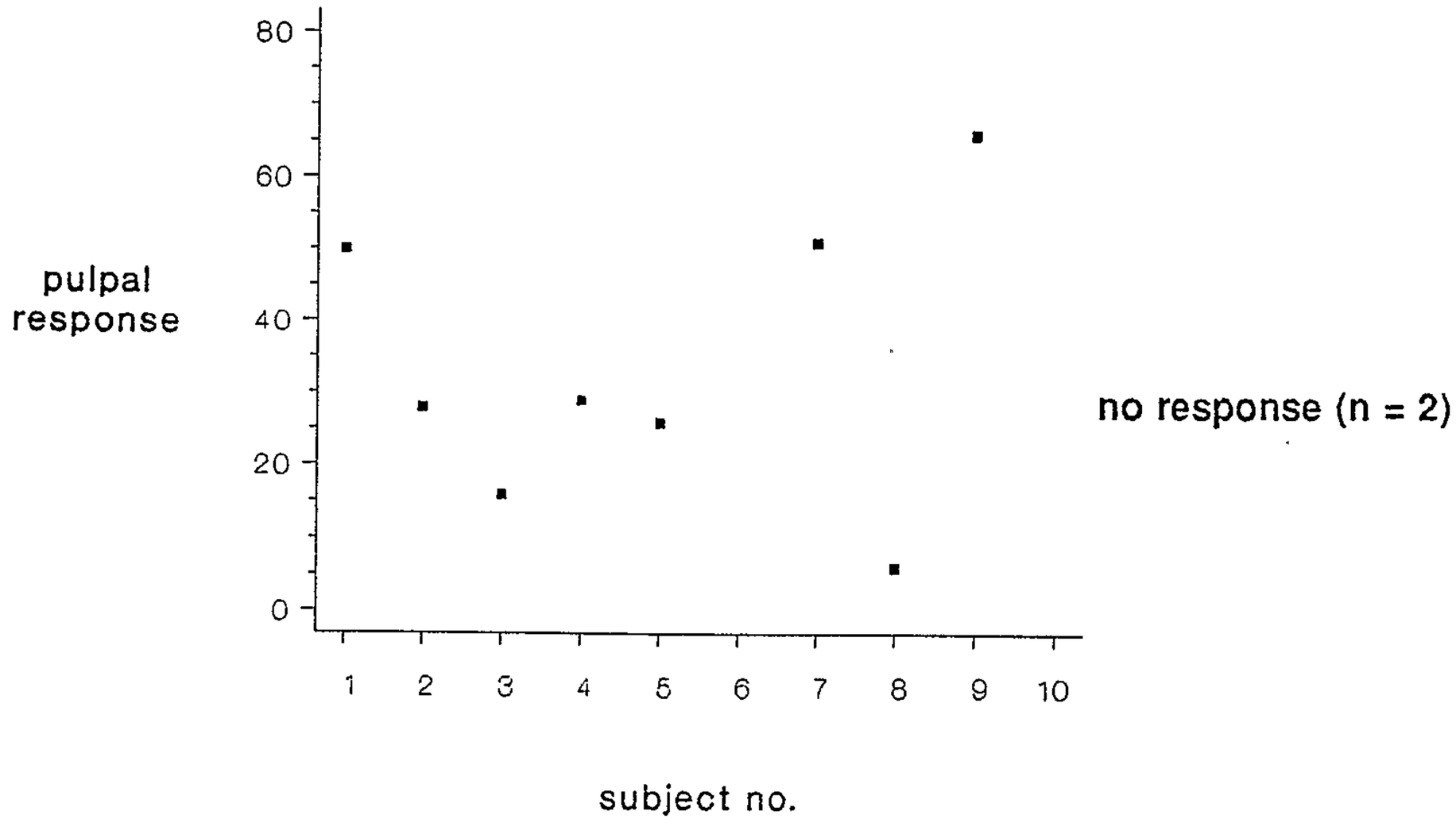

Fig. 11c. Placebo, 40 minute response

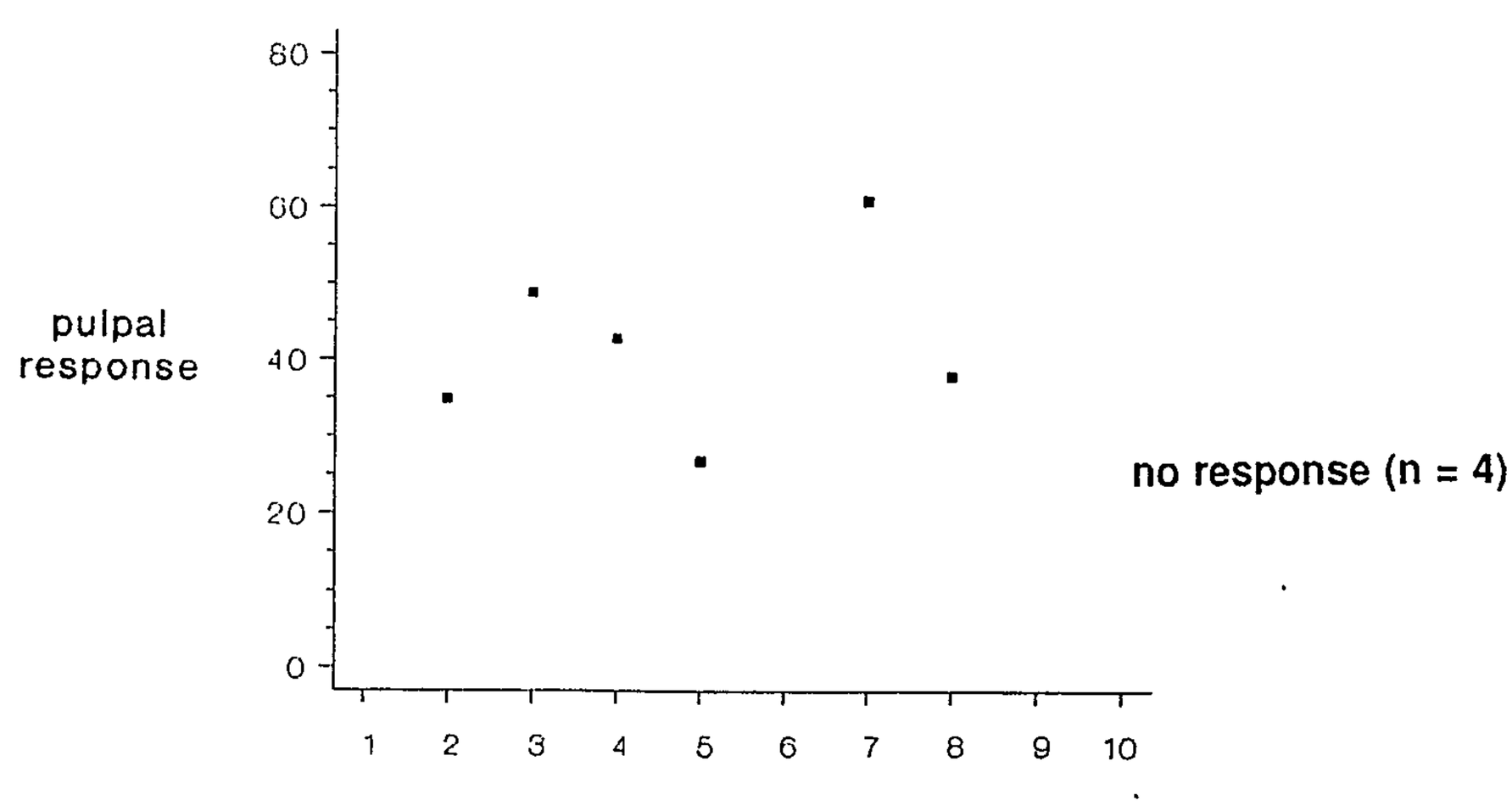

Table 7

Pulpal response, Group 1, Experiment 3.3

EMLA cream 5%

Subject	Sex	Baseline	15 minutes	30 minutes
1	male	48	80	32
2	female	22	80	80
3	female	21	53	80
4	male	15	80	80
5	female	20	80	48
6	male	30	30	80
7	female	22	46	80
8	female	25	66	51
9	male	29	76	80
10	male	31	80	80
11	male	38	80	80
12	male	20	80	80
13	male	31	80	80
	Mean	27	70	72
	S.D.	8.84	16.62	16.47

Table 8

Pulpal response, Group 2, Experiment 3.3

Xylocaine 10%

Subject	Sex	Baseline	15 minutes	30 minutes
1	male	17	22	52
2	female	7	34	80
3	male	17	60	25
4	male	32	43	58
5	male	31	80	56
6	female	24	54	80
7	male	21	66	75
8	female	24	59	79
9	male	35	30	56
10	male	2	80	80
11	male	34	80	42
12	male	26	32	9
	Mean	23	53	58
	S.D.	10.40	20.96	23.29

Table 9

Pulpal response, Group 3, Experiment 3.3

EMLA cream 5%

Subject	Sex	Baseline	10 minutes	40 minutes
1	male	3	24	80
2	male	38	80	73
3	male	14	35	53
4	male	29	80	26
5	male	16	12	63
6	female	25	23	72
7	male	23	67	55
8	female	4	24	66
9	female	22	74	66
10	male	12	24	80
11	male	13	42	53
12	female	21	19	53
	Mean	18	42	62
	S.D.	10.09	25.84	15.04

Table 10

Pulpal response, Group 4, Experiment 3.3

Placebo

Subject	Sex	Baseline	10 minutes	40 minutes
1	female	24	50	80
2	male	16	28	35
3	male	25	16	49
4	female	16	29	43
5	male	21	26	27
6	female	19	80	80
7	female	21	51	61
8	female	4	6	38
9	male	31	66	80
10	male	14	80	80
	Mean	19	43	57
	S.D.	7.30	26.22	21.43

3.4 " PLASMA CONCENTRATIONS OF LIGNOCAINE AND PRILOCAINE FROM ORAL MUCOSAL APPLICATION OF EMLA CREAM 5% "

3.4.1 Introduction

The use of EMLA in increased amounts for longer application times may result in high blood levels of the active constituents (lignocaine and prilocaine), as the rate and degree of absorption from oral mucosal application would be higher than from the skin. Only one study (Haasio et al.,1990) has been carried out to measure the plasma concentrations of lignocaine and prilocaine from oral mucosal application of EMLA.

It was felt that due to the potential applications of EMLA cream 5% in dentistry, plasma concentrations of lignocaine and prilocaine should be further evaluated in order to establish that these levels are within recommended safe limits and below known toxic levels. Results from Experiment 3.3 showed that highest levels of anaesthesia from EMLA occurred within a thirty minute application time. Therefore, blood level estimations for a sixty minute period from a thirty minute application of EMLA was considered appropriate for this study.

3.4.2 Aims

The aims of this study were twofold. First, to measure the range of plasma concentrations of lignocaine and prilocaine from a thirty minute application of EMLA cream 5% to the oral mucosa and second, to examine for any adverse local effects from the application of a large volume of EMLA when applied to the oral mucosa for a thirty minute period.

3.4.3 Patients and methods

Twelve volunteers (six males and six females) were recruited for this study. Subjects had an age range from twenty one to thirty six years and were in good general health. In particular, no subject suffered from bleeding problems or blood dyscrasias, kidney or liver problems. Known infectious patients and subjects with a history of allergy to local anaesthetics were excluded. The oral mucosa of each subject, in particular the cheek mucosa lining the vestibule, was clinically normal in appearance. Approval for this study was granted by the Ethical Review Committee (University of Sydney).

Prior to placement of EMLA the cheek pouches of each subject were cleared with cotton gauze to remove excess saliva. Four mL of EMLA was then applied to a Stomahesive bandage measuring three centimetres by three centimetres (9 cm^2) to allow for maximum coverage of the mucosa. The bandages carrying the EMLA cream were then placed and adapted against

the cheek mucosa which extended antero-posteriorly from the lip to the coronoid notch and vertically from the upper buccal vestibule to the lower buccal vestibule. A total of 8 mL of cream was used in each subject contacting 18 cm^2 of the cheek mucosa. High speed suction was carried out with care when pooled saliva in the floor of the mouth and oro-pharynx regions was observed. This was done to provide patient comfort and to eliminate the possibility of absorption of the preparation following ingestion. The application time of EMLA to the oral mucosa was thirty minutes. At thirty minutes both occlusive bandages were removed from the subject. The cheek mucosa was wiped free of residual cream by fresh cotton gauze. Following this the subject was asked to rinse the mouth thoroughly to remove any remaining cream. The mucosa was then examined for any adverse local effects from the application of EMLA.

The cubital fossa of the right or left arm was selected as the site for venous blood sampling. The area was cleaned with Skin Cleansing Swab[5], a sterile tissue containing 70 per cent isopropyl alcohol, prior to venous cannulation. A 21 gauge butterfly needle (Illustration 8) was then inserted into the vein and held in place by adhesive tape. Two mL of venous blood was drawn into a sterile 2.5 mL clear plastic syringe after the EMLA had been applied for ten minutes. Following the sampling a new syringe containing 1 mL of sterile heparinised saline was connected to the butterfly needle. The heparinised saline was slowly injected until the next sampling period to

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[5]Briemarpak, Victoria.

heparinised saline was slowly injected until the next sampling period to prevent clotting inside the cannula. Immediately prior to the next sampling time 0.5 mL of blood was withdrawn into the empty syringe that had contained the heparinised saline in order to remove diluted blood from the cannula. The volume of the cannula was 0.15 mL. Blood sampling alternating with the heparinised saline flushes was carried out at each interval during the experiment. New syringes were used for each blood sample and saline flush. The sampling times for each subject was at 10, 20, 30, 40, 50 and 60 minutes after the initial application of EMLA. This allowed for three blood samples (10, 20, 30 minutes) while the cream was in place and for a further three samples (40, 50 and 60 minutes) following the removal of EMLA from the test sites. The subject was placed in a comfortable seated position throughout the experiment. After the 60 minute sample had been taken the butterfly needle was removed and haemostasis achieved with cotton gauze and new adhesive tape.

Each blood sample was deposited and gently rolled in a 5 mL Vacutainer®[6] containing ethylene diamine tetra-acetate to prevent clotting. At the end of sampling for each subject, the samples were frozen at -10°C to prevent metabolism of lignocaine and prilocaine from microsomal enzymes in the blood.

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[6]Becton Dickinson VACUTAINER Systems, Rutherford, U.S.A.

Laboratory Methods

1. Each labelled vial containing frozen blood sample was thawed in a water bath at 20°C for twenty minutes. The vial was rolled gently between fingers for thirty seconds and 1 mL of blood pipetted using a 1 mL multi-dose micropipette with plastic disposable tips (Illustration 9). A new tip was used for each sample. Each sample was then placed in a clean 10 mL conical glass tube and sealed with a screw-on cap. To each tube was added 5 mL of ether and 0.2 mL of 0.1 M sodium hydroxide solution. Amethocaine standard 200 ng/mL (40 µL of 5 µg/mL) was then added using a 100 µL syringe to the tube. A plastic screw-on cap with teflon insert was then screwed on top of the glass tube to prevent loss of the ether during vortex-mixing.

2. Each tube was then vortex-mixed for one minute followed by centrifugation (3,000 rpm) for five minutes to allow for extraction of the drugs into the ether and the subsequent separation of the ether layer from the packed cell matter and plasma protein layer. The ether was then decanted by clean Pasteur pipettes into a new 10 mL conical glass tube with screw-on plastic cap and teflon insert.

3. To each tube containing the ether was added 0.2 mL of 0.01 M hydrochloric acid. The tube was again tightly sealed with the cap, vortex-mixed for one minute and centrifuged (3,000 rpm) for five minutes to allow for the extraction of the local anaesthetic agents from the ether to the acid. The ether

layer was then discarded by Pasteur pipette. The open tube with residual acid containing the drugs was then placed in a water bath of 70°C to evaporate off the remaining ether. The acid containing the local anaesthetics was then transferred by clean Pasteur pipette to a 250 µL conical glass vial for carousel placement and subsequent injection in a Waters HPLC (High Performance Liquid Chromatography) apparatus.

HPLC Conditions

The measurement of the plasma concentrations of the anaesthetic agents was carried out by Waters[7] chromatography equipment (Illustration 10). The system hardware and software consisted of -

1. 600E System controller with twin pump heads and sapphire pistons enabling solvent pressures of 0-6,000 psi through the column.
2. 700 Satellite Automatic WISP (Waters Intelligent Sample Processor) injector with variable dose needle (0-2,000 microlitres).
3. 486 ultra-violet wavelength detector (190-500 nm).
4. 386-SX IBM compatible computer running Baseline 815 chromatography software.

Recommended start-up and calibration procedures for the apparatus were carried out and included :-

1. Solvents prior to chromatographic use were vacuum filtered and then

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[7]Millipore Corp., Milford, U.S.A.

sparged with pure helium gas at 100 mL/min. for thirty minutes to remove dissolved extraneous gases.

2. The WISP injector was purged at the beginning of each set of sample runs.

3. The pump heads were primed with 10 mL of each solvent for the removal of air bubbles in each solvent line.

4. A linear calibration curve over a range of 20-500 ng/mL for prilocaine and lignocaine was established prior to the first sample run.

5. The minimum detectable peak height was set at three times the baseline noise.

The solvents used for the analysis were acetonitrile (HPLC grade) and 0.04 sodium dihydrogen phosphate buffer prepared using triple distilled and filtered water with 0.02 per cent triethylamine reagent. The pH of the buffer was five. The solvent ratio was set at 55 per cent acetonitrile and 45 per cent phosphate buffer. The run time of each sample was ten minutes over an isocratic gradient. The flow rate of the solvent mixture was 4.0 mL/minute with constant helium sparging (20 mL/minute) of both solvents during the run time and the lapse time of data acquisition following each sample run. The data acquired at the end of each sample run was stored on hard disk for peak integration. An injection of 80 µL from each vial was selected to analyse through a Waters 5 micron C18 fully end-capped (Resolve Radialpak) column subjected to constant external water pressure. Retention times for the anaesthetic agents was 5-5.5 minutes for prilocaine, 6-6.5 minutes for lignocaine and 8-8.5 minutes for the amethocaine internal standard. The ultra-

violet wavelength detector was set at 210 nm with positive polarity and detector sensitivity selected at 0.005 AUFS (absorbance units full scale).

Peak integration of each chromatogram was then carried out. This measured individual peak heights of the drugs. The direct ratio of the peak heights of lignocaine and prilocaine were compared to the peak height of the amethocaine standard and the resulting values were the plasma concentrations of lignocaine and prilocaine.

Results

All subjects had measurable concentrations of prilocaine and lignocaine except in two subjects (7; 8) at the ten minute sampling time where prilocaine was not detected. The plasma concentrations of the drugs for each subject are shown in Table 11. The maximum concentration for lignocaine and prilocaine in any subject was measured in subject 2 at the 30 minute period. The maximum concentration for lignocaine was found to be 418 ng/mL and for prilocaine 223 ng/mL.

The mean concentrations of lignocaine and prilocaine, and their concentration ratio for each sample period is shown in Table 12. Figs. 12 and 13 show the mean concentrations (± S.D.) of the group. Peak mean concentrations of 131 ng/mL for prilocaine and 221 ng/mL for lignocaine were found at the 40 minute sample time.

No adverse local effects were observed to the mucosal tissues from the thirty minute application of EMLA.

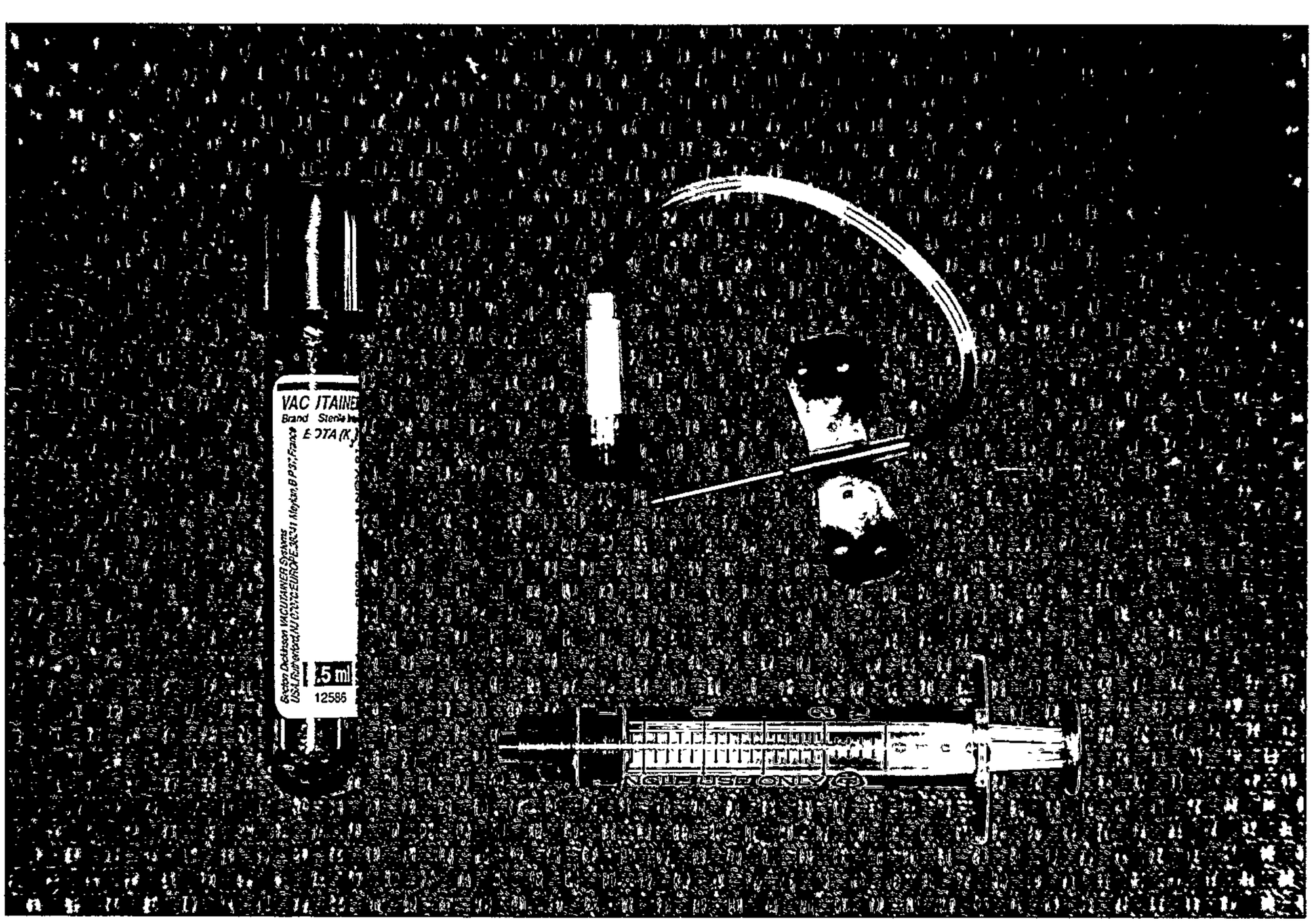
Discussion

A primary aim of this thesis was to assess that plasma concentrations of lignocaine and prilocaine from oral mucosal application of EMLA were below known toxic levels. Experiment 3.3 had shown that the highest levels of pulpal anaesthesia was achieved when EMLA was applied for up to 30 minutes. The purpose of Experiment 3.4 was to measure the concentrations from a thirty minute application using the maximum acceptable amounts of EMLA in the oral cavity and to assess its possible toxicity. The results of this study has shown that a large application (8 mL) of EMLA placed for thirty minutes produced maximum concentrations of lignocaine (418 ng/mL) and prilocaine (223 ng/mL) that were well below known toxic levels. The toxic levels for lignocaine and prilocaine were considered to be 4,400 ng/mL for lignocaine and 6,000 ng/mL for prilocaine (Malamed,1986).

The results show that prilocaine base is absorbed through the mucosa and therefore should have topical anaesthetic activity. However, plasma concentrations of prilocaine were lower when compared with lignocaine. In the case of EMLA, the role of prilocaine seems to be facilitating the eutectic form by lowering the melting point of the mixture. Lignocaine base therefore, appears as the main agent responsible for topical anaesthesia.

Illustration 8.

Blood sampling equipment, Experiment 3.4

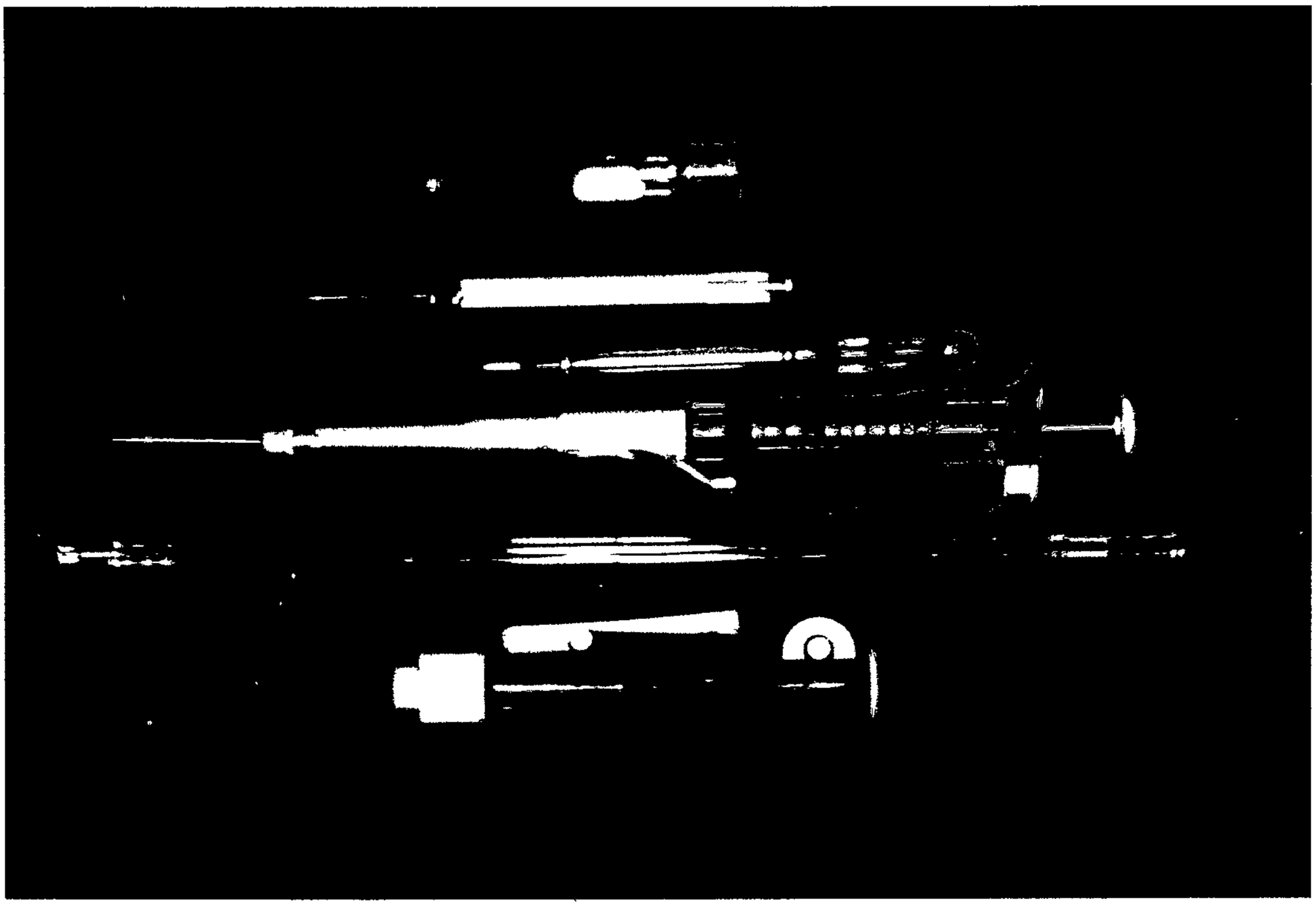


2
1
3

1. 5 mL Vacutainer (with EDTA) for blood sample.
2. 21 gauge butterfly needle with screw-on cap.
3. 2.5 mL syringe for collecting blood and for heparinised saline flushes.

Illustration 9.

Laboratory equipment, Experiment 3.4

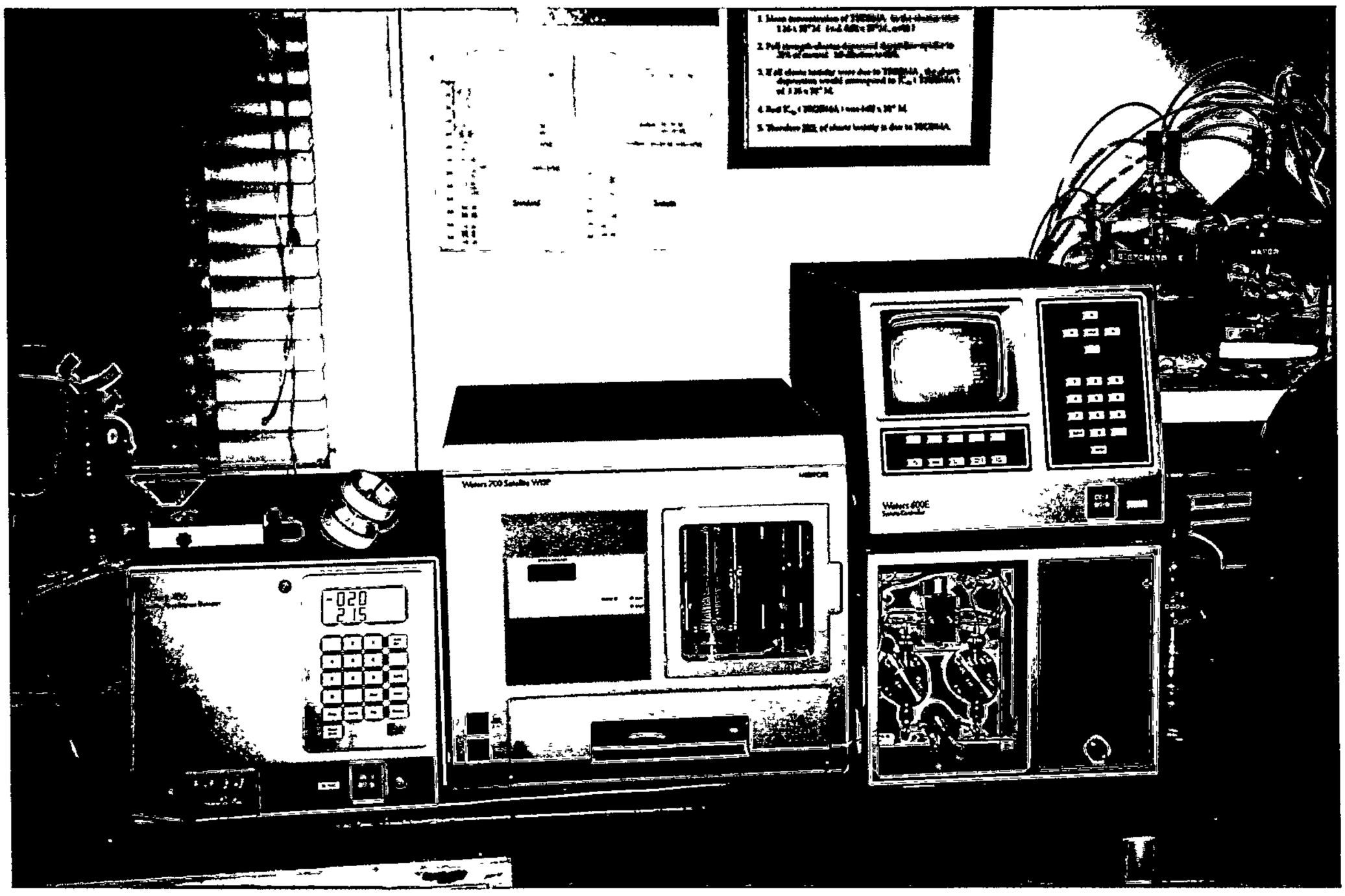


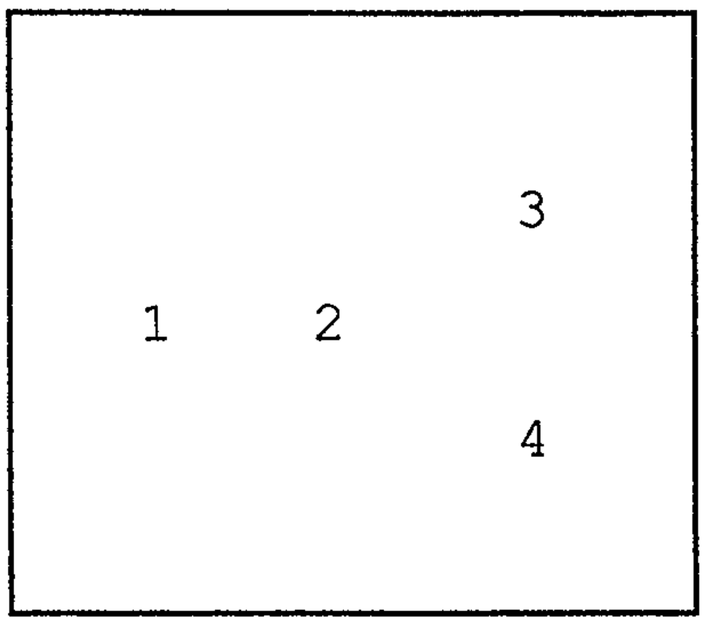
1
2
3
4
5
6

1. 10 mL glass vial with screw-on cap
2. 50 µL syringe
3. Pasteur pipette
4. Multi-dose micropipette
5. 5 mL pipette
6. Suction handle for 5 mL pipette

Illustration 10.

Chromatography (HPLC) equipment, Experiment 3.4





1. U.V. wavelength detector
2. 700 Satellite WISP injector
3. 600E system controller
4. Twin pumps with sapphire pistons

Table 11.

Plasma concentrations (EMLA cream 5%), Experiment 3.4

	Sample time (minutes)	Prilocaine concentration (ng/mL)	Lignocaine concentration (ng/mL)
subject 1	10	43	83
(DS) male	20	83	149
age 22	30	146	247
62 kg	40	108	207
	50	76	150
	60	38	77
subject 2	10	64	77
(DT) female	20	90	153
age 24	30	223	418
68 kg	40	158	337
	50	149	328
	60	144	313
subject 3	10	22	37
(PH) male	20	83	149
age 27	30	102	180
96 kg	40	89	175
	50	54	110
	60	20	31
subject 4	10	54	85
(TS) male	20	92	168
age 22	30	102	195
83 kg	40	211	359
	50	91	264
	60	53	225

Table 11 (Cont.)

	Sample time (minutes)	Prilocaine concentration (ng/mL)	Lignocaine concentration (ng/mL)
subject 5	10	10	51
(RV) male	20	64	96
age 36	30	68	111
73 kg	40	99	161
	50	122	240
	60	118	206
subject 6	10	9	19
(LR) female	20	70	112
age 32	30	93	197
58 kg	40	146	293
	50	56	144
	60	48	80
subject 7	10	-	69
(BT) female	20	33	94
age 34	30	113	246
72 kg	40	124	280
	50	67	108
	60	26	70
subject 8	10	-	24
(MM) male	20	62	78
age 24	30	79	118
56 kg	40	81	98
	50	60	95
	60	60	89

Table 11 (cont.)

	Sample time (minutes)	Prilocaine concentration (ng/mL)	Lignocaine concentration (ng/mL)
subject 9	10	43	72
(NH) female	20	92	140
age 22	30	111	146
60 kg	40	124	151
	50	99	201
	60	81	135
subject 10	10	38	46
(GL) female	20	86	130
age 25	30	111	134
62 kg	40	119	166
	50	141	161
	60	109	132
subject 11	10	20	33
(DW) male	20	118	171
age 21	30	181	250
60 kg	40	179	229
	50	102	174
	60	87	120
subject 12	10	66	140
(JN) female	20	71	150
age 28	30	116	173
60 kg	40	133	200
	50	99	189
	60	76	178

Fig. 12. Mean concentration of lignocaine (± S.D.)

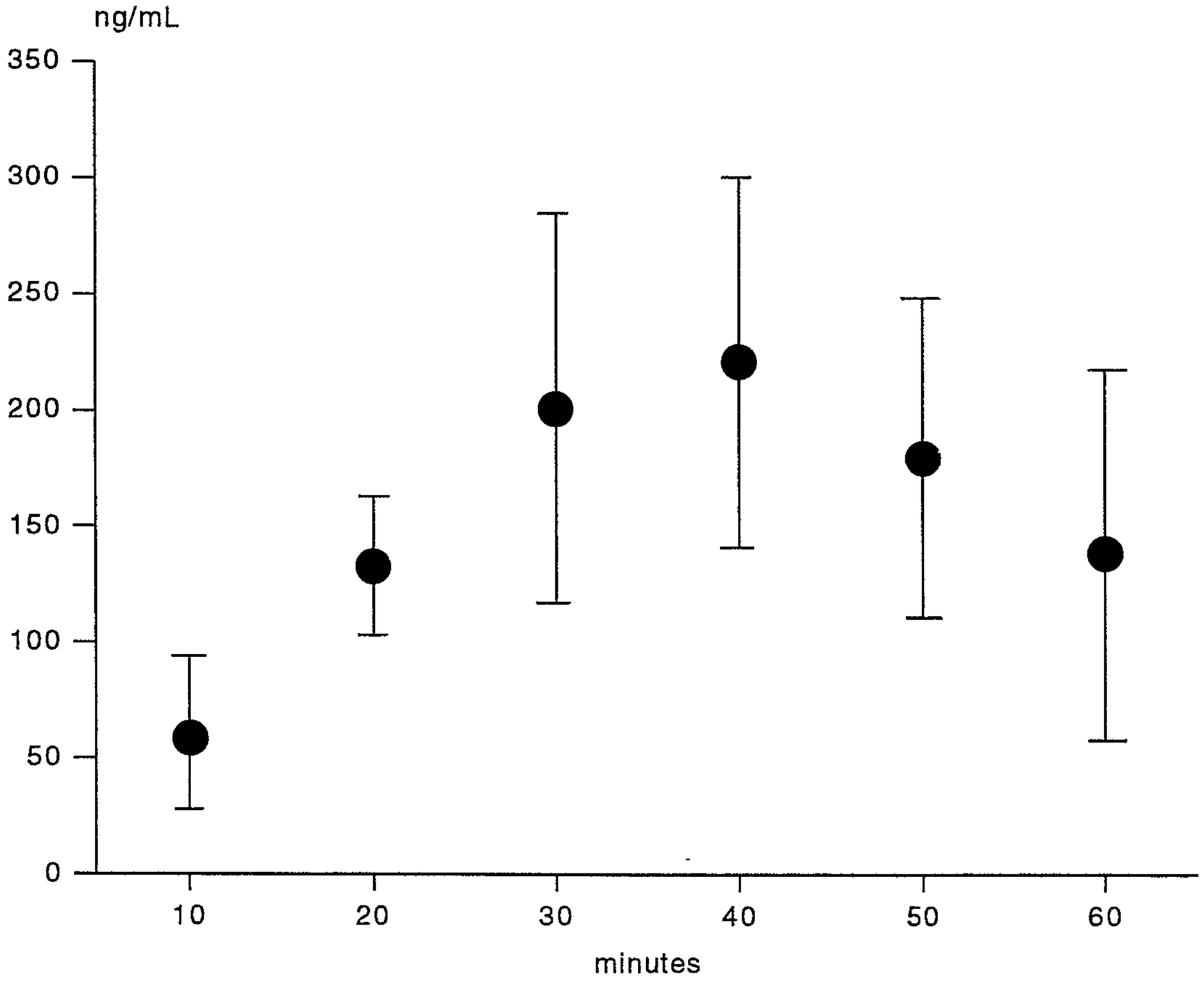
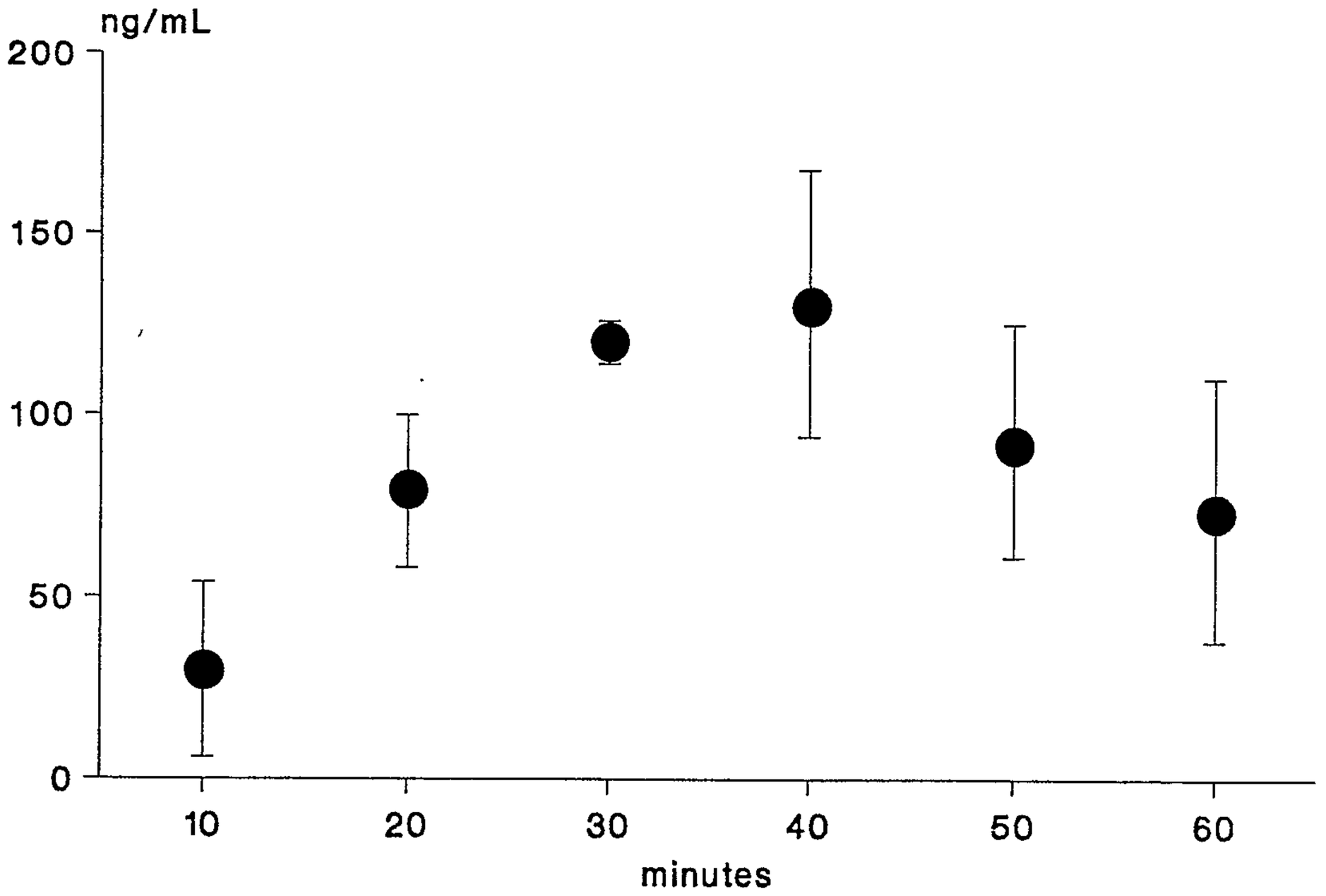


Fig. 13. Mean concentration of prilocaine (± S.D.)



Chromatograms for subject 2 (maximum plasma concentrations)

Retention times for peaks:

prilocaine (p) - 5 minutes; lignocaine (l) - 6 minutes; amethocaine (a) - 8 minutes

Fig. 14a 10 minute blood sample

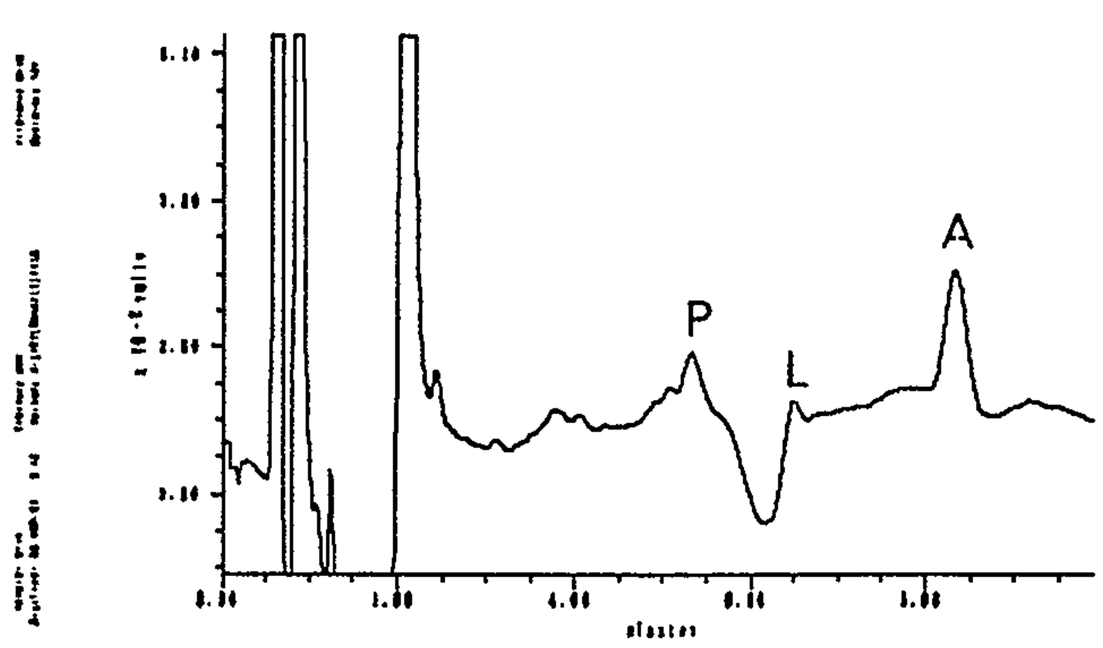


Fig. 14b 20 minute blood sample

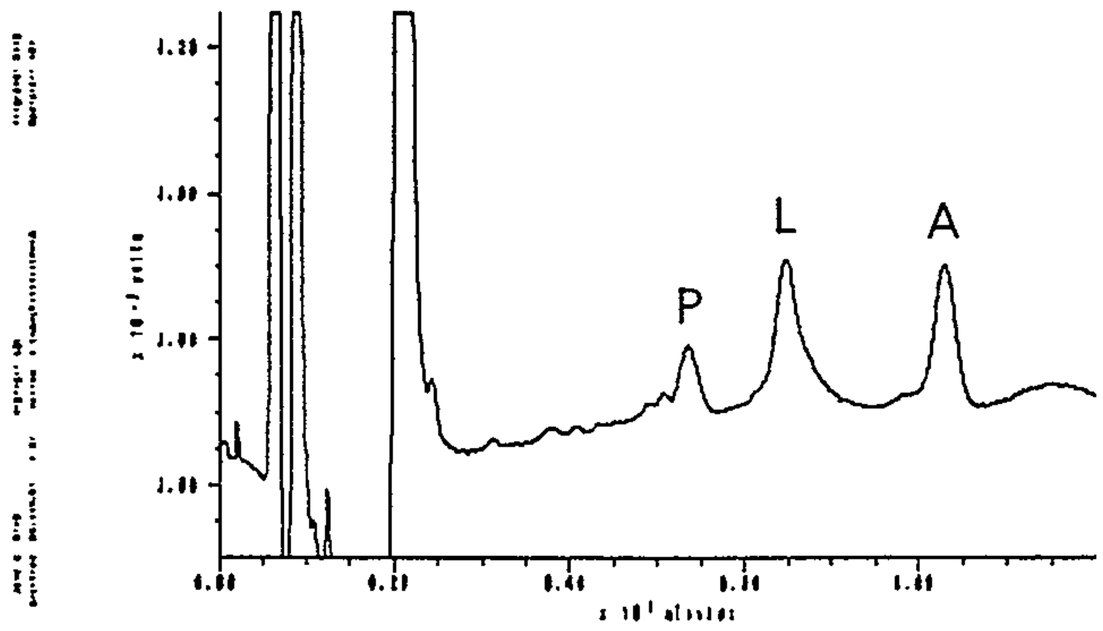


Fig. 14c 30 minute blood sample

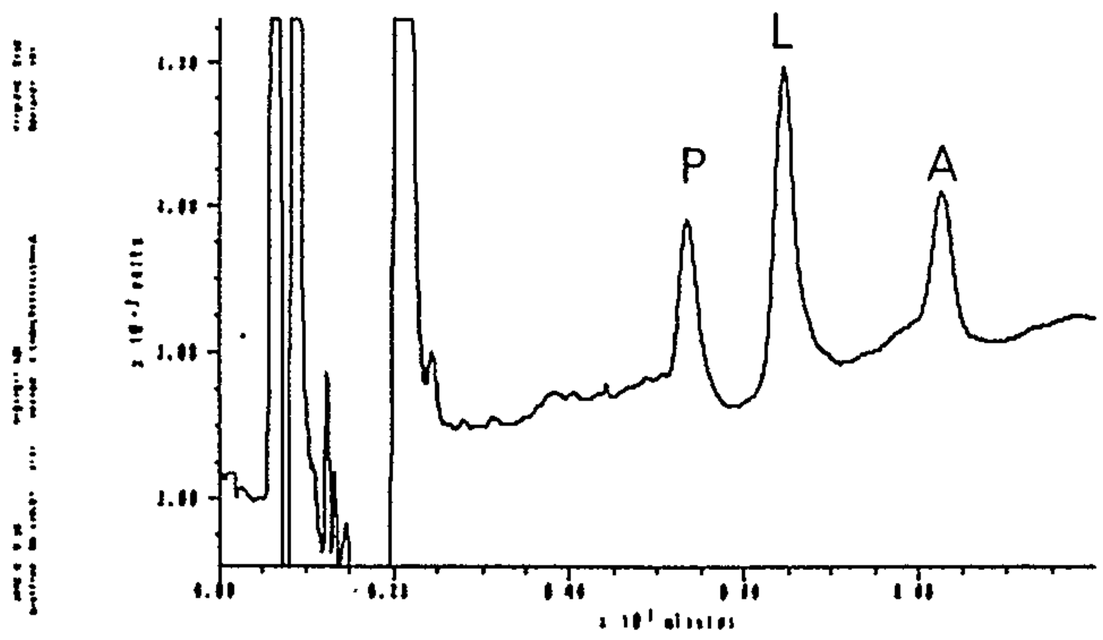


Fig. 14d 40 minute blood sample

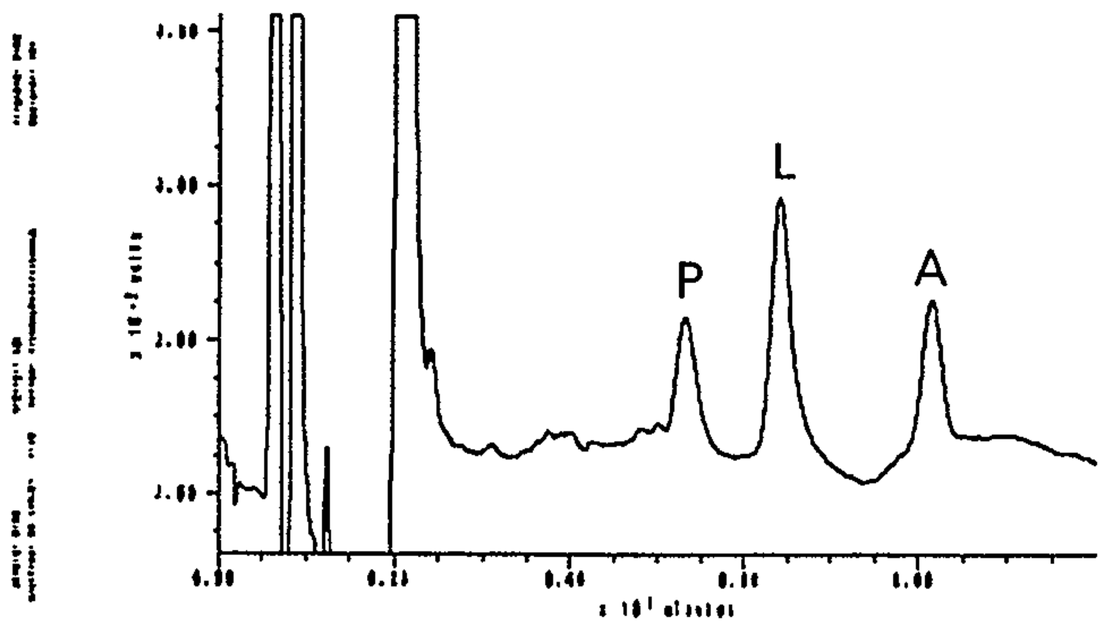


Fig. 14e 50 minute blood sample

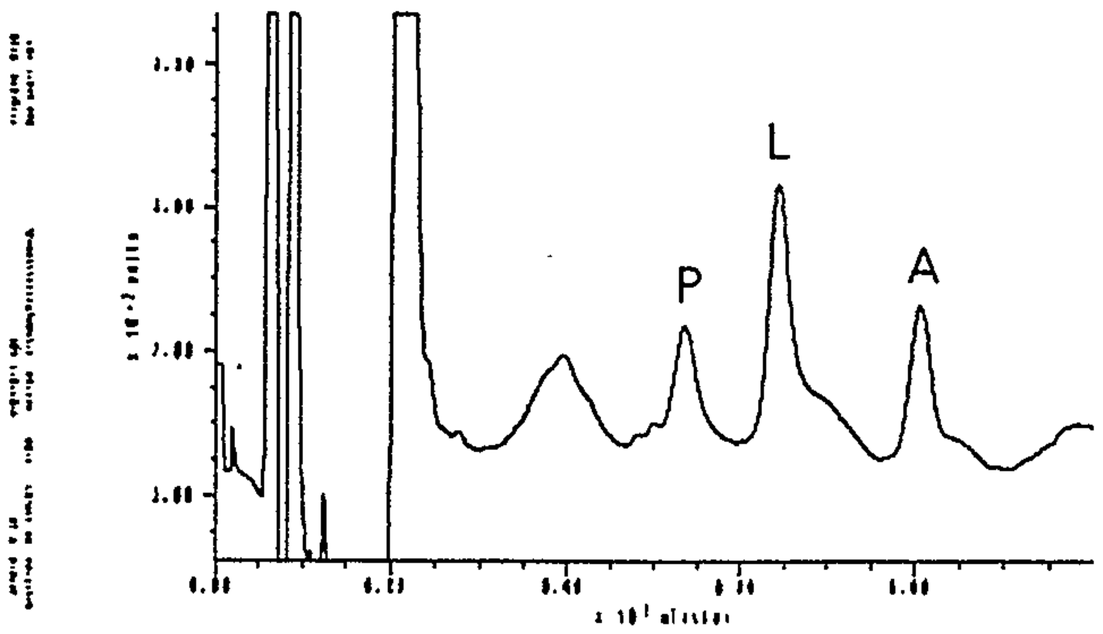
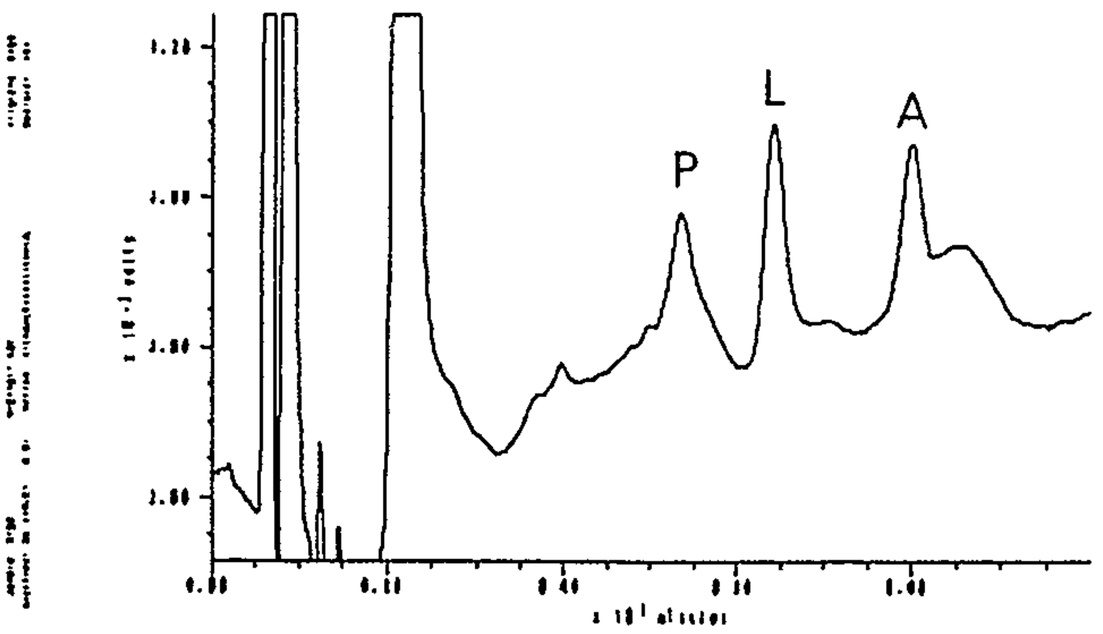


Fig. 14f 60 minute blood sample



CHAPTER 4

CONCLUSION

In concluding this thesis a number of statements can now be made based on the outcome of individual studies.

Study 3.1 confirmed all three topical anaesthetic agents tested (NUM, Xylocaine 5% and EMLA cream 5%) to be significant when compared with placebo in reducing the pain experience from needle insertion in the oral mucosa. This provides support that topical local anaesthesia is an essential adjunct in the administration of relatively pain-free injections in the mouth. However, this study did not test the area of the hard palate for a reduction in the pain experience from needle insertion. The hard palate, being highly keratinised, would require a longer application time for drugs to be absorbed in order to obtain a similar reduction in pain. This area requires further investigation.

Statistical results from study 3.1 showed that EMLA was the most effective agent of the three topical anaesthetics. However, in study 3.2, a direct pairing of Xylocaine 5% and EMLA showed that no one agent was superior to the other when applied for two minutes to the mucosa, thus

indicating that the duration of application for EMLA should be more than two minutes to obtain a greater depth of anaesthesia when compared to Xylocaine 5%. Support for this view was found in the results of study 3.3 when EMLA was found to be clearly superior to Xylocaine 10% when applied for longer periods as shown by electrical pulp testing. Results of study 3.3 showed that maximum pulpal anaesthesia occurred when EMLA was applied for up to thirty minutes, thus indicating that EMLA has the potential to anaesthetise the pulps of teeth during longer applications. Further clinical trials are indicated to evaluate the onset, duration and depth of pulpal anaesthesia following concurrent application of EMLA on the buccal and palatal/lingual aspects of teeth.

The plasma concentrations of lignocaine and prilocaine from a thirty minute application time of large quantities of EMLA were well below known toxic levels where adverse cardiovascular, central nervous system and methaemoglobin effects have been reported. This indicates that adverse systemic reactions are unlikely to occur with therapeutic applications of EMLA in the mouth.

No adverse local or systemic effects have been observed in any subject during the investigations. Most subjects also found the taste of the EMLA cream 5% to be bland, although several subjects reported a slight burning sensation when first applied, most likely due to the high pH (8.7-9.7) of EMLA. The investigator found the cream easy to apply after excess saliva had

been removed from the surface of the mucosa. EMLA adhered well to the mucosa and was conveniently localised by Stomahesive bandage although excess pressure on the bandage caused seepage of the cream from below the margins of the dressing.

From the results of the studies EMLA can be recommended for a number of clinical applications in dentistry. It would be the topical anaesthetic of choice due to its bland taste, ease of application and its efficacy in reducing the pain experience from dental needle insertion. Pilot studies have been undertaken based on the ability of the cream to achieve pulpal anaesthesia and intense anaesthesia of the mucous membrane. A clinical pilot study has been carried out to investigate EMLA as an alternative to infiltration anaesthesia for restorative procedures. In this study 0.5 mL of EMLA was occluded in the buccal sulcus of upper and lower anterior teeth that required restorations. The agent was left in place for fifteen to twenty minutes prior to high and low speed drilling. To date, a 75 per cent success rate (9/12 subjects) has been found (Appendix C1). If this success rate can be maintained then the following patient groups may benefit from this non-invasive anaesthetic technique :-

1. Haemophiliacs where the risk of haemorrhage can be avoided as there is no needle penetration into the tissues.

2. Patients with heart valve defects where prophylactic antibiotic cover may be unnecessary because of the nature of the operative procedure.

3. Patients who suffer from needlephobia.

4. The reduced risk of needle-stick injury when treating infectious patients.

A pilot study is also underway to assess the usefulness of EMLA for oral surgical procedures such as the removal of eyelet wires / archbars in jaw fracture patients and for the biopsy of lesions of the mouth. To date, three patients have undergone the removal of wires / archbars without the need for multiple local anaesthetic injections (Illustrations 11, 12 and 13) and two patients have undergone excisional biopsies on the oral mucosa without the need for an infiltration local anaesthetic (Illustrations 14, 15, 16 and 17). Although the biopsies were completed in the two cases reported, it was noted that the anaesthesia was short-lasting and the surgical field was bloodier. Both of these findings are attributable to the absence of vasoconstrictor at the surgical site. Further research should therefore be aimed at incorporating a potent vasoconstrictor, as this would certainly improve the potency of EMLA as a topical anaesthetic agent and reduce haemorrhage at the surgical site.

With the future of improved patient care in dental and medical procedures leading towards less invasive techniques, EMLA cream 5% deserves further clinical investigation for its potential applications in dentistry.

Illustration 11.

Clinical case 1. Eyelet wires imbedded through gingival tissue.

Illustration 12.

Application of EMLA to buccal gingival tissue.

Illustration 13.

Removal of wires carried out without multiple local anaesthetic injections.

Illustration 14.

Clinical case 2. Area of lichen planus situated on cheek mucosa.

Illustration 15.

Application of EMLA to lesion.

Illustration 16.

Excisional biopsy performed on lesion.

Illustration 17.

Primary closure of surgical site.

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Appendix A1.

Pain response sheet (visual analogue score and descriptive table response),

Experiment 3.1

Study: A Comparison of the Efficacy of Three Topical Anaesthetic Preparations on the Oral Mucosa.

Dr Vickers and Dr Moorthy.
Dept of Oral Medicine and Oral Surgery.

Date:.....
Subject's name:.....
Preparation code number:.....
Subject response:

1. Mark level of response on visual analogue scale-

```
+-----------------------------------+
no pain                             severe pain
```

2. Tick appropriate response:
   i) Total anaesthesia: no pain.....
  ii) Partial anaesthesia: mild to moderate pain.....
 iii) No anaesthesia: severe pain.....

Appendix A2.

Product acceptability and mucosal effects questionnaire, Experiment 3.1

Topical Anaesthetic Study

Subject's name:.....
Preparation code number: Left side..... Right side.....
Date:.....

1. List any local effects on the site of application-
Left side.....
Right side .....

2. List any systemic effects from the preparation-
.....
.....

3. Give comments regarding your acceptability to the preparation-
Left side .....
Right side .....

Appendix B1.

Journal of Dental Reseach (1990), Vol.69, No.4, p.937. (Abstract).

Vol. 69 No. 4 *Australian and New*

(33)

A Comparison of The Efficacy of Three Topical Anaesthetic Agents.

VICKERS, E.R.* and PUNNIA-MOORTHY, A. Department of Oral Surgery and Oral Medicine, Faculty of Dentistry, University of Sydney.

A double blind randomised study to compare the efficacy of Xylocaine 5% (lignocaine 5%), NUM (benzocaine 15% and amethocaine 1.7%) and EMLA 5% cream (eutectic mixture of 2.5% lignocaine and 2.5% prilocaine) was carried out on sixty volunteer subjects. Bilateral sites in the buccal sulcus adjacent to the upper premolar region were used. 0.2 mls of the agent and the placebo were placed for 2 minutes at the site of needle insertions. A standard 27 gauge needle was inserted to a depth of 5mm. Two methods of pain assessment were used; (i) visual analogue scale. (ii) descriptive table response. Mann-Whitney U test for the visual analogue scale and chi-squared test for the table response with confidence intervals of 95% were applied to test for significance.

In the first study the three agents were compared to placebo. The results of both assessment methods showed that Xylocaine 5% was significant in achieving a reduction in pain ($p<0.05$). EMLA 5% cream was significant in visual analogue scale ($p<0.05$) but not significant for the table response ($p>0.05$). NUM was significant in visual analogue scale ($p<0.05$) but not significant for table response.

A second study compared EMLA 5% cream and Xylocaine 5% for efficacy in twenty two volunteers using identical methods.

Results showed there was no significant difference in pain reduction between the two agents ($p>0.05$).

Appendix B2.

Journal of Dental Reseach (1991), Vol.70, No.4, p.650. (Abstract).

6 The Efficacy of Topical Anaesthetics to Produce Pulpal Anaesthesia. E.R. VICKERS* and A. PUNNIA-MOORTHY (Department of Oral Surgery, University of Sydney):

A single blind study was carried out to investigate the efficacy of topical anaesthetics in producing pulpal anaesthesia. Group 1 (8 male, 5 female subjects) was tested with EMLA^{TM} 5% Cream (Eutectic Mixture of 2.5% prilocaine and 2.5% lignocaine) and Group 2 (9 male, 3 female subjects) received Xylocaine 10% Special Adhesive (10% lignocaine). An electric pulp tester (Analytic Technology, U.S.A.) was used to measure pulpal response. The tester produces a pulse wave form and generates an increasing voltage (0-300 Volts) when applied to a tooth surface. It has a digital readout (0-80) representing a range 0-300V. The centre of the buccal aspect of the upper right central incisor was selected for testing and a base line response of the tooth was first established. 0.5mL of the test agent was then placed in the labial sulcus, adjacent to the tooth and kept in place by Stomahesive bandage^{TM}. Pulpal response was tested at 15 minute and 30 minute intervals. At 15 minutes, EMLA 5% Cream had produced profound pulpal anaesthesia to testing (no response at readout 80/300V) in 8 subjects, and at 30 minutes in another 4 subjects. For Xylocaine 10%, at 15 minutes, 3 subjects indicated no response to maximum setting and at 30 minutes in another 3 subjects. EMLA 5% Cream produced profound pulpal anaesthesia in 12/13 subjects tested within the intervals. This finding justifies further investigation for the use of EMLA 5% Cream in dentistry.

Appendix B3.

A.N.Z.A.O.M.S., Proceedings, 14th Clinical Meeting (1991),

Adelaide, Australia.

CLINICAL STUDIES EVALUATING THE EFFICACY OF EMLA 5% TOPICAL ANAESTHETIC CREAM

E.R.Vickers, A.Punnia-Moorthy

Department of Oral Surgery, University of Sydney.

EMLA 5% topical anaesthetic Cream (Astra Pharmaceuticals) has been shown to be effective in achieving anaesthesia of the skin for venous cannulation,harvesting of skin grafts and for the removal of genital warts. EMLA is a 1:1 oil/water emulsion of an eutectic mixture of lignocaine 2.5% and prilocaine 2.5%.

The aims of the studies were to evaluate clinical applications of EMLA in the mouth and to note any adverse effects from its application.

Study 1 compared the efficacy of EMLA 5% Cream, Xylocaine 5% (lignocaine 5%) and NUM (benzocaine 15%, amethocaine 1.7%) to placebo in reducing the pain experience during needle insertion. In a random, double blind study three groups of twenty volunteers each, had a paired topical anaesthetic/placebo placed bilaterally in the buccal sulcus of the upper premolar regions for two minutes, followed by the insertion of a standard 27 gauge needle to a depth of 5mm. Pain experience was measured with visual analogue scales. Results showed that all three agents significantly reduced pain when compared to placebo - EMLA ($p<0.002$); Xylocaine ($p<0.05$); NUM ($p<0.005$).

Study 2 examined the depth of anaesthesia from EMLA, Xylocaine 10% and placebo when applied to the oral mucosa for longer periods. This single blind study consisted of three groups, with each subject receiving 0.5 ml of test agent, applied to the buccal sulcus adjacent to the upper right central incisor. An electric pulp tester (Analytic Technology, U.S.A.) with a voltage range of 0-300 volts was used to measure the level of pulpal anaesthesia. It was found that EMLA had produced profound pulpal anaesthesia in 12/13 subjects within 30 min. as shown by electrical pulp testing. No adverse effects were observed from the studies.

Results of these studies indicate the need for further research into other possible applications of EMLA in the oral cavity.

Appendix B4.

I.A.D.R.(A.N.Z. Division), Proceedings, 31st Meeting (1991),

Brisbane, Australia.

50 Plasma Concentrations of Lignocaine and Prilocaine from Oral Mucosal Application of EMLA 5% Topical Anaesthetic Cream.

E.R.Vickers, A.Punnia-Moorthy. Department of Oral and Maxillofacial Surgery, University of Sydney.

EMLA 5% Cream (Astra Pharmaceuticals) has been shown to be effective in achieving anaesthesia of the skin for venous cannulation, harvesting of skin grafts and for the removal of genital warts. EMLA is a 1:1 oil/water emulsion of an eutectic mixture of lignocaine 2.5% and prilocaine 2.5%.

Studies carried out by the authors have shown EMLA to be superior in reducing pain experienced during needle insertion compared to Xylocaine 5% and NUM (Amethocaine 1.7%; Benzocaine 15%) topical anaesthetics. Further studies showed EMLA to produce profound pulpal anaesthesia of upper central incisors in 15-30 minutes when applied to the buccal sulcus.

Results of these studies indicated the need for measuring plasma concentrations of the anaesthetic constituents of EMLA. Venous blood samples were obtained from the decubital fossa following a 1 g application of EMLA to the oral mucosa. Levels of the anaesthetic agents were measured by High Performance Liquid Chromatography (HPLC). Results indicate low concentrations of lignocaine and prilocaine which were well below known toxic levels.

Appendix B5.

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Ref.: MS 852

12 December 1991

Dr A. Punnia-Moorthy
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2 Chalmers Street
SURRY HILLS NSW 2010

Dear Dr Punnia-Moorthy,

MS 852 - A clinical evaluation of three topical anaesthetic agents, by E.R. Vickers and A. Punnia-Moorthy.

Thank you for revising and returning your paper, 'A clinical evaluation of three topical anaesthetic agents'. It is now acceptable for publication, but as I reread the manuscript, it seemed to me that you could conveniently and meaningfully combine Figures 2a and 2b, 3a and 3b and 4a and 4b. This would make the comparisons easy to see and also would save journal space. I would also suggest more descriptive captions for these figures.

Yours sincerely,

John K. Harcourt

John K. Harcourt
Editor

Encl.

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Appendix C1.

A pilot study evaluating the efficacy of EMLA cream 5% (1 mL) in anaesthetising teeth for dental restorative procedures (including high speed and low speed drilling).

Subject	Operator	Teeth treated	Application time of EMLA (min.)	Successful
1	NM	25	15	yes
2	NM	11	20	yes
3	NM	12,13	not recorded	yes
4	LI	21	20	yes
5	PK	43	20	no
6	TT	15	22	no
7	TT	23	18	yes
8	TT	41	14	yes
9	TT	22	37	yes
10	TT	11	21	no
11	TT	12	22	yes
12	TT	21	32	yes

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