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STUDIES ON THE PHARMACOLOGY OF INJECTABLE ANAESTHETIC AGENTS

by

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SUMMARY

The development of techniques of anaesthesia, and the pharmacology of currently used injectable anaesthetic agents, have been reviewed to illustrate recent progress in medical and veterinary anaesthesia, and to identify areas where improvements in drugs and techniques could be sought.

In routine veterinary anaesthesia arterial blood pressure is rarely monitored, and the adequacy of ventilation is generally assessed by observation of the rate and depth of respiration. In this study, simple techniques, suitable for the measurement of arterial blood pressure and carbon dioxide tension in anaesthetised dogs, have been validated. Values of alveolar carbon dioxide tension, obtained with an infra-red analyser sampling end-tidal expired gas, were shown to correlate well with measurements made with a Severinghaus carbon dioxide electrode on samples of arterial blood; while the accuracy of indirect measurements of arterial pressure, made with a Newcastle infant sphygmomanometer, was shown to be dependent on the size of the occluding cuff.

The above techniques were used in a comparative study of the clinical efficacy, in dogs, of three neuroleptic and analgesic drug combinations. The incidence of excitatory side effects with all three mixtures was such that none could be considered as a reliable alternative to conventional general anaesthesia for major surgery. For use in minor procedures the combination of etorphine and methotrimeprazine produced

the most consistent analgesia, but also the greatest degree of respiratory depression. The respiratory depressant effect of fentanyl alone was shown to be greater than that produced by the combination of droperidol and fentanyl.

In medical anaesthesia, a renewed interest in techniques of total intravenous anaesthesia has been one of the consequences of recent concern about the possible deleterious effects of operating theatre pollution with waste anaesthetic gases and vapours. The profile of an ideal, short acting and non-cumulative drug has been outlined as the review of the properties of currently used injectable anaesthetic agents indicated that none could be considered to be entirely satisfactory when used to maintain anaesthesia by intravenous infusion.

Preliminary examination of the anaesthetic properties of a series of 2,6-dialkylphenols in mice and rabbits indicated that 2,6-diisopropylphenol (disoprofol) appeared to have a desirable pharmacological profile. Methods were developed to examine in detail the anaesthetic potency of this compound, its speed of onset, cumulative effect, cardiovascular and respiratory effects, and its interactions with ancillary drugs used in anaesthesia. Results obtained with disoprofol were compared with those of standard agents and the importance of using equianaesthetic doses and constant injection times was demonstrated. These results indicate that disoprofol is a rapidly acting agent with an anaesthetic potency similar to that of methohexitone. The duration of

anaesthesia produced by a single dose was short and, in marked contrast to thiopentone, minimal cumulation was seen when the drug was given by repeated injection to mice. The cardiovascular and respiratory effects produced by disoprofol in pigs were generally similar to those produced by thiopentone and 'Althesin'. A secondary pressor response followed the initial hypotensive effect of thiopentone and 'Althesin' but was not seen after the administration of disoprofol.

In human patients the anaesthetic, propanidid, has been reported to potentiate the neuromuscular blocking effects of suxamethonium. An animal model, in which this interaction can be demonstrated was developed and using this model it has been shown that a similar potentiation of the effect of suxamethonium does not occur with disoprofol, thiopentone, methohexitone or 'Althesin'.

Water solubility is accepted as a highly desirable property in an intravenous anaesthetic agent. Unfortunately disoprofol is a highly lipophilic compound which does not dissolve readily in water. This drug is currently solubilized with the aid of Cremophor EL, a surfactant which is present in the commercial preparation of propanidid and the mixture of anaesthetic steroids containing alphaxalone and alphadolone acetate.

In man a number of anaphylactoid reactions to Cremophor-containing anaesthetic agents have been reported and uncertainty still exists as to the role of the surfactant in these reactions.

An animal model has been developed, using the miniature pig, in which it has been possible to demonstrate a characteristic anaphylactoid response to Cremophor EL and Cremophor-containing anaesthetics, when a second injection has been given seven days after an initial dose. A similar response was not produced when propanidid was given in a non-Cremophor formulation, but the results obtained with alphaxalone and alphadolone suggest that the steroids and the surfactant may both contribute to the reactions to this agent seen in man. These findings indicate that Cremophor EL is unlikely to be a satisfactory agent for the formulation of disoprofol, but the results obtained in the pig appear to be sufficiently consistent to allow the model to be used for the evaluation of alternative surfactants for lipophilic intravenous anaesthetics.

INTRODUCTION

The development of new drugs for use in human anaesthesia has provided veterinary anaesthetists with the opportunity of examining the efficacy of these new agents in clinical veterinary anaesthesia. The opposite situation may also occur where the effects of new chemical compounds are first examined in experimental animals, to define the pharmacological profile of these compounds, before they are subjected, after the satisfactory completion of safety evaluation studies, to clinical trial in man.

In the selection of potential new anaesthetic agents a knowledge of the deficiencies of currently used agents and techniques is required. By comparing the pharmacological effects of new agents with those of established agents, the likely advantages of a new agent can be predicted from results obtained in a range of experimental animals.

The first part of this investigation describes the development of simple methods for the estimation of arterial blood pressure and carbon dioxide tension in anaesthetized dogs and a study of the clinical pharmacology and efficacy of some injectable anaesthetic agents which have been recently introduced into clinical veterinary anaesthetic practice. The second part is concerned with aspects of the detailed evaluation in experimental animals of a chemical compound, 2,6-diisopropylphenol, an agent which had not previously been used as an anaesthetic in man.

Over the years the basic aims of anaesthesia, i.e. to provide suitable conditions for surgery and to protect the patient from the effects of surgery, have remained unchanged. The concept of general

anaesthesia has however changed to a certain extent, largely due to evolution of new techniques and the development of new drugs, such that the components required for a satisfactory anaesthetic technique may now be provided by a number of alternative methods.

1. HISTORICAL DEVELOPMENTS IN ANAESTHESIA

1.1 Developments in medical anaesthesia

The merit of the discovery of general anaesthesia belongs to William Morton who, on October 16th, 1846, demonstrated the practicability of ether anaesthesia at Massachusetts General Hospital in Boston. In discussing the events which preceded this discovery Armstrong Davison (1965) has pointed out that, by the end of the eighteenth century, there had grown up a sufficient body of knowledge to have rendered anaesthesia practicable, had it been possible to draw the relevant facts together.

Paracelsus (von Hohenheim), who died in 1541, described the synthesis of ether in his 'Opera Medico-Chemica sive Paradoxa' which was first printed in 1605. This author even described experiments with ether: "....it has an agreeable taste, so that even chickens take it gladly and thereafter fall asleep for a long time, awakening unharmed. In view of the effect of this vitriol, I think it especially noteworthy that its use may be recommended for painful illnesses, and that it will mitigate the disagreeable complications of them." However, not until the circulation of the blood had been demonstrated by William Harvey in 1628 could there by any rational attempt at the administration of drugs by inhalation or by intravenous injection.

In the eighteenth century scientific advances continued with an increasing tempo and an important part of the scientific research of this period was directed towards work with gases. Hydrogen was isolated by Cavendish in 1766 and Joseph Priestly, closely followed by Scheele, produced oxygen in 1771 and nitric and nitrous oxide in 1772. Humphry Davy experimented with nitrous oxide and in his Researches Chemical and Philosophical, chiefly concerning Nitrous Oxide.....and its Respiration the following statement appears: "As nitrous oxide in its extensive operation appears capable of destroying physical pain, it may probably be used with advantage during surgical operations in which no great effusion of blood takes place".

The exhilarating effects of both ether and nitrous oxide became well known and were demonstrated at fairs in the United States of America by itinerant chemists. Horace Wells, a Connecticut dentist attended a demonstration of the intoxicating effects of nitrous oxide in 1844. He arranged to have one of his own teeth extracted under the influence of this gas and later satisfied himself that nitrous oxide was an effective anaesthetic by using it in a dozen cases. Wells attempted to demonstrate his technique to students and physicians at the Massachusetts General Hospital, but the patient was only partly anaesthetized and the audience were unimpressed.

William Morton witnessed the failure by Wells to produce anaesthesia with nitrous oxide and sought a more powerful agent. After consulting an eminent chemist, Charles Jackson, on the properties of ether, Morton initiated studies with this agent which culminated in his successful demonstration of anaesthesia in Boston in 1846. News of the discovery of anaesthesia spread quickly and in a short time ether was being widely used. In 1847 it was introduced into obstetric practice by Sir James Y. Simpson, Professor of Midwifery at Edinburgh. Simpson also experimented with chloroform, which had been discovered in 1831, and introduced this agent in his practice in 1847.

Nitrous oxide was later re-introduced and ether and chloroform, administered alone, or in combination with this gas remained for a time the most widely used anaesthetic agents. In 1933 Waters and his colleagues at Madison, Wisconsin introduced cyclopropane into clinical practice. Dennis Jackson of Cincinnati University re-described in 1934 the anaesthetic properties of trichloroethylene, which had first been used as an anaesthetic agent in animals by Lehmann in 1911 while working at Wurzburg University (Wylie and Churchill-Davidson, 1966). The pharmacological properties of halothane, a fluorinated hydrocarbon, were first described by Raventos (1956) and this agent, being non-flammable and stable in the presence of soda lime, rapidly achieved considerable popularity as an inhalation anaesthetic. Another fluorinated hydrocarbon, methoxyflurane, was first studied by Van Poznak

and Artusio (1960) and more recently the methyl ethyl ether, enflurane, has been introduced into clinical practice (Virtue, Lund, Phelps, Vogel, Beckwitt and Heron, 1966). Isoflurane, an isomer of enflurane, has been examined in animals (Byles, Dobkin, Ferguson and Levy, 1971) and in limited human studies (Dobkin, Byles, Ghanooni and Valbuena, 1971; Stephens, Cromwell, Halsey, Eger, Shakespeare and Bahlman, 1971) but has not yet been widely used in patients.

By 1853 the stage had been set for the development of intravenous medication by Alexander Wood of Edinburgh when he combined the hollow hypodermic needle developed by Francis Rynd, a Dublin surgeon, with a glass syringe with a tapered nozzle developed in the same year by Charles Gabriel Pravaz of Lyons (Dundee and Wyant, 1974). Intravenous anaesthesia was first undertaken in 1872 by Ore of Bordeaux with chloral hydrate, but the first successful use of this route depended on the development of the bariturate group, first synthesized by Fischer and von Mering in 1903. In 1932, Weese and Scharpff introduced hexobarbitone and this was quickly followed by thiopentone, introduced by Lundy at the Mayo Clinic in 1934 (Armstrong Davison, 1965). Despite some competition from other barbiturates, thiopentone has remained essentially unchallenged among the intravenous anaesthetics. Of the many other barbiturates examined, methohexitone, which was first given to human patients by Stoelting (1957), has been the only barbiturate to compete

with thiopentone and only in recent years have any practical alternatives to the barbiturates appeared. Propanidid was the first eugenol derivative to be used on a wide scale in anaesthesia. An early clinical comparison of propanidid with methohexitone and thiopentone in man soon established its main properties (Dundee and Clarke, 1964). A mixture of two steroids, alphaxalone and alphadolone acetate, was first given to human patients by Campbell, Forrester, Miller, Hutton, Kennedy, Lawrie, Lorimer and McCall (1971). An imidazole derivative, etomidate, is the latest intravenous induction agent to be introduced into clinical practice (Doenicke, 1974).

The first neuromuscular blocking drug to be used in anaesthesia was a relatively crude extract of curare (Griffith and Johnson, 1942). Gray and Halton (1946) described a series of over 1,000 patients to whom a pure crystalline alkaloid, d-tubocurarine chloride, had been administered. A significant milestone in the development of anaesthesia was the introduction by Rees and Gray (1950) of the concept which views anaesthesia as a triad, the components of which, narcosis, relaxation and analgesia, ideally are provided by specific drugs with selective actions. The technique, whereby thiopentone was used to induce sleep, muscular relaxation was produced with a neuromuscular blocking drug, and the response of the patient to noxious stimuli was reduced by the use of nitrous oxide and occasionally a more potent specific

analgesic drug, was described in more detail by Gray and Rees (1952).

Techniques aimed at the production of sedation or light sleep with intense analgesia were first devised in France between 1952 and 1954. A 'lytic cocktail' combining a potent analgesic and two tranquilizers was used and the resulting state was termed 'artificial hibernation'. De Castro and Mundeleer (1959) introduced the term 'neuroleptanalgesia' for a similar technique which combined the neuroleptic properties of certain butyrophenones with the profound analgesia produced by potent short acting analgesics. Recent developments in the use of both neuroleptics and analgesics have followed the trend set by Brown, Horton and Macrae (1963) and have been directed toward their inclusion with nitrous oxide and a muscle relaxant as part of a balanced anaesthetic technique.

Ketamine, a phenylcyclohexylamine derivative, was first given to human patients by Corssen and Domino (1966). These authors described the effect as "dissociative anaesthesia" as the drug produced a state characterized by catalepsy, amnesia and marked analgesia, with only superficial sleep. Anaesthesia with ketamine is accompanied by a high frequency of undesirable side effects, and emergence excitement and unpleasant dreaming have caused this drug to retain only a limited place in human anaesthetic practice (Dundee, Knox, Black, Moore, Pandit, Bovill, Clarke, Love, Elliot and Coppel, 1970).

The most recent development in the use of injectable anaesthetic agents has been the investigation of techniques for the maintenance of anaesthesia by the infusion of intravenous anaesthetic agents (Du Cailar, 1972; Savage, Ramsey, Curran, Cotter, Walling and Simpson, 1975; Mocavero, Gregoretti and Bosatra, 1976; Park and Wilson, 1978). One stimulus to developments in this direction has been the increasing concern in recent years that pollution of the operating room atmosphere with anaesthetic gases and vapours may have harmful effects on operating room personnel.

1.2 Developments in veterinary anaesthesia

General anaesthesia was first induced in animals by the inhalation of ether. Almost immediately after the publication of Morton's work on the administration of ether to human beings this agent was adopted as an anaesthetic for animals in the Royal Veterinary College, London. The introduction of chloroform as an anaesthetic in man was followed by its application in the horse throughout the United Kingdom (Hall, 1971).

Chloral hydrate was one of the earliest"basal narcotic" agents to be introduced into veterinary practice and this agent is still widely used in equine practice. Pentobarbitone was used as a general anaesthetic in veterinary surgery in America and Kreutzer (1931) and Haigler (1931) were amongst the first to record its use. In this country the use of pentobarbitone was described by Wright (1934). The drug was initially administered orally or by intraperitoneal injection and

entirely consistent results could not be obtained. Auchterlonie (1934) administered hexobarbitone to animals by intravenous injection and the later use of this route for the administration of pentobarbitone was one of the factors which made possible a considerable increase in general surgical work. The use of thiopentone for animal anaesthesia was first recorded in America by Sweebe (1936) and it has since been extensively employed throughout the world.

A technique of balanced anaesthesia for the dog and cat, using a combination of inhalation and injectable anaesthetic agents, was described by Hall and Weaver (1954) and Campbell and Lawson (1958) examined in detail the signs by which the level of anaesthesia in animals could be judged. Halothane was introduced into veterinary anaesthesia by Hall (1957) and Fisher and Jennings (1961) described its respiratory effects in cattle, horses, sheep and dogs.

In more recent years the development of new agents for use in human anaesthesia has continued to be followed by an examination of their utility in veterinary practice.

2. METHODS USED IN CLINICAL PHARMACOLOGICAL STUDIES OF INJECTABLE ANAESTHETICS IN DOGS

2.1 Indirect blood pressure measurement

Detailed investigations of the haemodynamic effects of new anaesthetic agents have in the past been restricted to studies in experimental animals. In early studies with phencyclidine (Chen, Ensor, Russell and Bohner, 1959), fentanyl and droperidol (Yelnosky and Gardocki, 1964) and with ketamine (McCarthy, Chen, Kaump and Ensor, 1965) blood pressure in a cannulated carotid or femoral artery was measured in anaesthetised dogs with a mercury manometer. The latter authors also examined the haemodynamic effects of ketamine in conscious dogs and measured arterial blood pressure in a previously isolated, skin covered, carotid loop with a strain gauge transducer. In an experimental study of the cardiovascular effects of etomidate, propanidid and thiopentone Patschke, Bruckner, Gethmann, Tarnow and Weymar (1975) used a pressure transducer to measure blood pressure in a cannulated femoral artery in dogs initially anaesthetised with piritramide. Blane, Boura, Fitzgerald and Lister (1967) also used a pressure transducer and cannulated an artery in pentobarbitone anaesthetised dogs in an experimental study of the actions of etorphine hydrochloride.

Although direct measurement of blood pressure, involving some degree of surgical intervention, can readily be employed in experimental situations, the use of this technique on a routine basis cannot be justified in investigations performed on clinical cases. Methods of

indirect blood pressure measurement are frequently employed in clinical studies in man but it is noteworthy that the early clinical studies with neuroleptanalgesic mixtures in dogs (Yelnosky and Field, 1964; Soma and Shields, 1964; Marsboom et al, 1964; Franklin and Reid, 1965; Crooks, Whiteley, Jenkins and Blane, 1970) provide no detailed information on the effects of the various drugs investigated on arterial blood pressure. The most probable explanation for this omission is the fact that techniques of indirect blood pressure measurement have not become established as part of the routine monitoring procedure during clinical veterinary anaesthesia. Numerous methods for indirect measurement of blood pressure in dogs have been described but technical difficulties and the inaccuracy inherent in many of the methods employed have prevented their more widespread use. The development of a simple and satisfactory technique for the indirect measurement of blood pressure was one of the objectives of this study.

2.2 Measurement of end-tidal carbon dioxide concentration

As a number of drugs used in anaesthetic practice are known to reduce metabolic rate and oxygen consumption (Jennett, 1968) measurement of alveolar ventilation alone may provide an inaccurate assessment of the degree of any respiratory depression present. With drugs which reduce oxygen consumption and carbon dioxide production, a reduced level of alveolar ventilation may be adequate to maintain a normal arterial carbon dioxide tension.

In this situation the direct measurement or estimation of arterial carbon dioxide tension provides a more accurate assessment of the adequacy of ventilation.

Although only a small and statistically insignificant reduction in basal metabolic rate was produced by the administration of a neuroleptanalgesic mixture containing fentanyl and droperidol in a study in human patients (Forbes, Ovassapian, Smith and Wollman, 1967) a 45 to 50 per cent reduction in oxygen consumption has been reported with neuroleptanalgesic techniques in dogs (Gemperle, 1965). While fentanyl has been shown to have little effect on heart rate in man (Prys-Roberts and Kelman, 1967) a marked reduction in heart rate to less than 50 per cent of control values has been noted in dogs (Marsboom et al., 1964). A marked reduction in myocardial oxygen consumption would be expected to accompany this degree of bradycardia in the dog and this factor may partly explain the conflicting results in the two species.

The method most frequently used for the measurement of arterial carbon dioxide tension ($PaCO_2$) involves electrometric measurement using a carbon dioxide electrode of the type developed by Severinghaus and Bradley (1958). The collection of arterial blood samples requires either repeated needle puncture of an artery or the placement of a catheter in an artery. The latter method is frequently employed in experimental studies and arterial blood samples

were collected in a study of the respiratory effects of neuroleptanalgesic mixtures in dogs reported by Short, Greenwald and Bendick (1970). It is rarely possible to collect numerous arterial samples during investigations performed on clinical cases and a method for the indirect measurement of $PaCO_2$ is required in this situation.

It is usually assumed that the average PCO_2 in alveolar gas equals that in arterial blood as the alveolar-capillary membrane offers no significant obstruction to the diffusion of carbon dioxide. Many investigators have found $PaCO_2$ to be nearly equal to that found in expired gas at the end of expiration (Suskind and Rahn, 1954; Severinghaus, Stupfel and Bradley, 1957; Evans, Hogg and Rosen, 1977; Littlejohn, 1969). Severinghaus and Stupfel (1957) noted an average arterial-alveolar PCO_2 difference of 3mmHg in normal dogs without anaesthesia or artificial respiration. In anaesthetised dogs these authors found that the arterial-alveolar PCO_2 difference followed closely any changes in alveolar dead space.

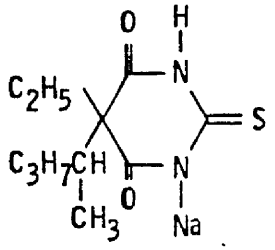
As the measurement of $PaCO_2$ in dogs has in the past been restricted to experimental studies, one objective of the present study was to assess the accuracy of end-tidal PCO_2 measurement in dogs as a method for the estimation of arterial PCO_2 during clinical anaesthesia when using a variety of anaesthetic techniques.

3. PHARMACOLOGICAL PROPERTIES OF CURRENTLY USED INJECTABLE ANAESTHETIC AGENTS

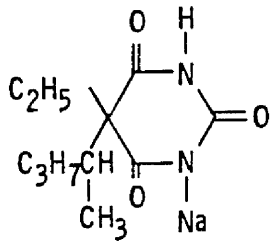
In any assessment of the utility of new anaesthetic compounds it is necessary to have a knowledge of the properties of, and the conditions provided by, established agents. A brief review will be provided of the pharmacological properties of the most widely used injectable anaesthetic agents and some of the drugs which have been more recently introduced into anaesthetic practice, and are relevant to the studies described in this investigation. The chemical structures of the compounds discussed are shown in Fig. 1.

3.1 Thiopentone sodium: sodium 5-ethyl-(1-methylbutyl)-2-thiobarbiturate

Thiopentone was the first thiobarbiturate to be used as an anaesthetic and is still the one in most widespread clinical use. It is a rapidly acting agent and the time taken to produce loss of consciousness is essentially the vein-to-brain circulation time. Thiopentone has a dissociation constant (pKa) of 7.4 (Bush, 1961) so that about 50% of the drug in blood is present in the non-ionised form. The non-ionised fraction has a high lipid solubility which allows it to pass rapidly into the brain (Price, Dundee and Conner, 1957; Bush, Berry and Hume, 1966).



Thiopentone Sodium



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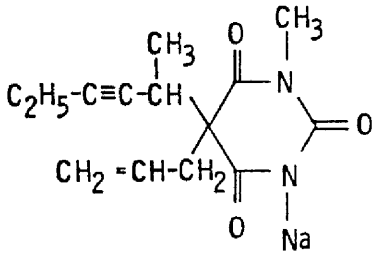
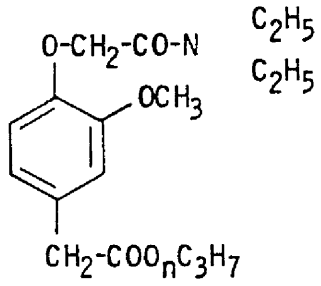


Methohexitone Sodium



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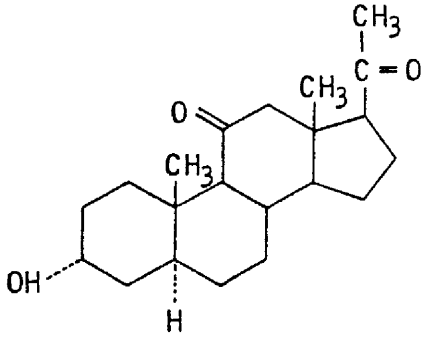
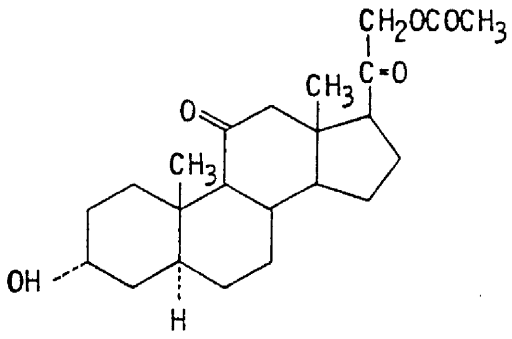
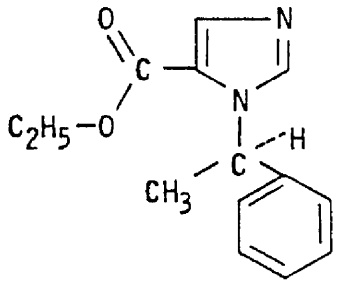


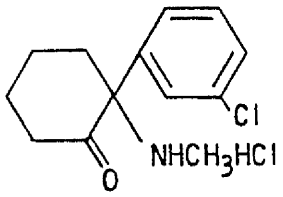
Alphaxalone



Alphadolone Acetate



Etomidate



Ketamine Hydrochloride

Fig. 1 Chemical structures of currently used injectable anaesthetics

It has been observed that when a large dose of thiopentone is used for the induction of anaesthesia, recovery of consciousness occurs at a greater plasma drug concentration than after a small dose (Dundee, Price and Dripps, 1956). This 'acute tolerance' is probably the explanation for the clinical observation that recovery from the rapid injection of a given dose of thiopentone occurs more quickly than would be the case if the same dose were to be given more slowly.

The immediate uptake of thiopentone by the brain is accompanied by a similar rapid uptake in other well perfused non-nervous, tissues such as the liver and kidneys and the plasma concentration falls quickly. The amount of drug reaching the brain is thus decreased and as the brain content falls anaesthesia lightens. With the exception of muscle and fat, the maximum tissue concentration of thiopentone is reached within one minute of a single intravenous injection. Equilibrium with muscle tissue is not attained until 15 minutes after injection, and despite its high affinity for fat, because of the poor blood supply, uptake of the drug in adipose tissues is slow and maximum deposition only occurs after about 2½ hours (Price, Knovat, Safer, Conner and Price, 1960). By this time the content of thiopentone in fat greatly exceeds that of other tissues and body fat content may be a factor in determining the duration of action of large doses of thiopentone.

When thiopentone is given by intermittent injection or infusion, the concentration in some tissues approaches equilibrium with the thiopentone in blood. Thus the body gradually loses its ability to remove thiopentone from the brain and recovery following the administration of large doses becomes more

dependent on metabolic degradation of the drug. This is a slow process as only 10-15 per cent of the drug in the body is metabolised per hour in man, and five per cent per hour in the dog (Brodie, Mark, Papper, Lief, Bernstein and Rovenstine, 1950). Thus recovery from thiopentone occurs when a large amount of drug remains unchanged in the body and a cumulative effect is observed after repeated doses. In a recent study of the pharmacokinetics of thiopentone in human patients (Christensen, Andreasen and Jansen, 1980) terminal serum half-lives were found to range from 4.5 - 22 hours in women and 4 - 8 hours in men following a single induction dose.

Thiopentone produces a progressive depression of the central nervous system and respiratory depression is marked, as the sensitivity of the respiratory centre to carbon dioxide is depressed in proportion to the depth of narcosis (Patrick and Faulconer, 1952).

A variable degree of hypotension is the usual sequel to the injection of thiopentone. Price, Dundee and Conner (1957) found a linear relationship between blood thiopentone concentration and the degree of arterial hypotension in man.

Thiopentone, like all barbiturates, has little, if any, analgesic action and reflex responses to stimuli are not abolished until an appreciably greater depth of anaesthesia is reached than is required with such agents as ether. With the barbiturates the clinical depth of anaesthesia is related to the intensity of the surgical stimulus as well as the degree of cerebral depression. In human patients Clutton-Brock (1960) and Dundee (1960) demonstrated an increased sensitivity to pain following the administration of subnarcotic doses of thiopentone.

An apparent increase in the sensitivity of the laryngeal reflexes also occurs under light thiopentone narcosis. This is probably due more to a failure of the barbiturate to depress the laryngeal reflexes rather than to any stimulant action.

Aqueous solutions of thiopentone are strongly alkaline (pH 10.5) and therefore irritant to tissues. If enough of the solution is placed extravenously it may cause necrosis of the subcutaneous tissue. Intra-arterial injection of thiopentone produces arterial spasm and thrombosis of the artery or its distal terminations may occur (Kinmonth and Shepherd, 1959).

The popularity of thiopentone is a consequence of the effectiveness of the drug in rapidly producing anaesthesia, the onset of which is usually smooth and unaccompanied by any movement or other excitatory side effects. One contraindication to the use of the drug in man is the condition of porphyria, where, in the latent stage of the disease, any barbiturate administration may cause an acute exacerbation with porphyrinuria, paralysis and frequently death.

Thiopentone is widely used in all of the domestic animal species and few absolute contraindications to its use exist. Although porphyria has been described in cats (Tobias, 1964) it is probably a very rare condition. The duration of effect is generally predictable in dogs but in a breed such as the greyhound, which has very little body fat, an impaired ability to reduce the concentration of thiopentone may cause narcosis to be prolonged (Stevenson, 1958; Bogan, 1970).

Significant disadvantages of thiopentone are its cumulative effect on repeated administration which limits its use to the induction of anaesthesia, its lack of analgesic properties, and its irritant effects following perivascular or intra-arterial injection.

3.2 Pentobarbitone sodium: sodium 5-ethyl-5-(1-methylbutyl)-barbiturate

Pentobarbitone is less lipid soluble than thiopentone and takes longer to cross the blood-brain barrier (Brodie, Kurz and Schanker, 1960). Hermann and Wood (1952) found that in rats the duration of narcosis was unaffected by the amount of body fat present and that the removal of pentobarbitone from plasma depended primarily on the rate of metabolic breakdown of the compound. In the dog the plasma concentration of pentobarbitone is reduced at a rate of 10-15 per cent per hour whereas in man the equivalent figure is about 4 per cent per hour (Brodie, Burns, Mark, Lief, Bernstein and Papper, 1953). The duration of anaesthesia in both man and animals is significantly greater than that produced by an equivalent dose of thiopentone.

Pentobarbitone is non-irritant if injected perivascularly but in other respects, including its effects on respiration and circulation, it has pharmacological properties similar to those of thiopentone.

In man, pentobarbitone is seldom used as an anaesthetic agent but it may be employed as a pre-operative sedative. In veterinary practice pentobarbitone continues to have some limited use as a sole anaesthetic agent in situations where facilities are not available to allow inhalation techniques to be used.

Difficulty in accurately controlling the depth of anaesthesia and a prolonged recovery period are the major disadvantages of pentobarbitone anaesthesia.

3.3 Methohexitone sodium: sodium α-(±)-5-allyl-1-methyl-5-(1-methyl-2-pentynyl)barbiturate

The properties of methohexitone in man have been described by Taylor and Stoelting (1960). It shares many of the properties of thiopentone but is about three times as potent and has a shorter duration of action. Respiratory depression is present at effective anaesthetic doses and may be greater than with equivalent doses of thiopentone. Laryngospasm and coughing are no more frequent than after other barbiturates but the incidence of hiccough is greater. Muscle movement and tremor are often reported as a complication of its use and their frequency and severity appear to be related to the premedication used. Moore and Dundee (1961) noted that the incidence of excitatory phenomena was reduced by premedication which had an analgesic component and was increased by premedication which increased sensitivity to somatic pain.

The administration of methohexitone produces pain at the site of injection in a high proportion of patients but the incidence of vascular damage is low and no untoward sequelae have followed its extravenous or intra-arterial injection.

The lower fat solubility of methohexitone, in comparison with thiopentone seems to be associated with less extensive localization of the drug in fat (Brand, Mark, Snell, Vrindten and Dayton, 1963) and Clarke and Dundee(1966) have confirmed that it is less cumulative than thiopentone when both drugs are given by intermittent injection. Full recovery after methohextione anaesthesia also occurs more rapidly than with thiopentone (Barry, Lawson and Davidson, 1967). This is supported by the finding of a relatively short elimination half-life of 70-125 minutes for methohexitone (Breimer, 1976), which indicates that the rate of hepatic metabolism is considerably greater than that of thiopentone.

Methohextione has been used in both large and small domestic animals and in general the anaesthetic properties of this agent are similar to those described in man. Unfortunately, in animals the recovery period is often complicated by muscle tremors or even frank convulsions (Fowler and Stevenson, 1961). These undesirable features are not seen if the animal is allowed to recover quite undisturbed, or if pethidine or a phenothiazine ataractic drug is used for premedication.

In comparison with thiopentone, the shorter duration of anaesthesia and the more consistent rapid awakening provided by methohexitone are significant advantages. Pain on injection and a high frequency of excitatory phenomena are the main disadvantages associated with the use of this compound.

3.4 Propanidid: propyl 4-diethyl-carbamoylmethoxy-3-methoxyphenyl acetate

Propanidid, a eugenol derivative, was the first non-barbiturate to be used widely as an intravenous anaesthetic agent. Though propanidid is an oil, a five per cent solution miscible with water can be achieved by dissolving it in a 20 per cent solution of the non-ionic surface active agent Cremophor EL. More recently Micellophor, a 16 per cent solution of the hydrophobic fraction of Cremophor EL has been used as the solvent.

The pharmacological properties of propanidid in animals have been described by Wirth and Hoffmeister (1965). Anaesthesia is induced in the vein-to-brain circulation time and potency is similar to that of thiopentone. The duration of anaesthesia is very short and animals return to normal rapidly. Blood pressure falls during the first few minutes, mainly as a result of peripheral vasodilatation, but usually returns to normal within 3 to 10 minutes. The early stages of anaesthesia are accompanied by hyperventilation which is followed in the higher doses by a period of apnoea. There is no evidence of analgesia or anti-analgesia, though propanidid has a definite local anaesthetic action when injected into tissues.

In the dog propanidid produces an anaphylactoid response with the release of histamine and other vaso-active

substances. Lorenz, Meyer, Doenicke, Schmal, Reimann, Hutzel and Werle (1971b) have demonstrated that the surface active agent, Cremophor EL, is responsible for this reaction.

Propanidid has not been used on a wide scale in veterinary anaesthesia because of the above response in the dog and because of its brevity of action in other species which makes anaesthesia very difficult to control.

Early studies with propanidid in man (Dundee and Clarke, 1964; Howells, Odell, Hawkins and Steane, 1964) indicated that the induction dose of 4.1 mg kg^{-1} was similar to that required with thiopentone. These workers also demonstrated that the duration of anaesthesia with propanidid was shorter than with thiopentone, with a more rapid return to clear headedness, and that no cumulation of effect occurred with repeated doses. Propanidid induced fewer excitatory phenomena than methohexitone but caused more postoperative emetic sequelae than methohexitone and thiopentone, when used with nitrous oxide:oxygen in patients undergoing gynaecological operations.

Respiratory effects in man were similar to those predicted from animal studies. There is general agreement than in man a 4 mg kg^{-1} dose of propanidid produces a minor degree of hypotension similar to that seen with the same dose of thiopentone. Above a dose of 8 mg kg^{-1} of propanidid the frequency and severity of hypotensive episodes increase beyond clinically acceptable levels

(Clarke, 1974). There is some electrocardiographic evidence (Johnstone and Barron, 1968) that an initial quinidine like cardiac depression may contribute to the hypotensive effect.

In a comparative study with thiopentone, methohexitone and Althesin (alphaxalone:alphadolone acetate), propanidid was found to produce a higher incidence of phlebitis and thrombophlebitis than the other three agents (Carson, Alexander, Hewitt and Dundee, 1972).

The extremely short duration of action of propanidid in man is the result of rapid hydrolysis of the active agent by plasma cholinesterase (Doenicke, Krumey, Kugler and Klempa, 1968).

Two features of propanidid anaesthesia will be discussed in more detail in later sections. The first is the interaction between propanidid and suxamethonium. Clarke, Dundee and Daw (1964) confirmed an earlier observation that a period of apnoea and respiratory depression produced by suxamethonium was greater following induction of anaesthesia with propanidid than was the case when thiopentone was the anaesthetic agent. The second is the occasional occurrence of hypersensitivity reactions, characterized by marked hypotension and cutaneous vasodilatation, following induction of anaesthesia with propanidid (Larard, 1970; Bradburn, 1970).

3.5 Alphaxalone and alphadolone acetate ('Althesin')"Saffan"

Although Selye (1941) demonstrated that certain steroids possess anaesthetic properties in animals it was not until hydroxydione was introduced by Laubach, P'an and Rudel (1955) that a steroid was used on a wide scale as an intravenous induction agent in man (Murphy, Guadagni and De Bon, 1955). Hydroxydione produced anaesthesia which was followed by smooth recovery but it had several disadvantages. The dose was large, induction of anaesthesia was slow and it produced a high incidence of thrombophlebitis in the injected vein (Robertson and Wynn Williams, 1961). The pharmacological properties in animals of the steroid combination Althesin were first reported by Child, Currie, Davis, Dodds, Pearce and Twissell (1971) and with this mixture the disadvantages of hydroxydione appear to have been avoided.

Althesin contains 9 mg ml^{-1} 3α-hydroxy-5α-pregnane-11, 20-dione (alphaxalone) and 3 mg ml^{-1} 21-acetoxy-3α-hydroxy-5α-pregnane-11,20-dione (alphadolone acetate) in an aqueous formulation containing 20 per cent of the surface-active agent Cremophor EL. In animals Child et al (1971) found that Althesin produced immediate induction of anaesthesia of short duration with rapid and uncomplicated recovery. It had a high therapeutic index (LD_{50}/ED_{50}) and was free from vascular irritation effects even when administered intra-arterially. In mice, repeated doses of Althesin were almost non-cumulative in marked contrast to thiopentone.

In a detailed comparative study of the cardiovascular and respiratory effects of intravenous anaesthetics in cats (Child, Davis, Dodds and Twissell, 1972) Althesin was found to produce less respiratory depression than other agents. The cardiovascular effects of Althesin were characterized by an initial short lasting tachycardia and fall in aortic blood pressure succeeded by a secondary depressor response. Althesin in a dose of 9.6 mg kg^{-1}* exerted a hypotensive effect similar to that produced by a comparable dose of thiopentone (24 mg kg^{-1}).

Evans, Aspinal and Hendy (1972) described the first clinical use of Althesin in cats by the intravenous or intramuscular routes. They concluded that a dose of 9 mg kg^{-1} induced anaesthesia of 10 to 12 minutes, sufficient for castration or dental procedures. The required level of anaesthesia could be maintained for longer periods with small supplementary intravenous doses as required. Satisfactory results following intramuscular injection were only obtained when a technique was adopted which ensured that the drug was not deposited into the fascial tissues surrounding muscles.

Although the effects of Althesin in horses, sheep and pigs have been investigated (Hall, 1972), the clinical use of the drug in veterinary practice has been largely restricted to its use in cats. Althesin cannot be safely used in dogs because of the ability of the solubilizing agent, Cremophor EL, to produce marked histamine release and hypotension in this species.

*total steroid dose

The use of Althesin in human patients was first described by Campbell et al (1971) and dose-response effects were subsequently investigated by Clarke, Montgomery, Dundee and Bovill (1971). The drug proved to be an effective and rapidly acting induction agent. The most suitable dose in adults was found to be 0.6 to 0.72 mg kg^{-1} and with this dose minimal effects on respiration were seen. Hypotensive effects at induction were similar to those seen with equivalent doses of thiopentone. Recovery was rapid and there was a low incidence of postoperative nausea and vomiting.

Slight muscle movement or tremor is seen in about 20 per cent of patients (Clarke, Dundee and Carson, 1972) but this occurs less frequently and is less marked than after an equivalent dose of methohexitone.

The lack of cumulative effect with Althesin suggests that the steroids are rapidly inactivated or eliminated from the body. Studies in the rat (Card, McCulloch and Pratt, 1972; Child, Gibson, Harnby and Hart, 1972) have shown that the plasma half life of ^{14}C-alphaxalone is six to eight minutes with the liver being the main site of metabolism. Approximately 80 per cent of the administered radioactivity was excreted in the bile during the first 3 hours after administration and subsequently appeared in the faeces. A later study with ^{14}C alphaxalone indicates that, in man, the major route of excretion is via the urine (Strunin, Strunin, Knights and Ward, 1977). These studies with radiolabelled drug did not distinguish between alphaxalone

and the metabolites formed. Recent work using a gas-liquid chromatographic method for the analysis of alphaxalone defined a plasma elimination half life of alphaxalone of 34 minutes (Simpson, 1978).

For a number of years Althesin was a popular induction agent in man, particularly for out patient procedures where rapid recovery from its effects was a distinct advantage. More recently its popularity has declined as a significant number of hypersensitivity reactions have been associated with its use (Clarke, Dundee, Garrett, McArdle and Sutton, 1975; Dodman, 1980).

3.6 Etomidate: R-(+)-ethyl-1-(α-methyl-benzyl)imidazole-5-carboxylate

Etomidate, the most recently introduced intravenous anaesthetic, has been more widely used in man in Europe than in the United Kingdom. Its animal pharmacology has been described by Janssen, Niemegeers, Schellekens and Lenaerts (1971) and Janssen, Niemegeers and Marsboom (1975). The drug is water soluble and was originally used as the sulphate salt. In rats etomidate is about 6 times more potent than methohexitone and 20 times more potent than thiopentone and propanidid. The therapeutic index of etomidate is also greater than that of the other three agents investigated and is similar to that of Althesin (Child et al, 1971).

Induction of anaesthesia with etomidate is rapid, duration of hypnosis is dose dependent, and recovery from its effects occurs very rapidly. In dogs anaesthesia was induced with a dose of 0.75 mg kg^{-1} etomidate and in this species myoclonic contractions and tremors were occasionally observed.

In a comparative study of the cardiovascular effects of intravenous anaesthetics Patschke et al (1975) found that in dogs, anaesthetised with piritramide and nitrous oxide, etomidate produced less alteration of cardiovascular haemodynamics than equivalent doses of thiopentone and propanidid. Dogs given propanidid were pretreated with an antihistamine drug to prevent the effects of histamine release produced by the solubilizing agent, Cremophor EL.

An early study with etomidate in man (Doenicke, 1974) confirmed that the drug produced very minor circulatory effects and little effect on respiration. A dose of 0.3 mg kg^{-1} was recommended for induction of anaesthesia and the duration of action of this dose corresponded to that following methohexitone 1.5 mg kg^{-1}. Uncontrolled and uncoordinated spontaneous movements were noted in 30 per cent of the patients in this study and pain on injection was also encountered.

Minimal cardiovascular and respiratory effects, and similar side effects, were also reported by Morgan, Lumley and Whitwam (1975). Attempts to modify the effects of etomidate by changes in formulation and opiate premedication (Holdcroft, Morgan, Whitwam and Lumley, 1976; Hendry, Miller and Lees, 1977; and Zacharias, Dundee, Clarke and Hegarty, 1979) have not been entirely successful in eliminating the undesirable side effects of pain on injection and myoclonic movements.

Etomidate has been given by repeated injection to maintain sleep in human patients and little evidence of cumulation has been seen (Kay, 1976). Van Hamme, Ghoneim and Ambre (1978) found a plasma elimination half life of 4.6 ± 2.6 hours in patients given a single dose of 0.3 mg kg^{-1} etomidate.

Etomidate has not yet been used to any significant extent in veterinary practice.

3.7 Dissociative anaesthetics

The characteristic property of dissociative anaesthetics which differentiates them from conventional anaesthetics is their ability to produce a state of catalepsy (Chen, 1965).

The first study in man with ketamine was described by Corssen and Domino (1966). In suggesting that the state induced by ketamine should be called 'dissociative anaesthesia' these authors believed that sensory input might reach cortical receiving areas but failed to be perceived in some of the association areas because

these were depressed by the drug.

Ketamine as the hydrochloride salt, is water soluble and was found to be well tolerated by tissues following intravenous or intramuscular administration. Surgical anaesthesia was established about 30 seconds after completion of the intravenous injection of doses of 1.0 to 2.0 mg kg^{-1} and five to eight minutes after the intramuscular injection of doses of 4 to 11 mg kg^{-1}. Administration of ketamine resulted in an increase in arterial pressure in the majority of adult patients. A brief period of respiratory depression followed induction of anaesthesia but respiratory exchange rapidly returned to normal. Protective pharyngeal and laryngeal reflexes were present during the course of anaesthesia and skeletal muscle tone was usually increased. Ketamine produced good analgesia of the extremities and skeleton but was less effective in blocking responses to visceral pain. Vivid dreaming, with or without psychomotor activity, was experienced by a number of adult patients but did not occur in infants and children.

Although ketamine has been described as a rapidly acting general anaesthetic, the rate of onset is slower than after methohexitone (Bovill, Coppel, Dundee and Moore, 1971). Plasma decay curves show a biphasic or triphasic decline with an initial fast phase up to about 45 minutes and a more slowly declining phase up to 12 hours.

The plasma elimination half life was found to be 2.5 hours in one study in human patients (Wieber, Gugler, Hengstmann and Dengler, 1975) and 3 hours in another (Clements and Nimmo, 1981).

Ketamine has now been widely studied in man and in general the initial clinical impressions have been confirmed. Knox, Bovill, Clarke and Dundee (1970) found that the chief complications at induction were hypertension and hypertonus, the incidence of which was only broadly related to dosage. Recovery of consciousness was rapid but marred by a high incidence of delirium at all dose levels. Hallucinations at this stage were so unpleasant that patient acceptance was much lower than with the barbiturates. Subsequent reports have been mainly concerned with attempts to minimise the undesirable side effects of ketamine with a variety of premedicant drugs. Pentobarbitone and droperidol were found to have little effect in improving the quality of the recovery period (Sadove, Hatano, Redlin, Thomason, Arastounejad and Roman, 1971).

Ketamine has retained more popularity in the United States of America than in the United Kingdom and this may be a reflection of differences in philosophy and standards of acceptability in the two countries. The desirable properties of the drug, in particular its efficacy by both the intravenous and intramuscular routes, its good analgesia, and the maintenance of laryngeal and pharyngeal reflexes, are such that it is likely to retain

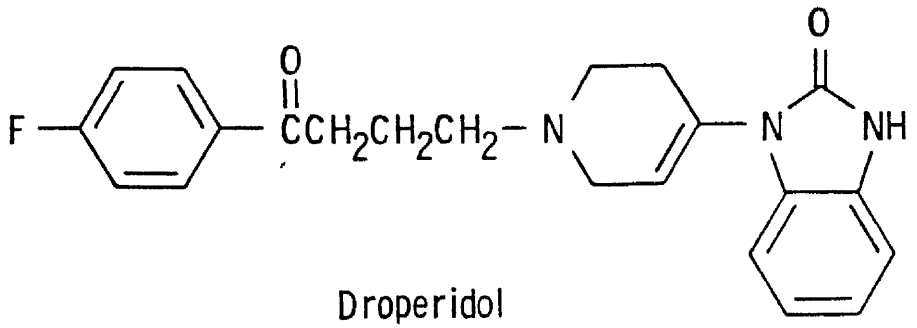
a limited place in anaesthetic practice. In children, where emergence problems are rarely encountered, it has been found to be particularly valuable for repeated burn dressings (Corssen and Oget, 1971) and cardiac catheterization studies (Coppel and Dundee, 1972). The drug may also find a place as an analgesic when given in subanaesthetic doses. In a recent experimental study, Grant, Nimmo and Clements (1981) found that pain tresholds were elevated 15 and 30 minutes after the intramuscular injection of 0.5 mg kg^{-1} ketamine. With this dose all subjects remained conscious and orientated.

In veterinary practice ketamine has been used in sheep (Taylor, Hopkins, Young and McFadyen, 1972), in pigs (Thurmon, Nelson and Christie, 1972), in cattle (Fuentez and Tellez, 1974), in horses, in combination with xylazine (Brouwer, Hall and Kuchel, 1980) and in cats. The use of the drug as an intramuscular anaesthetic for cats was first reported in America by Commons (1970) and Beck, Coppock and Ott (1971). The latter authors summarized the results of six separate clinical trials involving 442 cats, and found that doses under 22 mg kg^{-1} ketamine produced basic chemical restraint, whereas doses of 22 to 44 mg kg^{-1} provided cataleptoid anaesthesia.

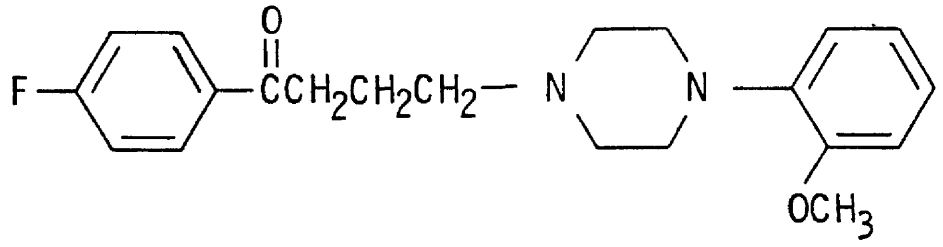
3.8 Neuroleptanalgesic drug combinations

The term 'neuroleptanalgesia' describes a neurophysiological state produced by the combination of a neuroleptic drug with an analgesic drug. This type of combination involving the use of a phenothiazine tranquilizer with an opiate analgesic has been a common component of balanced anaesthesia techniques for a number of years. The term has, however, come into more prominent use in recent years due to the development of more potent tranquilizers and analgesics and by using certain combinations it has been claimed that the neuroleptic component can potentiate the effect of the analgesic agent used (Yelnoski and Gardocki, 1964).

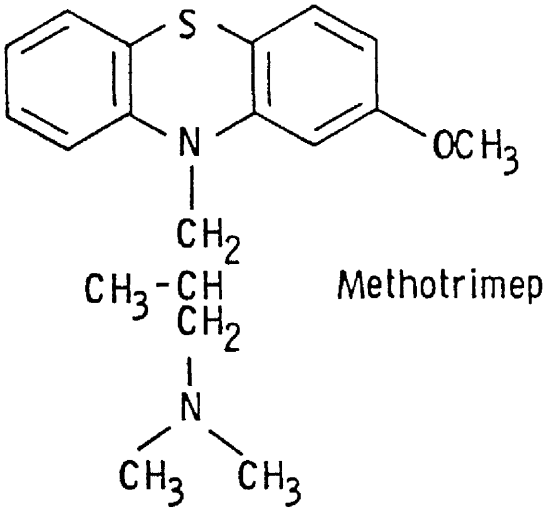
The new neuroleptic drugs are butyrophenone derivatives and have structures and actions similar to the phenothiazine class of tranquilizers. The most commonly used drugs of this type are droperidol and fluanisone (Fig. 2). The pharmacology of droperidol has been investigated in experimental animals by Janssen, Niemegeers, Schellekens, Verbruggen and Van Nueten (1963), Yelnosky, Katz and Dietrich (1964) and by Green (1972). Low doses of the drug reduce spontaneous motor activity in dogs while larger doses produce ataxia and sleep from which animals may be readily aroused by handling. Side effects associated with higher doses are muscle tremors and twitching. The butyrophenone neuroleptics have a greater cataleptigenic effect than chlorpromazine. A degree of alpha adrenergic blockade similar to that seen with chlorpromazine is produced. This contributes to the



Droperidol



Fluanisone



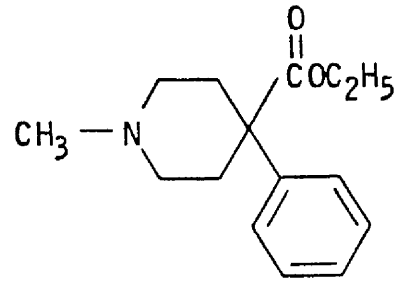
Methotrimeprazine

Fig. 2 Chemical structures of neuroleptic drugs used in neuroleptanalgesic techniques

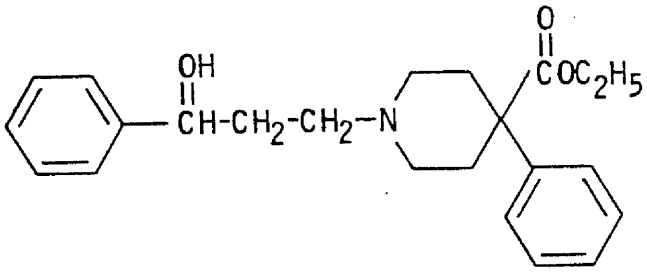
slight and transient hypotension produced by the drug, a reduction in total peripheral resistance, and an increase in peripheral blood flow. Slight respiratory stimulation may be seen and minimal myocardial depression is produced. Droperidol is also a very potent centrally acting anti-emetic.

The new analgesic drugs fentanyl and phenoperidine are similar in structure to pethidine (Fig. 3) but are much more potent. The pharmacological effects of fentanyl, in dogs, were investigated by Gardocki and Yelnosky (1964) who found that in addition to analgesia the drug produced sedation, bradycardia, hypotension and respiratory depression. The tone of the gastro-intestinal tract was increased and sphincters relaxed. The drug has some emetic activity in man but this has not been seen in dogs. Fentanyl is 500 times more potent than pethidine as an analgesic and has a relatively short duration of action. Phenoperidine is about 50 times more active than pethidine and has a longer duration of action than fentanyl (Nilsson, 1963).

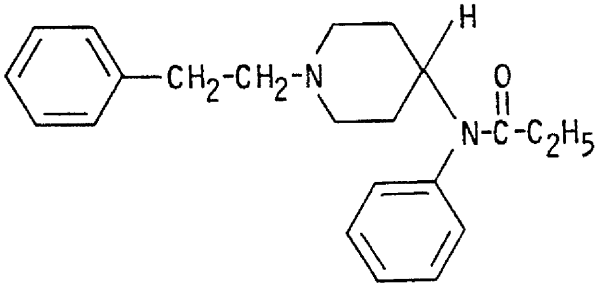
The potent analgesic, etorphine (Fig. 3), an oripavine derivative has been used in veterinary anaesthesia. When given intravenously it is about 150 and 2000 times more potent than morphine in the mouse and rat respectively. In laboratory animals etorphine produces a greater degree of catatonia and respiratory depression than equi-analgesic doses of morphine (Blane et al, 1967). Analgesia also develops more rapidly



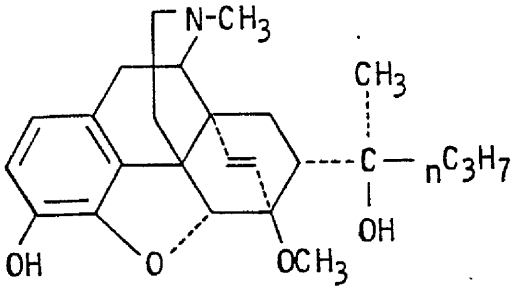
Pethidine



Phenoperidine



Fentanyl



Etorphine

Fig. 3 Chemical structures of analgesic drugs used in neuroleptanalgesic techniques

than with morphine.

When neuroleptanalgesia was first introduced into clinical practice in man (de Castro and Mundeleer, 1959) it was offered as an alternative to general anaesthesia and patients, although conscious, underwent even major surgery under the influence of droperidol and an analgesic alone. Although apparently well tolerated by patients this form of 'pure' neuroleptanalgesia has not gained wide acceptance in the United Kingdom. The technique has however proved satisfactory for the provision of general sedation for investigative techniques such as bronchography (Stevenson, Pandit, Dundee, McDowell and Morrison, 1972) and pneumoencephalography (Brown, 1969) and has been advocated as an effective method of anaesthesia for repeated burn dressings (Smith and Hollis, 1966).

A fixed ratio of 50:1 droperidol:fentanyl was used in an early study in man (Holderness, Chase and Dripps, 1963). These authors used additional sedative drugs for preanaesthetic medication and nitrous oxide was used as a maintenance supplement. Profound analgesia, minimal hypotension and a smooth post-operative course were described as appealing features of the technique. Because of the difference in the duration of action of the neuroleptic and analgesic components of neuroleptanalgesia, the two drugs are now rarely given together in a fixed ratio combination. The individual agents are generally given separately as required and are frequently used with neuromuscular blocking agents and nitrous oxide

as part of a balanced anaesthesia technique (Brown, Horton and MacRae, 1963).

In veterinary practice three neuroleptanalgesic mixtures have been advocated for use in the dog. The first of these, which was made available to the author for clinical trial studies, contained droperidol and fentanyl. This mixture has not been marketed for veterinary use in the United Kingdom but two other combinations, one containing fluanisone and fentanyl and another containing methotrimeprazine and etorphine have subsequently become available for clinical use in the dog. The neuroleptic, methotrimeprazine (Fig. 2) which is present in the latter formulation is a tranquilizer of the phenothiazine class which is also claimed to have significant anticholinergic properties (Blane, Boura and Dobbs, 1968).

The first clinical reports of the use of the combination of droperidol and fentanyl, in dogs, appeared in America. Yelnosky and Field (1964) used a mixture containing 20mg droperidol and 0.4mg fentanyl per ml and examined the clinical utility of this combination when given in a range of doses by the intramuscular route. These authors found that the most effective dose was 0.1 ml kg^{-1}. With this dose dogs became ataxic in two to three minutes, recumbent in five minutes and were unresponsive to painful and auditory stimuli in eight to 15 minutes. Laparotomies were attempted in

6 dogs at each dose investigated and a dose of 0.1 ml kg^{-1} was claimed to provide adequate operating conditions for 25 minutes while a dose of 0.05 ml kg^{-1} was found to be inadequate in one dog. With this lower dose it was also reported that three other animals showed some discomfort during suturing.

A fixed intramuscular dose of 0.1 ml kg^{-1} of the same 50:1 ratio of droperidol and fentanyl was also used by Soma and Shields (1964). These authors reported their experience with the mixture in a series of 100 dogs, in some of which neurolept-analgesia was supplemented with local infiltration anaesthesia or small amounts of thiobarbiturates. Sedation and ataxia were noted after 3 to 4 minutes and recumbency and maximum analgesia were obtained within 10 to 15 minutes. Bradycardia occurred in dogs not premedicated with atropine and some dogs defaecated. A slow rhythmic oscillation of the eyeballs was observed in 10 dogs, a vigorous eyelid reflex persisted and pupillary constriction occurred at the time of peak effect. These authors found that attempts to intubate animals could provoke swallowing whereas Yelnosky and Field (1964) claimed that intubation could be readily performed. A number of dogs responded to auditory stimuli with movements and in 5 dogs spontaneous movement of the head and limbs, unrelated to auditory or painful stimulation, was reported. Variable effects on respiration were noted with tachypnoea being seen in some animals and a reduced respiratory rate in others. No measurements of the effectiveness of respiration were made. Analgesia was found to be adequate for surgical interventions lasting

30 to 40 minutes and dogs were generally capable of maintaining sternal recumbency after 60 to 90 minutes. The effect of the analgesic component could be readily reversed with nalorphine but dogs remained sedated under the influence of droperidol. Although unconsciousness was not produced the technique was found to provide adequate conditions for diagnostic procedures and minor surgical interventions.

The advantages of the technique were found to be ease of administration with a wide margin of safety, a quiet post operative state, and the ability to antagonise the narcotic component of the mixture. The disadvantages which were encountered were, the variable response obtained in some breeds, particularly Australian Terriers and, in some cases, the occurrence of spontaneous movements and the need for supplementary local anaesthesia.

A brief report by Franklin and Reid (1965) described further studies with the same mixture of droperidol and fentanyl again given at a dose of 0.1 ml kg^{-1}. Little information is provided in this report on the operative procedures attempted and in a number of cases additional anaesthetic agents were used.

The clinical use of fluanisone and fentanyl in dogs was first described by Marsboom et al (1964). Two techniques were described: in the first fluanisone 5 mg kg^{-1} was given subcutaneously or intramuscularly

20 to 30 minutes before an intravenous injection of 0.1 mg kg^{-1} fentanyl. In the second technique both drugs were given in a single intramuscular injection. A variety of techniques including laparotomies were performed and the authors claimed that both methods could be accepted as fully adequate replacements for orthodox general anaesthesia despite the inclusion of the comment: "As loss of consciousness did not parallel analgesia, the animals were tied to the operating table to avoid possible sudden reactions of the animal to noises". In 15 per cent of the animals which received fentanyl intramuscularly, a supplementary injection of analgesic was necessary to obtain surgical anaesthesia and additional doses of fentanyl were sometimes given during the operative procedure. Although it was noted that defaecation occurred in most anaesthetized dogs few other side effects were described. Animals generally recovered within two hours but in some sleep lasted for up to 10 hours postoperatively.

Early studies in dogs with the neuroleptanalgesic combination containing etorphine and methotrimeprazine were described by Alford and Wozniak (1970) and Crooks et al (1970). The former authors conducted a dose finding study in experimental Beagles and found that a combination of 7.5µg kg^{-1} etorphine and 6 mg kg^{-1} methotrimeprazine provided successful neuroleptanalgesia;

and minor surgical procedures were performed without eliciting any painful response. With a combination containing 5 μg kg^{-1} etorphine and 2 mg kg^{-1} methotrimeprazine, 9 out of 10 dogs became cyanotic which suggested that the lower dose of methotrimeprazine failed to antagonise the respiratory depressant effect of etorphine. Transient bradycardia and hypothermia were also noted.

Crooks et al (1970) used a fixed ratio combination of etorphine and methotrimeprazine. The drugs were given intramuscularly and doses ranged from 3.75 μg kg^{-1} etorphine:1 mg kg^{-1} methotrimeprazine to 15 μg kg^{-1} etorphine:4 mg kg^{-1} methotrimeprazine. In a minority of cases halothane was used to supplement neuroleptanalgesia. The technique was deemed to be satisfactory in the majority of cases and was mainly used for diagnostic and minor surgical procedures. The drug combination was given to 131 dogs and slight cyanosis was reported in 9 animals. Defaecation or excessive salivation did not normally occur and emesis was never observed. In most cases the narcotic antagonist diprenorphine was given at the end of the procedure and animals were able to walk after 0.5 to 36 minutes. Recovery took 2 to 4 hours in two dogs not given the antagonist.

Whilst some authors have stated that the technique can provide suitable conditions for major surgery (Yelnosky and Field, 1964; Marsboom et al, 1964) others have found that the state obtained was suitable only for diagnostic and minor surgical procedures (Soma and Shields, 1964; Crooks et al, 1970). Differences have also been described in relation to the incidence of side effects and the degree of respiratory depression produced by the various combinations. Because of these discrepancies it seemed important to examine the effects produced by three different neuroleptanalgesic mixtures using a standardized protocol in the same population of dogs.

4. DESIRABLE PROPERTIES SOUGHT IN NEW INTRAVENOUS ANAESTHETIC AGENTS

No existing compound possesses all of the features which would be desirable in an ideal intravenous anaesthetic agent (Davis, 1975). The properties of an ideal compound can be listed as follows:

Stable in aqueous solution

Rapid induction

Wide safety margin

No cumulation of effect on repeated administration

Compatibility with other agents used in anaesthesia

Minimal respiratory or cardiovascular depression

Some degree of analgesia

Freedom from undesirable side effects:

- laryngospasm or bronchospasm
- pain on injection
- excitatory effects
- dreams or hallucinations
- local tissue or vascular damage
- anaphylactoid responses.

The requirement for many of the features listed is self evident but some additional comments can be added.

A major problem in the development of new intravenous anaesthetics has been the difficulty in obtaining water soluble compounds which are sufficiently lipid soluble to pass rapidly into the brain. Combination of the first two seemingly incompatible properties has been achieved in the past by adopting two different expedients. With the rapidly acting barbiturates, aqueous solutions are achieved by using sodium salts at a high pH, whereas with

propanidid and the steroid mixture of alphaxalone and alphadolone the surface-active agent Cremophor EL has been used to form water miscible solutions. A relatively high incidence of anaphylactoid reactions has been encountered with these Cremophor containing anaesthetics (Clarke et al, 1975) and it is possible that the solubulizing agent may be responsible for at least some of the sensitivity reactions reported. This topic will be discussed in more detail in a later section.

The absence of any cumulative effect is a particular requirement in agents which may be used to maintain anaesthesia when given by intermittent injection or infusion. A rekindling of interest in techniques of total intravenous anaesthesia has been one of the consequences of recent concern shown by anaesthetists about possible risks from inhalation of trace amounts of anaesthetic gases released into the theatre atmosphere. A number of studies have provided results which suggest that anaesthetic practice may be associated with an increased susceptibility to spontaneous abortion in female theatre personnel (Askrog and Harvald, 1970; Cohen, Bellville and Brown, 1971; Knill-Jones, Rodrigues, Moir and Spence, 1972). There is no direct evidence for the involvement of anaesthetic gases and vapours in the production of this effect, but as this possibility cannot be excluded a number of methods have been devised to reduce the amount of waste anaesthetic gases in the operating room environment. (Vaughan, Mapleson and Mushin, 1973; Parbrook and Monk, 1975; Davenport, Halsey, Wardley-Smith and Wright, 1976).

A retrospective study by Bruce, Eide, Linde and Eckenhoff (1968) showed a higher incidence of death from cancer of lymphoid tissue among anaesthetists compared with the general population but a later prospective study failed to substantiate this finding (Bruce, Bach and Arbit, 1974). Although early animal studies suggested there was some association between exposure to anaesthetic agents and foetal abnormalities in chick embryos (Smith, Gaub and Moya, 1965) and in rats (Fink, Shepard and Glandau, 1967), more recent studies, with concentrations of anaesthetics at least 500 times higher than are likely to be found in the atmosphere of an operating theatre, failed to demonstrate any evidence of teratogenicity or any significant increase in foetotoxicity (Lansdown, Pope, Halsey and Bateman, 1976).

Considerable attention has also been paid to the effects of trace concentrations of anaesthetics on the psychomotor performance of operating theatre personnel. Detrimental effects were demonstrated by Bruce, Bach and Arbit (1974) but the balance of experimental evidence indicates that concentrations of halothane much higher than 10 ppm are required before any impairment in performance can be detected (Smith and Shirley, 1978).

While debate continues on the possible detrimental effects of inhalation agents, an alternative solution to the removal of expired gases and vapours is the development of techniques where anaesthesia is maintained by the administration of intravenous drugs.

A technique of intravenous anaesthesia using a continuous infusion of alphaxalone and alphadolone (Althesin) has been described by Savage et al (1975). Although satisfactory operating conditions were obtained in a majority of patients, the principle complication of the technique was one of movement. In some cases movement occurred in response to painful stimuli despite the use of analgesic supplements. In other instances movement was apparently random and unrelated to surgical stimuli. The mean awakening time was 18 minutes after the end of surgery. As this is significantly longer than the recovery time which can be achieved with techniques utilizing inhalation agents, it would appear that Althesin is not an ideal agent for the maintenance of anaesthesia by intravenous infusion.

Only with an intravenous agent which is rapidly metabolised and does not accumulate in body tissues could one expect to achieve the fine control of anaesthetic depth and the rapid recovery of protective reflexes which can currently be obtained with inhalational techniques.

5. METHODS USED IN THE PHARMACOLOGICAL EVALUATION OF NEW INJECTABLE ANAESTHETICS IN LABORATORY ANIMALS

The search for improved intravenous anaesthetic agents involves the chemical preparation of new compounds and their pharmacological evaluation in animals. The discovery of anaesthetic activity in a series of alkylphenols presented an opportunity for the development of pharmacological models for the selection of the best compound from the series for detailed biological evaluation. Considerations of potency, speed of onset, duration and quality of anaesthesia in mice and rabbits indicated that 2,6-diisopropylphenol (disoprofol) had a desirable pharmacological profile. The factors considered in the selection of suitable methods and in the development of new methods, for this phase of the study, are discussed in the next section of the introduction. When disoprofol had been chosen for further investigation, subsequent experiments involved a comparison of its anaesthetic properties with those of currently used agents, and an evaluation of the role of the surfactant, Cremophor EL, in anaphylactoid reactions to anaesthetics formulated in this material.

5.1 Assessment of anaesthetic potency

Administration of anaesthetics by the intravenous route in mice provides information on the speed of induction, potency, duration of action and lethality. In the present context potency can be defined as the amount of drug required to produce a given depth of sleep. Before the properties of any new agent can be compared with those of established agents the relative potencies of agents used in comparative studies must be determined. Erroneous values can lead to fallacious impressions concerning the relative duration of action of drugs, such as occurred in human studies with thiopentone and buthalitone. Although early clinical impressions had suggested that buthalitone produced a shorter duration of sleep than thiopentone, a similar conclusion was not reached when doses were used which produced an equivalent electroencephalographic depth of anaesthesia (O'Mullane, 1957).

In studies of the relative potency of anaesthetic agents in mice the depth of anaesthesia can be readily standardized. The median hypnotic dose (HD_{50}) can be established as that dose which produces hypnosis in 50 per cent of animals. Hypnosis in mice has been defined by Carrington and Raventos (1946) as that condition in which it is not possible to elicit 'body righting reflexes'. The same authorsdefined anaesthesia as the state during which it is possible to make a cutaneous incision without evoking a response, and a

similar end point has been used by Gibson, Doran, Wood and Swanson (1959) in a study with methohexitone. The median anaesthetic dose (AD_{50}) determined in this way is well in excess of the HD_{50}. Child et al,(1971) in their examination of the potency of a range of anaesthetic agents in mice defined the AD_{50} as the dose required to produce loss of the 'righting reflex' in 50 per cent of mice and this dose can therefore be compared with the HD_{50} dose as defined by Carrington and Raventos (1946).

Atkinson, Davis, Pratt, Sharpe and Tanich (1965), when testing a series of anaesthetic steroids, chose the dose producing loss of righting reflex for 25 minutes in mice as their criterion for activity. This '25 minutes sleeptime' dose was selected as many of the compounds tested produced a slow induction of anaesthesia and with group mean sleep times below 15 minutes some of the mice did not sleep. Although this problem would be unlikely to be encountered with rapidly acting agents the requirement for an agent to produce sleep times of 15 to 25 minutes would mean that the potency of agents with a very brief duration of action would tend to be underestimated as a greater multiple of the HD_{50} would be needed to provide an extended period of sleep. Nevertheless, it has been found that the accuracy of the HD_{50} is improved when a short but finite minimum period of sleep is specified. In this investigation the HD_{50} is thus defined as that dose which produces loss of

righting reflexes for a minimum period of 30 seconds in 50 per cent of mice.

In previous studies of the relative potency of anaesthetic agents in mice little attention has been paid to the importance of standardizing the rate of injection to ensure that equipotent doses are given over the same injection period. As a greater depth of anaesthesia is likely to be produced when a given dose of a compound is injected more rapidly,the potency of a highly active drug is likely to be overestimated if drugs are injected at a given volume per unit time. Conversely the relative potency of a less potent agent, such as propanidid, is likely to be underestimated, as a greater injection time would be required to inject the larger HD_{50} dose of this drug. The influence of this effect can however be minimised by the use of more concentrated solutions of less active compounds. Carrington and Raventos (1946), in estimating the relative potency in mice of thialbarbitone, thiopentone and hexobarbitone, injected the drugs in 0.1 per cent and 1.0 per cent solutions at a constant rate of 0.1 ml per 10 seconds. As the drugs examined in this study were of similar potency it is unlikely that any significant discrepancy was introduced by the administration technique used. In the comparative study described by Child et al (1971), Althesin was injected into mice at a rate equivalent to 0.1 ml undiluted solution (1.2 mg) per kg per second. Although results obtained with a range of other anaesthetic agents are provided, the drug concentrations and injection rates used are not reported.

In studies designed to define the pharmacological properties of phencyclidine (Chen et al, 1959)and ketamine (McCarthy et al, 1965) in animals, no attempt was made to define the median anaesthetic dose, and this value is not relevant in connection with neuroleptanalgesic drug mixtures. Although Laubach, P'an and Rudel (1955) and P'an, Gardocki, Hutcheon, Rudel, Kodet and Laubach (1955) used the AD_{50} in describing the anaesthetic properties of hydroxydione, these authors did not provide information on the rate of injection used.

One method which can be adopted to ensure that equipotent doses of drugs are injected at an equivalent rate involves the use of a standard injection time for all of the doses given. This method was used by Janssen, Niemegeers and Marsboom (1975) in their investigation of the anaesthetic properties of etomidate. These authors used a standard injection time of 2 seconds and obtained valid comparative results with thiopentone, methohexitone and propanidid. A similar method was adopted in the present investigation and in the main comparative study all doses were given over an injection period of 10 seconds. In some preliminary experiments all doses were given over one to two seconds.

5.2 Assessment of speed of onset of anaesthesia

In clinical practice a rapid onset of anaesthesia is desirable to allow the dose administered to be titrated against the response of the patient. Differences in onset time between drugs can be accounted for by differences in lipid solubility of the undissociated drug molecule (Brodie, Kurz and

Shankar, 1960). These authors demonstrated that the greater lipid solubility of thiopentone, in comparison with that of pentobarbitone, was responsible for the more rapid passage of the former drug across the blood-brain barrier. With any particular drug, the speed of onset of anaesthesia is likely to be affected by the dose given, the rate of injection, and the arm-brain circulation time.

The effect of different injection techniques on the onset of thiopentone narcosis, in man, has been investigated by a number of authors. Egbert and Mitchell (1961) noted the importance of the rate of injection; a decrease in the rate of injection being associated with a delay in the onset of hypnosis. Onset of anaesthesia was also found by these authors to be slower when the drug was given into a vein on the dorsum of the hand rather than into an antecubital vein, but the difference in onset time obtained with the same dose of thiopentone given as a 2.5 or 3.5 per cent solution, into the same vein, was not statistically significant. In an early comparative study with methohexitone and thiopentone Wyant and Chang (1959) noted that onset of anaesthesia with methohexitone was slower than with thiopentone. In a later study (Wyant and Barr, 1960), in which both drugs were injected at the same rate, the difference in onset times was not confirmed and it was concluded that the discrepancy had resulted from the fact that, in the earlier study, methohexitone had been injected more slowly than thiopentone. The slower mean onset time obtained by Thomas (1967) with thiopentone (27.6 s) as compared with 10 to 11 seconds obtained by Clarke, Dundee, Barron and McArdle (1968) can also be attributed to the use of a slower rate of injection by the former author.

Arm-brain circulation time is likely to be increased in patients with a reduced cardiac output and can also be influenced by changes in ambient temperature. Dundee (1955) noted a faster onset time with thiopentone when the ambient temperature was 29 to 32°C in comparison with that obtained at a temperature of 22 to 23°C. In a study of the speed of onset and potency of Althesin in man, Carson, Dundee and Clark (1975) injected the drug during a period of reactive hyperaemia following temporary ischaemia of the forearm to reduce, and hopefully to standardize, the effect of the arm-brain circulation time on the speed of onset of anaesthesia.

In studies in laboratory animals one investigator (Butler, 1941) has paid considerable attention to the factors likely to influence anaesthesia onset time. In a comparative study with phenobarbital and glucochloralose speed of onset was determined using equivalent multiples of the AD_{50} of each compound. Although different rates of injection were used for the two drugs examined, all doses of each drug were given over a constant injection period. In other studies in animals little thought has been given to the factors affecting the rate of onset of anaesthesia (Child et al, 1971; Janssen, Niemegeers and Marsboom, 1975) despite the fact that, in the evaluation of the anaesthetic properties of a range of steroid compounds, Atkinson et al (1965) noted a significant delay in the onset of anaesthesia with a number of the agents examined. In this latter study the delay in the onset of anaesthesia can be attributed to the time required for the hydrolysis of ester pro-drugs to an active and more lipid soluble form.

In the evaluation of new anaesthetic compounds with unknown lipid solubility it is essential that a standardized technique is used for the evaluation of the speed of onset of anaesthesia. For this reason the relative importance of the effects of a number of factors which could influence speed of induction of anaesthesia in mice have been evaluated in the present investigation. The factors considered include drug dosage, rate of injection, drug concentration and ambient temperature.

5.3 Evaluation of cumulative effects

Although recovery after a single dose of thiopentone occurs rapidly and is dependent predominantly on redistribution of the drug into body compartments other than the brain (Price et al, 1960), the administration of repeated doses of thiopentone is likely to lead to prolonged anaesthesia. This occurs because of the accumulation of thiopentone in tissues, particularly fat, and the relatively slow rate of metabolism of thiopentone (Brodie et al, 1950). Agents which are metabolised rapidly are less likely to accumulate to a significant extent in body tissues and are more likely to allow a rapid recovery following their administration for maintenance of anaesthesia, by repeated injection or infusion. Pharmacokinetic studies have confirmed that an agent such as methohexitone,which may be used in this manner, has a much shorter elimination half life than thiopentone (Breimer, 1976).

In the development of new anaesthetic agents it is not generally possible at an early stage, when a large number of compounds may have to be examined, to obtain information on the pharmacokinetic profile of each compound. For this reason some

other method has to be used for the selection of compounds which are likely to be metabolised rapidly and unlikely to accumulate to a significant extent in body tissues.

Wyngaarden, Woods, Ridley and Seevers (1949) used the duration of action of repeated doses in dogs as a means of comparing the cumulative action of thiopentone with that of thiamylal. Doses of both drugs were adjusted to produce approximately the same duration of anaesthesia with the first injection and drug administrations were repeated at hourly intervals. Injections were stopped when one or more dogs in any group showed a duration of anaesthesia exceeding sixty minutes. The results obtained with this method indicated that thiamylal exhibited a lower rate of accumulation than thiopentone as evidenced by a smaller percentage increase in sleeping time with repeated doses, relative to the initial anaesthesia time.

A similar method has been used by Swanson and Chen (1953) to study a number of methylated thiobarbiturates and Dundee (1955) investigated the cumulative effects of four barbiturates in rats and dogs by noting the duration of narcosis with two doses of the same drug given at varying intervals to the same batch of animals in the case of rats, or to the same animal in the case of dogs. An appreciable increase in the duration of the second period of narcosis was noted with thiopentone, thioethanol, and thialbarbitone, if the interval between injections was less than 18 to 24 hours. With thiamylal a cumulative effect was not observed when the interval between injections was greater than 12 hours. Wyngaarden et al (1949) concluded that the lesser cumulative effect seen with thiamylal following the administration of equipotent doses of thiopentone and

thiamylal was due to the greater potency of thiamylal as it was assumed that both drugs were metabolised at an equal rate. In the study reported by Dundee (1955), when equal doses of the two drugs were given, thiamylal still caused less potentiation of a subsequent dose, thus suggesting that thiamylal was metabolised more rapidly than thiopentone.

Although the techniques described above have demonstrated a difference in the cumulative actions of thiopentone and thiamylal, the methods used would be unlikely to be sufficiently sensitive to detect relatively minor differences in the rate of accumulation of more rapidly metabolised drugs. It can be envisaged that when a significant interval is allowed between injections the tissue concentration of a drug, which accumulates to only a minor extent, could decline to an insignificant level. Accumulation could more readily be demonstrated with a shorter interval between repeated injections. The technique used by Child et al (1971) to compare the cumulative effects of Althesin with those of thiopentone and methohexitone conforms with this approach. In this method successive equal doses of the anaesthetic agents were administered to mice 30 seconds after the animals regained their righting reflex following a preceeding injection. A cumulative effect was readily demonstrated with thiopentone where sleeping time increased from 5.7 to 178 minutes after only four doses, whereas successive doses of the other two agents produced minimal increases in sleeping time. This method, which appeared to be particularly sensitive, was selected as one of the methods to be used for the evaluation of the cumulative action of disoprofol.

Some indication of the cumulative action of injectable anaesthetic agents can also be obtained from the slope of the dose/duration of effect curve obtained in animals. A compound such as thiopentone, which is metabolised slowly, has been shown to provide a much steeper curve than rapidly metabolised compounds such as etomidate and propanidid (Janssen, Niemegers and Marsboom, 1975). A possible limitation of this method, for use in a comparative study, is that differences in therapeutic ratio between compounds could, in some cases, prevent the examination of the effects of equivalent dose ranges.

Another satisfactory method, described by Dodds and Twissell (1973) utilised a comparison of recovery times after the maintenance of anaesthesia in cats for a period of three hours with supplementary doses of thiopentone or Althesin. With this method these authors demonstrated that Althesin accumulated to a much lesser extent than thiopentone as recovery occurred at a mean time of 100 minutes after the last dose of Althesin whereas 28 hours elapsed before the recovery of righting reflexes in cats given thiopentone. A modification of this latter method has also been used in the present investigation and recovery times were noted after maintenance of anaesthesia in rats with a constant continuous infusion of disoprofol for periods of one and two hours.

5.4 Evaluation of cardiovascular and respiratory effects

The testing of intravenous anaesthetic drugs in chloralose or pentobarbitone anaesthetized animals is unsatisfactory as the injection of a second drug which depresses the central nervous system may induce severe respiratory and circulatory depression in doses much smaller than those required to induce anaesthesia in the conscious animal. Lerman and Paton (1960) noted that the depressant actions of hydroxydione on respiration and blood pressure were increased five to ten fold in chloralose anaesthetized cats in comparison with unanaesthetized or decerebrate animals and Chen et al (1959) commented on the great sensitivity of the respiratory centre of the cat to the combined depressant action of phenobarbitone and phencyclidine. McCarthy et al (1965) described similar effects with ketamine in dogs anaesthetized with pentobarbitone or chloralose and a similar potentiation of the depressant actions of Althesin in cats anaesthetized with chloralose, pentobarbitone or inhalational anaesthetics has been observed by Child et al (1972a).

An alternative method which can be expected to give more reliable information involves the surgical implantation of polyvinyl or polyethylene catheters in a jugular vein and a carotid artery at some time prior to the investigation. When animals have recovered from the surgical preparation the effects of anaesthetic agents on blood pressure and respiration can then be examined in untreated and unrestrained subjects. The few studies in which the effects of intravenous anaesthetic drugs have been monitored in conscious intact animals include studies of 5-ethyl-6-phenyl-m-thiazine-2,4-dione and thiopentone (Cotton and Bay, 1956),

α-chloralose and pentobarbitone (Van Citters, Franklin and Rushmer, 1964); pentobarbitone (Olmsted and Page, 1966) and thiamylal and pentobarbitone (Goldberg, Linde, Gaal, Momma, Takahashi and Sarna, 1968).

The above studies were performed in dogs but surgically prepared unanaesthetized cats were used in a comparative study of the cardiovascular and respiratory effects of a range of intravenous anaesthetic drugs, described by Child et al (1972a). The cat was chosen by these authors as the Cremophor containing agents Althesin and propanidid were included in their study. Although some haemodynamic studies with propanidid have been undertaken in the dog (Conway, Ellis and King, 1968; Sankawa, 1965) this species would seem an inappropriate choice for the evaluation of any Cremophor containing formulation as this solubilizing agent is a potent liberator of histamine in the dog (Wirth and Hoffmeister, 1965). A significant degree of hypotension is much less likely to occur in the cat following the injection of Cremophor EL, but a small increase in plasma histamine concentration has been demonstrated in this species following the administration of this material (Lorenz et al, 1971b)and clinical signs indicative of histamine release have been noted in a number of cats following the administration of Althesin in veterinary practice (Dodman, 1980).

As the pig has been shown to be insensitive to the histamine releasing properties of Cremophor EL (Lorenz et al, 1971b)this species was chosen for the present investigation. The pig has been rarely used in the past for the investigation of the respiratory and cardiovascular effects of intravenous anaesthetics

but a recent study (Becker and Beglinger, 1980) investigated the effects of the intramuscular administration of the neuroleptanalgesic combination containing etorphine and acepromazine in surgically prepared, unanaesthetized pigs.

In animals with an implanted carotid artery catheter, respiratory effects of drugs can be readily examined by measurement of the partial pressures of oxygen and carbon dioxide in samples of arterial blood drawn anaerobically from the arterial catheter, and arterial blood pressure can be measured directly with a calibrated strain gauge transducer. The placement of a jugular venous catheter also allows test drugs to be given to the unrestrained animal.

5.5 Evaluation of interactions between injectable anaesthetics and ancillary drugs used in anaesthesia

As injectable anaesthetic drugs are frequently used in combination with ataractic, anticholinergic and analgesic drugs, inhalation anaesthetic agents, and neuromuscular blocking drugs, it is important that any significant interactions between these drugs are discovered at an early stage in the evaluation of new agents.

In the pharmacological investigation of propanidid, Wirth and Hoffmeister (1965) used mice to examine possible interactions between this agent and chlorpromazine, reserpine and iproniazid. In a study with Althesin, Child et al (1971) examined the effects of a range of pre- and postoperative medicaments on the course and duration of anaesthesia in cats. In the present investigation, a selection was made of agents likely to be used for

pre-anaesthetic medication and drugs were given subcutaneously to mice 30 minutes before induction of anaesthesia with an intravenous injection of disoprofol. By using mice it has been possible to use larger numbers of animals and to perform statistical analyses on the results.

Conventional anaesthetic techniques in cats, similar to those described by Child et al (1971) have been used in the present study to examine possible interactions between disoprofol and a range of inhalation anaesthetic agents and the neuromuscular blocking drugs suxamethonium, pancuronium and gallamine. As tubocurarine is known to produce histamine release in the cat (Hall, 1971) the combination of this agent with disoprofol was examined in the pig.

It is generally accepted that in clinical practice anaesthetic requirements are reduced in patients who have received ataractic or sedative premedication (Dundee, Nicholl and Moore, 1963). An experimental study in animals has demonstrated that premedication with benzodiazepine drugs, particularly diazepam, potentiates the hypnotic effect of steroid anaesthetic agents (Gyermek, 1974) and it was expected that some potentiation of the anaesthetic effect of disoprofol would be encountered when the drug was given in combination with large doses of sedative drugs.

An unexpected interaction between the neuromuscular blocking agent suxamethonium and the intravenous anaesthetic propanidid was discovered in the early clinical evaluation of propanidid in human patients (Clarke, Dundee and Daw, 1964; Howells et al, 1964). These authors found that the duration of apnoea and

respiratory depression produced by suxamethonium was increased when this drug was administered following induction of anaesthesia with propanidid, whereas no similar potentiation was found with methohexitone or thiopentone.

In human patients, potentiation of suxamethonium apnoea has also been observed following the co-administration of suxamethonium with quinidine (Grogono, 1963), procaine (Salgado, 1961; Foldes, McNall, Davis, Ellis and Wnuck, 1953) and eyedrops containing ecothiopate iodide, an agent with potent anticholinesterase activity (Gesztes, 1966; Pantuk, 1966). Hydrolysis by pseudocholinesterase is a major step in the metabolism of procaine (Kalow, 1951), suxamethonium (Lehmann and Silk, 1953) and propanidid (Doenicke et al, 1968). Substrate competition for this enzyme has been proposed as the mechanism responsible for the interaction between procaine and suxamethonium (Foldes et al, 1953) and a similar mechanism may, at least in part, explain the interaction between suxamethonium and propanidid. This theory gains support from the clinical observation that propanidid neither potentiates nor prolongs the neuromuscular block induced by decamethonium, another depolarizing relaxant, but one which is not dependent on hydrolysis by pseudocholinesterase for its elimination (Torda, Burkhart and Toh, 1972).

Interference with the activity of serum pseudocholinesterase cannot be the only mechanism responsible for the prolongation of suxamethonium apnoea as the neuromuscular effect of suxamethonium can still be potentiated by propanidid in an in vitro nerve-muscle preparation after the abolition of cholinesterase activity

(Ellis, 1968). This author concluded that propanidid was probably producing this effect by causing a partial depolarization of the muscle cell membrane, which would potentiate the depolarization produced by suxamethonium in the vicinity of the end plate.

Attempts to demonstrate an interaction between propanidid and suxamethonium in in vivo animal models have produced conflicting results. Howells et al (1964) found that propanidid did not potentiate the action of suxamethonium in a cat nerve-muscle preparation whereas this preparation has been used successfully by Cuthbert (1966) to demonstrate an interaction between both quinidine and procainamide and suxamethonium. Using the same preparation, Miller, Way and Katzung (1967) demonstrated an interaction between quinidine and both the non-depolarizing and depolarizing muscle relaxants in producing neuromuscular blockade. In one study, conducted in Italy (Musinu, Tagliamonte and Gessa, 1968) muscle relaxant drugs were given to rabbits after they had recovered from the anaesthetic effects of propanidid. A greater degree of muscle paralysis was produced in those animals given suxamethonium than in those which received tubocurarine. The administration of neuromuscular blocking drugs to conscious animals would not be condoned in this country and an attempt has therefore been made, in the present study, to define an animal model in which it may be possible to demonstrate an interaction between suxamethonium and propanidid in anaesthetized animals. To demonstrate the clinical relevance of the model it would also be necessary to

demonstrate that a similar potentiation of the effect of suxamethonium could not be produced by intravenous anaesthetics other than propanidid.

As significant serum pseudocholinesterase activity has been demonstrated in the mouse (Foldes, 1966) this species was chosen for this section of the work. This choice also allowed the use of previously determined equipotent doses of the intravenous anaesthetic agents.

6. THE ROLE OF CREMOPHOR EL IN ANAPHYLACTOID REACTIONS PRODUCED BY CREMOPHOR CONTAINING ANAESTHETICS IN MAN

Being a highly lipophilic compound, disoprofol, is virtually insoluble in water and is currently solubilized with the aid of a polyoxyethylated ricinoleic acid surfactant, Cremophor EL. This agent is also present in the commercial preparations of propanidid and Althesin.

In man an increasing number of apparent hypersensitivity reactions to intravenous anaesthetics have been reported recently, and the recorded frequency of reactions to Cremophor formulations of propanidid, Althesin (Clarke et al, 1975) and diazepam (Huttel, Schou Olesen and Stoffersen, 1980) suggest that this solubilizing agent may be responsible for at least some of the reported reactions to these compounds.

A prospective study of the frequency and causes of adverse responses to intravenous anaesthetic agents has been completed recently (Beamish and Brown, 1981). The results show that adverse reactions involving Althesin (13 in 7904) occurred more frequently than reactions to thiopentone (2 in 45546). Other

estimates of the frequency of reactions to Althesin range from one in 430 (Scott, 1979) to one in 19000 (Clarke et al, 1975).

The clinical manifestations of reactions include cutaneous hyperaemia, bronchospasm and hypotension. These signs resemble those produced by an immediate, immune mediated, hypersensitivity response (Watkins, 1979). However, as reactions have been reported following the first administration of an anaesthetic drug, it has been suggested, that unless evidence for definite antibody involvement exists, hypersensitivity-type reactions are best described as anaphylactoid (Watkins and Clarke, 1978). Four different pathological mechanisms, which could lead to an adverse clinical response, have been described by Watkins (1979) as follows:

i) Type 1 hypersensitivity response. These require previous exposure and involve IgE antibodies. There is direct histamine release from mast cells without involvement of complement.

ii) Immune reactions. The class of immunoglobulins involved may be in doubt, but histamine release occurs following activation of complement by the classical pathway.

iii) Alternate pathway activation of complement C3. These involve direct activation of C3 and this group appears to contain both patients apparently sensitized by previous exposure to the substance and first time responders.

iv) Pharmacological or chemical release of histamine. The injected substance causes histamine release from the mast cell without antibody or complement involvement.

Reactions to Althesin generally involve excessive activation of complement C3, leading to histamine release and are not reagin-antibody mediated, whereas reactions to barbiturates generally represent immediate, immune-mediated, hypersensitivity reactions (Watkins, 1979). The involvement of IgD in some instances has also been suggested, particularly in cases where close proximity of exposures to Althesin appears to be a factor predisposing to adverse response (Watkins, Allan and Milford-Ward, 1978).

Thus, although histamine is involved in the majority of reactions, direct pharmacological release of this amine is probably a minor factor in reactions occurring in human patients. It has been shown that the injection of thiopentone, propanidid and Althesin can produce histamine release in man (Lorenz, Doenicke, Meyer, Reimann, Kusche, Barth, Geesing, Hutzel and Weissenbacher, 1972; Doenicke, Lorenz, Beigl, Bezecny, Uhlig, Kalmar, Praetorius and Mann, 1972) but the amount released is normally insufficient to be of any clinical significance. The same authors were unable to demonstrate histamine release in man following the administration of Cremophor EL; and volunteers who had shown an increase in circulating histamine concentration following propanidid did not show a similar response when given Cremophor alone. These results suggest that Cremophor is unlikely to produce direct histamine release in man. However, they do not exclude the possibility that Cremophor could contribute

to an adverse response by one of the alternative mechanisms mentioned above.

In animals there are considerable species differences in susceptibility to the histamine releasing properties of Cremophor EL. In a study reported by Lorenz et al (1971b) the administration of Cremophor produced a 630 per cent increase in whole blood histamine concentration in dogs, a 90 per cent increase in cats, and no significant change in pigs. Lorenz, Reimann, Schmal, Dormann, Schwarz and Neugebauer, (1977) have used dogs to examine the histamine releasing capacity of several preparations of Cremophor EL, other non-ionic detergents and several components of Cremophor. Using this model these authors were able to demonstrate that oxyethylated oleic acid was the most important histamine releasing constituent in Cremophor.

The use of histamine release in the dog as a model for the evaluation of alternative surfactants has two serious limitations. Firstly the dog is particularly sensitive to the histamine releasing properties of Cremophor whereas this mechanism is only one of a number encountered in anaphylactoid reactions in man. Secondly the response in the dog requires no previous sensitization to the compound, as appears to be necessary in a proportion of adverse reactions in patients. An attempt has therefore been made to develop an animal model using pigs, a species which resembles man in being

insensitive to the direct histamine releasing effects of Cremophor. It was hoped that by examining the effects of repeated injections of a range of Cremophor-containing and Cremophor-free anaesthetics, some indication could be obtained of the importance of the surfactant in the pathogenesis of anaphylactoid reactions in man.

Chapter 1

AN EVALUATION OF A TECHNIQUE FOR THE INDIRECT MEASUREMENT OF BLOOD PRESSURE IN ANAESTHETISED DOGS USING THE 'NEWCASTLE' INFANT SPHYGMOMANOMETER

1. INTRODUCTION

The basic principle of indirect measurement of blood pressure involves the occlusion of an artery by an inflatable cuff and the detection of returning blood flow below or distal to the site of occlusion as the occluding pressure is reduced. Available methods differ only in the manner in which returning blood flow is detected. The auscultatory technique is the method most widely used in man to detect returning arterial pulsations distal to an occluding cuff. The conical shape of dog's limbs makes the proper application of an occluding cuff difficult but special cuffs have been devised which can be fixed around the thigh of dogs (Rule, 1944). Good correlation has also been shown between the auscultatory method and direct measurements with a capacitance manometer (Romagnoli, 1953). Disadvantages of the auscultatory technique are that access to the anaesthetised animal is required for auscultation, and in small animals the sounds may be difficult to detect.

The oscillometric technique has been used in animals by Romagnoli (1953) but this technique has the disadvantage that the systolic end-point is not clearly defined when a single cuff is used. Oscillations are detected at cuff pressures above systolic pressure, due to arterial pulsations impinging on the upper edge of the cuff.

Other methods of detecting peripheral pulses involve either palpation or the use of a condenser microphone transducer (Prioli and Winbury, 1960). Pulse monitors of this latter type have been tested in dogs by Campbell, Lawson and Sanford (1964)

who found the tail to be the best site for the occluding cuff and pulse monitor. Movement artefacts are readily picked up by this type of transducer and the systolic end-point is often difficult to detect. A similar technique using a tubular metal tail cuff has been described by Klemm and Hembrough (1966). These authors measured both systolic and diastolic pressure but most authors have found diastolic pressure difficult to measure by indirect methods using pulse monitors.

Photo-electric cell transducers may also be used as pulse monitors as arterial pulsations cause changes in the amount of light reflected onto the cell. This method is difficult to apply in animals with pigmented skins and is also very liable to display movement artefacts.

Pulse monitors based on the ultra-sonic doppler technique have been used by Valtonen and Eriksson (1970) and Garner, Hahn, Hartley, Hutcheson and Coffman (1975). The former authors used an occluding cuff obtained from a piece of cycle tube placed on the upper forelimbs while the latter workers measured pressure in a hind limb with an occluding cuff placed round the tibial area.

Apart from oscillometry, changes in the volume of a limb segment can be detected with a xylol pulse indicator as originally described by Neligan and Rogers (1959). With this instrument volume changes under a cuff placed distally to the occluding cuff are detected by the movement of a 'bead' of xylol in a glass capillary tube. The use of this instrument for the indirect measurement of blood pressure in dogs has been described by Wilson and Clarke (1964). As this system obviates

the need for an electronic transducer and appeared to be less sensitive to movement artefacts, it was selected for a detailed examination of its efficacy in different sizes of dogs.

The width of the occluding cuff has been shown to affect the accuracy of indirect measurements in studies in human adults (Karvonen,1962) and infants (Robinow, Hamilton, Woodbury and Volpitlo,1939; Schaffer, 1955). In infants a cuff which is too narrow for a given individual tends to overestimate the systolic pressure whereas an excessively wide cuff will tend to underestimate the true value. In adults, increasing the cuff width beyond 13cm has little influence on either the systolic or diastolic reading while narrower cuffs provide high values (Karvonen, 1962). The latter author has also shown that cuffs of insufficient length to completely encircle the limb used for the measurement will overestimate the true pressures. Differences in the efficiency of the transmission of cuff pressure to an artery are thought to be related to differences in limb circumference, the compressibility of tissues and the contour of the arterial pulse (Robinow et al, 1939; Holland and Humerfelt, 1964).

In only one study in dogs (Valtonen and Erikson, 1970) has serious consideration been given to the influence of cuff width on the accuracy of indirect measurements of pressure. Direct and indirect values were compared in 10 dogs and bodyweight and foreleg circumference were also noted. As only one dog weighed more than 20kg and as differences between direct and indirect measurements up to 60 mmHg were found in some instances these authors were unable to give any definite guidance on

the correct size of cuff for different dogs. They did confirm however that narrow cuffs gave high values in large dogs and wide cuffs gave erratic, often too low, values in small dogs.

In the present study the accuracy of indirect measurements obtained using a xylol pulse monitor and three different widths of occluding cuff has been investigated and an attempt made to relate the correct size of cuff for a given dog to foreleg circumference and body weight.

2. MATERIALS

2.1 Animals

Preliminary experiments were done in adult dogs kept for experimental purposes. When the criteria for the systolic end point and a suitable size of occluding cuff had been chosen, subsequent studies were done in dogs admitted for routine surgical interventions in the Department of Veterinary Surgery of the University.

2.2 Apparatus

Indirect blood pressure was measured with a 'Newcastle' sphygmomanometer (C.F. Thackray Ltd., Leeds). The use of this instrument in human infants has been described by Ashworth, Neligan and Rogers (1959). A schematic representation of the instrument is shown in Figure 4.

The apparatus incorporates an occluding cuff connected to a mercury manometer, and a second, pulse sensing, cuff attached to a pulse indicator. The pulse indicator consists of a straight glass capillary tube whose lumen contains an interrupted column of xylol. Beyond this column is a cushion of compressible air against which the xylol can be moved by pressure changes within the connecting cuff. This cuff and the pulse indicator can be isolated from the occluding cuff and the manometer with a clamp.

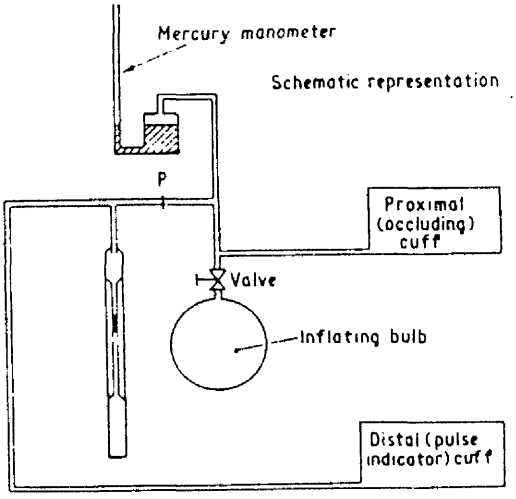


Fig. 4 Schematic representation of 'Newcastle' sphygomomanometer

The inflatable cuffs supplied with the instrument were made of polyvinyl chloride with a self adhesive 'tail' of the same material. Both cuffs were the same size and had an inflatable portion which measured 15 x 2.5 cm. This size was satisfactory for the pulse sensing cuff but early experiments indicated that complete arterial occlusion could not be produced in large dogs with a similar size of occluding cuff. A number of alternative cuffs were therefore obtained from the manufacturer of the instrument for use in the present investigation.

3. METHODS

3.1 TECHNIQUE OF INDIRECT BLOOD PRESSURE MEASUREMENT

In dogs the pulse sensing cuff was placed on the metacarpal region and the occluding cuff over the brachial artery, above the elbow, as described by Wilson and Clarke (1964). Cuffs were applied to the upper forelimb of anaesthetised dogs lying in the lateral position. In this situation artefacts caused by heart beats and respiratory movements being transmitted to the distal cuff were minimised. In all animals the trachea was intubated to avoid the occurrence of respiratory obstruction which would have accentuated the variations in arterial pressure which occur with different phases of respiration.

To record systolic pressure the whole system was first inflated to a pressure of about 60mmHg. The pulse indicator was then isolated from the rest of the system by clamping the rubber tubing at P (Figure 4). If the pulse indicator system was free from leaks it remained at a constant pressure until the clamp was released. When the pressure in the rest of the system, including the occluding cuff, was allowed to fall to zero by opening the control valve (Figure 4) movement of the xylol 'beads' in the capillary tube, representing the pulsation of the arteries of the foreleg, could be detected. The occluding cuff was then inflated until the xylol column ceased to move. Thereafter the pressure was allowed to fall slowly until the characteristic movements reappeared. Due to respiratory variations in systolic pressure, short runs of beats were noted before regular

arterial pulsations were detected at the systolic pressure end point. The diastolic end point occurred when the excursion of the xylol column in the capillary tube regained its maximum range. As this point was difficult to judge by eye no attempt was made to record diastolic pressure routinely.

When measurements were made over a prolonged period the distal cuff was deflated at intervals to prevent venous congestion and swelling of the foot.

3.2 EXPERIMENTS TO EXAMINE THE FACTORS INFLUENCING THE ACCURACY OF INDIRECT MEASUREMENTS

To validate the indirect technique, results obtained with the 'Newcastle' sphygmomanometer were compared with direct measurements of blood pressure taken from a femoral artery with a strain gauge transducer (Ether B.P. 15, Devices Sales Ltd., Welwyn Garden City, Herts). Percutaneous puncture of the artery was performed using a 19 or 21 B.W.G. needle connected to a short length of polythene tubing ('Butterfly INT' cannula, Abbott, Queenborough, Kent) and a 60 cm length of saline filled nylon manometer tubing (200/495/060, Portex Ltd., Hythe, Kent) was used to transmit pressure to the transducer. The transducer was calibrated with a mercury manometer and was placed at the same level as the upper forelimb to eliminate the effect of hydrostatic pressure on blood pressure readings. The direct pressure was displayed on a pen recorder (Polygraph, AEI, Scientific Apparatus Ltd., Urmston, Manchester) and, at the times when cuff pressure was read from the mercury manometer, an event marker on the recorder was activated. This allowed

simultaneous comparisons of the results obtained with the two techniques to be made. To avoid any bias when reading the direct pressure, the indirect pressures were not marked on the pressure record but were noted sequentially in a separate list.

3.2.1 Definition of systolic pressure end-point

When taking an indirect measurement of systolic pressure the manometer pressure may be read at the point when occasional pulses, due to respiratory variations in systolic pressure, are first noted. Alternatively it may be recorded at the point when regular arterial pulsations are first detected. In one experiment in a dog weighing 19kg, direct and indirect readings were taken at both end points to determine the most accurate technique for subsequent studies. Anaesthesia was induced with 26 mg kg^{-1} thiopentone ('Intraval', May and Baker, Dagenham, Essex) following premedication with 10mg droperidol ('Droleptan', Janssen Pharmaceutical, Marlow, Bucks), and was maintained by the inhalation of halothane ('Fluothane', ICI, Macclesfield, Cheshire). An occluding cuff measuring 25 x 3.75 cm was used and in total 27 comparisons of direct and indirect systolic pressure were made as blood pressure was altered by adjusting the inspired concentration of halothane.

3.2.2 Cuff length

In some preliminary experiments three sizes of occluding cuff were used. These were of varying lengths and widths and

measured: 15 x 2.5 cm

20 x 3.75 cm

25 x 5.0 cm

It was found that in a large dog weighing 25 kg with a forelimb circumference of 18 cm, the smallest cuff gave readings which were up to 50 mmHg higher than values obtained by direct measurement. This size of cuff did, however, give indirect values which agreed to within 10 mmHg with direct values in two dogs weighing 13 kg with forelimb circumferences of 12 and 13 cm respectively. To study the influence of cuff length, independent of cuff width, on the accuracy of indirect measurements a set of cuffs was obtained which measured:

15 x 2.5 cm

20 x 2.5 cm

25 x 2.5 cm

A dog weighing 12.6 kg with a foreleg circumference of 15 cm was anaesthetised with 25 mg kg^{-1} pentobarbitone ('Nembutal', Abbott Laboratories, Queenborough, Kent). At intervals during the experiment arterial pressure was increased by the intravenous injection of 10 mg bolus doses of methylamphetamine ('Methedrine', Wellcome Medical, Crewe, Cheshire) and decreased by the administration of halothane. Using three different lengths of occluding cuff, 23 comparisons of direct and indirect systolic pressure were made over a directly measured systolic pressure range of 82 - 180 mmHg.

3.2.3 Cuff width

To study the influence of cuff width on the accuracy of indirect measurements of systolic pressure, occluding cuffs with the following dimensions were obtained:

25 x 2.5 cm

25 x 3.75 cm

25 x 5.0 cm

Using these cuffs, 57 comparisons of direct and indirect pressure were made in a dog weighing 32 kg with a foreleg circumference of 20 cm. As in the previous experiment, anaesthesia was induced with 25 mg kg^{-1} pentobarbitone and arterial pressure was altered at intervals by the administration of methylamphetamine or the inhalation of halothane. In this experiment the directly measured systolic pressure varied between 120 and 210 mmHg.

3.2.4 Body weight and forelimb circumference

The results obtained in preliminary experiments indicated that the occluding cuff width was more important than cuff length in affecting the accuracy of indirect measurements of systolic pressure in an individual animal. To define the correct width of cuff for a given size of animal the study was continued in dogs of different sizes.

Simultaneous measurements of direct and indirect systolic pressure were made during clinical anaesthesia in 51 dogs. The anaesthetic technique used involved induction with thiopentone and maintenance with an inspired concentration

of 1-2% halothane in oxygen. In 15 dogs acepromazine ('Acetylpromazine', Boots Ltd., Nottingham) was given intramuscularly 30-45 min before induction of anaesthesia. Wider cuffs were used for large dogs and narrower cuffs for small dogs and, on a number of occasions, more than one size of cuff was used for the same animal. All animals were weighed and the circumference of the forelimb just above the elbow was measured by passing a nylon thread round the limb.

3.3 EVALUATION OF RESULTS

In experiments designed to define the systolic pressure end-point and the correct size of cuff for an individual animal the mean values of a number of estimates of indirect and direct pressure were calculated together with the mean of the difference between individual values. The correlation coefficient between a number of indirect and direct readings and equations of linear regression were also calculated.

In the study which attempted to define the correct width of cuff for a given animal the correlation of the difference between indirect and direct values with body weight and foreleg circumference was also calculated to quantify the influence of these variables on the accuracy of the indirect measurement. When the 'best' size of cuff for a given animal had been established a scatter diagram was drawn to display the results obtained

in animals in which an appropriate size of cuff had been used.

The significance of the correlations established was determined using the formula: $t = \sqrt{\frac{r^2(n-2)}{1-r^2}}$

where r = correlation coefficient

n = number of comparisons

4. RESULTS

4.1 EXPERIMENTS TO EXAMINE THE FACTORS INFLUENCING THE ACCURACY OF INDIRECT MEASUREMENTS

4.1.1 Definition of systolic pressure end-point

Values of indirect pressure obtained in a single animal and read from the mercury manometer at the point when occasional pulses, due to respiratory variations in systolic pressure (IdR) were first detected in the pulse detector, and when continuous pulses (IdC) were first seen, are given in Table 1. Simultaneous direct measurements (D) of systolic pressure made at each indirect pressure end point are also shown.

The accuracy of the indirect pressure readings appeared to be little influenced by the indirect systolic end-point used. A comparison of mean values of a number of indirect and direct pressure readings could be misleading as similar mean values would be obtained if high indirect values were balanced by other low values. In averaging the differences between the indirect and direct readings in Table 1 the arithmetic sign has been ignored and it can be seen that the mean difference between the two readings was marginally less when the end point defined by continuous pulses was used. The equations of linear regression and correlation coefficients (r) obtained with the two end points were as follows:

$D = 0.96\ IdR + 1.22;\quad r = 0.91$ Both correlations

$D = 1.02\ IdC + 2.17;\quad r = 0.92$ significant at 0.1% level

Table 1 Comparison of direct and indirect measurements of systolic pressure when two different indirect pressure end-points were used. All values in mmHg.

IdR (a)	D (b)	Difference IdR - D	IdC (c)	D	Difference IdC - D
132	130	+ 2	128	130	- 2
120	120	0	110	114	- 4
126	120	+ 6	115	120	- 5
118	116	+ 2	120	120	0
116	112	+ 4	110	113	- 3
110	108	+ 2	114	112	+ 2
114	100	+ 14	104	108	- 4
100	95	+ 5	110	100	+ 10
100	100	0	96	96	0
110	110	0	96	95	+ 1
115	114	+ 1	108	108	0
120	120	0	114	114	0
124	117	+ 7	115	116	- 1
			120	118	+ 2
Mean ± SD	115.7 ± 9.3	112.5 ± 9.8	3.3 ± 4.0	111.4 ± 8.8	111.7 ± 9.7	2.4 ± 2.7

(a) IdR = indirect pressure read when first occasional pulse due to respiratory variation in systolic pressure was noted

(b) D = direct pressure

(c) IdC = indirect pressure read when continuous pulses first noted

Using both end points a high degree of correlation was found between indirect and direct measurements. As no significant difference in the accuracy of the indirect reading was obtained by using one or other end-point an arbitrary choice was made. In all further experiments indirect pressure was noted when more than about four recognizable pulses in a row were seen.

4.1.2 Cuff length

Values of indirect systolic pressure obtained with occluding cuffs of three different lengths, and simultaneously measured direct values in a single animal, are given in Table 2. The correlation coefficients between indirect and direct values, and the equations of linear regression obtained with the three cuffs, were as follows:

$D = 0.98\ Id(15) - 4.62;\quad r = 0.94$

$D = 0.86\ Id(20) + 7.55;\quad r = 0.97$

$D = 1.12\ Id(25) - 21.4;\quad r = 0.95$

All correlations significant at 0.1% level

A good correlation between indirect and direct values of systolic pressure was found with all three cuffs. The indirect technique tended to overestimate the direct pressure in most instances but the error was not increased significantly when the smaller cuffs were used. As the standard deviation of the difference between indirect and direct values was smallest when the 25 cm cuff was used, this length of cuff was selected for subsequent studies.

Table 2 Indirect (Id) and direct (D) values of systolic pressure obtained with occluding cuffs of different lengths. All cuffs 2.5 cm wide.

Cuff length (cm)	15			20			25		
Systolic pressure	Id	D	Id-D	Id	D	Id-D	Id	D	Id-D
mmHg	80	82	-2	88	86	+2	94	80	+14
	130	120	+10	90	86	+4	98	95	+3
	155	123	+30	145	123	+22	130	120	+10
	160	140	+20	136	120	+16	145	123	+22
	168	168	0	170	150	+20	145	160	-15
	175	180	-5	170	165	+5	170	160	+10
	179	178	+1				170	165	+5
	184	178	+6				160	166	-6
							170	180	-10
Mean ± SD	154 ± 34	146 ± 36	9.25 ± 10.5	133 ± 37	121 ± 32	11.5 ± 8.8	142 ± 30	139 ± 35	10.5 ± 5.8

4.1.3 Cuff width

The mean values of indirect pressure, obtained in one dog, with occluding cuffs of three different widths and mean values of simultaneously measured direct pressure are given in Table 3 . The calculated equations of linear regression and the correlation coefficients between the direct and indirect values of systolic pressure are also shown. The individual values of direct and indirect pressure obtained from this animal are given in Appendix 1.

In contrast to the results obtained with occluding cuffs of different lengths it was found that changes in cuff width produced marked and more consistent changes in the accuracy of the indirect measurements. The mean values for simultaneously measured direct and indirect pressures indicated that the smallest cuff size tended to overestimate the true systolic pressure while the two wider cuffs tended to underestimate the direct reading. The greatest error was produced by the 5 cm cuff which underestimated the true reading by a mean value of 29 mmHg. When the calculated regression lines obtained from simultaneously measured direct and indirect pressures were plotted (Figure 5) this trend could be seen more readily and in addition it was noted that at values of direct pressure below 140 mmHg the 2.5 cm cuff also tended to underestimate the true pressure. The regression line obtained with the 3.75 cm cuff was closest to the 45^{o} line of equal values and this cuff also provided the highest correlation coefficient between diect and indirect pressure.

Table 3 Mean values of indirect (Id) and direct (D) systolic pressure, linear regression equations and correlation coefficients obtained using occluding cuffs of three different widths. All cuffs 25 cm in length.

Cuff width (cm)	2.5			3.75			5.0		
	Id	D	Id-D	Id	D	Id-D	Id	D	Id-D
Mean systolic pressure (mmHg)	166	157	10.7	147	156	9.3	128	157	29
± SD	± 25.3	± 17.9	± 10.3	± 20	± 22	± 7.1	± 17.8	± 18.6	± 8.8
Linear regression equation	D = 0.64 ID + 51			D = 1.07 Id - 1.44			D = 0.90 Id + 42		
r	0.90 *			0.95 *			0.86 *		

*Significant at 0.1% level

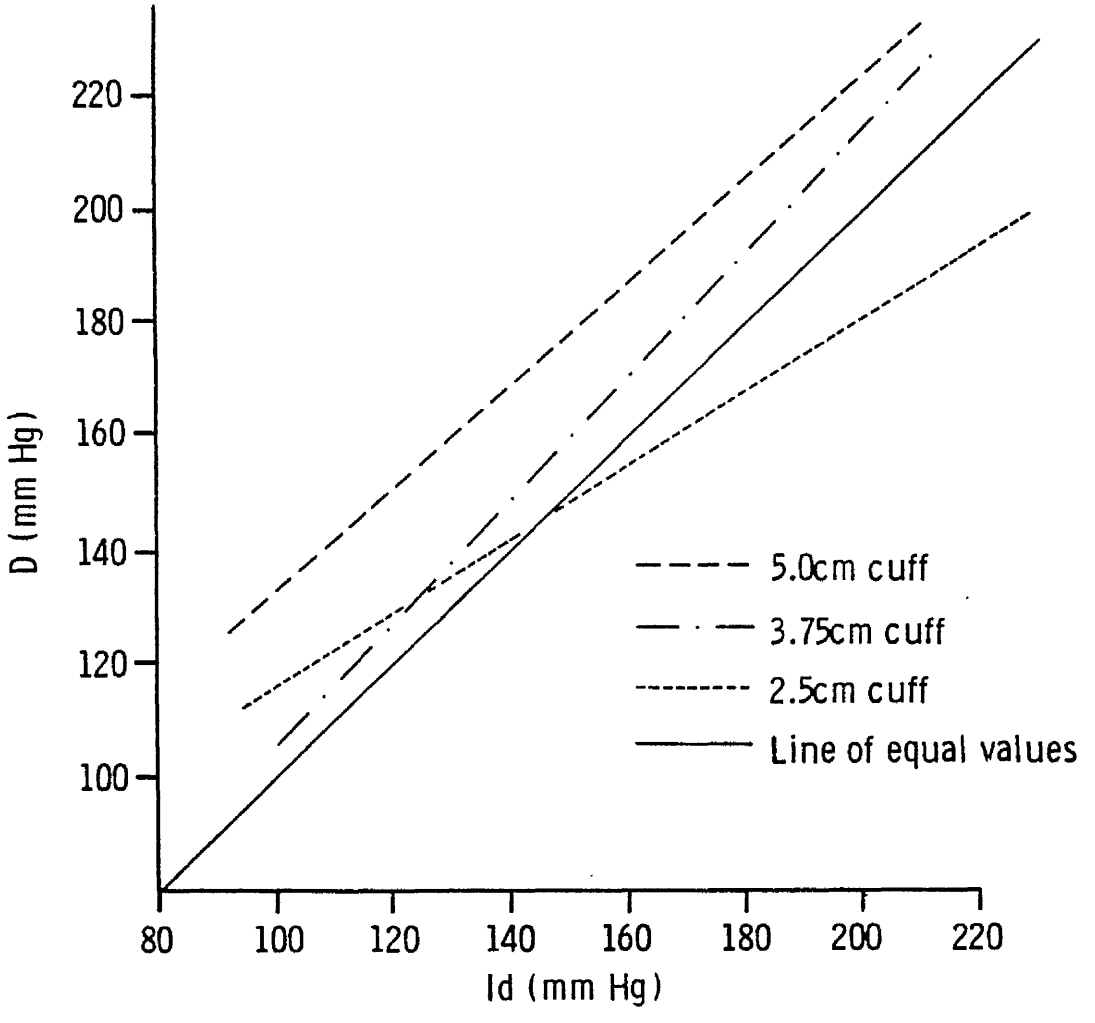


Fig. 5 Calculated regression lines obtained from simultaneous measurements of indirect (Id) and direct (D) systolic pressure

This experiment indicated that, for the dog used in this study, the 3.75 cm cuff gave the most accurate values of indirect pressure while the wider cuff gave readings which were much lower than the direct pressure and the narrower cuffs generally provided values in excess of the true pressure.

4.1.4 Body weight and forelimb circumference

Details of the number of comparisons of direct and indirect pressure made, and the correlation between the two measurements with different sizes of cuffs are given in Table 4. In 23 animals both the 2.5 and 3.75 cm cuffs were used and in a few animals the accuracy of all three cuffs was assessed. Simultaneously measured values of indirect and direct pressure obtained from all 51 dogs are given in Appendix 2 .

Table 4 Correlation between direct and indirect measurements of systolic pressure

Occluding cuff width (cm)	No. of comparisons	Weight range (kg)	Correlation coefficient	Equation of linear regression
2.75	27	6.5 - 29	0.87*	D = 0.94 Id + 5.39
3.75	46	8 - 47	0.91*	D = 0.88 Id + 20.49
5.0	12	23 - 39	0.96*	D = 1.08 Id + 0.95

*Significant at 0.1% level

All the cuffs gave values for indirect pressure which correlated well with directly measured pressures. In the 23 animals where both the 2.5 and 3.75 cm cuffs were used the wider cuff generally gave a lower reading.

In Table 5 the correlation of the difference between indirect and direct measurements (Id - D) with body weight is shown. Results obtained in individual animals are given in Appendix 3.

Table 5 Correlation between (Id - D, mmHg) and body weight (kg) obtained with occluding cuffs of three different widths

Occluding cuff width (cm)	No. of comparisons	Correlation coefficient	Equation of linear regression
2.5	27	0.63*	Id-D = 1.56 Wt - 21.72
3.75	46	0.58*	Id-D = 0.94 Wt - 26.01
5.0	12	0.31	Id-D = 0.86 Wt - 38.44

*Significant at 0.1% level

The accuracy of the results obtained with the 2.5 and 3.75 cm cuffs was significantly affected by body weight whereas a similar degree of correlation was not obtained with the 5 cm cuff.

The accuracy of the indirect readings obtained with the two smaller cuffs was also affected by changes in foreleg circumference (Table 6) but the degree of correlation obtained was no greater than that found with body weight.

Table 6 Correlation between (Id-D, mmHg) and foreleg circumference (F.C., cm) obtained with occluding cuffs of three different widths

Occluding cuff	No. of comparisons	Correlation coefficient	Equation of linear regression
2.5	27	0.58*	Id-D = 4.17 FC - 67.28
3.75	46	0.54**	Id-D = 2.82 FC - 59.56
5.0	12	0.21	Id-D = 1.06 FC - 36

*Significant at 1% level

**Significant at 0.1% level

To determine the correct width of cuff for a given size of animal the regression lines of the difference between indirect and direct measurements (Id-D, mmHg) and body weight, obtained with the 2.5 and 3.75 cm cuffs were drawn (Figure 6). It can be seen that the 2.5 cm cuff would be expected to provide the most accurate results (Id-D = 0) in animals weighing 14 kg. The results obtained with this size of cuff are liable to be greater than the true value in dogs heavier than 14 kg and less than the direct value in lighter dogs. Similar effects were found with the 3.75 cm cuff with the most accurate results being found in animals weighing 27.5 kg.

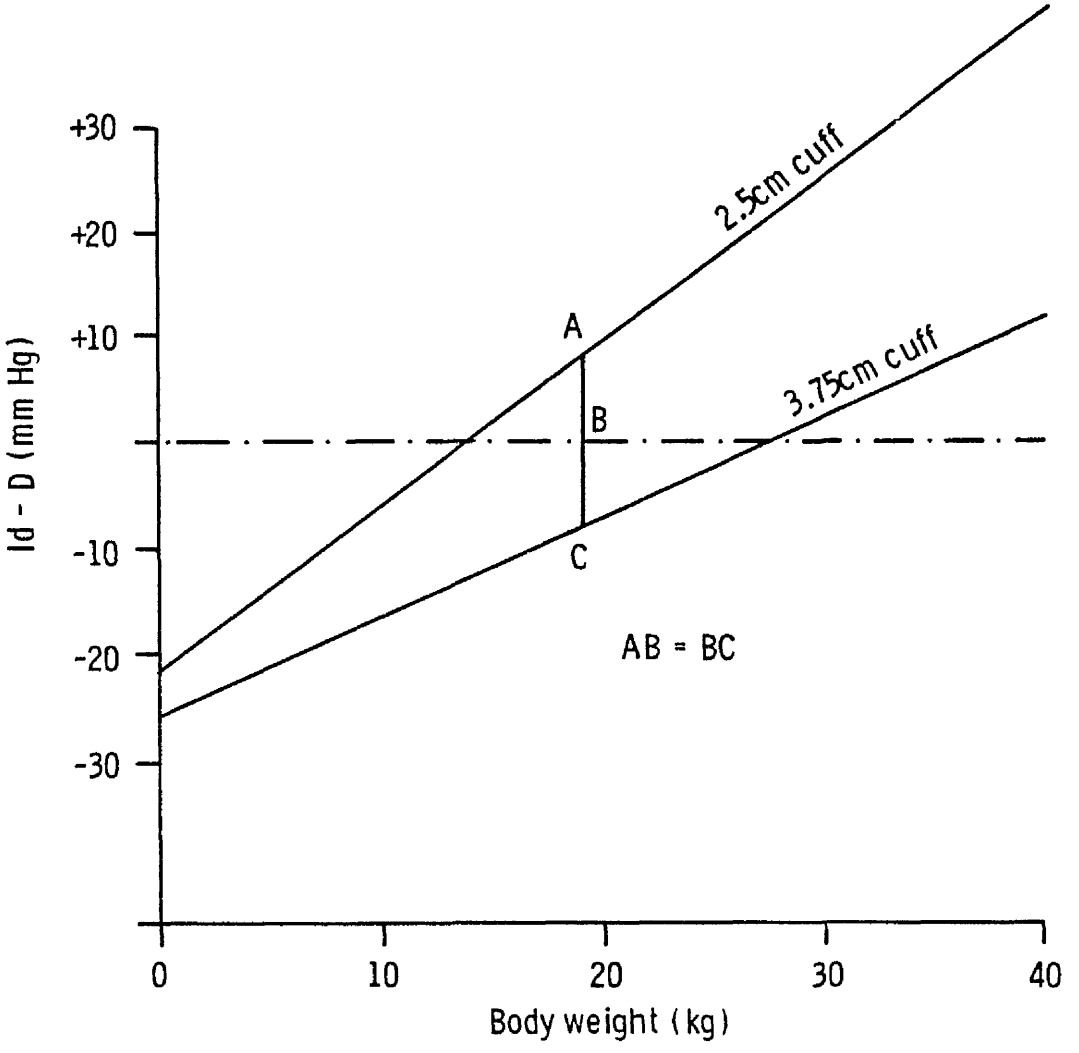


Fig. 6 Regression lines of the difference between indirect and direct measurements of systolic pressure (Id-D, mmHg) and body weight. At 19kg the positive error (AB) obtained with the smaller cuff is equal to the negative error (BC) incurred with the wider cuff

When dealing with a range of sizes of animals the 'best' weight at which to change the size of the occluding cuff is that weight where the positive error incurred by using the smaller cuff is equal to the negative error obtained when the larger cuff is used. In Figure 6 it can be seen that this crossover point is reached at a weight of 19 kg. It would appear therefore that the 3.75 cm cuff should be used for dogs heavier than 19 kg whereas the 2.5 cm cuff can be expected to give more accurate results in lighter dogs.

An examination of the results obtained in individual animals showed that indirect measurements had been made with a 2.5 cm cuff in 19 dogs under 19 kg and with a 3.75 cm cuff in 26 dogs over this weight. A scatter diagram of the results obtained from these 45 dogs is shown in Figure 7. The calculated regression line obtained with these values (D = 0.96 ID + 5.81) lay very close to the 45^{o} line of equal values and the correlation coefficient between the two values was 0.91. In only three dogs, where the 2.5 cm cuff was used, did the indirect pressure differ from the direct pressure by more than 15 mmHg. Two of these dogs were terrier breeds in which accurate placement of the occluding cuff was found to be more difficult. Excessively high indirect readings were obtained in some very large dogs where bulging of the 3.75 cm cuff occurred. In a few of these cases the 5 cm cuff produced more accurate results but in general this size of cuff tended to underestimate the directly measured pressure.

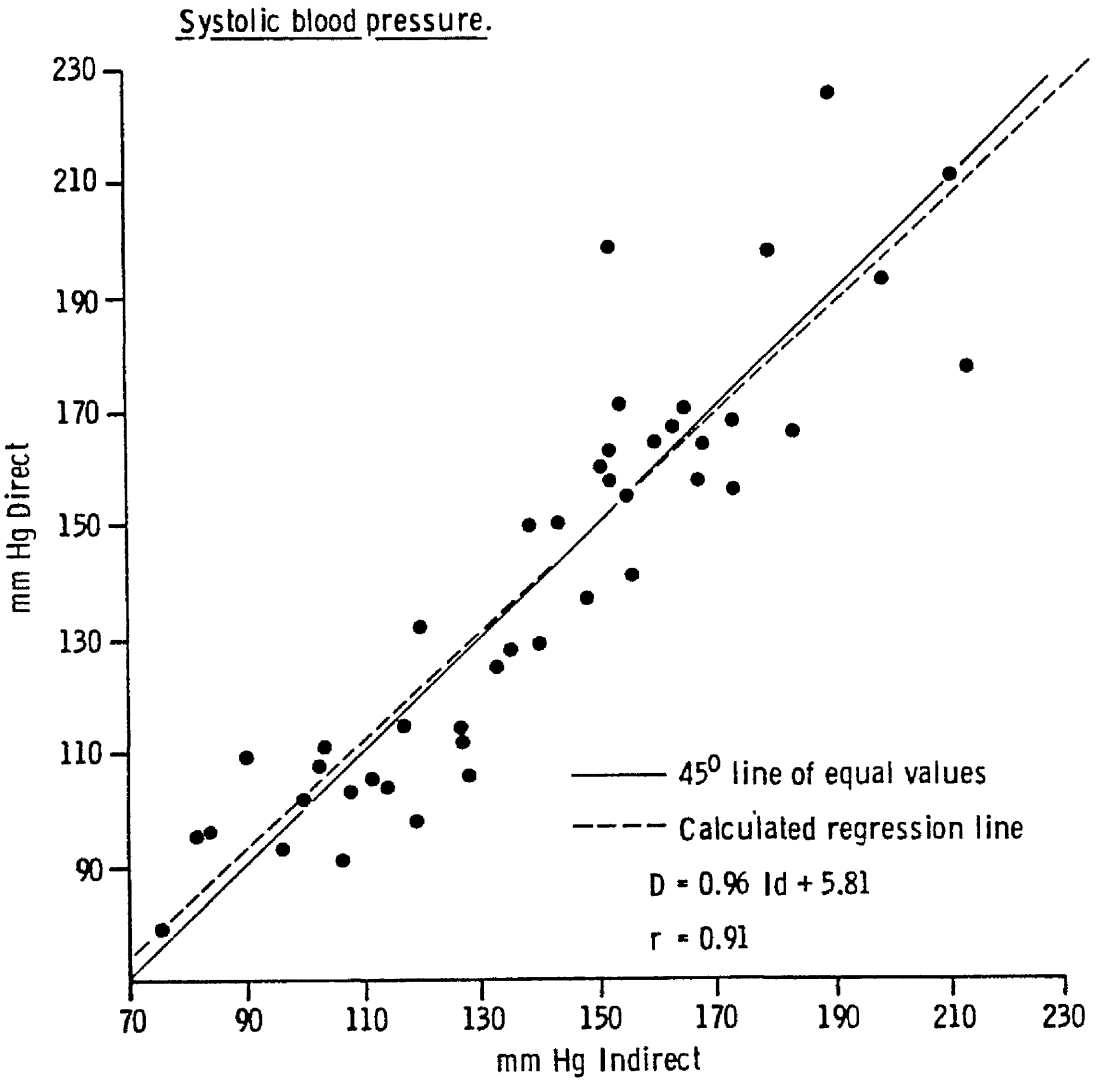


Fig. 7 Scatter diagram to illustrate the relationship between direct and indirect measurements in 45 dogs. A cuff 2.5cm wide was used for dogs up to 19kg and a 3.75cm wide cuff for heavier dogs

5. DISCUSSION

The assessment of the state of the circulatory system of the anaesthetised dog is usually restricted to the measurement of pulse rate, estimation of pulse volume and observation of the colour and capillary refill time in the oral mucous membranes. The measurement of arterial pressure can provide useful additional information and where accurate measurement is required direct techniques are usually employed. For routine use, indirect methods, which do not require cannulation or needle puncture of an artery are preferable.

A technique suitable for use in the anaesthetised dog should be shown to provide indirect values which correlate well with simultaneous direct measurements of arterial pressure. The apparatus used should be as simple as possible and the technique should be applicable to dogs of varying size and conformation. The pulse sensing device should be sensitive enough to detect small peripheral pulses but should not be so sensitive that movement artefacts obscure arterial pulsations (Campbell, Lawson and Sanford, 1964). Once apparatus has been set up no access to the anaesthetised animal should be necessary as this may be difficult in animals covered with surgical drapes.

The present study has shown that the 'Newcastle' infant sphygmomanometer with a xylol pulse indicator fulfills the

above requirements well. Indirect measurements of systolic pressure correlated closely with direct values in 51 dogs with a weight range of 6.5 - 47 kg. In an individual animal the accuracy of indirect measurements was found to be affected more by the width of the occluding cuff than by its length. In animals of varying size the accuracy of the indirect method was affected by body weight and a correlation with foreleg circumference was also established. From the calculation of linear regression equations derived from the difference between direct and indirect values and body weight it appears that a reasonably accurate estimate of systolic pressure can be made by using a cuff 2.5cm wide in dogs weighing less than 19kg and a 3.75cm wide cuff in heavier animals.

Wilson and Clarke (1964) have reported the only previous study in which a xylol pulse monitor was used to measure indirect blood pressure in dogs. These authors found a good degree of correlation between indirect and direct readings in a single 12kg dog. The width of the occluding cuff used was 3cm and was similar to that recommended in the present work for a dog of this size.

In the original report of the use of the 'Newcastle' sphygmomanometer, in human infants, Ashworth, Neligan and Rogers (1959) compared the accuracy of measurements made with the xylol pulse indicator with indirect values

obtained by auscultation or palpation of the peripheral pulse. Measurements made with the xylol indicator showed the smallest range of variation but indirect readings were not compared with directly measured pressures in these infants.

The results of the present study can be compared with those of Valtonen and Eriksson (1970) who used an occluding cuff placed on the upper forelimb, and detected peripheral pulsations with a condenser microphone. The influence of cuff width on the accuracy of their indirect measurements was examined by comparing the results obtained with seven cuffs, varying in width from 3 - 5.6cm, with direct measurements from a femoral artery in 10 dogs weighing between 6 and 26kg. It was found that narrow cuffs gave values greater than direct readings in large dogs while wide cuffs gave erratic values in small dogs. With the small number of animals examined it was not possible to give any definite guidance on the selection of the correct size of cuff for different dogs. The inflatable portion of the occluding cuff used by these authors was made from a cycle tube and cuffs of different widths were prepared by covering this tube with non-distensible cloth. With this design of cuff it is possible that cuff pressure may not have been transmitted equally to the edges of the larger cuffs.

In other investigations in dogs, where indirect and direct values have been compared, a single size of occluding cuff has generally been used and little attention has been paid to the effects of cuff width on the accuracy of the indirect reading (Rule, 1944; O'Connor, 1955; Prioli and Winbury, 1960; Klemm and Hemborough, 1966; Baum and Rowles, 1969; Garner et al., 1975). Cuffs were applied to the thigh in the studies reported by Rule (1944) and Romagnoli (1953). Klemm and Hemborough (1966) used a tubular metal tail cuff and tail cuffs 2 and 3cm wide were used by Baum and Rowles (1969) and Prioli and Winbury (1960) respectively. The latter authors also shaved the tail prior to the application of the cuff. Garner et al., (1975) used a 5cm wide cuff applied to the tibia of four dogs with a weight range of 20-27kg and O'Connor (1965) applied cuffs 1-2.5cm wide to the femoral artery enclosed in a skin loop, when investigating a technique for use in physiological studies in dogs. In clinical practice one is likely to encounter some dogs with an insufficient length of tail to retain an occluding cuff and the conical shape of the thigh makes accurate placement of a cuff at this site difficult. When a tibial cuff is used it is possible that the arterial vessels may be displaced between the two bony structures at this site and may thus be protected from the pressure exerted by the occluding cuff. As a variety of different sizes of cuff were used by the above

authors and no information on the circumference of the compressed area has been provided it is not possible to make a direct comparison of the results obtained with those of the present study.

To provide accurate indirect results in adult man it has been recommended that the inflatable portion of the occluding cuff should be 20% wider than the diameter of the limb on which it is placed (Kirkendall, Burton, Epstein and Freis, 1967). Ragan and Bordley (1941) compared readings taken in man with 13 and 20cm wide cuffs and found that values obtained with the wider cuff were significantly lower than directly measured pressures. Some correlation between the accuracy of the indirect reading and the circumference of the arm was found by these workers but Holland and Humerfelt (1964) were unable to demonstrate a similar correlation in a study in which cuff pressure was found to underestimate direct systolic pressure by a mean value of 24.6mmHg. As arm diameter is difficult to measure accurately Geddes and Whistler (1978) related the recommendation of Kirkendall et al. (1967) to arm circumference and suggested that an occluding cuff width equal to 40% of the arm circumference should be used. In a study which compared indirect values obtained with cuffs 9cm and 18cm wide with reference values obtained with a 12cm cuff these workers found that when the cuff width:arm circumference ratio was 0.34 blood pressure was

overestimated by about 5%. When the ratio was increased to 0.50 pressures were underestimated to a similar extent.

It can be calculated from the regression equations obtained in the present work that the 2.5cm and 3.75cm cuffs used would be most suitable in dogs with foreleg circumferences of 15cm and 21cm respectively. These figures provide cuff width:foreleg circumference ratios of 0.17 and 0.18. These ratios are much lower than those found to give satisfactory results in man and indicate that for a given limb circumference relatively narrower cuffs provide satisfactory indirect results in dogs.

A more relevant comparsion may be made between results obtained in dogs and in human infants. Robinow, Hamilton, Woodbury and Volpitto (1939) concluded that a cuff width of 2.5cm was suitable for newborn infants whereas a 5cm cuff was required to give accurate results in older infants. In another study, in newborn infants, Schaffer (1955) found that a cuff 4cm wide gave more accurate results than 2.5 or 8cm cuffs. Robinow et al. (1939) noted some correlation between the width of cuff required and arm circumference when the latter measured less than 14cm. These authors suggested that the tissues in the arm of a young infant were more compressible and were displaced laterally by inflation of the cuff such that the diameter of the tissue actually compressed was decreased. A similar mechanism may explain the need for a relatively narrower cuff

in dogs but further work is required to examine the compressibility of the tissues of the forelimb in this species.

Results obtained in the present work indicate that the length of the occluding cuff is of less importance than the cuff width. All three cuffs examined were sufficiently long to completely encircle the forelimb. Although it has been recommended for use in man that the inflatable portion of the cuff should be long enough to half encircle the limb (Kirkendall et al., 1967) it has been noted that a cuff which completely encircles the limb can reduce the scatter of the indirect readings obtained (Karvonen, 1962).

In addition to cuff size the accuracy of the indirect technique may be affected by a number of other factors. In this work and in the studies of Wilson and Clark (1960) and Valtonen and Eriksson (1970) indirect pressure was estimated in a brachial artery while direct pressure was measured in a femoral artery. The latter authors have shown that in dogs the femoral pressure may be up to 10mmHg greater than the brachial pressure. Although smaller differences between femoral and brachial pressures have been noted in man (Pascarelli and Bertrand, 1964; Bell, Nielsen, Lassen and Wolfson, 1973) a difference between pressure at the site of cuff placement and the reference pressure may be of more importance in studies where a tail cuff has been used (Klemm and Hemborough, 1966; Baum and Rowles, 1969; Prioli and Winbury, 1960).

It has also been suggested that indirect methods measure the lateral pressure against the wall of an artery, whereas the direct method determines the end-on thrust of pressure against the diaphragm of a transducer (Van Bergen, Weatherhead, Treolar, Dobkin and Buckley, 1954). However, these authors were unable to detect significant differences in brachial artery pressure when a needle was directed peripherally or centrally.

The frequency response of the catheter-recorder system is important for the accurate measurement of the direct pressure. Although this was not examined in the present work the diameter and composition of the catheters used were similar to those which were shown to be satisfactory by Stegall, Kardon and Kemmerer (1968). It is possible that the silicone rubber catheter used by Baum and Rowles (1969) may have caused the directly measured pressure to be damped excessively.

It has been shown that if a given size of cuff is applied more tightly the indirect pressure measured is reduced (Schaffer, 1955). Difficulty in applying cuffs in a uniform manner in dogs with widely differing conformation was a possible source of error in some of the dogs examined in the present study. However, in a given animal, and in the study as a whole, a good correlation was obtained between direct and indirect

values. This indicates that the method should be sufficiently accurate to detect changes occurring during anaesthesia in an individual animal.

Although indirect techniques have been used to measure arterial pressure in conscious dogs (Wilson and Clark, 1964; Prioli and Winbury, 1960; Romagnoli, 1953; O'Connor, 1955) the marked sinus arrhythmia seen in conscious animals, and the apprehension shown by untrained animals makes the procedure more difficult and less reliable than is the case in the anaesthetised dog.

Chapter 2

EVALUATION OF THE ACCURACY OF END-TIDAL CARBON DIOXIDE TENSION MEASUREMENT FOR THE ESTIMATION OF ARTERIAL CARBON DIOXIDE TENSION IN ANAESTHETISED DOGS

1. INTRODUCTION

It is generally accepted that measurement of the partial pressure of carbon dixoide in arterial blood ($PaCO_2$) provides a better indication of the effectiveness of respiration and the balance between carbon dioxide production and elimination than measurement of respiratory rate and tidal volume (Jennett, 1968). The velocity of diffusion of carbon dioxide is sufficiently rapid for pressure equilibrium to be established in the lung between alveolar gas and pulmonary capillary blood (Comroe, 1965). As pulmonary capillary blood has the same PCO_2 as arterial blood and as end-tidal expired gas can be expected to be representative of alveolar gas, measurement of end-tidal PCO_2 should provide an accurate estimation of $PaCO_2$. It has been found, however, in normal unanaesthetised dogs, that $PaCO_2$ is generally 3 mmHg greater than the alveolar value (Severinghaus and Stupfel, 1957). This difference was attributed to the presence of alveolar dead space resulting from the ventilation of some nonperfused alveoli.

In man, a significant increase in alveolar dead space has been found to follow induction of anaesthesia (Severinghaus, Stupfel and Bradley, 1957; Campbell, Nunn and Peckett, 1958) while in a study in dogs anaesthetised with pentobarbitone, Suskind and Rahn (1954) found that the alveolar-arterial carbon dioxide gradient did not change in any predictable manner.

In the present study the difference between alveolar (end-tidal) and arterial blood PCO_2 has been measured to assess the accuracy of the end-tidal values in the estimation of $PaCO_2$ during anaesthesia produced in dogs of various sizes with a range of anaesthetic drugs and techniques. The results should also provide information on the size of the alveolar dead space in these animals but no attempt has been made to standardize the techniques used to allow the influence of the various techniques on alveolar dead space to be examined.

2. MATERIALS

2.1 Animals

The 22 dogs (5-40 kg) used in this investigation were clinical cases requiring anaesthesia for routine surgical interventions in the Department of Veterinary Surgery of the University. All animals were free from any detectable abnormality in their respiratory and cardiovascular systems.

2.2 Apparatus

The carbon dioxide concentration in the expired air was measured with an infra-red analyser (Godart Capnograph, Cardiopulmonary Instruments, Rochester, Kent) and recorded continuously with a Polygraph pen recorder (AEI Scientific Apparatus Ltd., Urmston, Manchester). Arterial PCO_2 ($PaCO_2$) was measured with a Severinghaus electrode (Radiometer Type E5036, V.A. Howe and Co. Ltd., London).

In most cases anaesthesia was maintained with inhalation agents delivered with oxygen from a Boyle's anaesthetic apparatus. A 'to and fro' and a circle absorber were both used and in one instance gases were delivered with a Magill circuit.

2.3 Drugs

Thiopentone ('Intraval', May and Baker Ltd., Dagenham, Essex) was the agent most frequently used to induce anaesthesia and both halothane ('Fluothane' ICI, Macclesfield, Cheshire) and cyclopropane (BOC Medical, Harlow, Essex) were used as maintenance agents. In three dogs a mixture of etorphine and methotrimeprazine ('Immobilon', Reckitt and Coleman, Hull) was used. Eight dogs were premedicated with acepromazine

('Acetylpromazine', Boots Ltd., Nottingham) and in two cases atropine sulphate B.P. (Macarthy's Ltd., Romford, Essex) was given prior to induction of anaesthesia.

3. METHODS

3.1 Technique of end-tidal carbon dioxide measurement

In all animals the trachea was intubated with a cuffed endotracheal tube and end-tidal carbon dioxide partial pressure ($P_{ET}CO_2$) was calculated from the plateau obtained in the record produced by the infra-red analyser. End-tidal PCO_2 was calculated from the percentage figure obtained from the tracing using the formula:

$$PCO_2 \text{ (mmHg)} = \frac{(B-50) \text{ mmHg} \times CO_2\%}{100}$$

(B-50) = Barometric pressure - saturated water vapour pressure at 38°C.

The analyser sampled gas continuously from the endotracheal tube through a 4mm bore nylon catheter. Mixtures of 4% and 8% carbon dioxide in oxygen were used to calibrate the analyser and the zero adjustment was made with the carbon dioxide free anaesthetic gas mixture used in a given animal.

Preliminary experiments were undertaken to investigate the influence of different expired gas sampling techniques on the value obtained for $P_{ET}CO_2$ under steady state conditions in a given animal prior to the start of surgery.

3.1.1 Length of sampling catheter

Two lengths of catheter, one being 90cm and the other 180cm, were used to collect samples from the endotracheal tube. Although the longer catheter increased the delay in the analysis of the CO_2 content of a given expiration,

identical values of $P_{ET}CO_2$ were obtained with both catheters. In all subsequent studies a 90cm catheter was used.

3.1.2 Site of withdrawal of gas sample

The effect of sampling gas from the proximal (oral) and distal ends of the endotracheal tube was investigated. It was found to be important to sample gas from the distal end of the endotracheal tube in animals breathing air to prevent the entrainment of room air. When the endotracheal tube was connected to an anaesthetic circuit no consistent difference was noted when the position of the sampling catheter was altered.

3.1.3 Gas sampling flow rate

A gas sampling flow rate of 0.8 $\ell\ min^{-1}$ was found to be satisfactory in most instances. Variation of the sampling flow rate between 0.5 and 1.5 $\ell\ min^{-1}$ had little effect on $P_{ET}CO_2$ although the appearance of the trace was altered, with the faster flow rates producing a narrower expiratory plateau. In large dogs little difference in $P_{ET}CO_2$ was noted when different anaesthetic circuits were used or when fresh gas inflow into a semi-closed circuit was stopped (e.g. Table 7 and Figure 7).

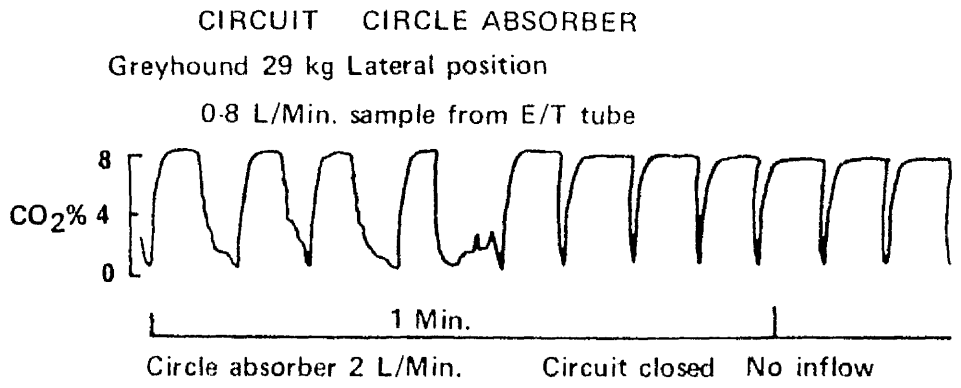


Fig. 7 Alteration in capnograph trace produced by stopping the inflow into a circle system

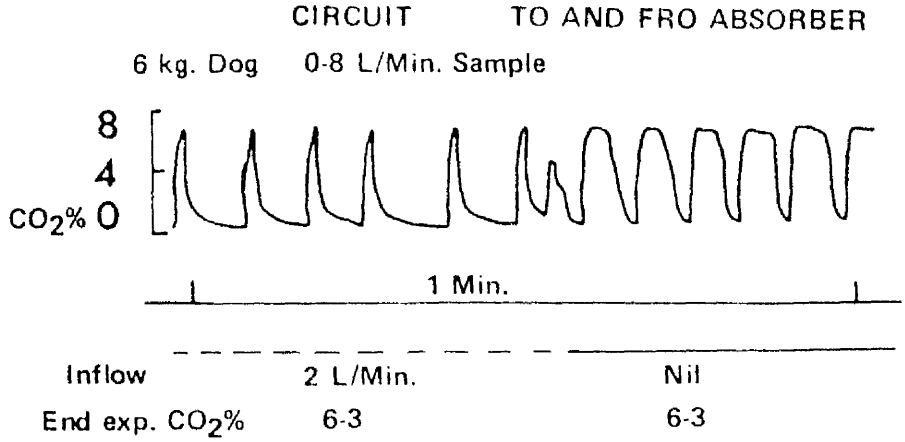


Fig. 8 Alteration in capnograph trace produced by stopping the inflow into a 'to and fro' system

Table 7 Effects of different circuits and gas inflow rates on values of $P_{ET}CO_2$ obtained in a 28kg dog

Anaesthetic circuit	Fresh gas flow $\ell\ min^{-1}$	$P_{ET}CO_2$ (mmHg)
Circle absorber	4	32
'To and fro' absorber	4	32
'To and fro' absorber	0	33
Magill	4	33.5

In smaller dogs (5-11kg), when the fresh gas flow was stopped the appearance of the trace was altered in such a way that a longer expiratory plateau was obtained but $P_{ET}CO_2$ was not increased (e.g. Figure 8). With small dogs on a T-piece system or in animals not connected to an anaesthetic circuit, sample flow rates in excess of 0.5 $\ell\ min^{-1}$ entrained room air and reduced $P_{ET}CO_2$ slightly.

3.2 COMPARISON OF END-TIDAL AND ARTERIAL VALUES OF PCO_2

Comparisons of $P_{ET}CO_2$ and $PaCO_2$ were made with dogs breathing spontaneously and lying in a lateral position. Samples of arterial blood were collected from a femoral artery by needle puncture and blood was allowed to flow into a heparinised syringe under its own pressure. Each sample was collected over three or four respiratory cycles. Syringes were capped and $PaCO_2$ was measured within 10 min of sample collection. The time of collection of an arterial sample was noted on the

continuous trace produced by the infra-red analyser, which sampled gas from the endotracheal tube, in order that simultaneous values of $P_{ET}CO_2$ and $PaCO_2$ could be compared. Where a single comparison was obtained in a given animal, the arterial sample was collected 20-30 min after induction of anaesthesia. In some animals a number of comparisons were made before surgery was begun.

3.3 EVALUATION OF RESULTS

Where more than one comparison was made in a given animal the mean values of $P_{ET}CO_2$ and $PaCO_2$ obtained in that animal were used in calculating the overall correlation between the two values in the whole group of dogs. Scatter diagrams were plotted to examine the relationship between $P_{ET}CO_2$ and $PaCO_2$ in an individual animal and in all 22 dogs. Correlation coefficients, equations of linear regression, and group mean values obtained with simultaneous measurements of $P_{ET}CO_2$ and $PaCO_2$ were calculated.

4. RESULTS

4.1 COMPARISON OF END-TIDAL AND ARTERIAL VALUES OF PCO_2

Simultaneous measurements of $P_{ET}CO_2$ and $PaCO_2$ were made on 52 occasions in the 22 dogs studied, over a range of $PaCO_2$ values of 30-70 mmHg. No more than 6 comparisons were made in any one dog. As an example of the results obtained in an individual animal the values measured in a 23kg Greyhound are given in Table 8.

Table 8 Simultaneously measured values of $PaCO_2$ and $P_{ET}CO_2$ in an individual animal

$PaCO_2$ (mmHg)	$P_{ET}CO_2$ (mmHg)	$PaCO_2$-$P_{ET}CO_2$ (mmHg)
70	64	6
70	64	6
54	48	6
62	60	2
66	61	5
36	31	5
Mean ± SD 59.7 ± 13.0	54.7 ± 13.0	5.0 ± 1.54

In this animal $P_{ET}CO_2$ consistently underestimated $PaCO_2$, the mean difference between the two values being 5mmHg.

When the equation of linear regression obtained with these results was calculated (Figure 9) the regression line was found to lie parallel to the 45^{o} line of equal values and the two measurements demonstrated a high degree of correlation (r=0.99) which was significant at 0.1% level.

The breeds and weights of the 22 dogs studied and the anaesthetic techniques and circuits used are given in Table 9. The values of $PaCO_2$ and $P_{ET}CO_2$ obtained from these animals are given in Table 10. The difference between the mean values obtained with the two techniques of measurement was 2.1mmHg with the value of $PaCO_2$ generally being greater than $P_{ET}CO_2$. $P_{ET}CO_2$ was greater than $PaCO_2$ in 7 dogs. The results from these 7 animals can be seen to lie below the 45^{o} line of equal values in the scatter diagram shown in Figure 10 while the majority of values and the calculated regression line demonstrate that in most instances the value of $PaCO_2$ is equal to or greater than $P_{ET}CO_2$. The correlation obtained between simultaneously measured $PaCO_2$ and $P_{ET}CO_2$ was statistically highly significant (r=0.85, significant at 0.1% level). In only two dogs was $a-_{ET}PCO_2 \geq$ 10mmHg. These two dogs were amongst the smallest studied but in another small dog (no. 19) the two values were identical. The weight range of the dogs studied and the diversity of anaesthetic drugs and circuits used was such that no detailed analysis of the influence of these factors on the magnitude of the arterial-end tidal PCO_2 gradient was attempted.

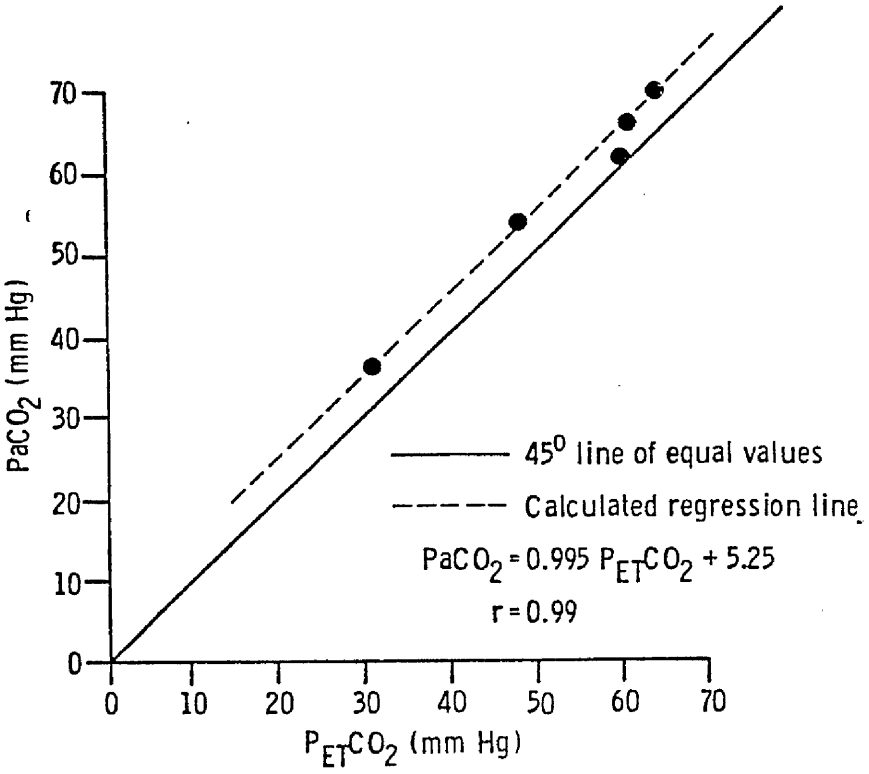


Fig 9 The relationship between simultaneous measurements of end tidal carbon dioxide tension ($P_{ET}CO_2$) and arterial tension ($PaCO_2$) in an individual dog

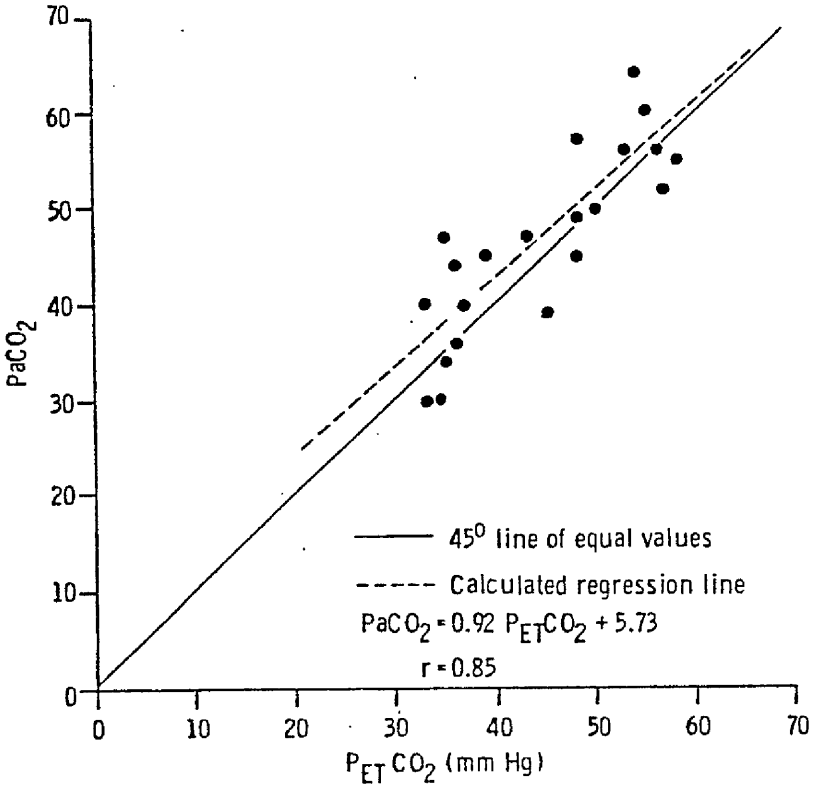


Fig. 10 The relationship between simultaneous measurements of end tidal carbon dioxide tension ($P_{ET}CO_2$) and arterial tension ($PaCO_2$) in 22 dogs

Table 9 Breeds and weights of dogs studied and anaesthetic techniques used in the comparison of $PaCO_2$ and $P_{ET}CO_2$

Dog No.	Breed	Wt. (kg)	Premedication	Anaesthetic drugs*	Anaesthetic circuit**
1	Alsation	37	-	Et/Me/Air	-
2	Greyhound	29	-	Et/Me/Air	-
3	Poodle	15.5	-	Et/Me/O_2	ScC
4	Greyhound	23	-	Thio/Cyclo/O_2	cC
5	Greyhound	23	-	Thio/Halo/O_2	ScC
6	Alsation	5	-	"	ScT
7	Greyhound	29	-	"	"
8	Retriever	18	-	"	ScC
9	Beagle	9.5	-	"	ScT
10	Sheltie	7.5	-	"	cT
11	Sheltie	5.5	-	"	"
12	Bull Mastiff	34	-	"	ScC
13	-	-	Atropine	"	cT
14	K.Charles Spaniel	9	"	"	ScT
15	Pointer	40	Acepromazine	"	Magill
16	Cairn Terrier	-	"	"	ScT
17	Doberman	23	"	"	"
18	Collie	11	"	"	"
19	Poodle	8.5	"	"	"
20	Alsatian	28	"	Halo/O_2	cC
21	Collie	-	"	Thio/Halo/N_2O/O_2	ScT
22	Retriever	28	"	Thio/Cyclo/O_2	cC

*Et/me = Etorphine methotrimeprazine
Thio = Thiopentone
Cyclo = Cyclopropane
Halo = Halothane

**C = circle absorber
T = 'to and fro' absorber
c = closed
Sc = semi-closed

Table 10 Simultaneously measured values of $PaCO_2$ and $P_{ET}CO_2$

Dog No.	No. of comparisons	$PaCO_2$ (mmHg)	$P_{ET}CO_2$ (mmHg)	$PaCO_2-P_{ET}CO_2$ (mmHg)
1	1	45	48	-3
2	3	55	58	-3
3	1	52	57	-5
4	6	56	53	3
5	6	60	55	5
6	2	64	54	10
7	6	56	53	3
8	1	56	56	0
9	1	49	48	1
10	4	47	43	4
11	5	57	48	9
12	5	40	37	3
13	1	45	39	6
14	1	47	35	12
15	1	40	33	7
16	1	39	45	-6
17	1	44	36	8
18	1	30	33	-3
19	1	36	36	0
20	2	30	34	-4
21	1	34	35	-1
22	1	50	50	0
Total	52	Mean	46.9	44.8	2.1
		$\pm$ SD	$\pm$ 9.6	$\pm$ 8.92	

5. DISCUSSION

In an animal with a constant metabolic rate any change in alveolar ventilation is reflected by a change in the tension of carbon dioxide in arterial blood ($PaCO_2$). An increase in alveolar ventilation leads to a decrease in $PaCO_2$ while underventilation causes $PaCO_2$ to increase. The measurement of $PaCO_2$ therefore provides a suitable method for the assessment of the effects of anaesthetic drugs on respiration.

As the collection of samples of arterial blood for the direct determination of $PaCO_2$ with a carbon dioxide electrode (Severinghaus and Bradley, 1958) is seldom possible during routine veterinary anaesthesia, an indirect method of measurement would have considerable advantages. One indirect technique which has been used in man involves the calculation of $PaCO_2$ from a measurement of mixed venous PCO_2 derived using a rebreathing technique (Campbell and Howell, 1960). This method would be difficult to apply to dogs of different sizes as a number of different rebreathing times and re-breathing bags of varying capacity would have to be employed. The method investigated in the present study involved the estimation of $PaCO_2$ from measurement of the concentration of carbon dioxide in end expired gas with a rapid infrared analyser (Collier, Affeldt and Farr, 1955; Burton, 1966 and 1969). The correlation between simultaneous measurements of end-tidal carbon dioxide tension ($P_{ET}CO_2$) and $PaCO_2$ was examined to assess the

accuracy of the end-tidal measurements.

In an individual animal a very good degree of correlation (r=0.99) between the two values was found and in all of the dogs studied, with body weights ranging from 5-40kg the correlation coefficient obtained with simultaneous measurements of $P_{ET}CO_2$ and $PaCO_2$ was 0.85. These results indicate that measurement of $P_{ET}CO_2$ should give a reasonably accurate estimation of $PaCO_2$ in dogs of different sizes and that changes in $PaCO_2$ in an individual animal are reflected in changes of similar magnitude in the value of $P_{ET}CO_2$.

These results can be compared with those obtained in a recent study where the accuracy of $P_{ET}CO_2$ measurements was assessed in 6 dogs weighing 22-28kg and anaesthetised with halothane (Hightower, Kiorpes, Butler and Fedde, 1980). When dogs were allowed to breath spontaneously or were ventilated with a constant pressure ventilator the overall correlation coefficient between end tidal and arterial values was 0.80. A lower correlation coefficient of 0.46 was obtained when dogs were ventilated by intermittent manual compression of a reservoir bag. The correlation obtained by these workers in spontaneously breathing dogs was slightly lower than that found in the present study in which animals with a much greater weight range were investigated.

In anaesthetised rabbits, with a mean weight of 2.96kg, Evans Hogg and Rosen (1977) found a good correlation between $P_{ET}CO_2$ and $PaCO_2$ over a wide range of ventilatory rates. In other studies in anaesthetised animals, rather than examine the correlation between $P_{ET}CO_2$ and $PaCO_2$, the difference between the two values has been measured. These investigations were primarily designed to examine the influence of a number of factors on alveolar dead space.

The presence of alveolar dead space results from the dilution of alveolar gas with gas from poorly perfused alveoli with a low carbon dioxide content. This causes the PCO_2 of mixed alveolar gas to be reduced below that of arterial blood (Severinghaus and Stupfel, 1957). The presence of alveolar dead space is thus one of the main reasons for differences in the absolute values of $P_{ET}CO_2$ and $PaCO_2$.

In a small number of experimental studies the difference between $PaCO_2$ and $P_{ET}CO_2$ has been examined in both conscious and anaesthetised dogs. Suskind and Rahn (1954) measured $P_{ET}CO_2$ with a thermo-conductivity analyser in three conscious dogs trained to lie in the supine position wearing individually fitted masks and found a mean difference between $PaCO_2$ and $P_{ET}CO_2$ of 1 mmHg. During anaesthesia with pentobarbitone the arterial-end-tidal gradient did not change in any predictable manner, the values obtained ranging from 4mmHg to -1mmHg. Severinghaus

and Stupfel (1957) reported a mean difference of 3mmHg with a range of 1-12mmHg in normal dogs with little change in alveolar dead space being produced by pentobarbitone or chloralose anaesthesia. In a further study, Severinghaus, Stupfel and Bradley (1957) claimed that anaesthesia produced no change in the $PaCO_2$-$P_{ET}CO_2$ gradient. A criticism of this study is that control values were obtained in conscious animals paralysed with succinylcholine. While this type of study should be condemned on humanitarian grounds it is also possible that the stress involved in paralysing conscious animals may have caused endogenous catecholamine release which would have produced an increase in arterial and pulmonary perfusion pressures and a decrease in alveolar dead space.

In some other investigations in anaesthetised dogs significant differences between $PaCO_2$ and $P_{ET}CO_2$ have been recorded. Bergman (1963) found gradients ranging from 3.5-8.4mmHg in dogs anaesthetised with pentobarbitone and paralysed with succinylcholine. In fourteen dogs (13-29kg) anaesthetised with thiopentone, Suwa, Hedley-Whyte and Bendixen(1966) found that physiological dead space (anatomical plus alveolar) increased when ventilation was controlled in comparison with results obtained in spontaneously breathing animals. The increase in dead space was correlated with a reduction in cardiac index and these authors concluded that differences in physiological dead space in dogs were probably due to

concomitant changes in the pulmonary circulation.

In the present investigation the mean difference between $PaCO_2$ and $P_{ET}CO_2$ was 2.1mmHg with a range of 12mmHg to -6mmHg. The mean figure obtained is in close agreement with the values reported in dogs by Suskind and Rahn (1954), Severinghaus and Stupfel (1957) and Bergman (1963). The range of values is however greater than has been previously reported and indicates that a variable amount of alveolar dead space was present in the dogs examined. In dogs with a positive $PaCO_2$-$P_{ET}CO_2$ gradient alveolar dead space may have been increased if the anaesthetic drugs used or the posture of the animals led to a reduction in the perfusion of ventilated alveoli (Bergman, 1963; Suwa et al., 1966). The relationship between $P_{ET}CO_2$ and $PaCO_2$ also depends on the pattern, rate and depth of respiration (Severinghaus, Stupfel and Bradley, 1957) and no attempt was made to control these variables.

The range of carbon dioxide gradients noted included a number where $P_{ET}CO_2$ was greater than $PaCO_2$. While it is probable that measurement inaccuracies may have contributed to the larger gradients it is also possible that genuine negative $PaCO_2$-$P_{ET}CO_2$ gradients may be obtained in some animals.

Uneven ventilation is likely to elevate $P_{ET}CO_2$ as poorly ventilated alveoli having a high PCO_2 usually empty last (Severinghaus, Stupfel and Bradley, 1957). It must also be noted that alveolar CO_2 concentration varies with the respiratory cycle. The concentration is reduced during inspiration and decreased during expiration as the relatively constant output of CO_2 is released into a lung volume which changes with the different phases of respiration (Collier et al., 1955). With large tidal volumes and slow respiratory rates $P_{ET}CO_2$ may more closely approximate $PaCO_2$ (Burton, 1966). Fletcher, Jonson, Cumming and Brew (1981) have noted negative $PaCO_2$-$P_{ET}CO_2$ gradients in some human patients and postulate that such a gradient implies compensation by perfusion for early emptying, overventilated alveoli. While mean alveolar PCO_2 is in equilibrium with $PaCO_2$, this mean value is a temporal and spatial mean and negative gradients need not imply carbon dioxide diffusion against a concentration gradient.

In the anaesthetised dogs investigated by Suskind and Rahn (1954) small negative gradients were noted and in dogs anaesthetised with halothane (Hightower et al., 1980) superimposition of a 45^{o} line of equal values on the scatter diagram shown demonstrates that a number of negative gradients were obtained.

In human patients anaesthetised with nitrous oxide, halothane or cyclopropane, $PaCO_2$ has generally been found to be slightly greater than $P_{ET}CO_2$ (Nunn and Hill, 1960; Askrog, Pender and Eckenhoff, 1964; Askrog, Pender, Smith and Eckenhoff, 1964; Burton, 1969). On the other hand, Frumin, Bergman and Holaday (1959) found that the arterial value was on average 2mmHg less than the end-tidal value.

Some of the discrepancies found in these studies and the wide range of gradients found in the present study may be attributed to minor inaccuracies in the measurement of $PaCO_2$ or $P_{ET}CO_2$. Correction of the value of $PaCO_2$ for a $1^{\circ}C$ reduction in an animals body temperature relative to the temperature of the CO_2 electrode would cause the true $PaCO_2$ to be reduced by a factor of 0.96 (Severinghaus, 1965). In animals breathing air while the infrared analyser was calibrated with CO_2:oxygen mixtures the value of $P_{ET}CO_2$ may have been overestimated by 0.4 per cent (Ramwell, 1957). Although nitrous oxide and cyclopropane were only used in a total of 3 dogs it is unlikely that the pressure broadening effect which can be produced by these gases (Powell, 1966; Severinghaus, Larsen and Eger, 1961) had a significant effect on the results as no consistent trend was noted in animals in which these gases were used.

The total measurement error is unlikely to have been greater than 4mmHg and as such would not invalidate the conclusion that measurement of $P_{ET}CO_2$ produces a reasonably accurate assessment of $PaCO_2$ in dogs of different sizes and anaesthetised with various anaesthetic agents and techniques. It is obvious however that particular attention must be paid to the accuracy of the measurements made to allow small changes in alveolar dead space to be detected. It has also been pointed out by Askrog, Pender, Smith and Eckenhoff (1964) that a comparison of the effects of different anaesthetic agents on alveolar dead space can only be made in normal unoperated patients with depth of anaesthesia and tidal volume being controlled precisely.

Chapter 3

STUDIES WITH NEUROLEPTANALGESIC MIXTURES IN DOGS

1. INTRODUCTION

In this section studies to examine the clinical efficacy of three different neuroleptanalgesic combinations are described. Although Marsboom et al, 1964, reported a study where the analgesic component of a fentanyl:fluanisone mixture had been given by intravenous injection following the intramuscular administration of fluanisone, none of the published studies has described the effects produced by the simultaneous intravenous administration of both components of a neuroleptanalgesic mixture. In the present work a comparison is made of the neuroleptanalgesic effects and side effects produced when two neuroleptanalgesic combinations are given by either the intravenous or intramuscular routes.

In the reports on the clinical use of neuroleptanalgesic mixtures in dogs few quantitative details have been provided on the respiratory and haemodynamic effects produced by the technique. Marsboom et al. (1964) gave some information on the different effects of neuroleptanalgesic drugs on heart rate; but effects on blood pressure and end-tidal carbon dioxide concentration have not been described. In some of the dogs examined in the present study systolic blood pressure has been estimated with an indirect method of measurement and end-tidal carbon dioxide concentration has been measured to provide more information on the respiratory effects of the mixtures examined.

Pharmacological studies with mixtures of fentanyl and droperidol have produced conflicting findings about the effects of this combination of drugs on respiration. Yelnosky and Gardocki (1964) found that in dogs the respiratory depressant effect of the mixture of droperidol and fentanyl was similar to that of fentanyl alone, whereas Greene (1972) concluded that droperidol potentiated the respiratory depressant effect of fentanyl in a study in mice. In a study in man, Foldes, Kepes, Torda, Bailey and Wulfsohn (1964) found that droperidol had no significant effect on the respiratory depression produced by fentanyl when anaesthesia was induced with thiopentone, but had a marked antagonistic effect on the action of fentanyl when the barbiturate was omitted from the anaesthetic technique. An experimental study in dogs was therefore planned to investigate further this aspect of the pharmacology of droperidol and fentanyl.

2. MATERIALS

2.1 Animals

The dogs in which the neuroleptanalgesic techniques were investigated were clinical cases presented to the Department of Veterinary Surgery of the University for minor surgical procedures, examination of painful conditions or for radiography. At the outset of the investigation it was envisaged that the technique would be extended for use in animals presented for major surgery if preliminary results were encouraging.

The investigation of the pharmacological properties of fentanyl and droperidol was performed in dogs maintained for experimental purposes in the Department.

2.2 Drugs

Supplies of droperidol:fentanyl ('Thalamonal Vet') and methotrimeprazine:etorphine ('Immobilon') mixtures were donated by Janssen Pharmaceutical Ltd. (Marlow, Bucks) and Reckitt and Colman (Hull) respectively. The third mixture examined contained fluanisone and fentanyl ('Hypnorn') and was purchased from Crookes Laboratories (Basingstoke, Hants).

The composition of the mixtures used was as follows:

'Thalamonal Vet'	droperidol	20 mg ml^{-1}
	fentanyl	0.4 mg ml^{-1}
'Hypnorn'	fluanisone	10 mg ml^{-1}
	fentanyl	0.2 mg ml^{-1}
'Immobilon'	methotrimeprazine	18 mg ml^{-1}
	etorphine	68 µg ml^{-1}

3. METHODS

3.1 COMPARISON OF THE EFFICACY OF THREE NEUROLEPTANALGESIC COMBINATIONS

3.1.1 Dosage and routes of administration

The combination of droperidol and fentanyl was examined in 113 dogs, that containing fluanisone and fentanyl in 25 animals, and the mixture containing methotrimeprazine and etorphine in 35 dogs. The manufacturers recommended that the drugs should be given by intramuscular injection but on a number of occasions the intravenous route was also used with the former two combinations. The mixtures, having a fixed ratio of neuroleptic and analgesic components were given in doses of 0.05 - 0.25 ml kg^{-1}. The doses and routes of administration used and the dose of each constituent contained in the volume of the mixture administered are given in Table 11.

Atropine premedication 0.1 mg kg^{-1} was given subcutaneously 5-15 minutes prior to the intramuscular administration of the neuroleptanalgesic mixture in 16 dogs given the droperidol and fentanyl combination and in 6 dogs which received fluanisone and fentanyl.

Table 11 Dosage and route of administration of neuroleptanalgesic mixtures

Neuroleptanalgesic mixture and dose (ml kg^{-1})	Route of administration	Neuroleptic dose	Analgesic dose
Droperidol:fentanyl 'Thalamonal Vet'		Droperidol mg kg^{-1}	Fentanyl mg kg^{-1}
0.05	i.v.	1.0	0.02
0.1	i.v.	2.0	0.04
*0.1	i.m.	2.0	0.04
Fluanisone:fentanyl 'Hypnorm'		Fluanisone mg kg^{-1}	Fentanyl mg kg^{-1}
0.25	i.v.	2.5	0.05
*0.5	i.m.	5.0	0.1
Methotrimeprazine:etorphine 'Immobilon'		Methotrimeprazine mg kg^{-1}	Etorphine µg kg^{-1}
0.05	i.m.	0.9	3.4
*0.1	i.m.	1.8	6.8

*Manufacturer's recommended dose

3.1.2 Evaluation of sedation, analgesia and side effects

The time interval between an injection and the production of a maximum degree of sedation and analgesia was noted. Sedative effects were assessed subjectively as 'good', 'fair' or 'poor' based on the degree of immobility and unresponsiveness produced. Analgesia was also assessed as 'good', 'fair' or 'poor' in relation to the response of an animal to the manipulation being performed. Analgesia was classed as 'good' when an animal failed to respond to any surgical stimulus, 'fair' when a mild response was seen and 'poor' when a marked response to a painful stimulus was seen. In some cases analgesia was not required as the neuroleptanalgesic technique was used in these instances to provide sedation and immobilization. In some of these cases analgesia was assessed by noting the response of an animal to compression of a toe nail.

Animals were observed prior to and throughout the period of immobilization and side effects such as spontaneous movement, sensitivity to noise, 'nystagmus', defaecation and salivation were noted when they occurred.

The duration of useful effect of the combinations examined was noted and in a number of animals the effects of the analgesic component in a mixture were antagonised with a narcotic antagonist. Nalorphine 1 mg kg^{-1} i.v. ('Lethidrone', Wellcome Medical, Crewe, Cheshire) was the antagonist used in dogs given the droperidol:fentanyl or fluanisone:fentanyl

mixtures. In those animals which received methotrimeprazine: etorphine, the antagonist used was diprenorphine 0.03 mg kg^{-1} i.v. ('Revivon', Reckitt and Colman, Hull).

3.1.3 Evaluation of effects on respiration

Respiratory rate was counted at intervals during the period of immobilization produced by the neuroleptanalgesic mixtures and the presence or absence of cyanosis in the tongue and oral mucous membranes was recorded. In eight dogs given the droperidol:fentanyl mixture expired minute volume was measured with a Wright's Respirometer attached to a cuffed endotracheal tube. Using the technique described in Chapter 2 end-tidal carbon dioxide tension was measured in 11 dogs given the methotrimeprazine:etorphine mixture and in 4 animals given fluanisone and fentanyl.

3.1.4 Evaluation of effects on circulation

Heart rate was counted from the femoral pulse and systolic blood pressure was measured in 15 dogs given droperidol:fentanyl, in 6 dogs given fluanisone:fentanyl and in 19 animals which received methotrimeprazine:etorphine. Blood pressure was measured with an indirect method as described in Chapter 1.

3.1.5 Assessment of adequacy of the neuroleptanalgesic technique

The overall assessment of the adequacy of the neuroleptanalgesic technique in a given animal was based on the degrees of sedation and analgesia obtained in relation to the particular requirements of the procedure performed. The presence or absence of significant side effects, including undesirable effects on the circulatory or respiratory systems were also taken into account in making this assessment.

3.2 COMPARISON OF CIRCULATORY AND RESPIRATORY EFFECTS OF FENTANYL GIVEN ALONE AND IN COMBINATION WITH DROPERIDOL

Nine experiments were performed in three dogs to examine some of the respiratory and circulatory effects of fentanyl alone, and in combination with droperidol, in dogs anaesthetised with 25 mg kg^{-1} pentobarbitone. With an interval of at least one week between experiments each of three dogs received either pentobarbitone alone, pentobarbitone followed after 15 min by 0.02 mg kg^{-1} fentanyl, or fentanyl plus 1.0 mg kg^{-1} droperidol. Heart rate was counted from the femoral pulse and respiratory rate was noted. Systolic blood pressure was measured with an indirect method as described in Chapter 1. The trachea was intubated with a cuffed endotracheal tube following the topical application of lignocaine to the larynx and end-tidal carbon dioxide tension in gas withdrawn from the endotracheal tube was measured as described in Chapter 2. The effects produced by the neuroleptic and analgesic drugs were compared with values obtained in all nine experiments 15 min after the administration of pentobarbitone.

3.3 EVALUATION OF RESULTS

The frequency of a particular finding in any two treatment groups was compared by the construction of 2×2 contingency tables and calculation of the χ^2 statistic. Students 't' test was used to compare quantified variables in different groups and in some cases with pre-dose values.

4. RESULTS

4.1 COMPARISON OF THE EFFICACY OF THREE NEUROLEPTANALGESIC COMBINATIONS

4.1.1 Body weight and age of dogs in which neuroleptanalgesic techniques were used

The mean body weight, age, and the ranges of these characteristics in each group investigated are given in Table 12. The overall age range was 0.2 - 16 years and body weights ranged from 2.0 to 68kg. The heaviest animals were found in the group given 0.05 ml kg^{-1} of the droperidol:fentanyl mixture while the oldest and some of the smallest dogs were present in the group given this mixture with atropine premedication. In other respects the groups were broadly similar.

4.1.2 Procedures performed with neuroleptanalgesia

The numbers of the various procedures performed with the three neuroleptanalgesic combinations are shown in Table 13. The technique was used most frequently for minor surgical procedures such as the suturing of superficial wounds or the removal of small skin tumours. Analgesia was not required when neuroleptanalgesia was used to provide immobilization for radiography but many of the diagnostic examinations involved the examination of painful joints or ears.

4.1.3 Onset and duration of sedation and analgesia

The times of onset of maximum effect and the useful duration of action following the intravenous or intramuscular injection of the three combinations examined are given in Table 14 .

Table 12 Body weight and age of animals in which neuroleptanalgesic techniques were examined

Neuroleptanalgesic drugs and dose (ml kg^{-1})	No.	Body weight Mean ± S.D. kg	Weight range	Age (yr) Mean ± S.D.	Age range (yr)
Droperidol:fentanyl					
0.05 i.v	21	30.3 ± 16.7	9.0 - 68	3.6 ± 3.8	0.5 - 14
0.1 i.v.	44	16.0 ± 9.2	4.5 - 29.5	3.7 ± 3.0	0.2 - 10
0.1 i.m.	32	17.5 ± 11.8	3.6 - 45	3.1 ± 2.9	0.25- 9.5
0.1 i.m + Atropine	16	13.3 ± 8.5	2.7 - 26	5.2 ± 5.7	0.2 - 16
Fluanisone:fentanyl					
0.25 - 0.5 i.v.	25	24.6 ± 10.4	2.0 - 41	4.3 ± 3.0	0.2 - 9
Methotrimeprazine:etorphine					
0.05 i.m.	14	24.0 ± 10.2	4.5 - 37	5.7 ± 3.9	1.0 - 11
0.1 i.m.	21	19.7 ± 11	3.6 - 45	4.3 ± 3.9	0.8 - 33

Table 13 Procedures performed with neuroleptanalgesia

	Droperidol: fentanyl		Fluanisone: fentanyl		Methotrimeprazine: etorphine	
	No.	%	No.	%	No.	%
Minor surgery	34	30	9	36	18	51
Diagnostic examination	26	23	10	40	6	17
Radiographic examination	26	23	3	12	5	14
Application or removal of plaster cast	10	9	2	8	3	9
Dental procedures	8	7			2	6
Replacement of dislocated hip	6	5			1	3
Premedication for general anaesthesia	3	3	1	4		
Totals	113	100	25	100	35	100

Table 14 Onset and duration of sedation and analgesia

Drugs and doses (ml kg^{-1})	Interval between injection and maximum effect (min) Range or mean ± SD	Useful duration of action (min)
Droperidol:fentanyl		
0.05 i.v.	1-3	10
0.1 i.v.	1-2	10-15
0.1 i.m.	7.5±2.5	15-30
Fluanisone:fentanyl		
0.25 i.v.	1-2	10-15
0.5 i.m.	7.2± 2.8	15-30
Methotrimeprazine:etorphine		
0.05 i.m.	5.6± 2.34	20-30
0.1 i.m.	6.1± 2.6	30-40

A maximum degree of sedation and analgesia was seen within 1-3 min following the intravenous administration of the drug mixtures whereas in some animals given the drugs by the intramuscular route peak effects were not noted until 10-15 min after an injection. No significant differences were found in the mean onset times obtained with the three different combinations when the intramuscular route was used.

The duration of action of the droperidol:fentanyl and the fluanisone:fentanyl mixtures was similar whereas a longer period of useful sedation and analgesia was obtained with the methotrimeprazine:etorphine combination.

4.1.4 Quality of sedation and analgesia obtained

The subjective assessment of the degrees of sedation and analgesia obtained with the various mixtures examined is given in Table 15. Sedation was classed as 'good' or 'fair' in the majority of animals. The only statistically significant differences between groups were those found between animals given the droperidol:fentanyl mixture at all doses and those given methotrimeprazine:etorphine at 0.1 ml kg^{-1} (Droperidol:fentanyl 0.1 ml kg^{-1} v. methotrimeprazine:etorphine 0.1 ml kg^{-1} i.m. $\chi^2 = 4.22$ $P < 0.05$), with a greater number of animals given droperidol:fentanyl showing 'good' sedation.

The most profound degree of analgesia was obtained following the intravenous injection of the droperidol:fentanyl mixture at 0.1 ml kg^{-1} or the intramuscular administration of the methotrimeprazine:etorphine combination. Both of these combinations produced 'good' analgesia in a significantly greater number of animals than was found in groups receiving droperidol:fentanyl 0.05 ml kg^{-1} intravenously or 0.1 ml kg^{-1} intramuscularly ($\chi^2 > 3.84 < 6.63$; $P < 0.05$).

Table 15 Sedation and analgesia obtained with neuroleptanalgesic drugs

Drugs and doses (ml kg^{-1})	No. examined	Sedation			No. examined	Analgesia		
		% good	% fair	% poor		% good	% fair	% poor
Droperidol:fentanyl								
0.05 i.v.	17	88	12		9	44	56	
0.1 i.v.	40	78	17	5	37	70	27	3
0.1 i.m.	29	76	17	7	27	41	48	11
0.1 i.m. + Atropine	14	85	7.5	7.5	12	58	25	17
Fluanisone:fentanyl								
0.25 i.v.	9	67	33		5	60	25	25
0.5 i.m.	7	72	14	14	6	50	33	17
0.5 i.m. + Atropine	4	50	50		4	50	50	
Methotrimeprazine:etorphine								
0.05 i.m.	14	57	36	7	14	64	28	7
0.1 i.m.	21	48	38	14	21	71	24	5

4.1.5 Incidence of side-effects

The percentage incidence of side effects noted prior to and following immobilization is summarized in Tables 16 and 17 respectively. In a few animals a degree of excitement was noted before a maximum degree of sedation was obtained. This effect which was shown as repeated attempts to rise, whining, or marked spontaneous movements, was seen most frequently in the dogs given the higher dose of methotrimeprazine:etorphine. Defaecation prior to immobilization was seen most frequently in the animals given the fluanisone:fentanyl mixture and was not completely eliminated by atropine premedication. A low frequency of defaecation was seen in dogs given methotrimeprazine and etorphine and in animals which received the droperidol:fentanyl combination by the intravenous route. Salivation and urination were side-effects which were only occasionally seen prior to immobilization.

During the period of recumbency spontaneous movement was the side-effect most frequently noted in all groups. This side effect was seen most frequently in dogs given methotrimeprazine:etorphine 0.1 ml kg^{-1} and least frequently in animals given droperidol:fentanyl 0.1 ml kg^{-1} i.m. ($\chi^2 = 7.66$, $P < 0.01$). On a number of occasions spontaneous movements occurred in response to a sudden noise but at other times muscle twitching unrelated to any obvious stimulus was noted.

Table 16 Side effects noted prior to immobilization with neuroleptanalgesic drugs

Drugs and doses (ml kg^{-1})	No. examined	Percentage incidence of side-effects. Actual numbers in parenthesi			
		Excitement	Defaecation	Urination	Salivation
Droperidol:fentanyl					
0.05 i.v.	21	0	14 (3)	0	0
0.1 i.v.	44	9 (4)	2 (1)	2 (1)	2 (1)
0.1 i.m.	32	3 (1)	25 (8)	0	0
0.1 i.m. + Atropine	16	0	12 (2)	6 (1)	0
Fluanisone:fentanyl					
0.25 i.v.	11	9 (1)	36 (4)	9 (1)	0
0.5 i.m.	8	0	62 (5)	0	0
0.5 i.m. + Atropine	6	0	12 (2)	6 (1)	0
Methotrimeprazine:etorphine					
0.05 i.m.	14	0	14 (2)	0	0
0.1 i.m.	21	24 (5)	5 (1)	0	5 (1)

Table 17 Side-effects noted during immobilization with neuroleptanalgesic drugs

Drugs and doses (ml kg^{-1})	No. examined	Percentage incidence of side effects. Actual numbers in parenthesis			
		Movement	Sensitivity to noise	'Nystagmus'	Defaecation
Droperidol:fentanyl					
0.05 i.v.	21	57 (12)	29 (6)	0	14 (3)
0.1 i.v.	44	36 (16)	32 (14)	23 (10)	14 (6)
0.1 i.m.	32	28 (9)	22 (7)	6 (2)	16 (5)
0.1 i.m. + Atropine	16	60 (10)	36 (6)	6 (1)	18 (3)
Fluanisone:fentanyl					
0.25 i.v.	11	55 (6)	27 (3)	9 (1)	18 (2)
0.5 i.m.	8	50 (4)	38 (3)	25 (2)	0
0.5 i.m. + Atropine	6	67 (4)	67 (4)	50 (3)	17 (1)
Methotrimeprazine:etorphine					
0.05 i.m.	14	57 (8)	14 (2)	71 (10)	14 (2)
0.1 i.m.	21	67 (14)	24 (5)	62 (13)	10 (2)

Oscillatory eye movements differed from normal nystagmus in that the direction and timing of the rotational movements did not follow any regular pattern. These eye movements were seen most frequently in the dogs given methotrimeprazine:etorphine.

During the period of immobilization defaecation occurred in a few animals with a similar frequency being seen in all groups except those dogs given 0.5 ml kg^{-1} fluanisone:fentanyl i.m. where a greater frequency of defaecation had been noted prior to immobilization.

4.1.6 Effects on respiration

The minimum respiratory rates, the frequency with which cyanosis was noted, and measured values of end-tidal carbon dioxide tension are given in Table 18. Both the droperidol: fentanyl and fluanisone:fentanyl mixtures produced variable effects on respiratory rate with tachypnoea and respiratory rates in excess of 60 min^{-1} being frequently seen. In these groups mean respiratory rate was calculated only from results obtained in animals where regular respiration was present. Respiratory rate varied widely in these groups but no statistically significant differences were produced by the different dose rates used. The methotrimeprazine:etorphine mixture produced a more consistent slowing of respiratory rate with the larger dose producing a significantly slower rate than the smaller dose of this combination ($P < 0.05$).

Table 18 Effects on respiration produced by neuroleptanalgesic drugs

Drugs and doses (ml kg^{-1})	Minimum resp. rate min^{-1} (mean ± SD)	Percentage incidence of cyanosis (Actual numbers in parenthesis)	$P_{ET}CO_2$ (mmHg, mean ± SD)
Droperidol:fentanyl			
0.05 i.v.	Tachypnoea	0	
0.1 i.v.	16.3 ± 5.6	9 (4)	
0.1 i.m.	14.2 ± 7.9	3 (1)	
0.1 i.m. + Atropine	14.7 ± 3.9	6 (1)	
Fluanisone:fentanyl			
0.25 i.v.	20.8 ± 14.5	0	
0.5 i.m.	17.8 ± 8.3	25 (2)	48 ± 3.4
0.5 i.m. + Atropine	29 ± 11.4	33 (2)	
Methotrimeprazine:etorphine			
0.05 i.m.	16.4 ± 5.0	28 (4)	49 ± 3.8
0.1 i.m.	10.7 ± 5.8*	57 (12)	54 ± 6.3

*P < 0.05 Significant difference between two doses of methotrimeprazine:etorphine

Cyanosis was noted most frequently in the dogs given 0.1 ml kg^{-1} methotrimeprazine:etorphine, the number of animals showing cyanosis in this group being significantly greater than the number in the groups given droperidol:fentanyl intravenously ($\chi^2 = 17.6$, $P < 0.001$). The number of animals showing cyanosis in the two groups given the higher dose of fluanisone:fentanyl and the lower dose of methotrimeprazine:etorphine was not significantly different from the number showing this side effect following the higher dose of this latter mixture.

End-tidal carbon dioxide tension was increased above normal values in all the animals in which measurements were made. The greatest values were found in the group given the higher dose of methotrimeprazine:etorphine but no statistically significant differences were found between the mean values obtained from different groups.

Respiratory rates and tidal volumes measured in 8 dogs given the droperidol:fentanyl mixture are shown in Table 19. These results illustrate the wide variation in respiratory effects seen with this combination. In dogs which showed tachypnoea minute volume was greatly increased and tidal volume (ml kg^{-1}) was somewhat decreased.

4.1.7 Effects on heart rate and blood pressure

The mean heart rates obtained prior to the injection of drugs and at the time of their maximum effect are given in Table 20. Mean results obtained from the animals in which systolic pressure was measured are also shown.

Table 19 Respiratory rates and tidal volumes in 8 dogs given droperidol:fentanyl mixtures

Dose (ml kg^{-1})	Time after injection (min)	Minute volume (L)	Resp. Rate min^{-1}	Tidal Volume (ml)	Tidal Volume ml kg^{-1}
0.05 i.v.	5	2.3	13	176	16.6
	20	2.5	15	166	16.0
0.1 i.m.	20	10	70	143	7.0
0.1 i.v.	25	3	15	200	14.7
0.1 i.v.	45	20	70	285	10.6
0.1 i.v.	10	1.0	12	83	15.3
0.1 i.v.	8	50	200	250	9.3
	15	60	200	250	11.0
0.1 i.v.	8	7	12	580	21.5
	10	7	14	580	21.5
0.1 i.v.	4	8	60	133	11.8
	8	20	140	143	12.6
	10	23	160	144	12.7

A marked degree of bradycardia was produced in all groups in which no atropine premedication was given, the greatest reductions in heart rate being found in the groups given the droperidol:fentanyl and methotrimeprazine:etorphine mixtures. Atropine premedication produced a marked increase in heart rate in both groups in which it was given. All changes in heart rate from the pre-injection values were statistically significant.

Mean values for systolic pressure ranged from 138 to 152 mmHg and no statistically significant difference was found in the different groups examined.

4.1.8 Recovery times

Recovery times to head lift and until animals were able to walk again, following the intravenous injection of an antagonist, are given in Table 21. Head lift occurred most rapidly in animals given the mixtures containing fentanyl and was delayed slightly in those which received the methotrimeprazine:etorphine mixture. Animals which had been given droperidol:fentanyl were able to walk within a shorter interval than those which had received one of the other two neuroleptanalgesic combinations. Dogs remained sedated by the neuroleptic component in the mixture for 1-2 hours but any residual analgesia or respiratory depression was reversed by the narcotic antagonist drug. When an antagonist was not used full recovery was not evident until 2-4 hours after induction of neuroleptanalgesia.

Table 20 Effects on heart rate and blood pressure produced by neuroleptanalgesic drugs

Drugs and doses ($ml\ kg^{-1}$)	Heart rate min^{-1} (mean ± SD) Pre-injection	Heart rate min^{-1} (mean ± SD) At time of maximum effect	Systolic blood pressure (mmHg, mean ± SD) During immobilization	
Droperidol:fentanyl				
0.05 i.v.	118 ± 16.8	71 ± 15.8***		
0.1 i.v.	114 ± 17.2	74 ± 17.3***		
0.1 i.m.	115 ± 24.7	69 ± 17.7***	138 ± 21.5	(n=15)
0.1 i.m. + Atropine	121 ± 28	182 ± 31***		
Fluanisone:fentanyl				
0.25 i.v.	109 ± 21	81 ± 10.6*		
0.5 i.m.	127 ± 31	93 ± 22.1*	152 ± 16.9	(n=6)
0.5 i.m. + Atropine	120 ± 28	202 ± 24**		
Methotrimeprazine:etorphine				
0.05 i.m.	114 ± 18.9	74 ± 14.5***	141 ± 22	(n=19)
0.1 i.m.	113 ± 17	67 ± 18.1***	139 ± 17.8	

*P < 0.05
**P < 0.01
***P < 0.001

Values significantly different from pre-injection values

Table 21 Recovery times following the administration of a narcotic antagonist drug

Drugs and doses (ml kg^{-1})	No. given an antagonist	Time to head lift (min) mean ± SD	Time to walk (min) mean ± SD
Droperidol:fentanyl			
0.05 i.v.	15	0.6 ± 0.2**	2.8 ± 3.9
0.1 i.v.	19	0.6 ± 0.2**	2.1 ± 1.4
0.1 i.m.	16	0.7 ± 0.7*	1.8 ± 1.3
0.1 i.m. + Atropine	6	0.5 ± 0.5*	2.3 ± 0.5
Fluanisone:fentanyl			
0.25 i.v.	8	0.9 ± 0.5	38% > 5
0.5 i.m.	4	1.0 ± 0.9	50% > 5
0.5 i.m. + Atropine	5	0.8 ± 0.3*	40% > 5
Methotrimeprazine:etorphine			
0.05 i.m.	12	3.0 ± 1.5	50% > 5
0.1 i.m.	18	2.1 ± 1.2	39% > 5

*P < 0.05 Recovery times significantly shorter than methotrimeprazine:etorphine 0.1 ml kg^{-1}

**P < 0.01

4.1.9 Overall assessment of adequacy of the neuroleptanalgesic techniques

The overall assessment of the adequacy of the techniques investigated is summarized in Table 22. The group given 0.1 ml kg^{-1} droperidol:fentanyl intravenously achieved the greatest number of 'good' assessments. Animals in this group were generally well sedated, a good degree of analgesia was frequently present and the incidence of excitatory side effects and defaecation was relatively low. Analgesia was present less consistently in the sub-groups given this mixture intramuscularly or at a lower dose intravenously, and in two of the sub-groups more spontaneous movements were seen.

Animals given the fluanisone:fentanyl mixture showed the highest frequency of defaecation prior to immobilization and during the period of recumbency spontaneous movements were often noted.

The degree of analgesia produced by the methotrimeprazine: etorphine mixture was similar to that achieved with the intravenous administration of 0.1 ml kg^{-1} droperidol:fentanyl but unacceptable respiratory depression was present in a large number of the animals which received the former mixture together with a high incidence of spontaneous movement and other excitatory side-effects.

Table 22 Overall assessment of the adequacy of the neuroleptanalgesic techniques employed

Drugs and doses (ml kg^{-1})	No. Examined	% Good	% Fair	% Poor	Main reasons for Fair or Poor assessment
Droperidol:fentanyl					
0.05 i.v.	21	48	52		Movement
0.1 i.v.	44	66	25	9	Movement
0.1 i.m.	32	28	56	16	Defaecation, movement
0.1 i.m. + Atropine	16	50	37	13	Movement
Fluanisone:fentanyl					
0.25 i.v.	11	36	55	9	Movement
0.5 i.m.	8	25	75		Defaecation during induction
0.5 i.m. + Atropine	6	33	67		Movement
Methotrimeprazine:etorphine					
0.05 i.m.	14	44	36	20	Movement
0.1 i.m.	21	14	62	24	Respiratory depression

Supplementary anaesthesia with thiopentone or an inhalational agent was required to allow completion of the procedure being undertaken in 5 dogs (4.4%) given droperidol:fentanyl mixtures, in 2 (8%) given fluanisone:fentanyl and in 6 animals (17%) which received the methotrimeprazine:etorphine mixture.

4.2 COMPARISON OF SOME CARDIOVASCULAR AND RESPIRATORY EFFECTS OF FENTANYL GIVEN ALONE AND IN COMBINATION WITH DROPERIDOL

4.2.1 End-tidal carbon dioxide tension

In Figure 11 it can be seen that fentanyl 0.02 mg kg^{-1} given alone produced a greater increase in $P_{ET}CO_2$ than did the combination of an equal dose of fentanyl with 1.0 mg kg^{-1} droperidol. The increase in $P_{ET}CO_2$ produced by fentanyl, over that which was obtained 15 min after induction of anaesthesia with pentobarbitone, was statistically significant for 40 min after injection whereas the combination of droperidol and fentanyl produced an increase in $P_{ET}CO_2$ which was significantly elevated for only 10 min.

4.2.2 Respiratory rate

Both fentanyl and the fentanyl:droperidol combination produced some reduction in respiratory rate (Figure 12). The decrease, particularly at the earlier time points, appeared to be greater following droperidol:fentanyl but the wide variation in respiratory rates seen during pentobarbitone

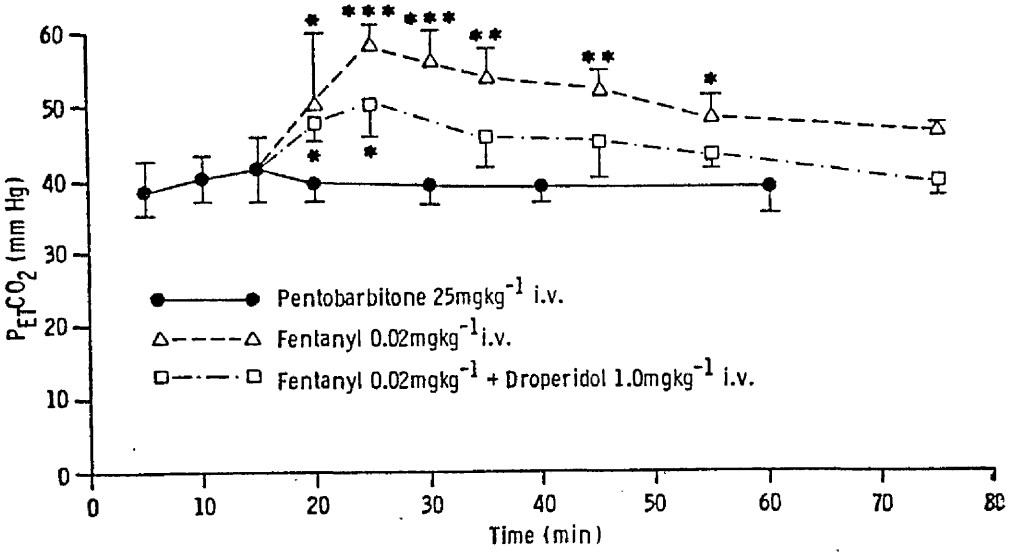


Fig. 11 Effects of fentanyl and fentanyl:droperidol on end-tidal PCO_2 in pentobarbitone anaesthetised dogs. Significant differences from 15 min. value: *P < 0.05; **P < 0.01; ***P < 0.001.
Mean ± SD
5-15 min, n=9; 15 min+, n=3.

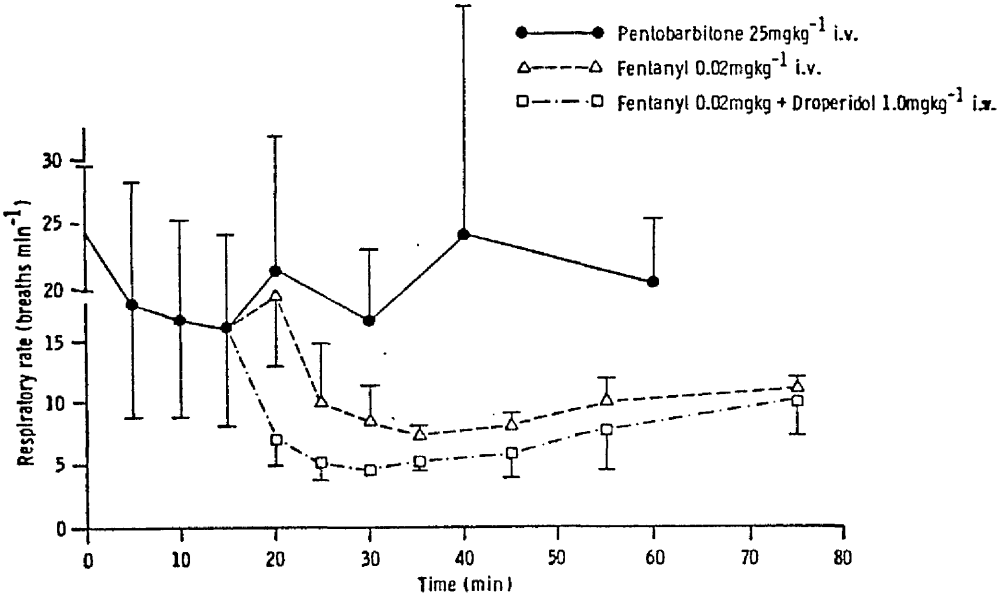


Fig. 12 Effects of fentanyl and fentanyl:droperidol on respiratory rate in pentobarbitone anaesthetised dogs
Mean ± SD
5-15 min, n=9; 15 min+, n=3.

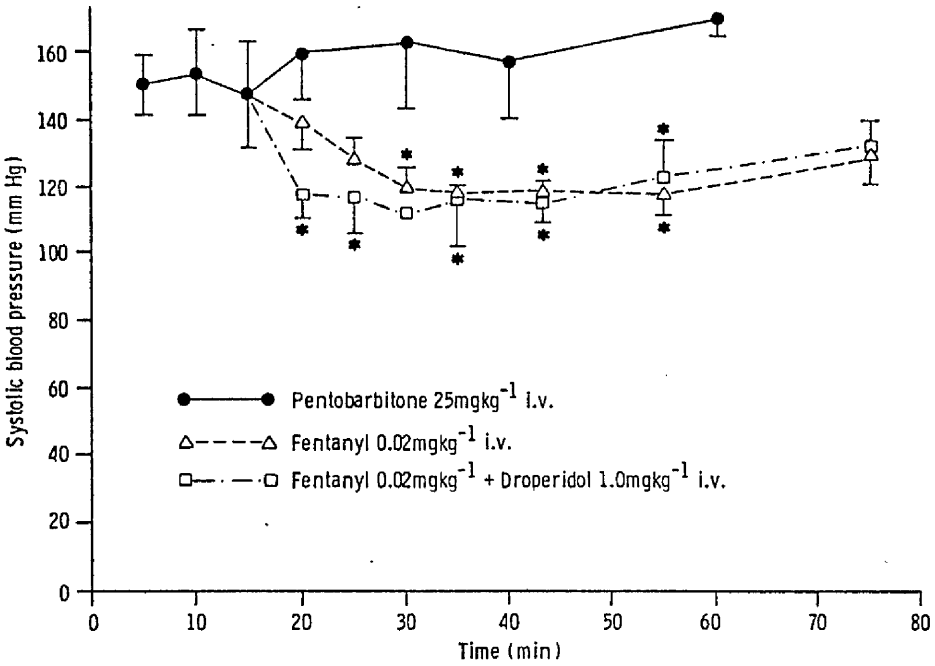


Fig. 13 Effects of fentanyl and fentanyl:droperidol on systolic blood pressure in pentobarbitone anaesthetised dogs. Significant differences from 15 min value: *P < 0.05.
Mean ± SD
5-15 min, n=9; 15 min+, n=3.

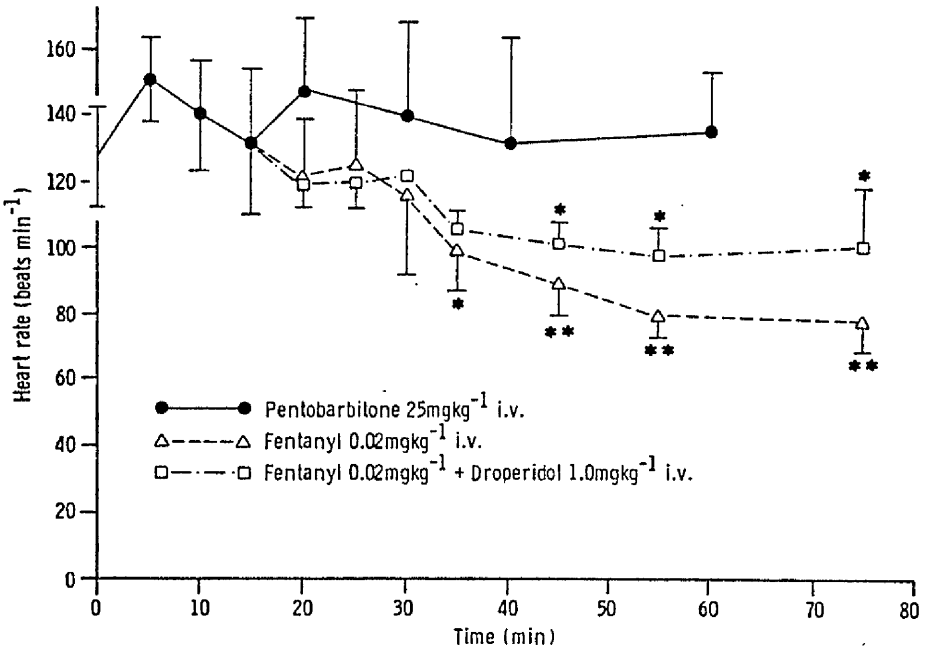


Fig. 14 Effects of fentanyl and fentanyl:droperidol on heart rate in pentobarbitone anaesthetised dogs. Significant differences from 15 min. value: *P < 0.05; **P < 0.01.
Mean ± SD
5-15 min, n=9; 15 min+, n=3.

alone did not allow any statistically significant reduction in respiratory rate to be demonstrated.

4.2.3 Systolic blood pressure

The droperidol:fentanyl mixture produced a reduction in systolic blood pressure which was statistically significant 5 and 10 minutes after injection (Figure 13). A significant reduction in blood pressure following fentanyl alone did not occur until 15 min after injection and thereafter a similar degree of hypotension was seen in both test groups.

4.2.4 Heart rate

Both fentanyl and the fentanyl:droperidol combination produced similar reductions in heart rate for the first 20 minutes after their injection (Figure 14). After this time little further decrease was seen in dogs given droperidol with fentanyl but a further reduction to a mean rate of 77 min^{-1} was noted in animals given fentanyl 0.02 mg kg^{-1} alone.

5. DISCUSSION

Previous clinical studies with neuroleptanalgesic drugs in dogs have generally been restricted to an examination of the effects produced by a single mixture, given intramuscularly at the manufacturers recommended dose. The clinical use of the droperidol:fentanyl combination has been described by Yelnosky and Field (1964), Soma and Shields (1964) and Franklin and Reid (1965). The combination of fluanisone and fentanyl has been evaluated in dogs by Marsboom et al. (1964) and the methotrimeprazine:etorphine combination has been examined by Alford and Wozniak (1970) and Crooks et al. (1970).

The studies described in this chapter were performed sequentially as the various neuroleptanalgesic mixtures became available for veterinary use in this country. By examining each mixture in a standard manner it has been possible to compare the clinical efficacy of three different neuroleptanalgesic combinations in animals in which analgesia and sedation were required for minor surgical interventions or diagnostic procedures.

Each combination contained a different neuroleptic agent. In two mixtures, in which droperidol or fluanisone were present, the neuroleptic belonged to the butyrophenone class, while methotrimeprazine, a phenothiazine derivative was included in the third. The analgesic drug, fentanyl, was present in two mixtures and the third contained the potent oripavine derivative, etorphine. This study

thus provided an opportunity to compare the effects produced by different, but related, neuroleptic and analgesic components. In the two mixtures containing fentanyl the recommended dose provided 0.04 mg kg^{-1} of this agent in one instance (droperidol: fentanyl) while 0.1 mg kg^{-1} fentanyl was included in the recommended dose of the fluanisone:fentanyl mixture. The effects produced by doses outside the recommended dose range were also examined and in addition the mixtures containing fentanyl were given by both intravenous and intramuscular injection.

In assessing the clinical efficacy of the neurolept-analgesic techniques investigated it is important to consider the effects produced in relation to the sedation and analgesia required for the surgical and diagnostic procedures which were performed. It is also necessary to compare the results with those which can be obtained with alternative anaesthetic techniques.

The incidence of excitatory side effects, such as spontaneous movement and sensitivity to noise, with all three combinations was such that none could be considered as a reliable alternative to conventional general anaesthesia for major surgical interventions as advocated by Yelnosky and Field (1964) and Marsboom et al. (1964).

Where a brief period of analgesia and sedation was required for minor surgical interventions, satisfactory operating conditions were obtained on a number of occasions but troublesome side effects were frequently seen. For procedures such as radiography where sedation only was required, neuroleptanalgesia facilitated positioning but a number of animals, being sensitive to sudden noises, moved at the critical time when a radiograph was being taken.

The main advantage of the technique was the ability to antagonise the narcotic component of the mixture such that immobilization was reversed and dogs were able to walk again within 2-3 minutes. While early ambulation may be considered a convenient feature of the technique, particularly for out-patients, where this is not required neuroleptanalgesia appears to offer few advantages over conventional techniques.

Alternative methods which can be employed for minor surgical procedures include the intravenous use of a short acting barbiturate, either alone or in combination with inhalation anaesthesia, and local anaesthesia in conjunction with sedation provided by a neuroleptic drug. The degree of sedation produced by a neuroleptic drug alone is less profound than that obtained with neuroleptanalgesic mixtures (Yelnoski and Gardocki, 1964) and full recovery may be prolonged if large doses of neuroleptic agents are employed. Recovery from thiopentone anaesthesia is more gradual and prolonged than that achieved by antagonizing the narcotic component of a neuroleptanalgesic

mixture, and while recovery from methohexitone anaesthesia is generally rapid, marked excitement may accompany recovery from this agent in dogs (Fowler and Stevenson, 1961). It would appear, therefore, that where early ambulation is required, neuroleptanalgesic techniques offer an advantage over alternative techniques.

Significant differences were noted in the degree of sedation and analgesia obtained, and in the incidence of side effects, with the different neuroleptanalgesic mixtures examined and with the various doses and routes of administration used. When the drugs were given by intramuscular administration at their recommended doses, the methotrimeprazine:etorphine mixture provided more reliable analgesia than the other two combinations. The greater narcotic potency of etorphine, relative to fentanyl, was also reflected in the greater number of animals in the methotrimeprazine:etorphine group in which significant respiratory depression was noted. While the analgesic potency of narcotic drugs is generally paralleled by respiratory depressant effects (Blane et al., 1967) it is also possible that in animals given the fentanyl containing mixtures the neuroleptic component more effectively antagonised the respiratory depressant effect of the analgesic component (Alford and Wozniak, 1970).

Good sedation was obtained less consistently with the methotrimeprazine:etorphine mixture than with the other two combinations. This could indicate either that etorphine possesses a greater central excitatory effect than fentanyl or that methotrimeprazine was less effective than the butyrophenone neuroleptics in suppressing central excitatory effects. It has been proposed by Lees and Hillidge (1976) that the increased muscle tone produced by etorphine in the horse may be produced by a central excitatory effect; and in cats excitement is the predominant behavioural effect produced by etorphine (Blane et al., 1967).

Many of the dogs given methotrimeprazine:etorphine showed oscillatory eye movements. These movements have not been recorded in previous studies with this combination (Alford and Wozniak, 1970; Crooks et al., 1970) but rhythmic oscillation of the eyeballs was observed by Soma and Sheilds (1964) in 10 per cent of animals given the droperidol:fentanyl mixture. As this side effect was also noted in some dogs given fentnayl-containing mixtures in the present investigation it seems probable that this phenomenon is another manifestation of narcotic induced central excitement. Occasional nystagmus was also recorded by Marsboom et al. (1964) in dogs given fluanisone:fentanyl.

Defaecation was a troublesome side effect which was noted most frequently prior to immobilization in dogs given fluanisone:fentanyl. This side effect can be

attributed to a cholinergic effect of the analgesic component in the mixtures (Gardocki and Yelnosky, 1964; Blane et al., 1967). The lower frequency of defaecation in dogs given methotrimeprazine: etorphine may be related to the anticholinergic properties of methotrimeprazine (Blane, Boura and Dobbs, 1968) while the lower dose of fentanyl in the droperidol:fentanyl mixture may explain the reduced incidence of defaecation in this group. Atropine given subcutaneously 5 min before the neuroleptanalgesic drugs, as used by Soma and Shields (1964), Yelnosky and Field (1964) and Franklin and Reid (1965), failed to abolish defaecation in all instances. It is possible that atropine premedication would be more effective in preventing defaecation if given about 30 min prior to the neuroleptanalgesic drugs.

Intravenous administration of the fentanyl-containing mixtures had the advantage that sedation and analgesia were obtained more rapidly. In the whole series the best results were achieved when the droperidol:fentanyl mixture was given intravenously at a dose of 0.1 ml kg^{-1}. The analgesia obtained with intravenous administration was more profound but was accompanied by a greater frequency of excitatory side effects. As the maximum effect of fentanyl is produced more rapidly than that of droperidol (Yelnoski and Gardocki, 1964; Yelnosky, Katz and

Dietrich, 1964) it is possible that excitatory effects could be minimised by giving fentanyl at the time of maximum effect of the neuroleptic. Studies on neuroleptanalgesia in horses have shown that fewer excitatory effects are seen when the analgesic component is given some time after the neurleptic agent (MacKenzie, 1977). Defaecation was rarely seen following the intravenous administration of droperidol:fentanyl and the reduced incidence in dogs which received fluanisone:fentanyl by this route may be related to the lower dose used.

The more frequent occurrence of respiratory depression in dogs which received the methotrimeprazine:etorphine combination has already been referred to. Cyanosis was noted in 57 per cent of the dogs given this mixture intramuscularly at the recommended dose rate. Using the same dose, Crooks et al. (1970) recorded cyanosis in only 8 per cent of treated dogs. This difference must be attributed to differences in the subjective criteria used to assess the presence or absence of cyanosis. This side effect was noted in a large number of dogs given a similar dose of this mixture by Alford and Wozniak (1970), but when these workers increased the proportion of methotrimeprazine in the combination cyanosis was not observed. As reported previously by others (Soma and Shields, 1964; Yelnosky and Field, 1964) the effects of the fentanyl-containing mixtures on respiration were more variable with a number of

animals showing marked tachypnoea. Cyanosis was noted least frequently in animals given the droperidol:fentanyl mixture and was not observed when lower doses of the fentanyl combinations were given intravenously.

The presence of significant respiratory depression in animals given fluanisone:fentanyl or methotrimeprazine: etorphine, intramuscularly, was confirmed by the demonstration of elevated end-tidal carbon dioxide tensions. In an experimental study, Short,Greenwald and Bendick (1970) found that arterial PCO_2 increased from a control value of 30 mmHg in unanaesthetised dogs to 45 mmHg in animals given methotrimeprazine:etorphine and to 36mmHg in dogs given droperidol:fentanyl. The greater respiratory depressant effect of the methotrimeprazine:etorphine mixture is in agreement with the clinical findings in the present study.

Although it has been claimed by Blane, Boura and Dobbs (1968) that the anticholinergic effect of metho-trimeprazine should reduce the bradycardia produced by the vagal action of etorphine, a similar degree of bradycardia was noted with all three neuroleptanalgesic combinations. Atropine premedication produced marked tachycardia and it would seem reasonable to withold atropine unless a marked bradycardia develops. In resting dogs, heart rate is influenced by a marked vagal inhibitory action (Dodds, Dolamore and Twissell, 1981) and normal heart rates of 80 min^{-1} and 66 min^{-1}

have been reported (Shepard and Whitly, 1964; Long, Truex and Friedman, 1955).

It was not possible to measure blood pressure in conscious animals but systolic pressure was similar in all three groups during the period of immobilization.

The cardiovascular effects produced by neuroleptanalgesic drugs in the dog are similar to those found in the pig (Becker and Beglinger, 1980) but contrast markedly with results obtained in the horse where hypertension and tachycardia are consistently found (Hillidge and Lees, 1971; Daniel and Ling, 1972; MacKenzie, 1977).

In the study in dogs anaesthetised with pentobarbitone, it was found that the combination of droperidol and fentanyl produced less respiratory depression than fentanyl alone. This difference was established by measuring end-tidal carbon dioxide tension and no significant difference was noted in the respiratory rates produced by the two treatments. Janssen et al. (1963) found that, in anaesthetised dogs, droperidol stimulated respiration by increasing the sensitivity of the respiratory centre to carbon dioxide and, in man, a beneficial effect of droperidol on fentanyl induced respiratory depression has been noted (Foldes et al., 1964; Corssen and De Kornfeld, 1966). The possibility that neuroleptic drugs can antagonise, to some extent, the respiratory depressant effect of narcotic analgesics is also supported by

the findings of Alford and Wozniak (1970) with etorphine and methotrimeprazine. Conflicting results have however been obtained by Yelnoski and Gardocki (1964) in anaesthetised dogs and by Greene (1972) in mice. These authors monitored respiratory minute volume and respiratory rate respectively. As neuroleptanalgesic drugs can produce a significant reduction in metabolic rate in the dog (Gemperle, 1965) it is possible that the measurement of end-tidal carbon dioxide tension in the present investigation provided a more sensitive evaluation of the respiratory effects of these compounds.

In this study it was also found that the combination of droperidol and fentanyl produced a greater hypotensive effect than fentanyl alone for the first 15 min. after administration. This difference may be attributed to a reduction in peripheral resistance due to the α-adrenoceptor blocking activity of droperidol (Janssen et al., 1963; Schaper, Jageneau and Bogaard, 1963; Yelnosky, Katz and Dietrich, 1964). The hypotensive effect of fentanyl alone in pentobarbitone anaesthetised dogs has been noted previously by Gardocki and Yelnoski (1964). These authors found a significantly smaller reduction in heart rate in vagotomised dogs but the hypotensive effect persisted.

The changes in heart rate produced by fentanyl alone and fentanyl:droperidol, in the present investigation, were similar for the first 20 min after administration. The smaller reduction in rate seen at later time points in animals given droperidol:fentanyl may possibly be attributed to baroreceptor reflex activity in response to the vasodilatation induced by droperidol (Schaper et al., 1963).

Chapter 4

ALKYLPHENOLS AS INTRAVENOUS ANAESTHETIC AGENTS

1. INTRODUCTION

While there is general agreement that, with the inhalational anaesthetic agents, anaesthetic potency can be predicted from lipid solubility (Meyer, 1937; Miller et al, 1972; Kaufman, 1977) the search for new agents still depends largely on an empirical approach. This is particularly the case with injectable agents where compounds must have the right structure as well as being lipid soluble (Davis, 1975). Although structure-activity relationships have been investigated within the barbiturate series (Sharpless, 1970; Dundee and Wyant, 1974) no comprehensive information relating the potencies of barbiturates to their partition coefficients is available. The steroid anaesthetics also have definite structure activity relationships (Gyermek and Soyka, 1975; Phillips, 1975) and it has been shown that, within this class of compounds, minor changes in molecular configuration, which would not be expected to alter lipid solubility, can have marked effects on anaesthetic activity. It can be seen that structural changes which affect anaesthetic potency, duration of action and toxicity in one class of compounds, are generally only relevant to that particular series of related compounds.

Many of the lipophilic compounds which have been synthesised in the past have never been examined in tests for anaesthetic activity because of their low solubility in aqueous solutions. The recent availability of the surface active agent Cremophor EL as a solubilizing agent

has removed this obstacle and has allowed the investigation of previously untested lipophilic compounds.

During the testing of compounds selected on a random basis it was found by the author that 2,6-diethylphenol produced anaesthesia when injected intravenously in mice. Induction of anaesthesia was not immediate but the activity shown by this compound was sufficiently interesting to justify an evaluation of the anaesthetic activity of a series of related alkylphenols.

Compounds were provided by Mr. R. James of the Chemistry Department of ICI Pharmaceuticals Division. Some were obtained from commercial sources and purified and others were synthesised by the introduction of alkyl groups into the ortho position of an appropriate phenol.

The synthesis and preliminary structure-activity considerations of this group of alkylphenols have been reported by James and Glen (1980). In the present study more detailed experiments, designed to examine the anaesthetic potency and speed of onset of anaesthesia of a selected group of 2,6 dialkylphenols are described. On the basis of the results obtained in these studies, 2,6-diisopropylphenol (disoprofol) was selected for further comparative studies with currently used injectable anaesthetic agents.

2. MATERIALS

2.1 Animals

Mice

Specific pathogen free Albino mice were housed in wire mesh cages and allowed free access to a standard pelleted mouse diet (P.M.D.) and tap water.

Rats

Specific pathogen free Albino rats were housed in wire mesh cages and allowed free access to a standard pelleted rat diet (P.R.D.) and tap water.

Rabbits

Dutch rabbits were purchased from an accredited breeder and housed individually in metal cages. Rabbits were allowed free access to a standard pelleted diet (R.G.P.) and tap water.

Cats

Domestic cats were purchased from an accredited breeder and housed in pairs in metal and wire mesh cages. Cats were fed once daily with kennel meat (Spillers) and fresh milk.

Pigs

Young domestic pigs (Large White X Landrace) were purchased locally and housed individually in concrete and tubular metal pens. They were allowed free access to tap water and fed twice daily with Sow Breeder Meal.

2.2 Drugs

Cremophor EL (BASF, Germany) a polyoxyethylated castor oil with surfactant properties, in a 10% (v/v) concentration in water was used to prepare solutions containing 10mg ml^{-1} of an alkylphenol test compound for intravenous injection. Test compounds were provided by Mr. R. James of ICI Pharmaceuticals Division, Macclesfield, Cheshire . In some instances ethyl alcohol (6% v/v) and dimethyl sulphoxide (6% v/v; Hopkin and Williams, Chadwell, Essex) were required as additional solubilizing agents. Disoprofol ('Diprivan', ICI, PLC, Macclesfield, Cheshire) was prepared in a standard formulation containing 10mg ml^{-1} in 16% (v/v) Cremophor EL and 8.66% (v/v) ethyl alcohol. In rats, cats and pigs this formulation was used undiluted but for some experiments in mice the formulation was diluted with isotonic saline to obtain a preparation containing 5mg ml^{-1} disoprofol.

A research sample of etomidate was donated by Janssen Pharmaceutical Ltd., Marlow, Bucks, and the following drugs were obtained commercially:

Thiopentone sodium ('Intraval', May and Baker Ltd., Dagenham, Essex); alphaxalone/alphadolone acetate* ('Althesin', Glaxo Laboratories, Greenford, Middlesex); methohexitone

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*'Althesin' contains 9mg ml^{-1} alphaxalone and 3mg ml^{-1} alphadolone acetate. For the sake of brevity the trade name 'Althesin' is used throughout this thesis and doses are based on the total steroid content of 12mg ml^{-1}.

sodium ('Brietal', Lilly Eli & Co. Ltd., Basingstoke, Hants); propanidid ('Epontol', Bayer UK Ltd., Haywards Heath, West Sussex); ketamine hydrochloride ('Ketalar', Parke Davis & Co., Pontypool, Gwent); pentobarbitone sodium ('Nembutal', Abbott Laboratories Ltd., Queenborough, Kent); droperidol ('Droleptan'. Janssen Pharmaceutical Ltd., Marlow, Bucks); chlorpromazine hydrochloride ('Largactil', May and Baker Ltd., Dagenham, Essex); diazepam ('Vallium', Roche Products Ltd., Welwyn Garden City, Herts); atropine sulphate injection B.P. and hyoscine hydrobromide injection BP (Macarthy's Ltd., Romford, Essex); pethidine injection BP (Roche Products Ltd.); pentazocine hydrochloride ('Fortral', Winthrop Laboratories, Surbiton, Surrey); halothane ('Fluothane') and trichloroethylene ('Trilene', ICI PLC); cyclopropane (British Oxygen Co., Harlow, Essex); enflurane ('Ethrane', Ohio Medical Products, Madison, Wisconsin); methoxyflurane ('Penthrane', Abbott Laboratories Ltd., Queenborough, Kent); di-ethyl ether (MacFarlane Smith Ltd., Edinburgh); chloroform (Hopkin and Williams Ltd., Chadwell Heath, Essex); lignocaine hydrochloride ('Xylocaine spray', Astra Chemicals Ltd., Watford, Herts); suxamethonium chloride injection BP ('Anectine', Wellcome Medical Division, Crewe, Cheshire); gallamine triethiodide ('Flaxedil', May and Baker Ltd.); pancuronium bromide ('Pavulon', Organon Laboratories Ltd., Morden, Surrey);

tubocurarine chloride injection BP ('Tubarine' (miscible) injection, Wellcome Medical Division); neostigmine injection BP ('Prostigmin', Roche Products Ltd.).

Drug doses are all expressed as mg kg^{-1} body weight and where drugs were administered in the form of a salt the doses refer to the amount of the relevant salt.

3. METHODS

3.1 ASSESSMENT OF ANAESTHETIC POTENCY AND THERAPEUTIC RATIO

Test compounds were administered intravenously to groups of male mice weighing 18-22g. All injections were given over a standard injection period of 10 seconds. A range of 6-8 doses was given, including a dose producing 100% lethality and one which failed to produce loss of righting reflexes for a minimum period of 30 seconds. Mice which lost their righting reflexes following the injection of a test compound were placed in a heated box in which the ambient temperature was maintained at 35°C during the period of sleep.

3.1.1 Preliminary screening tests

In preliminary tests, designed to select the best compound from the alkylphenol series, groups of 5 mice were used at each dose tested. The median hypnotic dose (HD_{50}) was estimated by interpolation from the dose-response curve as that dose which would be expected to cause loss of righting reflexes for a minimum period of 30s in 50% of mice. This technique is based on the method of probit analysis of Miller and Tainter (1944). To achieve an accurate estimate with this method it is necessary to examine doses producing effects in the range of 40% to 60%. In some instances, because of the steep slope of the dose-response lines obtained, these estimates were expressed as a dose range giving 0 and 100% effects with closely spaced doses.

The median lethal dose (LD_{50}) was estimated in the same way, as that dose which would be expected to produce a lethal effect in 50% of mice, and therapeutic ratio was calculated as the ratio LD_{50}/HD_{50}. The definition of HD_{50} and LD_{50} values within a 5-10 mg kg^{-1} dose range was deemed to be sufficiently accurate for a preliminary assessment of the activity and toxicity of a range of compounds to be made.

In addition to the assessment of potency and therapeutic ratio, a number of additional observations were made in these preliminary screening tests. Note was taken of the speed of onset of anaesthesia and the duration of the period of loss of righting reflexes. Since these features were noted at doses equal to 2 x HD_{50}, the HD_{50} being taken in some instances as the median dose within a defined dose range, it was not always possible to be certain that these observations were made at exactly equipotent doses. For this reason, limits were defined within which speed of induction was classed as immediate (<10s; i.e. mice lost righting reflexes by the end of an injection), slow (10-15s), or very slow (>15s) and duration of action was classed as brief (<5 min), moderate (5-10 min), or long (>10 min). A non-linear increase in the duration of anaesthesia with increasing doses indicated that a particular compound was accumulating in body tissues to a significant extent.

Analgesia was assessed by noting the response to tail pinching. Muscle tremors and myoclonia were noted as excitatory side effects and compounds deemed to produce poor muscle relaxation were those which failed to abolish muscle tone during anaesthesia. Mice were observed for 10 days following the test to allow any delayed deaths to be noted.

3.1.2 Comparative studies with disoprofol and standard agents

When disoprofol had been selected for further evaluation a more accurate assessment of anaesthetic potency and therapeutic ratio was required. In these experiments groups of 10 mice were used at each dose examined. The HD_{50} and LD_{50} as defined above were estimated by fitting parallel logit lines derived from the dose response results (Ashton, 1972). With this method it was also possible to derive 95% confidence limits for the estimates of HD_{50}, LD_{50} and therapeutic ratio.

Experiments were done with disoprofol, thiopentone, 'Althesin', methohexitone and propanidid to allow the results obtained with disoprofol to be compared with those produced by some currently used injectable anaesthetic agents.

3.2 ASSESSMENT OF SPEED OF ONSET OF ANAESTHESIA

3.2.1 Method development

In order to develop a standardized technique for the evaluation of the speed of onset of anaesthesia, the relative importance of the effects of a number of factors which could influence the speed of induction of anaesthesia were evaluated in mice.

Groups of male mice (18-22g) were studied and drugs were injected into a tail vein while the mice were restrained in a conical perspex holder. It was possible to observe mice through the transparent restraining apparatus and induction time was measured with a stop watch as the interval between the beginning of an injection and the occurrence of head drop. Preliminary experiments were performed with thiopentone to investigate the effect of variations in speed of injection, environmental temperature, drug dosage and concentration, on the speed of onset of anaesthesia with this agent.

a) Speed of injection

A standard dose of 40mg kg^{-1} of thiopentone, prepared at a concentration of 5 mg ml^{-1} was given to groups of 5 mice over injection times ranging from 5 to 40 seconds.

The speed of injection was found to have a marked effect on anaesthesia onset time as mean induction time was found to increase in a linear fashion from 5s to 26.2s as the duration of injection was increased from 5 to 40s (Table 23).

Table 23 The effect of variations in the speed of injection on the speed of induction obtained with 40 mg kg^{-1} thiopentone in mice. Drug concentration 5 mg ml^{-1}

Duration of injection (s)	5	10	15	20	25	30	35	40
Induction time (s) Mean ± SD (n=5)	5.0 ±0.70	8.0 ±0.70	11.0 ±0.70	14.4 ±1.14	16.2 ±1.30	17.8 ±1.30	22.4 ±1.81	26.2 ±1.09

b) Drug concentration

A standard dose of 40 mg kg^{-1} of thiopentone, prepared in concentrations ranging from 2 to 10 mg ml^{-1}, was given to groups of 10 mice over a constant injection time of 10 seconds.

Although there was a slight reduction in induction time with the highest concentrations used (Table 24), the influence of variation in drug concentration on induction time was very much less than the influence of speed of injection noted in the previous experiment.

Table 24. The effect of variations in drug concentration on the speed of induction obtained with 40 mg kg^{-1} thiopentone in mice. All doses given over 10 s.

Drug concentration (mg ml^{-1})	2	4	6	8	10
Induction time (s)	7.9	7.6	7.5	7.0	6.6
Mean ± S.D. (n=10)	±0.87	±0.69	±0.52	±0.81 *	±0.50 **

*P < 0.05 Induction time significantly different from that
**P < 0.001 obtained with 2 mg ml^{-1}. Student's unpaired t test.

c) Environmental temperature

A range of doses of thiopentone, prepared at a concentration of 5 mg ml^{-1}, was given to groups of 10 mice over injection times of 1s or 10s. One set of experiments was performed at room temperature (23°C), whereas in a second series mice were held in a warming box at 36°C before induction of anaesthesia.

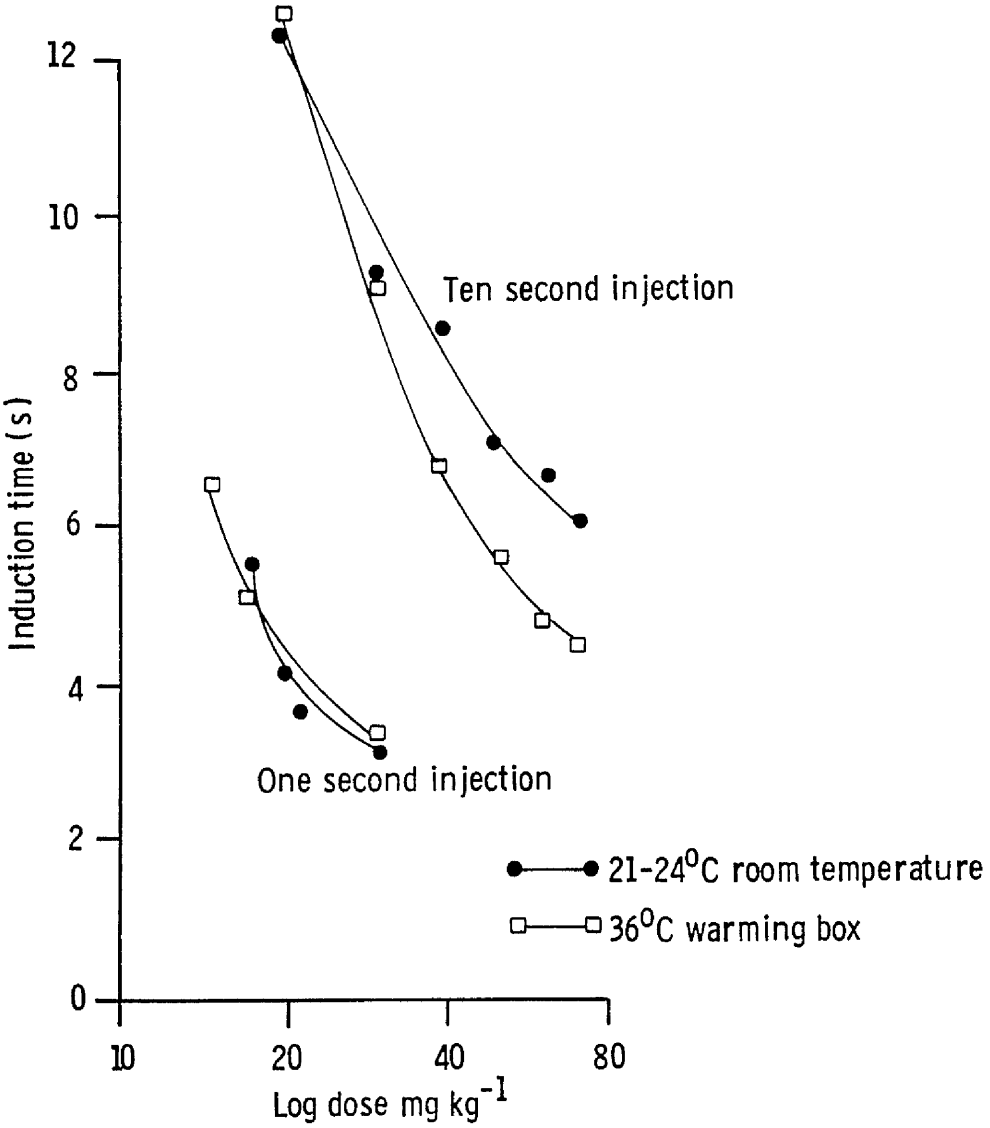


Fig. 15 Effect of environmental temperature on induction time with thiopentone.

Induction time was found to decrease as the dose of thiopentone given was increased (Figure 15). Alteration of the environmental temperature produced no apparent effect on induction time when a 1-s injection was used. Following the 10-s injection, mice in which the body temperature was maintained at 36°C showed significantly faster induction times ($P<0.002$) at doses greater than 40 mg kg^{-1} in comparison with mice held at room temperature before induction of anaesthesia (Figure 15). In all subsequent studies mice were kept in a warming box at 36°C prior to induction of anaesthesia.

d) Drug dosage

The results obtained in the above experiments indicated that drug dosage and speed of injection were the most important factors influencing the speed of induction of anaesthesia. To investigate the importance of drug dosage further experiments were performed with thiopentone and methohexitone using standard injection times of 1s or 10s.

The median hypnotic dose (HD_{50} as defined in Section 3.1) of each agent was calculated using the graphical method of Miller and Tainter (1944). Ten mice were used at each dose given for the estimation of the HD_{50}. The HD_{50} values obtained in this part of the study are given in Table 25.

Table 25 HD_{50} values obtained with thiopentone and methohexitone using two injection times

	HD_{50} mg kg^{-1} ± SEM 1-s injection	HD_{50} mg kg^{-1} ± SEM 10-s injection
Thiopentone	19.5 ± 0.56	21 ± 0.90
Methohexitone	7.0 ± 0.60	8 ± 0.43

With both drugs the HD_{50} figures obtained with the 10-s injection were slightly greater than those found with the 1-s injection time. This indicates that with the slower injection a certain amount of drug dilution and redistribution occurs during the course of the injection such that the dose given has to be increased slightly to produce the same degree of depression of the central nervous system. As the apparent potency of a drug can be influenced by the rate of injection, injection times the same as those used in the calculation of HD_{50} were used in subsequent estimations of induction times.

Having established an HD_{50} for each agent, increasing doses up to 2 x HD_{50} were then given to further groups of 10 mice and mean induction times were plotted against the dose given (Figure 16). From the dose response lines thus obtained and the previously calculated HD_{50}, it was then possible to compare induction times at any given multiple of the HD_{50} (Figure 17).

The results shown in this figure indicated that the use of a rapid injection minimised the influence of dose on induction

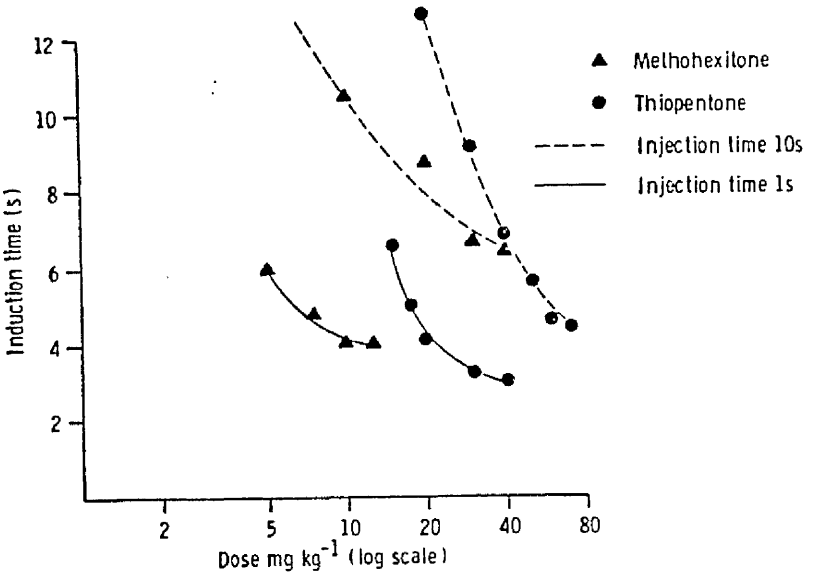


Fig. 16 Influence of drug dosage on anaesthesia induction time in mice.

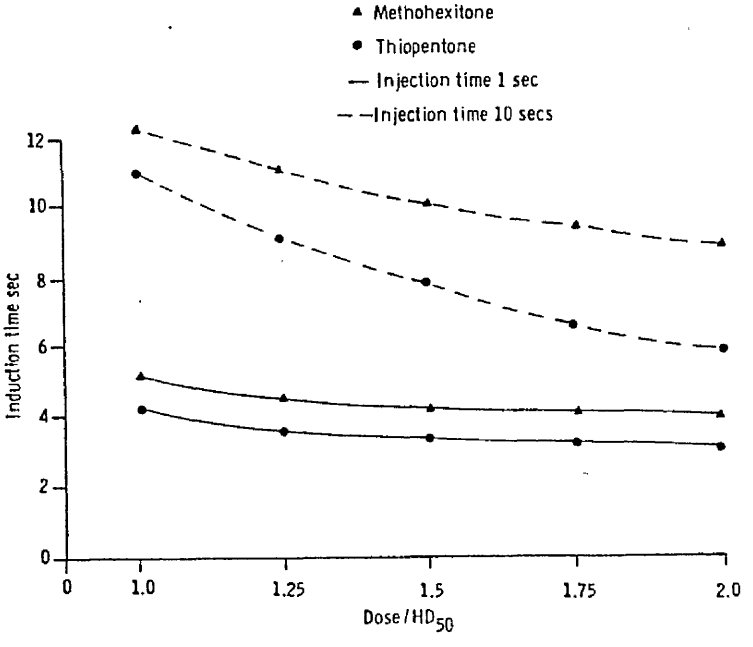


Fig. 17 Anaesthesia induction times with equipotent doses of thiopentone and methohexitone in mice.

time and virtually parallel dose response lines were obtained. A technique involving the use of a 1-s injection time was therefore preferred for studies where the prime objective was the comparison of the rate at which different compounds were able to penetrate the blood-brain barrier.

3.2.2 Comparison of disoprofol induction time with that of standard agents

The above technique, utilizing standardized injection times of 1s or 10s for the estimation of HD_{50} and induction times, was used to compare the induction time obtained with disoprofol with that of standard agents. On referring to the dose-response curves used in the potency estimations, the minimal dose which anaesthetized 100% of mice (HD_{100}) was found to be close to 1.25 x HD_{50}. This point was therefore selected as the equianaesthetic dose at which induction times were noted. The speed of induction obtained with disoprofol was compared with that achieved with pentobarbitone, thiopentone, propanidid, ketamine, methohexitone, 'Althesin' and etomidate.

3.2.3 Detailed comparison of structure-activity relationships within a group of 2,6-dialkylphenols

Having established a satisfactory technique for the measurement of the speed of induction of anaesthesia, a number of alkylphenols were examined in more detail. Compounds were selected partly on the basis of results obtained in primary screening tests and partly to provide groups of related

compounds with which to further define structure-activity relationships within a series of 2,6-dialkylphenols.

In addition to the estimation of speed of induction, duration of action and respiratory effects were also examined in the same animals. Groups of 10 male mice (18-24g) were given a range of doses of each test compound. A 1-s injection time was used for all doses and the HD_{50} was calculated by probit analysis as described in Section 3.1. Duration of action was noted following the administration of a dose equal to 2 x HD_{50} and respiratory rates were counted with the aid of a low pressure transducer (Devices UP1) connected to a 2ml syringe barrel, one minute after the administration of this dose. The use of this technique for the evaluation of the respiratory effects of drugs in mice has been described by Bradshaw, Biswas and Pleuvry (1973). Induction times were calculated as described in the previous section at 1.25 x HD_{50}.

3.3 EVALUATION OF ANAESTHETIC PROPERTIES IN RABBITS

Results obtained in primary screening tests with alkylphenols were used to select compounds for further evaluation in rabbits. Compounds without serious side effects, with an $HD_{50} \leq 20$ mg kg^{-1} and a therapeutic ratio $\geq$ 4 in mice were selected for further testing in rabbits. Test agents were prepared as aqueous solutions containing 25 mg ml^{-1} in Cremophor EL 10% (v/v), and injected into a marginal ear vein over a period of 20s. A range of doses was examined to give some

indication of minimum hypnotic and lethal doses, and to assess the quality of anaesthesia obtained. Each dose was generally given to only a single rabbit and four to six rabbits were used to study each compound. Speed of onset, duration of anaesthesia, muscle relaxation, and any excitatory effects were noted. In addition, compounds which produced a period of respiratory arrest lasting more than 20s at anaesthetic doses were classed as potent respiratory depressants. Thiopentone sodium was used as a standard drug in this test.

3.4 DURATION OF ACTION, RECOVERY TIMES AND RESPIRATORY EFFECTS OF DISOPROFOL AND STANDARD COMPOUNDS

Doses approximately equivalent to 2 x HD_{50} were given to groups of 10 male mice (18-24g) over an injection period of 10s. Respiratory rates were counted as described in Section 3.2.3 one minute after induction of anaesthesia. The duration of loss of righting reflex was noted in each mouse as was the time required after righting before mice were willing and able to walk from the centre to the periphery of a 30cm diameter disc. Co-ordination was tested with a metal rod walking test, mice being classed as co-ordinated when after a maximum of four attempts they either remained on the rod for 20s or walked to the end of the rod within a shorter period.

Experiments were performed with disoprofol, thiopentone, 'Althesin' and methohexitone.

3.5 EVALUATION OF CUMULATIVE EFFECTS

3.5.1 Cumulation in mice

The cumulative effect of repeated doses of disoprofol was evaluated using the technique described by Child et al (1971). Five mice were each given an initial dose of disoprofol which produced a period of sleep of about 5 minutes. A dose of 25 mg kg^{-1} given over 10s was found to be suitable. When righting reflexes returned 30s were allowed to elapse before a further injection of the same dose was given. The sleeping time between repeated injections was noted and the experiment was continued until a total of 10 doses of disoprofol had been given. During the experiment mice were kept at an ambient temperature of 36°C.

For comparative purposes similar experiments were done with thiopentone and 'Althesin', the doses used being 40 mg kg^{-1} and 12 mg kg^{-1} respectively.

3.5.2 Cumulation in rats

Cumulative properties were examined in rats by investigating the effects produced by the continuous infusion of disoprofol, given at a constant rate of 0.9 ml hr^{-1} (10 mg ml^{-1} concentration) for periods of one and two hours. Two groups of five female rats (200-225g) were used.

A 25 B.W.G. 'Butterfly' cannula (Abbott Laboratories, Queenborough, Kent) was filled with saline containing 100 i.u. heparin ml^{-1}, and attached to a 3-way tap. The cannula was introduced into a tail vein and held in position with adhesive tape. Heparinised saline was then injected through the cannula to confirm its correct placement. An induction dose of disoprofol was then given over 20s, through the 3-way tap, followed by a flushing injection of saline. The dead space of the cannula was filled with disoprofol and the tap was connected to a primed nylon infusion catheter (Manometer line,200/3495/060, Portex Limited, Hythe, Kent) attached to a syringe infusion pump ('Unita' Braun, Melsungen, Germany). Rats were placed on a heated pad and body temperature was maintained at $38^{\circ}C \pm 1^{\circ}C$ during the period of anaesthesia and recovery.

At 10 minute intervals during the maintenance period respiratory rate was counted visually and heart rate was derived from a Lead II electrocardiograph signal with an electronic ratemeter. The degree of muscle relaxation produced by the anaesthetic and the presence or absence of palpebral and pedal withdrawal reflexes were also recorded. After the end of an infusion, the time intervals until the animal could lift its head, and later regain the ability to walk, were noted.

3.6 EVALUATION OF CARDIOVASCULAR AND RESPIRATORY EFFECTS

The objective of the study was to examine some of the cardiovascular and respiratory effects of disoprofol in conscious, unrestrained, and unpremedicated animals. Comparative studies were performed with thiopentone and 'Althesin'.

A group of 7 pigs (four females and three castrated males) weighing 17-40kg was used. One week prior to the start of the experiment, polythene cannulae were surgically implanted in a carotid artery and a jugular vein under halothane anaesthesia. The cannulae were passed subcutaneously to emerge on the dorsal aspect of the neck. Heparinised saline was injected to ensure their patency and they were closed with the crimped shaft of a hypodermic needle.

Preliminary experiments had previously been performed to determine equivalent anaesthetic doses of the three agents in the pig. A minimal anaesthetic dose was established as that dose which was just sufficient to produce a brief period (3-5min) of anaesthesia. This dose and a dose equal to twice the minimum anaesthetic dose were chosen for the subsequent study. The doses selected were as follows:

Disoprofol	2.5 mg kg^{-1}	5.0 mg kg^{-1}
Thiopentone	10 mg kg^{-1}	20 mg kg^{-1}
'Althesin'	1.8 mg kg^{-1}	3.6 mg kg^{-1}

The experiment had a cross-over design in that each pig received both doses of the three anaesthetic agents. At least two clear days were allowed between successive experiments in the same animal.

The pigs were found to settle easily in a wooden box with a wire mesh lid. Saline filled polythene cannulae were passed through this mesh and connected to the cannulae which had been surgically implanted. Anaesthetic drugs were injected from outside the box, through the catheter connected to the venous cannula, over a standard injection time of 30 seconds. The arterial catheter provided samples for blood-gas analysis and was otherwise connected to a Bell and Howell L221 strain gauge transducer for the continuous measurement of arterial pressure. Mean arterial pressure was calculated as diastolic pressure plus 1/3 pulse pressure. Samples of arterial blood were collected anaerobically into heparinised syringes prior to anaesthesia and three and 20 minutes after induction. Standard Radiometer electrodes were used to measure PO_2 PCO_2 and pH in these samples. Base excess was calculated from the pH and PCO_2 values using the Henderson-Hasselbalch equation. Heart rate was derived electronically from the blood pressure signal and respiratory rate was counted visually. Arterial blood pressure and heart rate were recorded continuously with

Devices M19 heated stylus recorder. Recording was continued until the animals regained consciousness.

3.7 EVALUATION OF INTERACTIONS BETWEEN DISOPROFOL AND ANCILLARY DRUGS USED IN ANAESTHESIA

3.7.1 Interactions with drugs used in preanaesthetic medication

Studies were carried out in male mice weighing 18-20g. The drugs used were chosen as representative examples of the ataractic, sedative, analgesic and anticholinergic classes of drug, frequently administered prior to anaesthesia (Riding, 1965; Foster, 1966). The doses originally chosen were expected to allow each class of drug to exhibit its pharmacological effect in mice and were much greater than the doses normally used for preanaesthetic medication in man. In a preliminary experiment the drugs were administered subcutaneously, and 30 minutes later the gross behavioural effects produced were noted. The drugs and doses used and the effects produced were as follows:

Chlorpromazine hydrochloride	3 mg kg^{-1}	deep sedation
Diazepam	3 mg kg^{-1}	deep sedation
Pentobarbitone sodium	10 mg kg^{-1}	very slight ataxia
Droperidol	1 mg kg^{-1}	deep sedation
Pethidine hydrochloride	10 mg kg^{-1}	no apparent effect
Pentazocine hydrochloride	10 mg kg^{-1}	no apparent effect
Atropine sulphate	1 mg kg^{-1}	no apparent effect
Hyoscine hydrobromide	1 mg kg^{-1}	no apparent effect

In order to quantify the degree of sedation produced by the ataractic drugs, and to allow equipotent doses to be chosen for interaction studies further experiments were done in mice with chlorpromazine, diazepam and droperidol. These drugs were examined in an open field test (Turner, 1965) in which spontaneous or exploratory activity is measured, and in a test in which evidence of muscle relaxation is shown by the inability of a mouse to draw up its hind legs onto a wire supporting its forelegs. Five groups of 10 mice were used in each test, four groups receiving increasing doses of one of the test agents, given 30 minutes prior to testing, and a control group injected with saline.

The results obtained in these two tests are shown in Figures 18 and 19. All the doses of chlorpromazine and droperidol produced marked reductions in spontaneous activity. Diazepam at 1.5 mg kg^{-1} increased activity slightly but at 3.0 mg kg^{-1} produced a very large reduction in activity. All the drugs produced dose-related muscle relaxation, the most marked effects being produced by the highest doses of chlorpromazine and diazepam. The doses chosen for interaction studies were those which produced approximately 50% reduction in spontaneous activity and/or muscle relaxation. These doses were as follows:

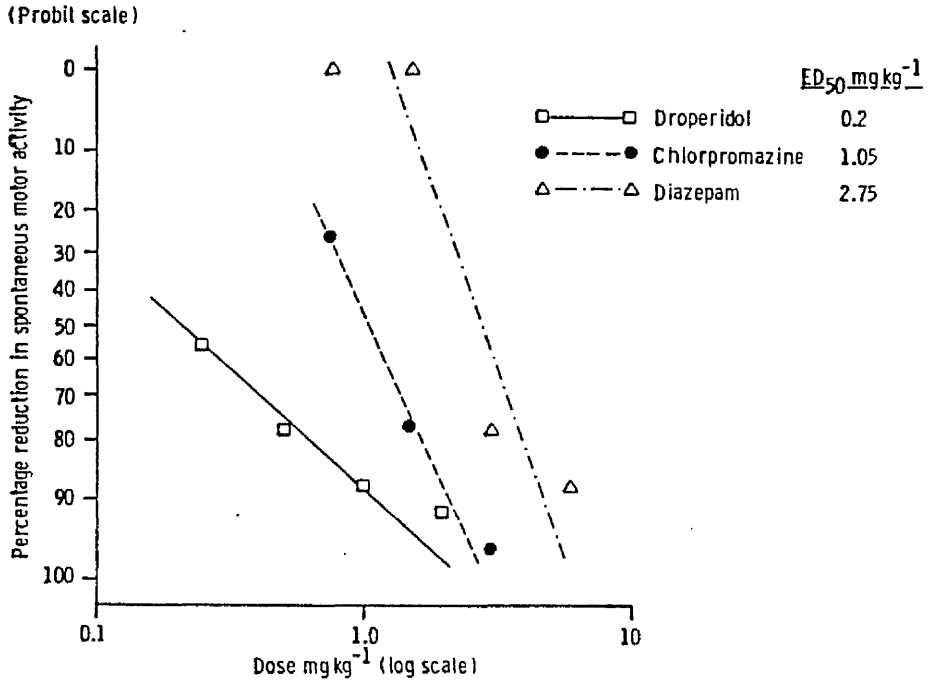


Fig. 18 Inhibition of spontaneous motor activity by premedicant drugs in mice

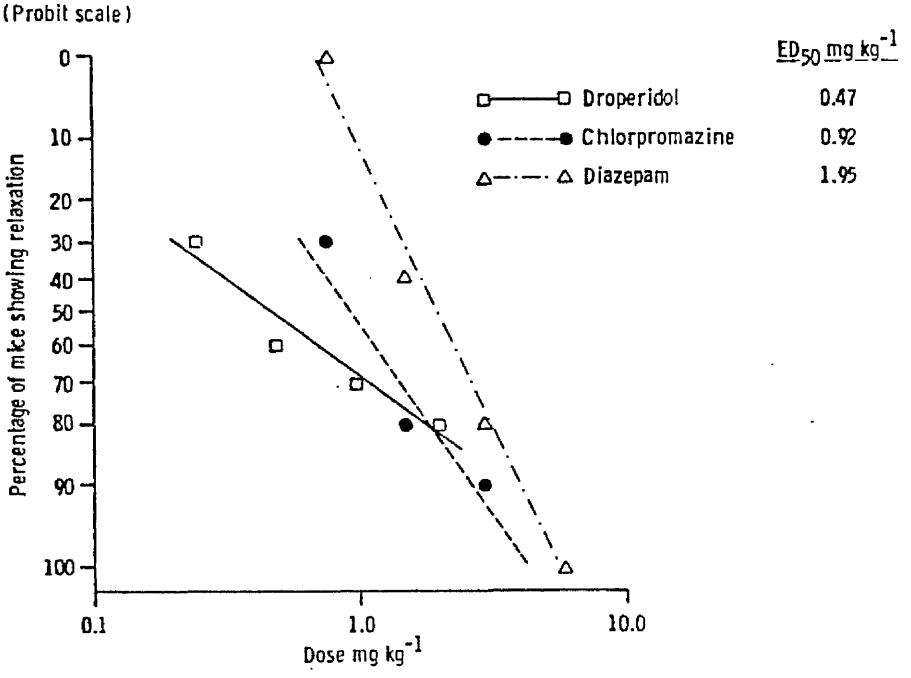


Fig. 19 Muscle relaxation produced by premedicant drugs in mice

Chlorpromazine hydrochloride	1.0 mg kg^{-1}
Diazepam	2.0 mg kg^{-1}
Droperidol	0.25mg kg^{-1}

As no significant behavioural effects had been produced by the other premedicant drugs examined in the preliminary experiment, the doses originally selected were used in the interaction studies.

To examine the interaction of the premedicant drugs with disoprofol, each drug was given subcutaneously to a group of 10 mice, 30 minutes prior to induction of anaesthesia with an intravenous injection of 25 mg kg^{-1} disoprofol, given over 10 seconds. Mice were kept in a warming box at an ambient temperature of 35-37^{o}C. A control group of 10 mice was given disoprofol alone.

Duration of loss of righting reflex and the intervals between recovery of righting reflexes and the ability to walk and co-ordinate were observed. Co-ordination was deemed to have returned when a mouse was able to maintain balance on a 1cm diameter metal rod fixed horizontally 10cm above bench level. The duration of any period of apnoea at induction, and respiratory rate one minute following the injection of disoprofol were also measured in control and premedicated mice using the methods described in Section 3.2.3.

As the results obtained in this experiment indicated that chlorpromazine, diazepam, droperidol and atropine produced significant changes in the sleeping time

produced by disoprofol, and in the recovery indices examined, a further experiment was designed to compare the potentiation seen with disoprofol with that produced by the combination of these premedicant drugs with thiopentone and 'Althesin'.

a) Comparative studies with thiopentone and 'Althesin'

Chlorpromazine (0.25 - 2.0 mg kg^{-1}), diazepam (0.5 - 4.0 mg kg^{-1}), droperidol (0.125 - 0.5 mg kg^{-1}) or atropine (1.0 mg kg^{-1}) were given subcutaneously to mice 30 minutes prior to induction of anaesthesia with disoprofol thiopentone or 'Althesin'. A group of 10 male mice (18-20g) received each dose of premedicant drug. The anaesthetic drugs were given at a single dose rate equivalent to twice their HD_{50} as previously determined (Section 3.1).

Sleeping times, recovery times and respiratory rates were measured as described in the previous section.

3.7.2 Interactions with inhalation anaesthetics

The combination of disoprofol with a range of inhalation anaesthetic agents was examined in a group of 6 adult cats (three male and three female) weighing between 1.8-2.3kg. All the cats were anaesthetised on more than one occasion but an interval of at least one week was allowed between successive experiments.

Each cat was given an induction dose of 7.5mg kg^{-1} disoprofol and the trachea was intubated with an uncuffed tube following the aerosol application of 10 mg lignocaine hydrochloride to the laryngeal mucosa. Anaesthesia was then maintained for one hour with one of a variety of inhalation agents. The inhalation agents examined were halothane, nitrous oxide, trichloroethylene, cyclopropane, enflurane, methoxyflurane, diethyl ether and chloroform. Halothane was vapourized in a 50:50 mixture of nitrous oxide and oxygen while the other agents were mixed solely with oxygen. Three cats were used for each agent investigated. The inspired concentration of each agent was adjusted to maintain a light level of anaesthesia, the lower concentrations used being those required for maintenance of anaesthesia in this species.

Cats were unpremedicated with the exception of the group given diethyl ether. In this case 0.2mg atropine sulphate was administered intramuscularly 30 minutes prior to induction of anaesthesia with disoprofol.

Halothane was vaporized in a Mark III 'Fluotec' vapouriser. All the other volatile agents were vapourized in a universal vapourizer (Raventos, 1956), the concentration of vapour delivered being calculated from the density and molecular weight of the compound. The delivered concentration could be readily altered by changing the flow rate of oxygen passing through the saturated vapour in the vapourizer. Cyclopropane

mixtures in oxygen were prepared by passing the two gases through calibrated rotameters at a flow rate calculated to provide final cyclopropane concentrations in the range 20% - 30%.

All agents, with the exception of cyclopropane, were in the first instance delivered to the cats with an Ayre's T-piece circuit, with a fresh gas flow rate ranging from 1.5 - 4 ℓ min^{-1} depending on the concentration of agent being produced. When inspired concentrations, suitable to maintain light surgical anaesthesia, had been determined in this manner, one or two experiments in each group were carried out with the anaesthetic mixtures prepared in large nylon reservoir bags. With these bags it was possible to administer the agents with a non-rebreathing circuit utilizing unidirectional valves. A pneumotachograph was incorporated on the inspiratory side of these valves to allow measurement of tidal volume with an electronic integrator. In all cats given cyclopropane reservoir bags were used instead of a continuous flow T-piece circuit.

Following induction of anaesthesia with disoprofol, respiratory rate was counted at 10 min intervals and leads were attached to monitor the electrocardiogram and to measure heart rate with a cardiotachometer. The P-R interval in the Lead II electrocardiogram was measured prior to and following inhalation of each vapour or gas mixture. Any abnormalities in the electrocardiogram were noted as they occurred. At the end of the maintenance

period the duration of the recovery phases was noted.

3.7.3 Interactions with neuromuscular blocking drugs

a) Experiments in cats

Male and female cats were used to investigate any possible interactions between disoprofol and the neuromuscular blocking drugs suxamethonium, gallamine and pancuronium. All drugs were given in a manner which simulated their use in clinical practice. The cats, weighing 1.8 - 2.4kg were given 0.2mg atropine sulphate intramuscularly 30 minutes prior to induction of anaesthesia with 7.5 mg kg^{-1} disoprofol.

Throughout the period of relaxant induced muscle paralysis, anaesthesia was maintained with 0.5 - 2.0% halothane in oxygen, delivered with an Ayre's T-piece circuit. A fresh gas flow of 3 ℓ min^{-1} was used and in all cases the trachea was intubated with an uncuffed endotracheal tube. When muscle paralysis proved to be short lasting, anaesthesia was maintained for a minimum period of 30 minutes. In other instances anaesthesia was maintained beyond this point if spontaneous respiration was not present. During the maintenance period Lead II electrocardiograms were monitored and heart rates counted. During the period of respiratory paralysis, positive pressure ventilation was provided by intermittent occlusion of the expiratory limb of the T-piece circuit.

On a number of occasions the neuromuscular blocking drugs were antagonized with 0.1mg neostigmine methyl sulphate given with 0.2mg atropine sulphate. In some animals the non-depolarizing drugs were given when spontaneous respiration had returned following the administration of suxamethonium.

b) Experiments in pigs

Because of the histamine releasing properties of tubocurarine in the cat (Hall, 1971) the effects produced by the combination of this relaxant with disoprofol were investigated in pigs. Male and female pigs weighing between 12 and 22 kg were given 0.3mg atropine sulphate and 2.5mg droperidol, intramuscularly, 30 minutes prior to induction of anaesthesia with 5 mg kg^{-1} disoprofol. A Magill semi-closed circuit was used during maintenance of anaesthesia with 0.5 - 2.0% halothane given in a total fresh gas flow of 6 ℓ min^{-1} of a 50:50 mixture of nitrous oxide and oxygen. Cuffed endotracheal tubes were used in pigs, with 2 mg kg^{-1} suxamethonium being given in each case to facilitate endotracheal intubation. Tubocurarine (0.15 - 3.0 mg kg^{-1}) was given when spontaneous respiration had returned and a stable level of anaesthesia had been achieved. The duration of the period of respiratory paralysis produced by tubocurarine, and recovery times following the discontinuation of halothane anaesthesia were noted.

c) Experiments in mice

It is known that, in man, the intravenous anaesthetic, propanidid, potentiates the period of apnoea and respiratory depression produced by the neuromuscular blocking drug, suxamethonium (Clarke, Dundee and Daw, 1964). Experiments were performed in mice in an attempt to develop an animal model in which it would be possible to demonstrate this interaction. The combination of disoprofol and other standard intravenous anaesthetics, with suxamethonium, could then be examined in this model to see if any similar interaction might be encountered.

i) Method development

Preliminary experiments were performed with thiopentone and suxamethonium to select doses of these agents which would produce anaesthesia for about 5 minutes and a period of relaxant induced apnoea of less than 1 minute. It was hoped that if a suitable dose could be found, an equivalent dose of propanidid could then be combined with suxamethonium to see if a longer period of apnoea and respiratory depression could be demonstrated.

An intravenous dose of 50 mg kg^{-1} thiopentone alone produced no apnoea and a 6 minute period of sleep. When this dose of thiopentone was followed immediately by an intravenous dose of 0.5 mg kg^{-1} or 1.0 mg kg^{-1} suxamethonium, sleeping times were extended but apnoea was not consistently produced. Increasing the dose of suxamethonium to 1.5 mg kg^{-1} produced persistent apnoea and death. A similar result was

obtained when the dose of thiopentone was reduced to 40 mg kg^{-1}. The results of these preliminary experiments are summarized in Table 26 .

Table 26 Dose finding experiments with thiopentone and suxamethonium

Thiopentone dose mg kg^{-1} i.v.	Suxamethonium dose mg kg^{-1} i.v.	Result
50	-	6 min sleep
50	0.5 - 1.0	no apnoea
50	1.5	apnoea/death
40	1.5	apnoea/death

In a further experiment 100% oxygen was administered to mice following induction of anaesthesia with 40 mg kg^{-1} thiopentone. Suxamethonium 1.5 mg kg^{-1} was then given and oxygen supplementation was continued until regular respiration returned. In this experiment the mean apnoea time induced by suxamethonium was 22.5 $\pm$ 5.4 s. All mice survived but sleeping time was extended to 14.2 $\pm$ 3.1 min.

When the same doses of thiopentone and suxamethonium were mixed together and given in a single injection, and oxygen supplementation given as in the previous experiment, the mice failed to survive.

The next modification of the technique involved reducing the dose of suxamethonium to 1.0 mg kg^{-1} and giving this dose in a single injection mixed with 40 mg kg^{-1} thiopentone. When oxygen administration was stopped on the return of regular respiration, apnoea time was 22.5 $\pm$ 8.2 s, but sleeping time varied from 8 min to more than 1 hour. When oxygen was given for 2.5 min after the injection of the mixture of thiopentone and suxamethonium mean apnoea time was 29.5 $\pm$ 2.3 s and sleeping time was still prolonged but more consistent (8.8 $\pm$ 2.2 min). The administration of oxygen throughout the period of sleep produced the desired result with mean apnoea time being 40 $\pm$ 19.3 s and mean sleep time 5.6 $\pm$ 1.6 min. The effects produced by these different periods of oxygen supplementation are summarized in Fig.[20] .

As this technique appeared to give satisfactory results with thiopentone and suxamethonium, further experiments were performed with a range of equivalent doses of thiopentone and propanidid. The doses used were multiples of the previously determined HD_{50} (Section 3.1).

The anaesthetic drugs were mixed with a fixed dose of 1 mg kg^{-1} suxamethonium and injected into a tail vein over 20s. Groups of 10 male mice (18-20g) received the anaesthetic/suxamethonium combinations while others were given the anaesthetic agents alone. The dose volume was 0.1ml in mice which received the anaesthetic alone and 0.2 ml when one of the mixtures was given. Sleeping time (loss of righting reflex) and the duration of apnoea,

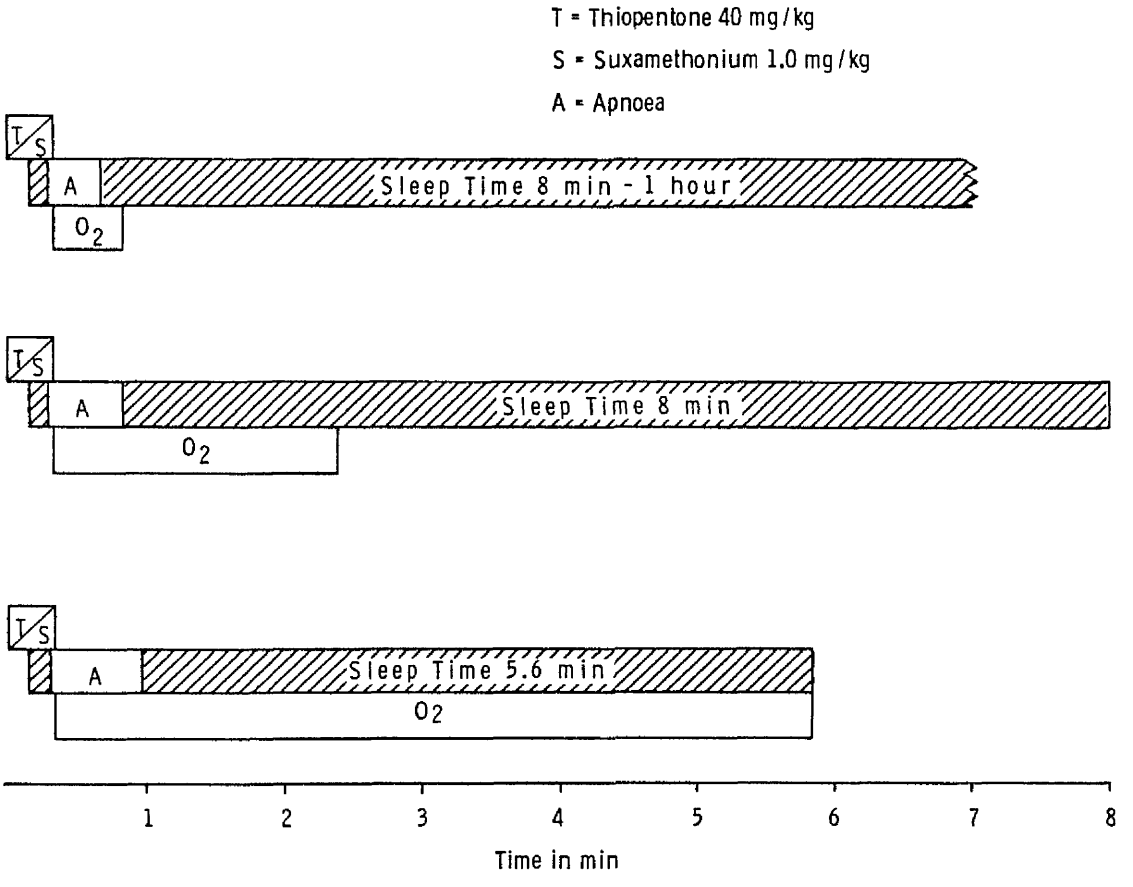


Fig. 20 The effect of supplementary oxygen on thiopentone/ suxamethonium interactions in mice.

ending with the return of regular respiration, were noted. Respiratory rate was measured 2 min after an injection by the method described in Section 3.2.3. Mice were kept in a warm box at an ambient temperature of 35 $\pm$ 1°C and 100% oxygen was delivered at a flow rate of 1 ℓ min^{-1} with a face mask throughout the period of sleep.

The results obtained in this experiment are given in Table 27 and Fig. 21. At the doses examined neither of the anaesthetics, when given on their own, produced any apnoea. At equivalent doses (2 x HD_{50}) both drugs, when combined with 1 mg kg^{-1} suxamethonium produced a similar period of apnoea. In this respect the model failed to demonstrate any significant difference between the thiopentone and propanidid combinations. On the other hand, marked differences were noted in the respiratory rates measured 2 min after induction of anaesthesia. Respiratory rates were reduced slightly in mice given thiopentone and suxamethonium whereas much greater reductions were noted in those given propanidid and suxamethonium.

This difference between the two agents indicated that the model would be suitable for the examination of any possible interaction between new anaesthetic agents and suxamethonium. The above method was therefore used to investigate the effects produced by the combination of disoprofol and suxamethonium. Comparative experiments were also done with thiopentone, propanidid,

TABLE 27 RESPIRATORY EFFECTS PRODUCED BY THIOPENTONE AND PROPANIDID IN MICE WHEN GIVEN ALONE AND IN COMBINATION WITH SUXAMETHONIUM

Anaesthetic Agent	Dose mg kg^{-1}	Suxamethonium 1 mg kg^{-1}	Duration of apnoea (s)	Respiratory rate 2 min after injection
Thiopentone	30	-	-	193 ± 17.2
	40 ($2xHD_{50}$)	-	-	197 ± 27.6
	50	-	-	194 ± 36.5
	30	+	28 ± 11.6	195 ± 57.9
	40	+	33 ± 13.6	172 ± 39.7
	50	+	61 ± 29.9	124 ± 29.2***
Propanidid	40 ($2xHD_{5}$)	-	-	219 ± 24.9
	50	-	-	205 ± 18.6
	60	-	-	193 + 29.7
	40	+	38 ± 11.6	91 ± 30.5***
	50	+	47 ± 14.3	37 ± 10 ***
	60	+	56 ± 13.6	34 ± 16.5***

***P < 0.001. Significant difference from group given anaesthetic alone at same dose.

Students unpaired t test.

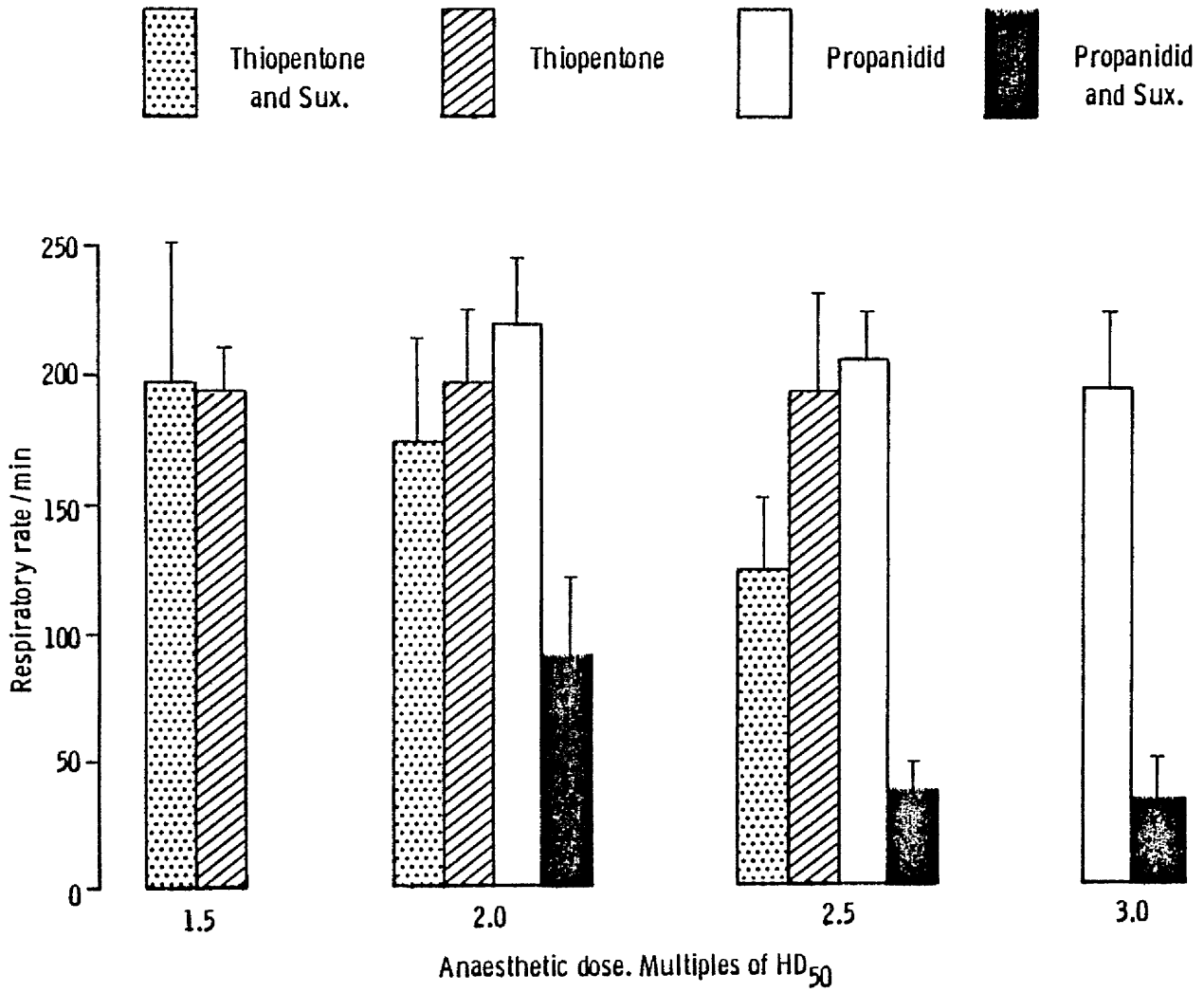


Fig. 21 Effects on respiratory rate produced by equipotent doses of thiopentone and propanidid in combination with suxamethonium.

methohexitone and 'Althesin', with all the anaesthetic drugs being given at a dose equal to twice their HD_{50} and the dose of suxamethonium being standardized at 1 mg kg^{-1}.

3.8 STATISTICAL ANALYSIS

Student's unpaired 't' test was used to compare the means of results obtained from different groups of animals. A paired 't' test was used when more than one measurement was obtained from the same animal in the cardiovascular studies where the measured variables were compared with pre-anaesthetic values.

4. RESULTS

4.1 THE SELECTION OF DISOPROFOL

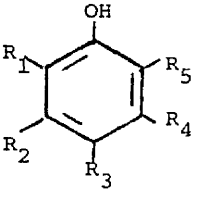
4.1.1 Primary screening tests

Following the discovery by the author of anaesthetic activity in the compound 2,6-diethylphenol, a further 188 alkylphenols were examined in the primary screening test in mice. The most interesting compounds were found in the 2,6-dialkyl series whereas monosubstituted phenols as a class showed only moderate activity and therapeutic ratios were low. Corresponding para-isomers proved toxic as did the parent compound, phenol. The results obtained with 28 compounds from the 2,6-dialkyl series are summarized in Table 28.

The 2,6-di-n-alkylphenols from 2,6-dimethylphenol (1) to 2-n-butyl-6-n-propylphenol (6) exhibited similar activity with moderate potencies and therapeutic ratios. Induction of anaesthesia with compounds (3), (4) and (6) was slow and with doses greater than 2 x HD_{50} recovery was prolonged.

In the 2-n-alkyl-6-sec alkyl subgroup, compounds (7) - (18), potency was found to increase as the total number of carbon units (ΣC) in the side chains increased, reaching a maximum at ΣC=7-8, e.g. (8), (13), (15), (17) and (18). The introduction of a cycloalkyl moiety, e.g. (9) resulted in decreased activity in comparison with the sec-alkyl derivative (8).

Table 28 Results obtained in primary screening tests with 2,6-dialkylphenols



$R_2 = R_3 = R_4 = H$

Compound No.	R_1	R_5	HD_{50} mg kg^{-1}	HD_{50} mg kg^{-1}	Onset (a)	Duration (b)
1	CH_3	CH_3	20-30	80	I	B
2	CH_3	C_2H_5	30	100	I	B
3	C_2H_5	C_2H_5	15-20	100	S	B
4	C_2H_5	$n\text{-}C_3H_7$	20-40	100	VS	B
5	$n\text{-}C_3H_7$	$n\text{-}C_3H_7$	20-30	100	I	B
6	$n\text{-}C_3H_7$	$n\text{-}C_4H_9$	20-40	100-200	VS	B
7	CH_3	$CH(CH_3)_2$	20-30	80	I	B
8	CH_3	$CH(CH_3)\text{-}n\text{-}C_5H_{11}$	15-20	100	VS	L
9	CH_3		30-40	140-160	VS	B
10	CH_3	$C(CH_3)_3$	40-60	120-140	I	B
11	C_2H_5	$CH(CH_3)_2$	15-20	60-80	I	B
12	C_2H_5	$CH(CH_3)C_2H_5$	10-15	80	I	B
13	C_2H_5	$CH(CH_3)\text{-}n\text{-}C_3H_7$	10-20	100-120	I	B
14	$n\text{-}C_3H_7$	$CH(CH_3)_2$	20-30	80	I	B
15	$n\text{-}C_3H_7$	$CH(CH_3)C_2H_5$	10-20	120	S	B
16	$n\text{-}C_3H_7$	$CH(CH_3)\text{-}n\text{-}C_4H_9$	50	140	VS	M
17	$n\text{-}C_4H_9$	$CH(CH_3)_2$	20	100	I	B
18	$n\text{-}C_4H_9$	$CH(CH_3)C_2H_5$	20-30	120	VS	M
19	$CH(CH_3)_2$	$-c\text{-}C_3H_5$	20-30	120-140	I	B

Cont'd.....

Table 28 Cont'd.

Compound	R_1	R_5	HD_{50} mg kg^{-1}	HD_{50} mg kg^{-1}	Onset (a)	Duration (b)
20	$CH(CH_3)_2$	$CH(CH_3)_2$	5-10	50-60	I	B
21	$CH(CH_3)_2$	$CH(CH_3)C_2H_5$	5-10	50	I	B
22	$CH(CH_3)_2$	$CH(CH_3)$-n-C_3H_7	10-15	80	S	L
23	$CH(CH_3)_2$	$CH(CH_3)$-n-C_4H_9	10-15	100-120	S	L
24	$CH(CH_3)_2$		30-40	100-120	S	L
25	$CH(CH_3)C_2H_5$	-c-C_3H_5	20-40	120-140	S	B
26	$CH(CH_3)C_2H_5$	$CH(CH_3)C_2H_5$	5-10	60	S	M
27	$CH(CH_3)$-n-C_3H_7	$CH(CH_3)$-n-C_3H_7	15-20	160-180	VS	L
28	$C(CH_3)_3$	$C(CH_3)_3$	80-100	120		

(a) Speed of onset of anaesthesia I= immediate, <10s; S= slow. 10-15s; VS = very slow, > 15s.

(b) Duration of anaesthesia B = brief, < 5 min; M = moderate, 5-10 min; L = long, > 10 min.

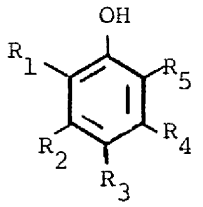
When $\Sigma C \geq 8$ e.g. (8), (16) and (18) induction of anaesthesia was slow, and at higher doses prolonged recovery was seen. In the rest of this group, with the exception of compound (15), therapeutic ratios were good, and induction and recovery were smooth and rapid.

The most interesting compounds were found in the group of 2,6-di-sec-alkylphenols - compounds (19) - (27). Activity was generally high, though cyclic analogues were again less active than their sec-alkyl equivalents, e.g. (19) cf. (20), (25) cf. (21) and (24) cf. (23). Potency increased with increasing chain length of the substituents, reaching a maximum at ΣC = 6-8 e.g. (20) - (22) and (26). Therapeutic ratios were good and induction of anaesthesia was rapid when $\Sigma C \leq 7$ but became slower when the side chains were further increased in length. Analgesia was noted during the period when the righting reflex was lost with a large proportion of the compounds in this series and the overall quality of anaesthesia with compounds (20) - (23), (26) and (27) was good. The compound 2,6-di-tert-butylphenol, included here for comparison was essentially inactive as an anaesthetic.

4.1.2 Detailed comparison of structure-activity relationships within the group of 2,6-dialkylphenols

The results obtained in the more detailed study of selected compounds are summarized in Table 29. In most respects the

TABLE 29 DETAILED COMPARISON OF STRUCTURE-ACTIVITY RELATIONSHIPS WITHIN THE GROUP OF 2,6-DIALKYLPHENOLS



$R_2=R_3=R_4=H$

Compound No.	R_1	R_5	HD_{50} mg kg^{-1} (a)	Induction time (s) (b)	Duration of sleep (min) (c)	Respiratory rate min^{-1} (c)	Percentage showing apnoea (c)	Duration of apnoea (s) (c)
1	CH_3	CH_3	26 ± 0.64	9.0	3.0 ± 0.6	358 ± 31	20	12.5
2	CH_3	C_2H_5	27.5 ± 0.57	5.5	2.2 ± 0.5	300 ± 57	40	9.8 ± 4.5
3	C_2H_5	C_2H_5	21 ± 0.25	5.4	2.7 ± 1.2	219 ± 38	100	5.7 ± 1.3
4	C_2H_5	C_3H_7	30 ± 0.48	5.3	2.8 ± 0.5	200 ± 21	100	6.5 ± 3.8
5	C_3H_7	C_3H_7	26.5 ± 0.37	10	4.3 ± 1.5	224 ± 20	70	4.5 ± 2.8
6	C_3H_7	C_4H_9	33 ± 0.51	13.4	3.9 ± 1.6	196 ± 11	50	3.8 ± 3.5
7	CH_3	$CH(CH_3)_2$	21.5 ± 0.68	6.6	3.0 ± 0.7	260 ± 58	80	6.0 ± 3.4
11	C_2H_5	$CH(CH_3)_2$	14 ± 1.02	5.9	3.6 ± 0.7	174 ± 26	100	5.7 ± 1.9
14	C_3H_7	$CH(CH_3)_2$	23 ± 0.63	6.9	3.6 ± 1.3	207 ± 24	100	4.3 ± 1.7
17	C_4H_9	$CH(CH_3)_2$	21.5 ± 0.75	9.7	5.2 ± 1.0	190 ± 20	100	5.6 ± 2.4
20	$CH(CH_3)_2$	$CH(CH_3)_2$	7.5 ± 0.64	4.8	3.0 ± 0.9	270 ± 48	100	4.3 ± 1.6
21	$CH(CH_3)_2$	$CH(CH_3)C_2H_5$	6.0 ± 0.74	8.3	2.9 ± 0.8	247 ± 46	80	4.4 ± 0.9
22	$CH(CH_3)_2$	$CH(CH_3)C_3H_7$	9.2 ± 0.52	7.8	4.7 ± 1.0	213 ± 48	100	5.1 ± 1.4
26	$C_2H_5(CH_3)CH$	$CH(CH_3)C_2H_5$	4.8 ± 0.27	8.8	4.7 ± 2.1	245 ± 45	60	5.3 ± 0.8

(a) HD_{50} ± SE (Miller and Tainter, 1944)

(b) Calculated at 1.25 x HD_{50}

(c) Calculated at 2 x HD_{50}. Mean ± SD, n=10

results confirmed those obtained in the primary screening test.

a) Di-n-alkylphenols

In the group of di-n-alkyl substituted compounds the most active compound was again found to be 2,6-diethylphenol (3) and induction of anaesthesia with this compound was more rapid than had been expected on the basis of the primary test result. The slow onset of anaesthesia seen with 2-n-butyl-6-n-propylphenol (6) was confirmed and extended induction times were also found with 2,6-dimethylphenol (1) and 2,6-di-n-propylphenol (5). The duration of anaesthesia was similar with all the compounds in this group and the death of 50% of the animals given 2,6-dimethylphenol (1) at 2 x HD_{50} confirmed the lower therapeutic ratio of this compound. With the exception of 2,6-di-n-propylphenol (6) respiratory depression appeared to increase as ΣC increased, and apnoea was seen most consistently with the two compounds - compounds (3) and (4) - which showed the most rapid onset of anaesthesia.

b) 2-n-alkyl-6-sec-alkylphenols

Of the four compounds from this subgroup examined in the detailed test, 2-ethyl-6-isopropylphenol (11) was found to be the most active and to produce the most rapid induction of anaesthesia, and the greatest degree of respiratory depression. Sleeping times were found to increase as ΣC increased.

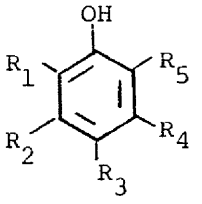
c) 2,6-di-sec-alkylphenols

The compounds in this subgroup were again found to be more active than those in the other two groups. Although compounds (21) and (26) were more active than 2,6-diisopropylphenol (20), a slow induction of anaesthesia was seen with the former two compounds. The duration of anaesthesia with compounds (22) and (26) was slightly longer than that seen with compounds (20) and (21). No significant differences in the respiratory effects of these compounds could be demonstrated although 2,6-diisopropylphenol (20) appeared to be among the group of compounds from all three subgroups which showed least respiratory depression. The compounds from other subgroups, which showed minimal respiratory depression - compounds (1), (2) and (7) - were much less active than 2,6-diisopropylphenol (20).

d) Correlation of anaesthetic effects with lipophilicity

Partition coefficients between octanol and water (log P) for selected analogues were determined by Mr. P.J. Taylor using a high pressure liquid chromatographic technique (Mirrlees Moulton, Murphy and Taylor, 1976). As the results obtained indicated that, to a first approximation, log P α ΣC, the compounds in Table 29 have been rearranged in Table 30. Compounds with equal numbers of carbon atoms in their side chains, and thus expected to

TABLE 30 RESULTS GIVEN IN TABLE 29 REARRANGED IN ORDER OF INCREASING SIDE-CHAIN LENGTH (ΣC)



$R_2=R_3=R_4=H$

Compound No.	ΣC	R_1	R_5	HD_{50} mg kg^{-1}	Induction Time(s)	Duration of sleep (min)	Respiratory rate min^{-1}	Percentage showing apnoea	Duration of apnoea(s)
1	2	CH_3	CH_3	26 ±0.64	9.0	3.0±0.6	358±31	20	12.5
2	3	CH_3	C_2H_5	27.5±0.57	5.5	2.2±0.5	300±57	40	9.8±4.5
3	4	C_2H_5	C_2H_5	21 ±0.25	5.4	2.7±1.2	219±38	100	5.7±1.3
7		CH_3	$CH(CH_3)_2$	21.5±0.68	6.6	3.0±0.7	260±58	80	6.0±3.4
4	5	C_2H_5	C_3H_7	30 ±0.48	5.3	2.8±0.5	200±21	100	6.5±3.8
11		C_2H_5	$CH(CH_3)_2$	14 ±1.02	5.9	3.6±0.7	174±26	100	5.7±1.9
5	6	C_3H_7	C_3H_7	26.5±0.37	10	4.3±1.5	224±20	70	4.5±2.8
14		C_3H_7	$CH(CH_3)_2$	23 ±0.63	6.9	3.6±1.3	207±24	100	4.3±1.7
20		$CH(CH_3)_2$	$CH(CH_3)_2$	7.5±0.64	4.6	3.0±0.9	270±48	100	4.3±1.6
6	7	C_4H_9	C_3H_7	33 ±0.51	13.4	3.9±1.6	196±11	50	3.8±3.5
17		C_4H_9	$CH(CH_3)_2$	21.5±0.75	9.7	5.2±1.0	190±20	100	5.6±2.4
21		$CH(CH_3)_2$	$CH(CH_3)C_2H_5$	6.0±0.74	8.3	2.9±0.8	247±46	80	4.4±0.9
22	8	$CH(CH_3)_2$	$CH(CH_3)C_3H_7$	9.2±0.52	7.8	4.7±1.0	213±48	100	5.1±1.4
26		$CH(CH_3)C_2H_5$	$CH(CH_3)C_2H_5$	4.8±0.27	8.8	4.7±2.1	245±45	60	5.3±0.8

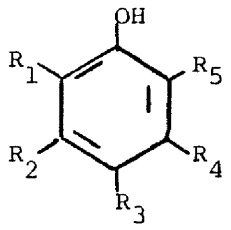
have a similar degree of lipophilicity, are grouped together. It can be seen in Table 30 that there does not appear to be a direct relationship between lipophilicity, based on the total number of carbon atoms in the substituents and anaesthetic potency. Within the groups containing 5-8 carbon atoms quite marked differences in anaesthetic potency were found despite the fact that, within any particular group, the compounds would be expected to have a similar degree of lipophilicity. Significant differences in induction time were also found in some groups of compounds with similar lipophilicity and no significant correlation between sleeping times, respiratory effects and lipophilicity could be demonstrated.

4.1.3 Evaluation of anaesthetic properties in rabbits

The most active compound in the 2,6-di-n-alkylphenol subgroup, 2,6-diethylphenol (3) had a low therapeutic ratio in the rabbit and induction of anaesthesia with a dose of 20 mg kg^{-1} was slow in this species.

Four compounds - (18), (11), (12) and (15) - from the 2-n-alkyl-6-sec-alkyl subgroup were selected for evaluation in rabbits but were subsequently eliminated from further testing because of slow induction of anaesthesia, sometimes accompanied by excitement and poor muscle relaxation. The compound, 2-ethyl-6-(1-methylbutyl)phenol (13) was also examined in this species and found to produce good anaesthesia, but profuse salivation at higher doses was noted as a significant side effect.

Table 31 Results obtained with 2,6-di-sec-alkylphenols in rabbits



$R_2 = R_3 = R_4 = H$

Compound No.	R_1	R_5	Summary result
20	$CH(CH_3)_2$	$CH(CH_3)_2$	7.5-15 mg kg^{-1}: rapid induction, good muscle relaxation, short duration of anaesthesia (4-7 min) and rapid recovery. 20 mg kg^{-1}: lethal.
21	$CH(CH_3)_2$	$CH(CH_3)C_2H_5$	5 mg kg^{-1}: slow induction with some excitement. 15 mg kg^{-1}: lethal.
22	$CH(CH_3)_2$	$CH(CH_3)C_3H_7$	5-10 mg kg^{-1}: rapid induction, poor muscle relaxation. 20 mg kg^{-1}: lethal.
23	$CH(CH_3)_2$	$CH(CH_3)C_4H_9$	10 mg kg^{-1}: excitement at induction, poor muscle relaxation. 15 mg kg^{-1}: lethal.
26	$CH(CH_3)C_2H_5$	$CH(CH_3)C_2H_5$	5 mg kg^{-1}: rapid induction, prolonged duration of action (13 min). 10 mg kg^{-1}: lethal.
27	$CH(CH_3)C_3H_7$	$CH(CH_3)C_3H_7$	5-15 mg kg^{-1}: marked excitement at induction, poor muscle relaxation. 30 mg kg^{-1}: lethal.
thiopentone sodium			10 mg kg^{-1}: righting reflex retained. 20-30 mg kg^{-1}: rapid induction, good muscle relaxation, moderate duration of action (6-11 min). 30 and 46 mg kg^{-1}: lethal.

The results obtained in rabbits with the best compounds in the 2,6-di-sec alkyl series, as identified in the primary screening test, are summarized in Table 31. As with all phenols tested in this species, therapeutic ratios were generally lower than those obtained in mice. Excitement was frequently seen on induction and muscle relaxation was sometimes poor. However, 2,6-diisopropylphenol (20) was exceptional in that it produced smooth, rapid induction, and recovery with good muscle relaxation and a short but useful period of anaesthesia.

On the basis of this result in rabbits, and the confirmation in mice that this compound produced a more rapid induction of anaesthesia than two other more potent compounds - (21) and (26) - 2,6-diisopropylphenol (disoprofol) was selected for further evaluation in comparative studies with standard anaesthetic compounds.

4.2 STUDIES TO COMPARE THE PHARMACOLOGICAL PROPERTIES OF DISOPROFOL WITH THOSE OF STANDARD ANAESTHETIC AGENTS

4.2.1 Anaesthetic potency and therapeutic ratio

The results obtained with disoprofol and the standard agents, thiopentone, 'Althesin', methohexitone and propanidid are given in Table 32. The activity of disoprofol in mice was found to lie between that of thiopentone and methohexitone being closer to the latter. In the mouse, disoprofol was found to be 1.8 times more potent than thiopentone and

propanidid while 'Althesin' was 3.6 times more active than disoprofol. The therapeutic ratio obtained with disoprofol was similar to that found with thiopentone and much lower than that of 'Althesin'.

Table 32 Activity and lethality of disoprofol and standard i.v. anaesthetics in mice

	HD_{50} mg kg^{-1}	LD_{50} mg kg^{-1}	Therapeutic ratio (LD_{50}/HD_{50})
Disoprofol	11.8 (10.86 - 12.98)	40.45 (36.79 - 44.47)	3.40 (2.99 - 3.88)
Thiopentone	21.68 (20.65 - 22.77)	84.69 (80.62 - 88.95)	3.91 (3.65 - 4.18)
'Althesin'	3.3 (3.13 - 3.55)	57.69 (53.94 - 61.7)	17.31 (15.79 - 18.98)
Methohexitone	7.93 (7.24 - 8.68)	37.66 (34.23 - 41.41)	4.75 (4.17 - 5.41)
Propanidid	21.19 (20.25 - 22.17)	118.0 (112.08 - 124.23)	5.57 (5.21 - 5.96)

95% confidence limits in parenthesis

4.2.2 Speed of onset of anaesthesia

The HD_{50} values obtained with disoprofol and the standard agents examined in this study, using two injection times, are given in Table 33.

Table 33 Median hypnotic doses (HD_{50} mg kg^{-1} ± SEM) of i.v. anaesthetics in mice, estimated using two standard injection times

	HD_{50} 1-s injection	HD_{50} 10-s injection	$\frac{HD_{50}\ (10s)}{HD_{50}\ (1s)}$
Pentobarbitone	21.5 ± 0.63	20.6 ± 0.60	0.96
Thiopentone	19.5 ± 0.56	21.0 ± 0.90	1.08
Propanidid	15.4 ± 0.52	21.5 ± 0.63	1.40
Ketamine	9.6 ± 0.36	12.2 ± 0.49	1.27
Disoprofol	7.5 ± 0.44	12.0 ± 0.57	1.6
Methohexitone	7.0 ± 0.60	8.0 ± 0.43	1.14
'Althesin'	2.5 ± 0.14	3.3 ± 0.11	1.32
Etomidate	1.1 ± 0.09	1.25 ± 0.05	1.14

With the exception of pentobarbitone, HD_{50} values were greater when a slower rate of injection was used. The greatest differences between the figures obtained with the different injection times were found with disoprofol and propanidid.

When a range of doses up to 2 x HD_{50} of each agent was given using a 1s-injection, the induction times shown in Figure 22 were obtained. Induction times with pentobarbitone were too slow to be included in this figure.

Similar experiments were done using a 10s injection time. When induction times were plotted against equal multiples of the HD_{50} for each agent the values for induction times at 1.25 x HD_{50} shown in Table 34 were derived.

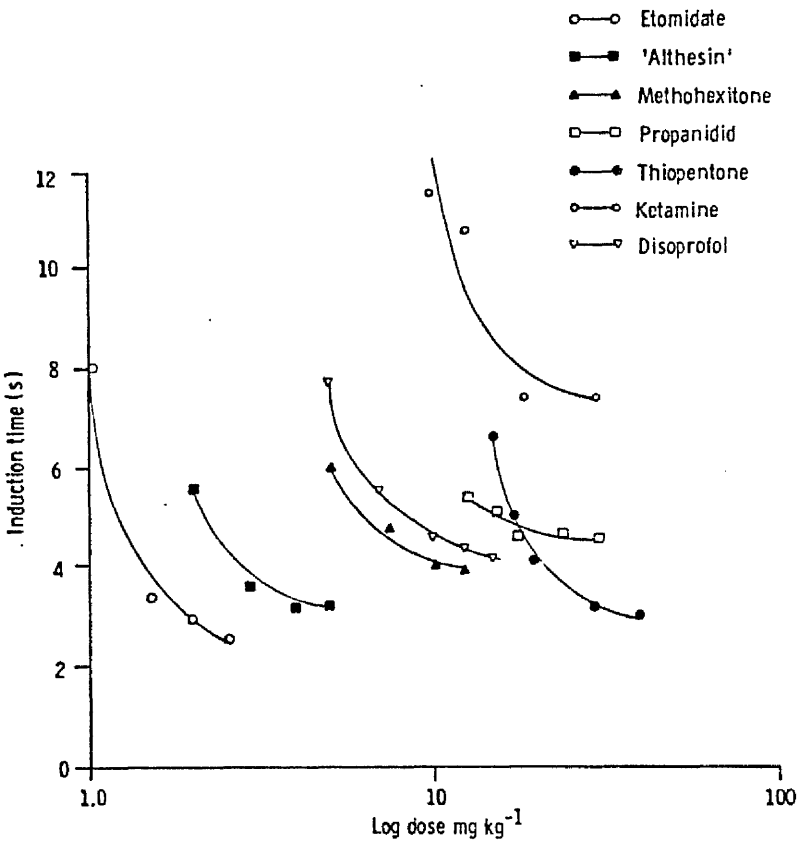


Fig. 22 The relationship between anaesthesia induction time and dose. All doses given over one second.

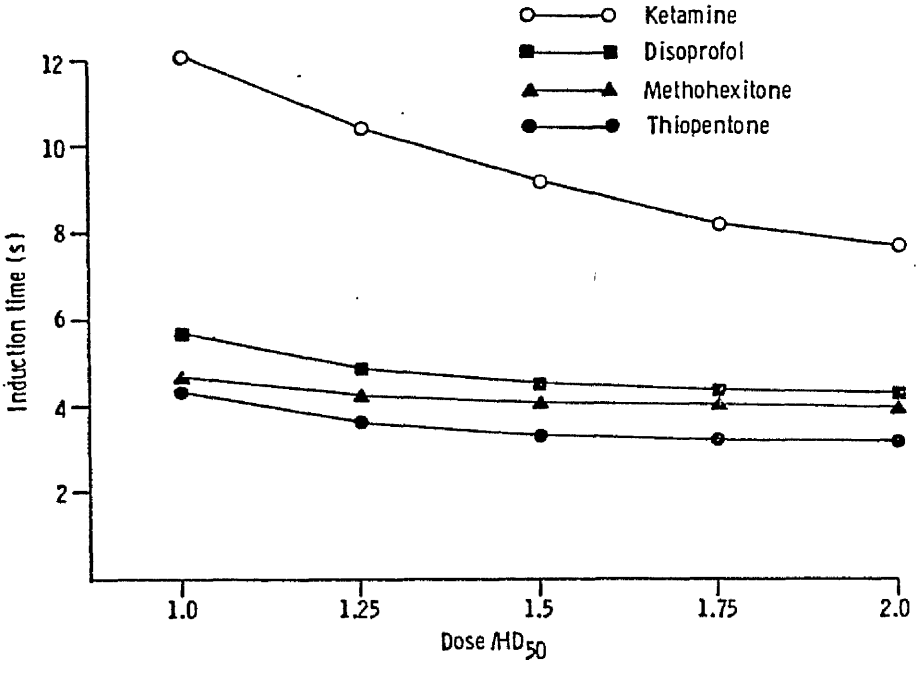


Fig. 23 Anaesthesia induction times in mice with equipotent doses of intravenous anaesthetics. All doses given over one second.

Examples of the induction time-dose response relationships obtained using a 1-s injection time with disoprofol, thiopentone, methohexitone and ketamine are shown in Figure 23.

Table 34 Comparison of induction times with i.v. anaesthetics in mice. Induction times compared at 1.25 x HD_{50}

	Induction time (s)	
	1-s injection	10-s injection
Thiopentone	3.6	10.0
'Althesin'	3.6	12.4
Methohexitone	4.25	11.0
Etomidate	4.4	10.8
Propanidid	4.7	11.2
Disoprofol	4.8	10.0
Ketamine	10.4	14.0
Pentobarbitone	42.0	62.0

With the 1-s injection time the shortest induction times were found with thiopentone and 'Althesin'. Induction time with disoprofol was similar to that obtained with propanidid. With ketamine, and particularly with pentobarbitone, induction times were much longer than those of the other standard agents.

Following the 10-s injection, similar induction times were found with disoprofol and the standard agents, with the exception of ketamine and pentobarbitone where induction was again found to be prolonged.

4.2.3 Duration of action, recovery times and respiratory effects of disoprofol and standard compounds

The results obtained with doses equivalent to 2 x HD_{50} of disoprofol and the standard agents examined in this study are given in Table [35]. Mean sleeping time with disoprofol was similar to that obtained with thiopentone and slightly longer than that found with 'Althesin' and methohexitone. Recovery of walking ability returned after a similar period with all the drugs, but the slight difference between disoprofol and the barbiturates was statistically significant. Following anaesthesia with disoprofol, methohexitone and 'Althesin', mice regained co-ordination rapidly, whereas almost 50 minutes elapsed before those mice given thiopentone had recovered completely. Recovery from the effects of disoprofol and thiopentone was free from excitatory side effects, whereas mice recovering from methohexitone and 'Althesin' showed some muscle tremor and twitching, particularly when being handled during the co-ordination test.

Table 35 Sleeping time, respiratory rate and recovery to walking and co-ordination in mice given i.v. anaesthetics

	Sleeping time (min)	Interval between righting and walking (min)	Interval between righting and co-ordination (min)	Resp. rate min^{-1} 1 min post injection
Disoprofol 26 mg kg^{-1}	5.6 ± 1.3	1.3 ± 0.9	3.3 ± 1.1	169 ± 21
Thiopentone 40 mg kg^{-1}	9.9 ± 12.5	0.6 ± 0.4 *	47.8 ± 6.5 *	167 ± 22
Methohexitone 16 mg kg^{-1}	2.0 ± 0.5 ***	0.5 ± 0.2 *	2.8 ± 0.6	186 ± 26
'Althesin' 7 mg kg^{-1}	3.5 ± 0.6 ***	0.8 ± 0.4	3.7 ± 0.9	193 ± 17 **

Mean values ± SD n=10

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Values significantly different from those obtained with disoprofol. Student's unpaired t-test.

Respiratory rates obtained with thiopentone and disoprofol were slower than those found with methohexitone and 'Althesin', but only in the comparison with 'Althesin' was this difference statistically significant.

4.2.4 Evaluation of cumulative effects

a) Cumulation in mice

The sleeping times produced in mice, following the repeated injection of equivalent anaesthetic doses of disoprofol, thiopentone and 'Althesin' are given in Table 36 . The results demonstrate that in comparison with thiopentone, the sleeping times produced by disoprofol and 'Althesin' were only extended to a slight extent as repeated injections were given. Initial sleeping times with all three drugs were similar and while the period of sleep produced by disoprofol and 'Althesin' increased to 15 min and 18 min respectively, that produced by thiopentone increased to more than 3 hours after a fourth dose.

This result indicates that, in the mouse, disoprofol resembles 'Althesin' in showing minimal cumulation in this species. In contrast, the prolonged sleeping time obtained following the repeated injection of thiopentone demonstrates that this compound accumulates to a significant extent in body tissues.

Table 36 Sleeping time (min) with repeated doses of disoprofol, thiopentone and 'Althesin' in mice (mean ± S.D.; n=5)

Dose	Disoprofol 25 mg kg^{-1}	Thiopentone 40 mg kg^{-1}	'Althesin' 12 mg kg^{-1}
1	4,6 ± 0.56	3.86 ± 0.79	5.03 ± 0.85
2	7.1 ± 0.89	30.3 ± 9.81	8.6 ± 1.34
3	8.4 ± 1.37	148.6 ± 57.5	9.5 ± 1.77
4	10.0 ± 2.13	>180	13.7 ± 3.3
5	10.8 ± 1.67		10.4 ± 1.08
6	10.2 ± 1.9		18.2 ± 11.76
7	10.8 ± 1.2		16.2 ± 5.4
8	11.7 ± 2.53		17.6 ± 7.9*
9	13.5 ± 2.9		18.7**
10	15.4 ± 4.57		18.3***

* n=4
** n=3
*** n=2

Remaining mice could not be given further injections because of mechanical damage to their tail veins.

b) Cumulation in rats

Mean infusion dose rates and recovery times to head lift and the return of the ability to walk are given in Table 37. Using a standard infusion rate of 0.9 ml hr^{-1} of disoprofol in rats weighing 200-225g the mean doses administered were 0.69 ± 0.01 mg kg^{-1} min^{-1} in the rats given a one hour infusion and 0.71 ± 0.04 mg kg^{-1} min^{-1} in those where the infusion was

Table 37 Infusion of disoprofol in rats. Dose rates used and recovery times obtained.

Wt. (g)	Infusion dose rate (mg kg^{-1} min^{-1})	Total dose mg kg^{-1}	Disoprofol 'utilization rate' mg kg^{-1} min^{-1}	Recovery time (min) to Head lift	walk
1 hour infusion					
220	0.68	48.4	0.73	5.7	11.0
220	0.68	48.4	0.74	5.6	6.2
210	0.71	50.5	0.76	6.0	11.8
215	0.70	49.3	0.79	2.0	9.0
220	0.68	48.6	0.73	6.5	12.0
Mean 217	0.69	49.03	0.75	5.08	10.0
± SD ± 4.5	± 0.01	± 0.88	± 0.025	± 1.78	± 2.43
2 hour infusion					
200	0.75	97.5	0.72	15	27
220	0.68	89.3	0.70	7	11.2
200	0.75	97.5	0.75	8.3	14.3
215	0.70	86.5	0.68	15	41
225	0.67	87.3	0.64	8	15
Mean 212	0.71	91.62	0.70	10.66	21.7
± SD ± 11.5	± 0.04	± 5.46	± 0.41	± 3.99	± 12.35

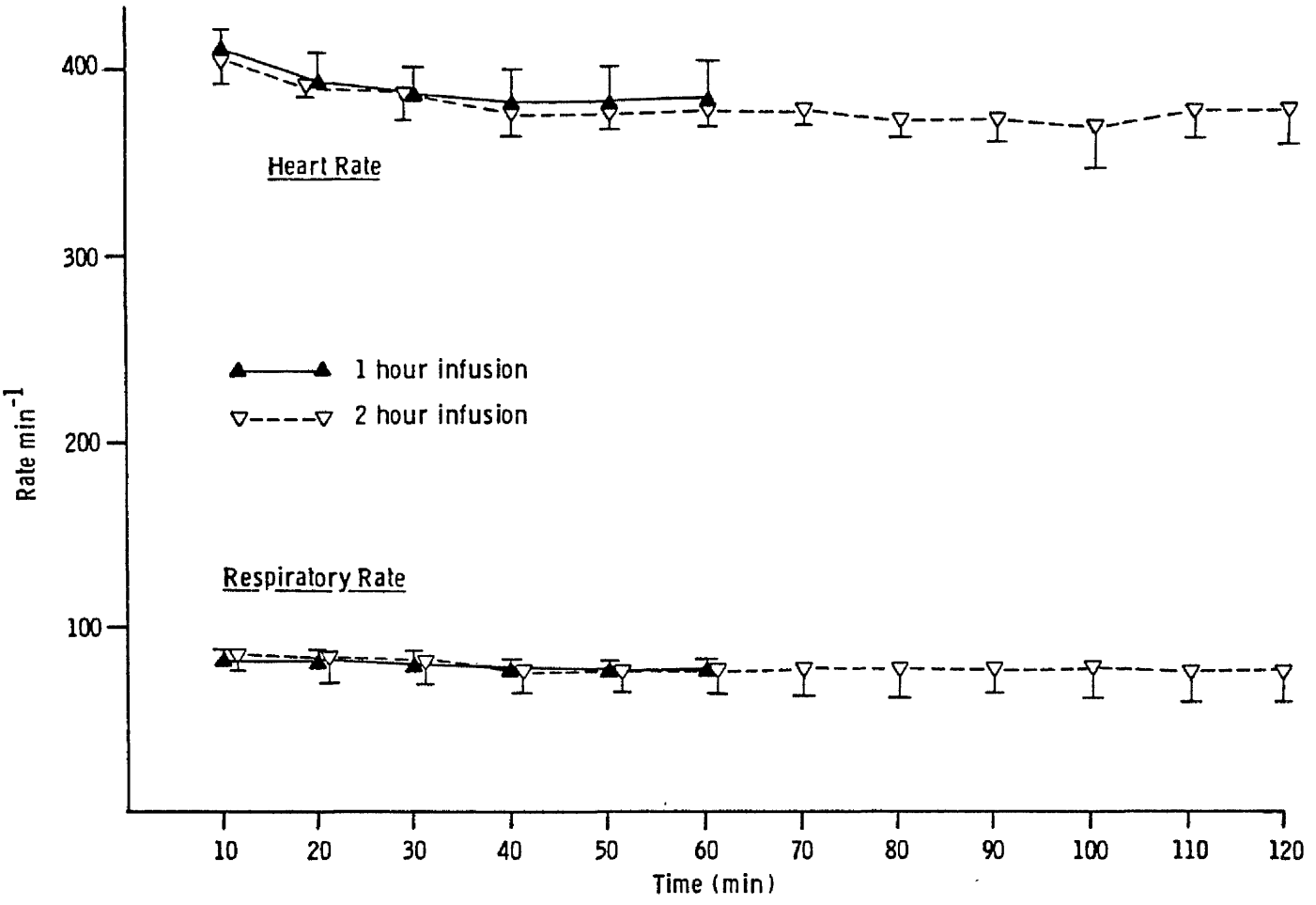


Fig. 24 Heart and respiratory rates in rats during the infusion of disoprofol.

maintained for two hours. Head lift occurred 5.08 $\pm$ 1.8 minutes after the end of the shorter administration and 10.66 $\pm$ 3.99 minutes after the two hour infusion.

The drug 'utilization rate' was calculated as the total dose of disoprofol given, including the induction dose, divided by the total period of sleep until head lift occurred. The slight increase in recovery time following the longer infusion was reflected in the slightly lower drug 'utilization rate' in this group.

Mean respiratory and heart rates recorded from the two groups of rats are shown in Figure 24. Although the rates of both variables decreased slightly during the first hour of infusion, no significant further decline was encountered in those animals where the infusion was continued for a further hour. The presence of the palpebral reflex and a weak pedal withdrawal reflex indicated that a light level of anaesthesia was maintained in both groups of animals.

As clinical signs of deepening anaesthesia and increased depression of respiratory and heart rates were not seen, and as recovery times were only moderately increased in the animals given the two hour infusion, it can be concluded that only minimal tissue accumulation of disoprofol had occurred.

4.2.5 Evaluation of cardiovascular and respiratory effects in pigs

The use of animals with chronically implanted arterial and venous cannulae allowed the cardiovascular and respiratory variables measured during anaesthesia to be compared with values obtained in the resting animal prior to induction of anaesthesia. The effects produced by disoprofol, thiopentone and 'Althesin' on mean arterial pressure and heart rate are summarized in Tables 38 and 39 respectively. The percentage changes in these variables, with the two doses used, are shown in Figures 25 and 26 and the results obtained in individual animals are given in Appendices 4 and 5.

Similar mean values of mean arterial pressure, within the range 89-92 mmHg were obtained prior to anaesthesia in all the groups investigated. All three agents produced an initial hypotensive effect which was significant one minute after injection. Following the administration of the lower dose of each agent there were no significant differences between agents in the degree of hypotension produced. With the larger dose, both disoprofol and thiopentone produced a more profound hypotensive effect than 'Althesin' but only in the comparison of disoprofol with 'Althesin' was this difference statistically significant ($p < 0.05$). After this initial hypotensive effect, both thiopentone and 'Althesin' produced increases in blood pressure. In the case of pigs given the larger dose of thiopentone (20 mg kg^{-1}) the mean arterial

TABLE 38 EFFECTS OF DISOPROFOL, THIOPENTONE AND 'ALTHESIN' ON MEAN ARTERIAL PRESSURE (mmHg) IN PIGS

	Minutes after injection						
	Pre-dose	1	2	3	4	5	20
Disoprofol 2.5 mg kg^{-1}	90 ± 5.9	71 ± 10.9 **	92 ± 24.7	91 ± 23.1	85 ± 8.9	84 ± 8.1 *	86 ± 10.3
Thiopentone 10 mg kg^{-1}	92 ± 12.0	72 ± 8.6 ***	98 ± 15.0	101 ± 17.6	98 ± 15.3	97 ± 13.7	94 ± 17.8
'Althesin' 1.8 mg kg^{-1}	91 ± 4.4	78 ± 9.1 *	95 ± 10.9	93 ± 8.9	93 ± 10.0	95 ± 10.8	95 ± 5.2
Disoprofol 5.0 mg kg^{-1}	91 ± 5.9	64 ± 10.5 ***	77 ± 10.8 *	88 ± 7.8	90 ± 5.4	90 ± 4.7	88 ± 9.0
Thiopentone 20 mg kg^{-1}	91 ± 2.7	63 ± 13.5 **	101 ± 9.6	109 ± 13.9 *	105 ± 9.7 *	104 ± 10.6	98 ± 5.4 *
'Althesin' 3.6 mg kg^{-1}	89 ± 7.3	74 ± 9.3 ***	99 ± 22.8	102 ± 11.9 *	100 ± 11.6	95 ± 6.9	89 ± 4.0

Mean values ± S.D.; n=7

*P < 0.05
**P < 0.01
***P < 0.001

Values significantly different from pre-dose values.
Students paired t test

TABLE 39 EFFECTS OF DISOPROFOL, THIOPENTONE AND 'ALTHESIN' ON HEART RATE MIN^{-1} IN PIGS

	Pre-dose	Minutes after injection 1	2	3	4	5	20
Disoprofol 25 mg kg^{-1}	112 ± 19.1	153 ± 16.4 **	153 ± 25.5 **	149 ± 23.0 ***	143 ± 24.2 ***	133 ± 17.5 ***	113 ± 17.1
Thiopentone 10 mg kg^{-1}	117 ± 21.7	185 ± 13.3 ***	149 ± 26.8 ***	152 ± 28.5 **	150 ± 31.3 **	147 ± 28.5 **	124 ± 28.5
'Althesin' 1.8 mg kg^{-1}	136 ± 17.7	193 ± 28.6 **	303 ± 21.6 ***	205 ± 23.4 ***	208 ± 25.9 ***	202 ± 25.5 ***	155 ± 19.4
Disoprofol 5.0 mg kg^{-1}	113 ± 17.3	180 ± 21.6 ***	163 ± 29.4 ***	157 ± 32.2 ***	147 ± 30.6 ***	143 ± 28.2 ***	115 ± 19.8
Thiopentone 20 mg kg^{-1}	124 ± 20.6	179 ± 15.6 ***	180 ± 22.1 **	175 ± 29.4 **	171 ± 28.9 **	169 ± 30.1 ***	144 ± 26.8 *
'Althesin' 3.6 mg kg^{-1}	105 ± 24.9	171 ± 25.5 ***	170 ± 35.5 ***	170 ± 31.7 ***	171 ± 22.4 ***	159 ± 17.0 ***	131 ± 22.3 **

Mean values ± SD; n=7

*P < 0.05
**P < 0.01
***P < 0.001

Values significantly different from pre-dose values.
Students paired t test

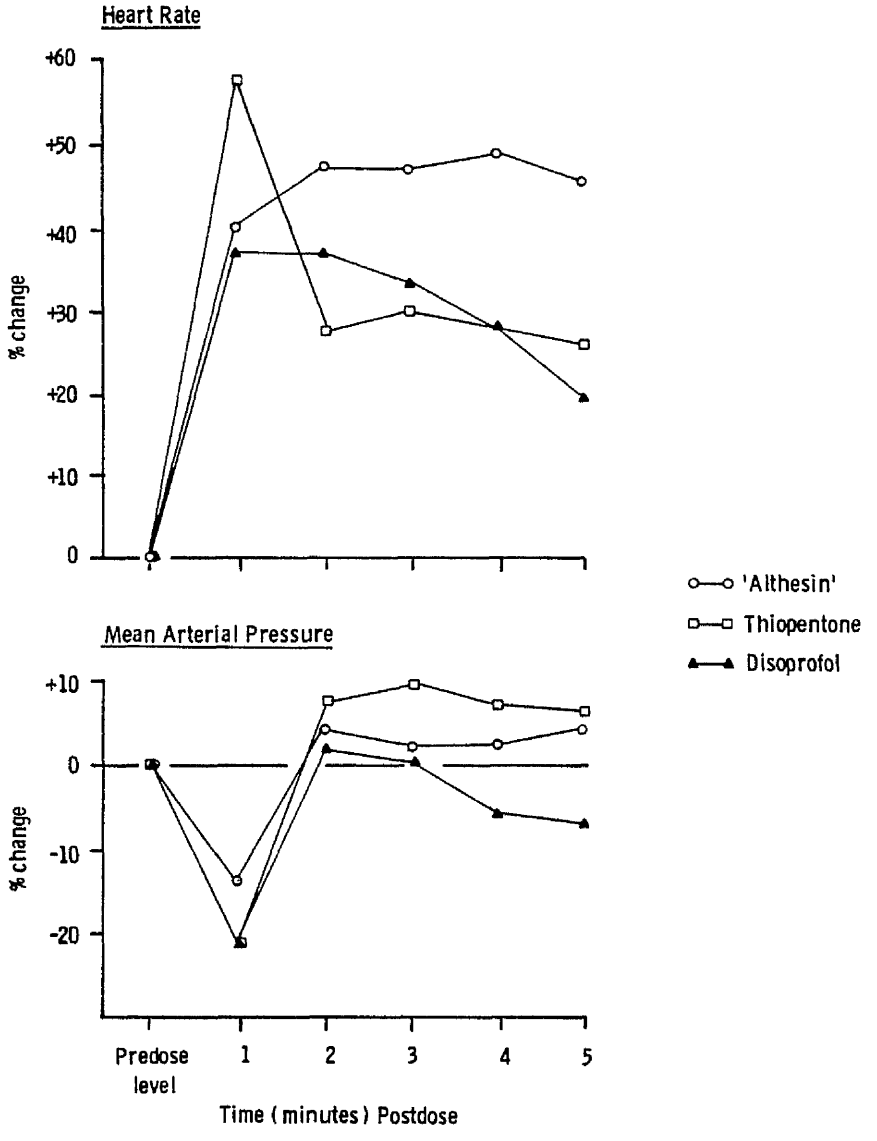


Fig. 25 Percentage changes in heart rate and mean arterial pressure in pigs given minimum anaesthetic doses of 'Althesin', thiopentone and disoprofol.

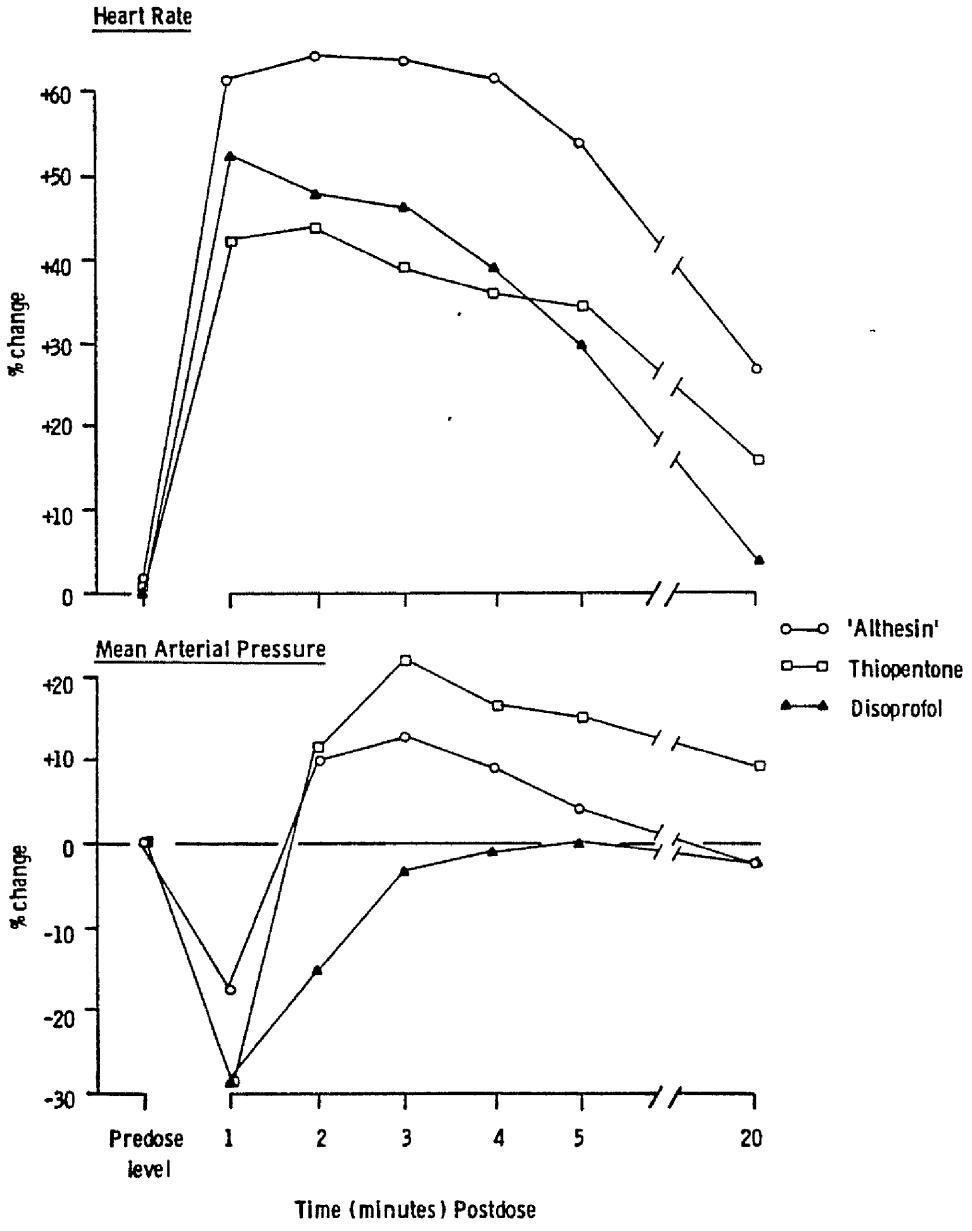


Fig. 26 Percentage changes in heart rate and mean arterial pressure in pigs given 2 X minimum anaesthetic doses of 'Althesin', thiopentone and disoprofol.

pressure 3, 4 and 20 minutes after induction was significantly greater than the pre-anaesthetic value. The increase at 3 minutes after injection in pigs given 3.6 mg kg^{-1} 'Althesin' was also statistically significant. The absence of a pressor effect with disoprofol allowed a more rapid return to pre-dose values with this agent.

The initial hypotensive effect produced by all three agents was accompanied by tachycardia which was most marked in the animals given thiopentone and 'Althesin'. With the larger doses of the latter agents, heart rates were still significantly greater than pre-dose values 20 minutes after induction of anaesthesia. At this same time point, in the pigs given disoprofol, heart rate had returned to the base line value. When the heart rates produced by the different agents were compared in the same animals, the tachycardia produced by the low dose of 'Althesin' was significantly greater than that produced by 2.5 mg kg^{-1} disoprofol for the first 5 minutes after induction of anaesthesia. The difference between disoprofol and thiopentone was only statistically significant at 1 minute. Following the administration of the larger dose of each agent, all three anaesthetics produced an initial degree of tachycardia. At 5 and 20 minutes after induction the tachycardia produced by thiopentone was significantly greater than that caused by disoprofol but the difference between 'Althesin' and disoprofol was not statistically significant.

TABLE 40 ARTERIAL BLOOD GAS VALUES IN PIGS ANAESTHETISED WITH DISOPROFOL, THIOPENTONE AND 'ALTHESIN'

	Pre-dose			3 min after injection			20 min after injection		
	pH	PO_2 (mmHg)	PCO_2 (mmHg)	pH	PO_2 (mmHg)	PCO_2 (mmHg)	pH	PO_2 (mmHg)	PCO_2 (mmHg)
Disoprofol 2.5 mg kg^{-1}	7.44 ± 0.03	84.5 ± 12.7	32.8 ± 3.8	7.39 ± 0.04 *	79.6 ± 11.8	34.4 ± 5.3	7.42 ± 0.05	82.5 ± 11.3	33.4 ± 3.9
Thiopentone 10 mg kg^{-1}	7.45 ± 0.02	85.8 ± 4.4	34.7 ± 2.9	7.41 ± 0.04 *	78.0 ± 14.7	38.6 ± 4.1	7.44 ± 0.02	84.7 ± 10.1	34.5 ± 4.3
'Althesin' 1.8 mg kg^{-1}	7.42 ± 0.04	85.6 ± 13.6	33.4 ± 3.9	7.40 ± 0.03	84.4 ± 10.9	33.5 ± 1.9	7.41 ± 0.04	78.7 ± 11.5 *	32.7 ± 2.8
Disoprofol 5.0 mg kg^{-1}	7.44 ± 0.04	87.9 ± 8.0	36.8 ± 4.5	7.34 ± 0.06 ***	75.3 ± 11.7 *	39.9 ± 4.6	7.43 ± 0.03	81.9 ± 7.8	34.0 ± 6.2
Thiopentone 20 mg kg^{-1}	7.42 ± 0.06	86.6 ± 7.9	32.6 ± 2.5	7.38 ± 0.04 *	72.8 ± 9.6 *	40.4 ± 4.9 ***	7.43 ± 0.03	81.8 ± 8.5 **	35.3 ± 3.9
'Althesin' 3.6 mg kg^{-1}	7.46 ± 0.02	84.8 ± 10.8	33.3 ± 4.2	7.40 ± 0.03 ***	70.9 ± 9.4 *	36.8 ± 6.8	7.44 ± 0.02 *	78.4 ± 8.3 *	32.7 ± 4.9

Mean values ± S.D.; n=7

*P < 0.05
**P < 0.01
***P < 0.001

Values significantly different from pre-dose values Students paired t test

The mean results obtained from blood gas analyses are given in Table 40. Three minutes after induction of anaesthesia, arterial pH values were significantly reduced in animals given the lower dose of thiopentone and disoprofol. At the 20 minute time point the only significant change, in animals receiving the lower dose, occurred in the pigs given 1.8 mg kg^{-1} 'Althesin' where arterial PO_2 values were slightly reduced. Following the larger dose of all three agents significant reductions in arterial pH were produced 3 minutes after injection. Values for arterial PO_2 were significantly reduced at this time point but a significant increase in arterial PCO_2 was seen only in the pigs given 20 mg kg^{-1} thiopentone. Slight reductions in PO_2 remained at 20 minutes in animals given thiopentone and 'Althesin' but no statistically significant change at this time was seen in the pigs given disoprofol. Although a number of statistically significant changes in arterial pH and blood gas tensions were found the actual changes were small. Calculation of base excess values from the mean values obtained for pH and arterial PCO_2 indicated that, in the animals given thiopentone, the reduction in pH was almost entirely attributable to an increase in arterial PCO_2 whereas in those given disoprofol a mild metabolic acidaemia contributed to the reduction in pH. Calculated values for base excess are given in Table 41.

Table 41 Calculated values for base excess in pigs anaesthetised with disoprofol, thiopentone and 'Althesin'

	Base excess ($mmol.\ell^{-1}$)		
	Pre-dose	3 min after injection	20 min after injection
Disoprofol 2.5 mg kg^{-1}	-1	-3.5	-2
Thiopentone 10 mg kg^{-1}	1	0	0
'Althesin' 1.8 mg kg^{-1}	-2.5	-3.5	-3
Disoprofol 5.0 mg kg^{-1}	1.2	-4	-1
Thiopentone 20 mg kg^{-1}	-2.5	-0.5	0
'Althesin' 3.6 mg kg^{-1}	0.5	-1.5	0

It can be concluded that the cardiovascular and respiratory effects measured indicate that the properties of disoprofol are generally similar to those of thiopentone and 'Althesin'. At the higher dose examined disoprofol produced a greater degree of arterial hypotension than 'Althesin', but the hypotensive effect of disoprofol was accompanied by a significantly smaller degree of tachycardia. Although none of the agents examined produced marked respiratory depression as indicated by the blood gas measurements, a reduction in respiratory rate was noted 1 minute after injection of the higher dose of all three agents. Respiratory rates had returned towards the pre-dose

values by the time the first arterial sample was collected for blood-gas measurements 3 minutes after injection.

In this study in pigs one animal given 2.5 mg kg^{-1} disoprofol showed an unexpected response characterized by paddling limb movements and the appearance of patchy erythema over the abdomen and neck. These patches later became cyanotic and the skin rash disappeared after 10 minutes and recovery was uneventful. This pig had previously been anaesthetised with thiopentone and 'Althesin', the latter being given 5 days before disoprofol. The same animal was subsequently given the solubilizing agent Cremophor EL alone and two further doses of disoprofol. No untoward reactions occurred on these occasions and it was presumed that the adverse response might have been caused by a pulmonary embolus derived from the implanted venous cannula.

4.2.6 Evaluation of interactions between disoprofol and ancillary drugs used in anaesthesia

a) Interactions with drugs used in pre-anaesthetic medication

In Table 42 the sleeping times and recovery times obtained with disoprofol given alone and in combination with a range of drugs used in pre-anaesthetic medication are given. The respiratory effects of these drug combinations are summarized in Table 43.

Of the premedicant drugs examined only chlorpromazine and diazepam produced highly significant increases in sleeping time in comparison with that produced by disoprofol alone. Droperidol

TABLE 42 SLEEPING AND RECOVERY TIMES WITH DISOPROFOL GIVEN IN COMBINATION WITH DRUGS USED FOR PREANAESTHETIC MEDICATION

	Disoprofol alone 25 mg kg^{-1}	Disoprofol 25 mg kg^{-1} given 30 min after preanaesthetic medication							
		Droperidol 0.25mg kg^{-1}	Chlorpromazine 1.0 mg kg^{-1}	Diazepam 2.0mg kg^{-1}	Pentobarbitone 10 mg kg^{-1}	Atropine 1mg kg^{-1}	Hyoscine 1mg kg^{-1}	Pethidine 10mg kg^{-1}	Pentazocine 10 mg kg^{-1}
Sleeping time (min)	4.7 ± 1.4	5.9 ± 0.9 *	7.2 ± 1.0 ***	9.6 ± 2.2 ***	5.6 ± 1.1	5.4 ± 0.9	5.1 ± 0.7	6.0 ± 0.8 *	5.7 ± 1.0 *
Interval between righting and walking (min)	1.3 ± 0.7	4.9 ± 1.6 ***	4.8 ± 3.3 **	7.4 ± 3.5 ***	1.5 ± 0.5	2.7 ± 1.2 **	1.4 ± 1.4	3.6 ± 1.6 ***	2.4 ± 0.9 **
Interval between righting and coordination	2.8 ± 0.5	6.5 ± 1.4 ***	7.9 ± 6.8 *	18.6 ± 4.0 ***	3.1 ± 0.9	4.6 ± 1.4 ***	5.6 ± 2.7 **	3.9 ± 1.5	3.0 ± 0.9

Mean values ± SD; n=10

*P < 0.05
**P < 0.01
***P < 0.001

Values significantly different from disoprofol alone.
Students unpaired t test.

TABLE 43 RESPIRATORY EFFECTS WITH DISOPROFOL GIVEN IN COMBINATION WITH DRUGS USED FOR PREANAESTHETIC MEDICATION

	Disoprofol alone 25 mg kg^{-1}	Disoprofol 25 mg kg^{-1} given 30 min after preanaesthetic medication							
		Droperidol 0.25 mg kg^{-1}	Chlorpromazine 1.0 mg kg^{-1}	Diazepam 2.0mg kg^{-1}	Pentobarbitone 10 mg kg^{-1}	Atropine 1 mg kg^{-1}	Hyoscine 1 mg kg^{-1}	Pethidine 10mg kg^{-1}	Pentazocine 10 mg kg^{-1}
Respiratory rate min^{-1}	162 ± 21	180 ± 33	185 ± 18 *	144 ± 10 *	179 ± 25	200 ± 10 ***	212 ± 28 ***	158 ± 16	150 ± 10
Duration of apnoea (s)	9.5 ± 3.7	10.5 ± 0.8	14.5 ± 5.0 *	14.5 ± 6.4	11.0 ± 4.6	0 ***	0 ***	11.5 ± 4.1	7.5 ± 4.2

Mean values ± SD; n=10

*P < 0.05
***P < 0.001

Values significantly different from disoprofol alone
Students unpaired t test

also produced a slight prolongation of sleeping time. A number of drugs, in particular droperidol and diazepam, produced some delay in the recovery of walking ability and co-ordination and a short but statistically significant delay in the recovery of co-ordination was also seen following atropine and hyosine premedication.

The respiratory effects of disoprofol were altered most markedly by the prior administration of atropine orhyoscine. These drugs abolished the short period of apnoea normally produced by disoprofol and respiratory rates were significantly greater than those produced by disoprofol alone. Chlorpromazine also produced a slight increase in respiratory rate while diazepam premedication increased the respiratory depressant effect of disoprofol to a slight extent. The increase in respiratory rate produced by chlorpromazine 1 minute after induction of anaesthesia followed a slight prolongation of the period of apnoea produced by this combination.

i) Comparative studies with thiopentone and 'Althesin'

Dose-response effects on sleeping time, recovery of co-ordination and respiratory rate produced by premedication with diazepam, droperidol and chlorpromazine prior to anaesthesia with equianaesthetic doses of disoprofol, thiopentone and 'Althesin' are shown in histogram form in Figures 27, 28 and 29. The effects produced by a single dose of atropine are also included in these figures.

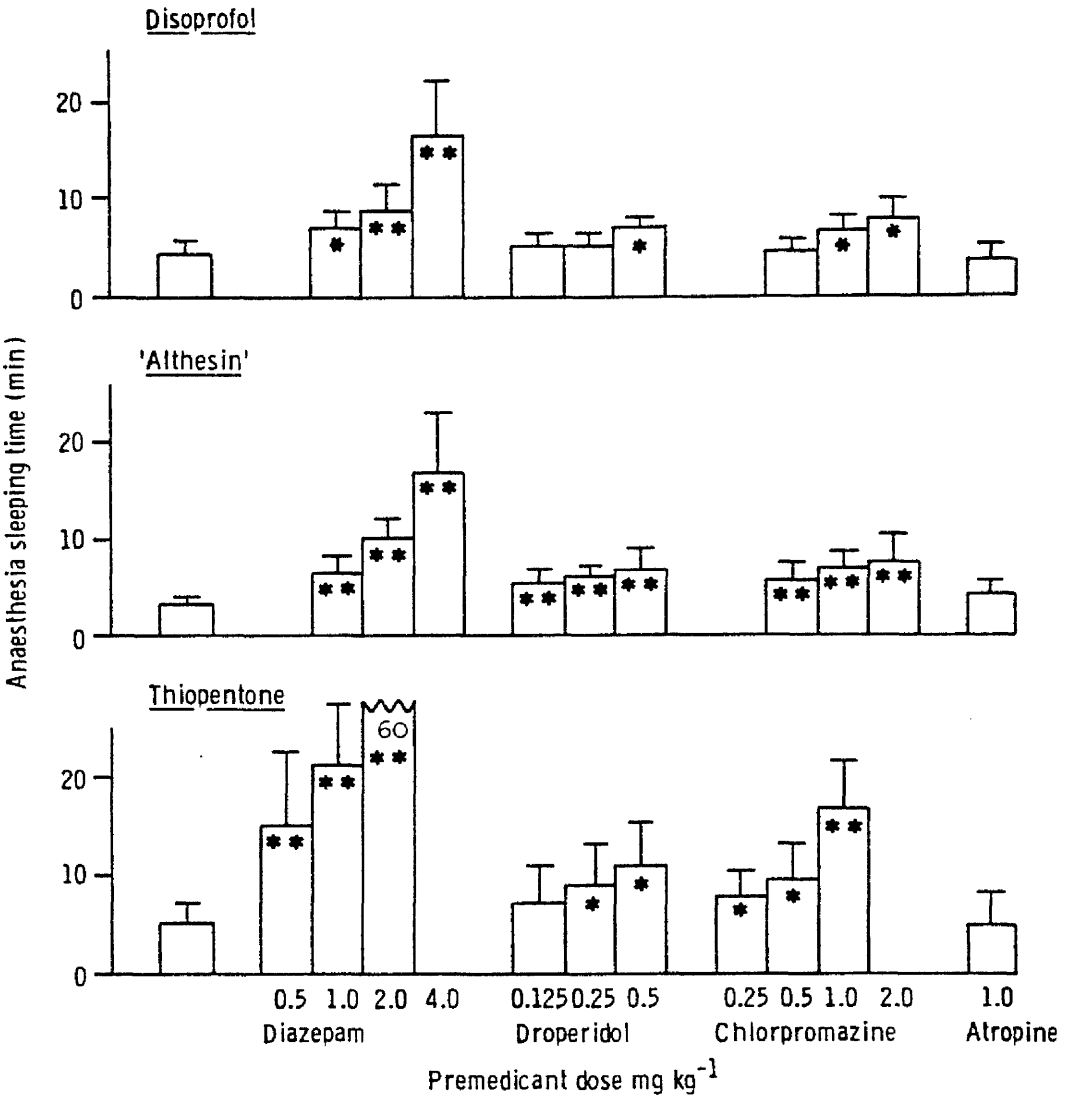


Fig. 27 The influence of premedicant drugs on anaesthesia sleeping time in mice. Mean values ± SD, n=10

*P < 0.01 Significant differences from unpremedicated mice. Students unpaired t test.

**P < 0.001

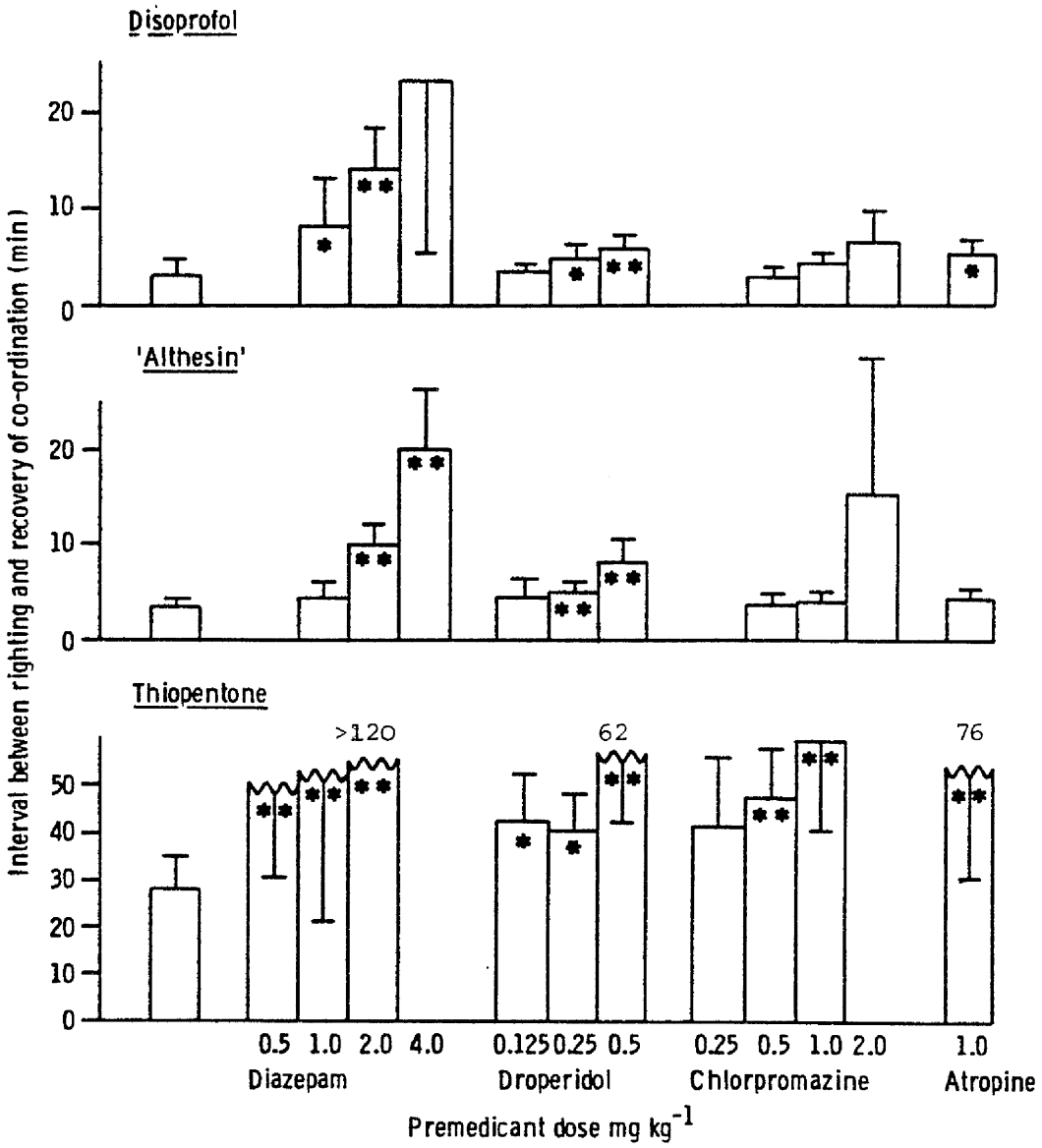


Fig. 28 The influence of premedicant drugs on the recovery of co-ordination following anaesthesia in mice. Mean values ± SD, n=10

*P < 0.01 Significant differences from unpremedicated mice. Students unpaired t test.

**P < 0.001

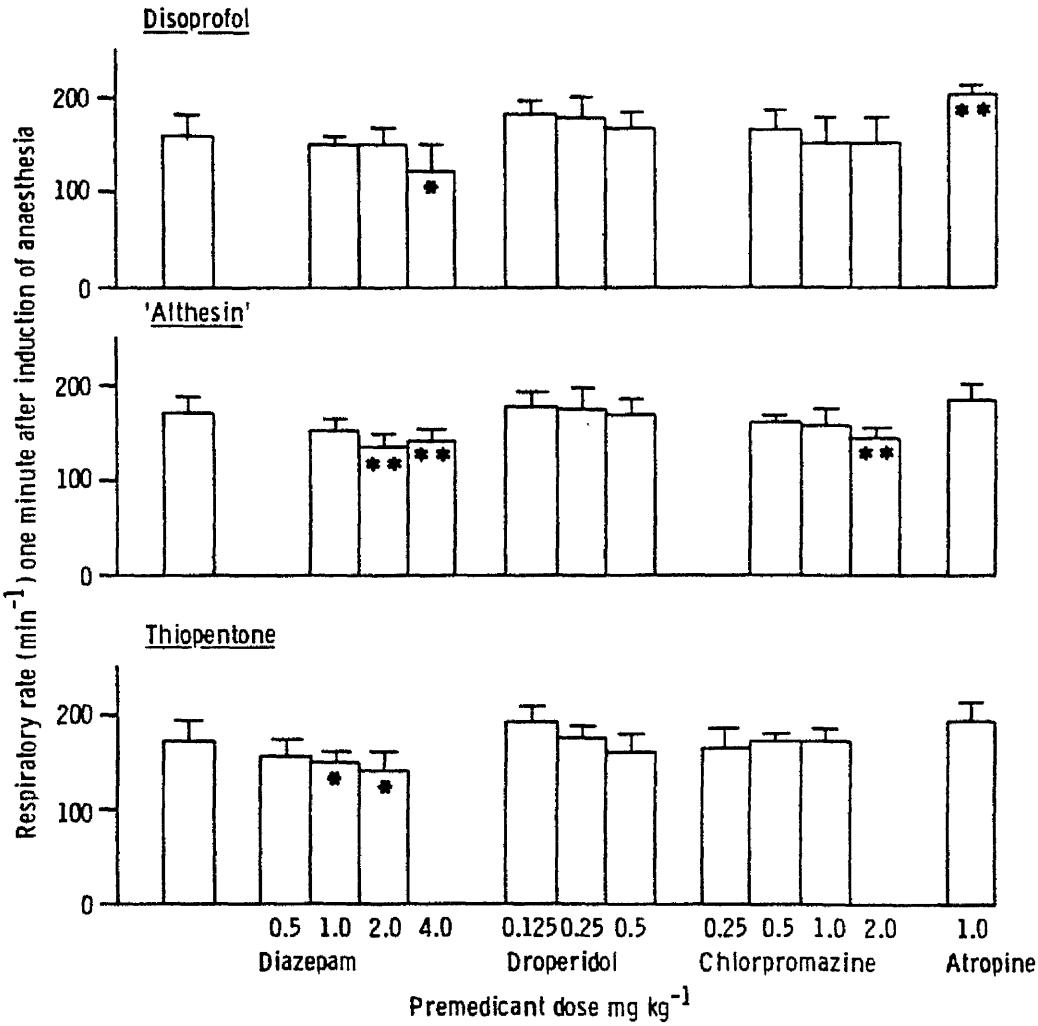


Fig. 29 The influence of premedicant drugs on respiratory rate during anaesthesia in mice Mean values ± SD, n=10

*P < 0.01 Significant differences from unpremedicated mice.

**P < 0.001 Students unpaired t test.

The prolongation of sleeping and recovery times noted in the previous experiment when disoprofol was given following premedication with diazepam, droperidol or chlorpromazine was seen again. A more marked increase in sleeping time and delay in the recovery of co-ordination was found in mice anaesthetised with thiopentone following premedication with the same compounds, while similar effects were seen in mice anaesthetised with disoprofol or 'Althesin'. Of the three premedicant drugs,given over a range of doses diazepam potentiated all three anaesthetics to the greatest extent. Because of the marked potentiation produced by this agent and by chlorpromazine in mice anaesthetised with thiopentone the maximum doses of these premedicant drugs were reduced in animals receiving thiopentone. Two mice given thiopentone after premedication with 1.0 mg kg^{-1} chlorpromazine died as did a similar number in the group given disoprofol following chlorpromazine 2.0 mg kg^{-1}.

Premedication with atropine 1.0 mg kg^{-1} produced a significant delay in the recovery of co-ordination in mice anaesthetised with thiopentone and disoprofol but not in those given 'Althesin'.

The higher doses of diazepam produced significant reductions in respiratory rate with all three anaesthetic agents. Premedication with droperidol and chlorpromazine had a minimal effect on respiratory rate during anaesthesia but the highest dose of chlorpromazine showed a significant depressant effect in mice anaesthetised with 'Althesin'. The lower doses of droperidol produced a slight increase in respiratory rate with all three anaesthetic agents but

this effect was not statistically significant. Atropine premedication also tended to increase respiratory rate during anaesthesia but only in the case of the group given disoprofol was the change consistent.

These results indicated that premedicant drugs which produced significant effects in mice anaesthetised with disoprofol also induced qualitatively similar effects in mice anaesthetised with thiopentone and 'Althesin'. A similar degree of potentiation of sleeping and recovery times was seen with disoprofol and 'Althesin' whereas greater effects were seen when the premedicant drugs were given in combination with thiopentone.

b) Interactions with inhalation anaesthetics

A summary of the main findings obtained when anaesthesia was maintained in cats with a range of inhalation anaesthetics, following induction of anaesthesia with 7.5 mg kg^{-1} disoprofol is given in Table 44.

Anaesthesia was maintained satisfactorily with all of the inhalation agents tested and no unexpected interactions were observed. The responses of the cats during maintenance reflected the pharmacological properties of the inhalation agents employed. Ventricular extrasystoles were noted during maintenance of anaesthesia with trichloroethylene and chloroform. Only minor changes in P-R interval were seen and these were consistent with the observed changes in heart rate. Respiratory depression was noted with halothane, cyclopropane, enflurane and methoxyflurane. With halothane a reduced tidal volume was accompanied

TABLE 44 COMBINATION OF DISOPROFOL WITH INHALATIONAL AGENTS IN CATS

Inhalational Agent	Vol %	Carrier Gases	Disoprofol Alone		Parameters measured at the end of one hour maintenance period		Other Observations	Recovery (mins)	
			Heart Rate/ min	Resp. Rate/ min	Heart Rate/ min	Resp. Rate/ min		Head Lift	Walking
Halothane	0.5 - 2.0	O_2/N_2O 50/50	165 ± 24	43 ± 10	125 ± 32	58 ± 7.2	Uneventful maintenance period	9 ± 1.7	11 ± 1
Trichloroethylene	1.25 - 1.9	O_2	175 ± 16	40 ± 7	159 ± 42	97 ± 20	Multifocal ventricular extrasystoles in all three cats	25 ± 5	36 ± 7.5
Cyclopropane	20 - 30	O_2	168 ± 11	37 ± 2.3	115 ± 13	27 ± 5	Uneventful light level of anaesthesia	3 ± 1	4.5 ± 0.3
Enflurane	1.7 - 3.8	O_2	149 ± 10	41 ± 6	127 ± 23	25 ± 9	Uneventful light anaesthesia with 1.7%	5 ± 3	7 ± 3.6
Methoxyflurane	0.6 - 1.5	O_2	183 ± 32	41 ± 2.3	147 ± 31	29 ± 16	Uneventful maintenance period	19 ± 7	24 ± 7
Diethyl Ether*	5.0 - 10	O_2	197 ± 12	43 ± 6	205 ± 9	65 ± 18	Uneventful maintenance period	12 ± 5	31 ± 18
Chloroform	1.25 - 2.0	O_2	165 ± 24	38 ± 10	139 ± 33	95 ± 44	Ventricular extrasystoles in 2 cats. Bradycardia in one cat	10 ± 3.4	27 ± 8

*Premedicated with 0.2mg Atropine Sulphate 30 minutes prior to induction of anaesthesia.

Figures are means from each group of three cats.
± SD

by an increase in respiratory rate. With the other depressant agents both tidal volume and respiratory rate were decreased. Trichloroethylene and di-ethyl ether produced characteristic tachypnoea.

Recovery times were, as expected, very rapid with cyclopropane and enflurane, rapid with halothane and considerably slower with the other agents.

c) Interactions with neuromuscular blocking drugs

i) Experiments in cats

Following induction of anaesthesia with 7.5 mg kg^{-1} disoprofol suxamethonium provided excellent muscle relaxation for intubation whereas the onset of relaxation with pancuronium and gallamine was slower. When gallamine was used topical anaesthesia was required to allow reasonably rapid intubation. A summary of the results obtained in this study is given in Table 45.

All the animals used in these experiments recovered uneventfully and in a number of instances the effects of different neuromuscular blocking drugs were compared in the same animal. Limb movements were noted in one cat at the time of injection of pancuronium. These were attributed to the fact that the level of anaesthesia was insufficient to prevent spontaneous movement when the animal was disturbed during the injection of the neuromuscular blocking drug. One cat developed a transient period of bradycardia (92 beats min^{-1}) 15 min after induction of anaesthesia when suxamethonium 1 mg kg^{-1}

TABLE 45 COMBINATION OF DISOPROFOL WITH NEUROMUSCULAR BLOCKING AGENTS IN CATS

Neuromuscular Blocking Agent	Dose mg kg^{-1}	No. of cats	I.P.P.V. Required (min.)	Duration of Maintenance Anaesthesia (min)	Recovery Times (min) Head Lift	Walking	Comments
Suxamethonium chloride	0.5	1	4	30	15	16	Transient bradycardia in 1 cat given 1.0 mg kg^{-1}
	1.0	2	7, 19	30	19	23	
Pancuronium	0.06	1	24	30	5	8	
	0.08*	1	54	56	8	10	
Gallamine triethiodide	1.0	1	6	30	17	18	
	1.5	1	12	30	8	15	
Suxamethonium chloride + Pancuronium**	0.5 0.06	4	5.1 ± 0.82 36 ± 3.4	61 ± 5.31	9.8 ± 5.6	16 ± 6.8	Movement at time of injection of pancuronium in 1 cat
Suxamethonium chloride + Pancuronium**	0.5 0.06*	3	8.5 ± 7.4 27.3 ± 1.5	52 ± 2.88	7.7 ± 2.3	10.7 ± 1.2	
Suxamethonium chloride + Gallamine	0.5 1.0*	1	2.5 21	50	10	12	

* Residual relaxation reversed with atropine 0.2 mg Neostigmine methyl sulphate 0.1 mg

** Relevant figures given as means ± SD

had been given. Two other cats given suxamethonium and pancuronium showed periods of nodal rhythm during the inhalation of halothane. Recovery times were increased slightly in cats given suxamethonium and gallamine, when anaesthesia was discontinued after 30 minutes.

No unexpected interactions were observed which could be related to the combination of disoprofol with any of the neuromuscular blocking drugs investigated.

ii) Experiments in pigs

The results obtained in pigs are summarized in Table 46. The dose of 0.3 mg kg^{-1} of tubocurarine produced a period of apnoea longer than would be desirable for clinical purposes. In a pig anaesthetised with thiopentone 0.2 mg kg^{-1} tubocurarine also produced an extended period of apnoea. With either disoprofol or thiopentone, when the dose of tubocurarine was reduced to 0.15 mg kg^{-1} a suitable duration of muscle relaxation was obtained and residual relaxation was readily reversed with neostigmine in two pigs.

All the animals used in this study recovered uneventfully and no unexpected interactions were observed which could be related to the combination of disoprofol with tubocurarine.

TABLE 46 COMBINATION OF DISOPROFOL AND THIOPENTONE WITH TUBOCURARINE IN PIGS

I.v. agent	Neuromuscular Blocking Agents	Dose mg kg^{-1}	No. of pigs	I.P.P.V. Required (min.)	Duration of Maintenance Anaesthesia (min)	Recovery Times (min.) Head Lift	Standing	Comments
Disoprofol 5 mg kg^{-1}	Suxamethonium chloride + Tubocurarine chloride	2.0 0.3	1	2 119	135		60	Dose of tubocurarine was excessive. Similar period of apnoea with 0.2 mg kg^{-1} with thiopentone
	Suxamethonium chloride + Tubocurarine chloride	2.0 0.15	2	4 50	70	12.5	35	
	Suxamethonium chloride + Tubocurarine chloride	2.0 0.15	2	5 29	59	15	27.5	Residual relaxation reversed reversed with Atropine 0.3 mg + Neostigmine 1.25 mg
Thiopentone 20 mg kg^{-1}	Suxamethonium chloride + Tubocurarine chloride	2.0 0.2	1	77	97		60	
	Suxamethonium chloride + Tubocurarine chloride	2.0 0.15	1	31	61		60	Residual relaxation reversed with Atropine 0.3 mg + Neostigmine 1.5 mg

TABLE 47 EFFECTS PRODUCED BY THE COMBINATION OF INTRAVENOUS ANAESTHETICS WITH SUXAMETHONIUM

	Disoprofol 25 mg kg^{-1}		Thiopentone 40 mg kg^{-1}		'Althesin' 7 mg kg^{-1}		Methohexitone 16 mg kg^{-1}		Propanidid 40 mg kg^{-1}	
	Alone	+ Sux.	Alone	+ Sux.	Alone	+ Sux.	Alone	+ Sux.	Alone	+ Sux.
Sleep Time (min)	3.8 ± 0.8	5.8 ± 1.0 *	3.3 ± 0.8	5.6 ± 1.6 *	2.6 ± 0.3	3.6 ± 0.6 *	1.6 ± 0.3	3.3 ± 1.0 *	1.0 ± 0.2	3.2 ± 0.9 *
Duration of apnoea(s)	0	37 ± 12.5	0	40 ± 19.3	0	34 ± 9.4	0	32 ± 14.6	0	38 ± 11.6
Resp. rate min^{-1} 2 min after induction	172 ± 23	180 ± 25	203 ± 20	149 ± 23 *	158 ± 11	172 ± 37	190 ± 23	174 ± 36	220 ± 25	91 ± 31 *

Mean values ± SD; n=10

*P < 0.001 Values with suxamethonium significantly different from those obtained with the anaesthetics alone. Students unpaired t test.

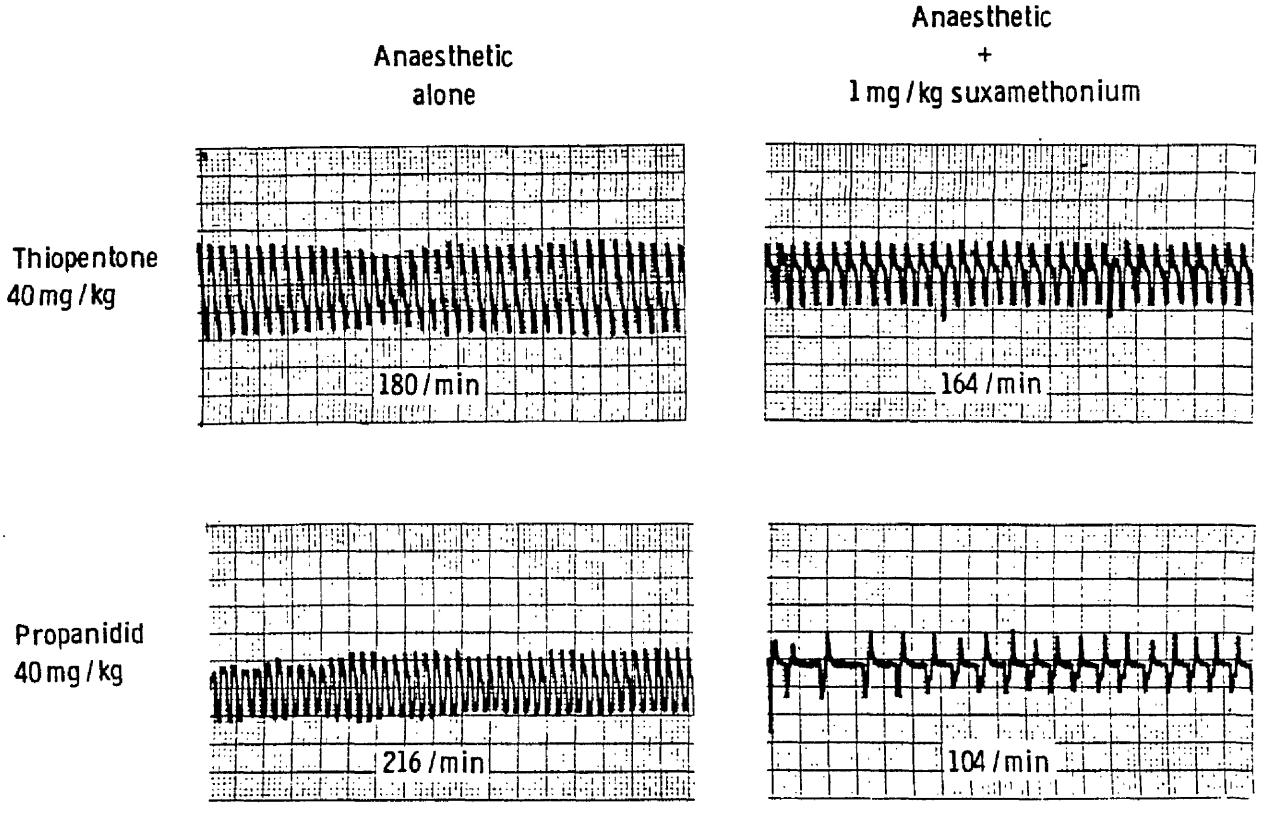


Fig. 30 Respiratory rates in mice two minutes after injection of thiopentone or propanidid alone and in combination with suxamethonium.

iii) Experiments in mice

The effects produced by suxamethonium given in combination with disoprofol, thiopentone, 'Althesin' and methohexitone are summarized in Table 47. A similar period of apnoea was produced by suxamethonium in every case. Some decrease in respiratory rate, measured 2 min after induction of anaesthesia, was seen in mice given thiopentone but a much more marked effect was seen in mice anaesthetised with propanidid. An example of a record obtained from mice given thiopentone or propanidid is shown in Figure 30.

Some prolongation of sleeping time was seen with all of the anaesthetic agents but again the most marked effect was noted in mice given propanidid.

The failure of suxamethonium to produce a significant reduction in respiratory rate in mice anaesthetised with disoprofol suggests that this combination of drugs should be unlikely to produce a prolonged period of respiratory depression as seen with propanidid and suxamethonium in man.

5. DISCUSSION

The discovery of anaesthetic activity in the alkylphenol series has allowed the structure-activity relationships within this series to be examined in more detail in this chapter. Preliminary considerations of structure-activity relationships (James and Glen, 1980) indicated that, in general, potency and kinetics appeared to be a function of both the lipophilic character and the degree of steric hindrance exerted by ortho substituents. The more detailed examination of selected alkylphenols described in this study confirmed the observation that anaesthetic potency was not correlated directly with lipophilicity. In a group of compounds, all of which contained 6 carbon atoms in their side chains, and would be expected to be equally lipophilic, HD_{50} values ranged from 7.5 mg kg^{-1} (2,6-diisopropylphenol) to 26.5 mg kg^{-1} (2,6-di-n-propylphenol). The presence of lipophilic groups close to the phenolic-OH group is likely to reduce non-specific binding and to increase anaesthetic potency (R. James, personal communication). The influence of this effect was confirmed but further rationalization of structure-activity relationships must await accurate measurements of the hydrogen bonding properties of selected compounds.

It was also found that speed of induction was not solely dependent on anaesthetic potency. Optimum potency, speed of onset, minimal respiratory effects and absence of excitatory effects were obtained only with 2,6-diisopropylphenol (disoprofol).

Comparative studies with disoprofol and standard anaesthetic agents were designed to predict the likely behaviour of the new compound in man. Results obtained in mice indicate that disoprofol is slightly less potent than methohexitone and 1.8 times more active than thiopentone. The therapeutic ratios obtained with standard agents in this study were lower than those reported in a previous investigation in mice (Child et al, 1971). This discrepancy can be explained by the use of a standard injection time and the definition of a finite minimum sleeping time in the present work. The relative potencies of the standard drugs obtained with this method reflect more closely results obtained in man. As in this study, Clarke et al (1968) and Stella, Torri and Castiglioni (1979) found thiopentone to be approximately equipotent with propanidid whereas Child et al, (1971) noted AD_{50} figures of 13.2 mg kg^{-1} and 22.9 mg kg^{-1} for thiopentone and propanidid respectively.

The importance of using standardized injection times was confirmed in studies designed to compare the speed of onset of disoprofol anaesthesia with that of standard agents. In this investigation the HD_{50} of disoprofol decreased from 12.0 mg kg^{-1} to 7.5 mg kg^{-1} when injection time was decreased from 10 seconds to 1 second. A similar increase in potency was seen with propanidid when a faster injection rate was used. This indicates that a certain amount of dilution, redistribution, and possibly even metabolism, occurs during the course of the slower injection. When equipotent doses of the anaesthetic drugs were injected over 1 second, the speed of onset of disoprofol anaesthesia was similar to that obtained with propanidid and marginally slower than the onset of thiopentone and 'Althesin' anaesthesia. Induction times with ketamine and pentobarbitone were much greater than those of the other standard agents. The ranking of induction times in mice was similar to that reported in man by Dundee (1957) and Carson, Dundee and Clarke (1975). The absolute induction times obtained with a 1-s injection time in mice were shorter than those found in man when a similarly rapid injection was given. This difference is probably due to a shorter circulation time in the mouse. When a 10-s injection was used in mice, onset times with the rapidly acting agents were similar to those reported in man following a rapid injection (1-2 s) during a period of reactive

hyperaemia produced by temporary ischaemia of the forearm (Clarke et al, 1968). These authors, in estimating the relative potency of thiopentone, methohexitone and propanidid made the assumption that all three drugs have a similar speed of onset. While this assumption may be valid with these particular agents, this technique would tend to underestimate the potency of a drug which was marginally slower in onset as a larger dose would be required to reach the desired end-point in the limited time set. This criticism emphasises the interrelationship which exists between anaesthetic potency and induction time, both of which are influenced by rate of injection. As the results obtained with standard agents in mice are similar to those obtained in man it can be predicted that disoprofol should be a rapidly acting agent in man.

In assessing the duration of anaesthesia produced by disoprofol and standard anaesthetics, doses equal to twice HD_{50} were used. As different compounds may not have parallel dose response curves (Stella, Torri and Castiglioni, 1979; Kissin, 1980) it is difficult to make an accurate comparison in this way. However, the results obtained with standard drugs were in general agreement with those found in man (Dundee and Wyant, 1974). The duration of anaesthesia produced by disoprofol was longer than that obtained with methohexitone and

'Althesin' and shorter than that produced by thiopentone. While early recovery from a single dose of thiopentone occurs reasonably rapidly, EEG analysis (Doenicke, Kugler and Laub, 1967) and simulated driving tests (Korttila et al, 1975) indicate that full recovery requires several hours. In mice it was possible to demonstrate a significant delay in recovery of co-ordination following thiopentone in comparison with disoprofol, methohexitone and 'Althesin'.

In the experiment in which the cumulative properties of disoprofol were compared in mice with those of thiopentone and 'Althesin' the results obtained with the standard agents were similar to those of Child et al (1971). Disoprofol resembled 'Althesin' in that sleeping times were extended to a slight extent with repeated injections whereas marked increases in sleeping time were noted with thiopentone. The continuous infusion of disoprofol in rats provided further evidence of a minimal cumulative effect. Heart and respiratory rates remained stable when the duration of the infusion was increased from 1 to 2 hours and a minimal increase in recovery time was noted after the longer infusion. These results suggest that the blood concentration of disoprofol is kept reasonably constant during an infusion due to rapid metabolism of the drug in the liver. Additional experiments (Glen and Growcott,

unpublished observations) have shown that disoprofol sleeping time in rats can be decreased by the induction of liver drug metabolizing enzymes with phenobarbitone. A similar result has been obtained in rats with 'Althesin' (Novelli, Marsili and Lorenzi, 1975; Sear and McGivan, 1980). These results confirm the significant role that hepatic metabolism plays in limiting the duration of anaesthesia following disoprofol and 'Althesin'. It can be predicted that disoprofol should show minimal cumulative effects in man and in this respect should resemble methohexitone, etomidate 'Althesin' and propanidid (Clarke and Dundee, 1966; Kay, 1976).

With the exception of the study by Becker and Beglinger (1980), in which the cardiovascular effects of neurolept-analgesic drugs were examined in pigs, this species has not been used previously in investigations of the haemodynamic effects of anaesthetic drugs. In the present investigation the use of surgically prepared animals allowed control values to be obtained in conscious, unrestrained animals and avoided problems in the interpretation of results as encountered when control values have been obtained in anaesthetised animals (Chen et al, 1959; Lerman and Paton, 1960; McCarthy et al, 1965).

In pigs disoprofol, thiopentone and 'Althesin' all produced an initial hypotensive effect and an increase in heart rate. The magnitude of the reduction in arterial pressure seen immediately after the injection of thiopentone depends on the change of the balance between cardiac output and total peripheral resistance (Conway and Ellis, 1969). Cardiac output may be reduced by a reduction in the contractile force of the left ventricle (Price and Helrich, 1955; Cotten and Bay, 1956). The increase in heart rate can be attributed to a baroreceptor reflex response to hypotension (Schaper et al, 1963; Cox, 1972) and possibly also to central depression of normal vagal tone (Dodds, Dolamore and Twissel, 1981). In man, Fieldman, Ridley and Wood (1955) and Flickinger, Fraimow, Cathcart and Nealon (1961) found that thiopentone produced an increase in total peripheral resistance, while variable effects were noted by Elder, Nagano, Eastwood and Harnagel (1955) and no significant changes were observed by Dobkin and Wyant (1957). Decreases in arterial pressure, stroke volume and systemic vascular resistance with a slight increase in cardiac output as a result of compensatory tachycardia have been recorded in human patients given 'Althesin' (Campbell et al, 1971; Leary, Coleman, Downing and Moyes, 1972; Savege, Blogg, Foley, Ross, Lang and Simpson, 1973; Sear and Prys-Roberts, 1979). In surgically prepared goats a small dose of 'Althesin' produced a greater degree of tachyardia than a larger dose (Foex and Prys-Roberts, 1972) and in cats, Child et al (1972a)

noted transient hypotension and tachycardia with 'Althesin' and thiopentone.

The results obtained with thiopentone and 'Althesin' are therefore similar to those described in man and other animal species. The greater degree of tachycardia seen with thiopentone and 'Althesin', in comparison with that noted with disoprofol, may indicate a greater degree of baroreceptor sensitivity with the former two drugs and may explain the later increases in blood pressure seen with these agents. Alternatively, these drugs may produce a different effect on total peripheral resistance. This possibility is supported by results obtained in further experiments where cardiac output was measured (Glen, Hunter and Lees, unpublished observations). In these experiments, thiopentone and 'Althesin' produced an increase in peripheral resistance, measured 2 minutes after injection, whereas disoprofol produced a slight reduction in this variable.

The potentiation of disoprofol sleeping time produced by premedication with droperidol, diazepam and chlorpromazine was expected as these drugs have previously been shown to potentiate barbiturate and steroid anaesthesia in mice (Janssen, 1963; Gyermek, 1974). The delay in recovery of co-ordination following premedication with atropine or hyoscine may be a reflection of the central depressant effect which can be produced by high doses of these

compounds (Stewart, 1965). Comparative studies with thiopentone and 'Althesin' confirmed that the potentiating effect of the premedicant drugs on disoprofol anaesthesia was not unique as similar or greater effects were found with the standard agents.

Diazepam also produced some potentiation of the respiratory depressant effect of disoprofol. A similar potentiation of barbiturate induced respiratory depression has been noted with diazepam in animals (Southgate and Wilson, 1971) and in man (Prensky, Raff, Moore and Schwab, 1967). The increase in respiratory rate produced by chlorpromazine and droperidol premedication may have been due to the ability of these compounds to increase the sensitivity of the respiratory centre to carbon dioxide (Janssen et al, 1963).

An unexpected finding was the ability of atropine to reverse the respiratory depression induced by disoprofol. This suggests that the respiratory depressant effect is cholinergically mediated. Stewart (1965) has observed that in man respiratory depression induced by premedicant doses of morphine may be counteracted by the concurrent administration of atropine. Cholinergic mechanisms also appear to be responsible for the respiratory depression produced in animals poisoned with organophosphorous cholinesterase inhibitors (Harris, Fleisher, Vick and

Cliff, 1969). In these animals treatment with atropine reversed the respiratory depressant effect.

The combination of disoprofol with a range of inhalation anaesthetics and neuromuscular blocking drugs produced few unexpected interactions. The movement seen in one cat given pancuronium was attributed to inadequate anaesthesia but may have been due to the initial neuromuscular stimulant action of the non-depolarizing agent as sometimes seen in cats (Payne, 1961). No interaction with suxamethonium was demonstrated and in this respect disoprofol differs from propanidid, an agent known to potentiate suxamethonium apnoea (Clarke, Dundee and Daw, 1964; Howells et al, 1964).

Chapter 5

THE ROLE OF CREMOPHOR EL IN ANAPHYLACTOID REACTIONS PRODUCED BY CREMOPHOR-CONTAINING ANAESTHETICS

1. INTRODUCTION

It has been shown that injection of the intravenous anaesthetic agents thiopentone, propanidid and 'Althesin' can produce histamine release in man (Lorenz et al, 1972; Doenicke et al, 1973). The amount of histamine released is normally insufficient to be of any clinical significance but a large increase in plasma histamine concentration has been found to be a common factor in patients who have developed an anaphylactoid reaction following the administration of the Cremophor containing anaesthetic, propanidid (Lorenz et al, 1972). In human volunteers, these authors were unable to demonstrate histamine release following the administration of the hypnotic agent etomidate, or the solubilizing agent Cremophor EL when given on its own (Doenicke et al, 1973).

The original aim in this study was to find an animal model in which to evaluate the histamine-releasing propensity of Cremophor EL and Cremophor-containing anaesthetics. The unsuitability of the dog as a species in which to examine the ability of Cremophor-containing compounds to produce histamine release has been referred to in the introduction (Section 6). In the dog the intravenous administration of Cremophor almost invariably induces a massive release of histamine (Lorenz et al, 1971b). The pig resembles man in being insensitive to the histamine

releasing properties of Cremophor EL (Lorenz et al, 1971b)and was selected as a suitable species in which to evaluate the effects of Cremophor-containing anaesthetics.

Preliminary studies in pigs failed to demonstrate any significant increase in plasma histamine concentration following a single injection of the Cremophor-containing anaesthetic 'Althesin'. At this time, the finding by Watkins et al (1976) of a high frequency of subclinical "hypersensitivity" reactions in man, following single and repeated injections of 'Althesin', propanidid and methohexitone, prompted an examination of the effect of repeated doses of 'Althesin' in the pig. The reactions in man were characterized by significant changes in white cell counts. In early studies in pigs it was found that the repeated administration of 'Althesin' produced a rapid and marked reduction in the number of circulating polymorphonuclear leucocytes and that this effect was accompanied by an abnormal clinical response. Further experiments were therefore designed to examine the effects of repeated injections of a range of Cremophor-containing and Cremophor-free anaesthetics. In this way it was hoped that the role of the surfactant Cremophor EL in producing anaphylactoid reactions could be investigated.

2. MATERIALS

2.1 Animals

Miniature pigs (Göttingen derived strain) of both sexes and weighing 12-20kg were used. They were housed individually in concrete and tubular metal pens, fed twice daily with Sow Breeder Meal, and allowed free access to tap water.

2.2 Drugs

One water soluble anaesthetic, thiopentone sodium ('Pentothal, Abbott) and two surfactant-containing anaesthetics, 'Althesin' (Glaxo) and 'Epontol' (Bayer), were examined. Thiopentone was prepared as a 2.5% w/v solution in water for injection B.P. 'Althesin' contains a mixture of two steroids, Alphaxalone 0.9% w/v and alphadolone acetate 0.3% w/v solubilized in 20% w/v polyoxyethylated castor oil (Cremophor EL, BASF), whereas 'Epontol' contains propanidid 5% w/v solubilized in 20% w/v Micellophor, a butanol extract of Cremophor EL. The active agents, alphaxalone/ alphadolone and propanidid were also obtained from their respective manufacturers and formulated in a non-Cremophor solution containing 10% w/v ethyl alcohol and 25% w/v propylene glycol BP (Boots).

3. METHODS

3.1 Experimental plan

One week before the first administration of a test agent, carotid and jugular cannulae were surgically implanted to allow arterial pressure to be measured and to facilitate the administration of test agents and the withdrawal of blood samples. For this procedure pigs were sedated with 0.2 mg kg^{-1} droperidol and anaesthesia was induced and maintained with halothane.

When test agents were administered arterial pressure was measured, before and during the period of anaesthesia, with a Bell and Howell strain gauge transducer. Heart rate was derived electronically from the arterial pressure input and records of both measurements were obtained on a Devices M19 recorder. Rectal temperature was measured during anaesthesia with a mercury thermometer. Abnormal clinical responses and sleeping times to head lift were noted.

Samples of arterial blood were collected before the administration of a test agent and at 2, 5, 10 and 20 min after injection. Arterial pH, PO_2 and PCO_2 were measured in heparinized blood samples using standard Radiometer or Corning electrodes. Samples for plasma histamine measurement were collected into EDTA and centrifuged at 1500g for 60 min. An equal volume of trichloracetic acid was added to the supernatant, to precipitate protein, and plasma histamine concentration was measured using an automated fluorimetric assay (Evans, Lewis and Thomson, 1973). Total and

differential white cell counts were performed by standard methods.

The doses of the anaesthetic agents investigated were as follows: thiopentone 10 mg kg^{-1}, alphaxalone/alphadolone 0.15 ml kg^{-1} (1.8 mg kg^{-1} total steroids) and propanidid 10 mg kg^{-1}. These were the minimum doses required to produce unconsciousness in the miniature pig. The use of an alternative solvent system containing 10% ethyl alcohol and 25% propylene glycol allowed a comparison to be made of the effects of Cremophor-free preparations of alphaxalone/alphadolone and propanidid with those of the commercially available preparations of 'Althesin' and 'Epontol' containing Cremophor EL and Micellophor respectively. Solvents, when examined alone, were given in a volume and concentration equal to that contained in the anaesthetic formulations. All injections were given over 30 seconds.

In most instances an interval of 1 week was allowed between the administration of each dose of a test agent. Additional experiments were performed with 'Althesin' using intervals of 4, 14 and 21 days and Cremophor was also readministered after a 14-day interval. Different groups of pigs (3-6 per group) were used for each agent or interval examined. Thus each pig received only two injections of the same agent.

3.2 Statistical evaluation of results

The significance of differences occurring within an experiment was estimated with Student's paired t-test. A similar test was also used to compare differences obtained on the first and second exposures to a test agent in the same group of pigs.

4. RESULTS

The results obtained in the first series of experiments where a 1-week interval was allowed between the administration of two doses of a test agent are summarized in Table 48 and Appendix 6.

4.1 Abnormal clinical responses

Cremophor EL and the Cremophor and Micellophor-containing agents 'Althesin' and 'Epontol' all produced frequent reactions when a second administration was given 7 days after the first exposure. On only one occasion, when alphaxalone and alphadolone were given in alcohol and propylene glycol, was an abnormal response seen on the first administration of any agent. The second administration of alphaxalone and alphadolone in the same solvent produced abnormal clinical responses in two of four pigs. No such responses were seen when propanidid was administered in alcohol and propylene glycol or when this solvent mixture was given alone. No abnormal responses were seen following the repeated administration of thiopentone.

Pigs showing an abnormal response developed an initial cutaneous hyperaemia and frequently showed convulsive limb and head movements. Peripheral cyanosis or a transient urticarial rash appeared later (Fig. 31). The responses produced by different agents were generally similar. Convulsive kicking and peripheral cyanosis were noted most frequently in the animals receiving Cremophor EL or 'Epontol', while the overt signs of a response were less marked following administration of the alphaxalone/alphadolone mixture in the alternative solvent. Three of the pigs which responded

TABLE 48 RESPONSES OBTAINED IN MINI-PIGS ON THE SECOND ADMINISTRATION OF ANAESTHETICS AND SOLVENTS, 7 DAYS AFTER AN INITIAL EXPOSURE

Agent	No. of pigs	Abnormal clinical response	Hypertensive response 50mmHg	Decrease in polymorph count 50%	Increase in plasma histamine 50%
Thiopentone	6	0	0	0	2
'Althesin'	4	3	3	3	2
'Epontol'	6	5	5	6	3
Alphaxalone/ Alphadolone*	4	2	3	1	1
Propanidid*	4	0	0	0	0
Cremophor EL	5	4	5	5	4
Ethyl alcohol/ propylene glycol	3	0	0	0	0

*Solubilized in ethyl alcohol and propylene glycol

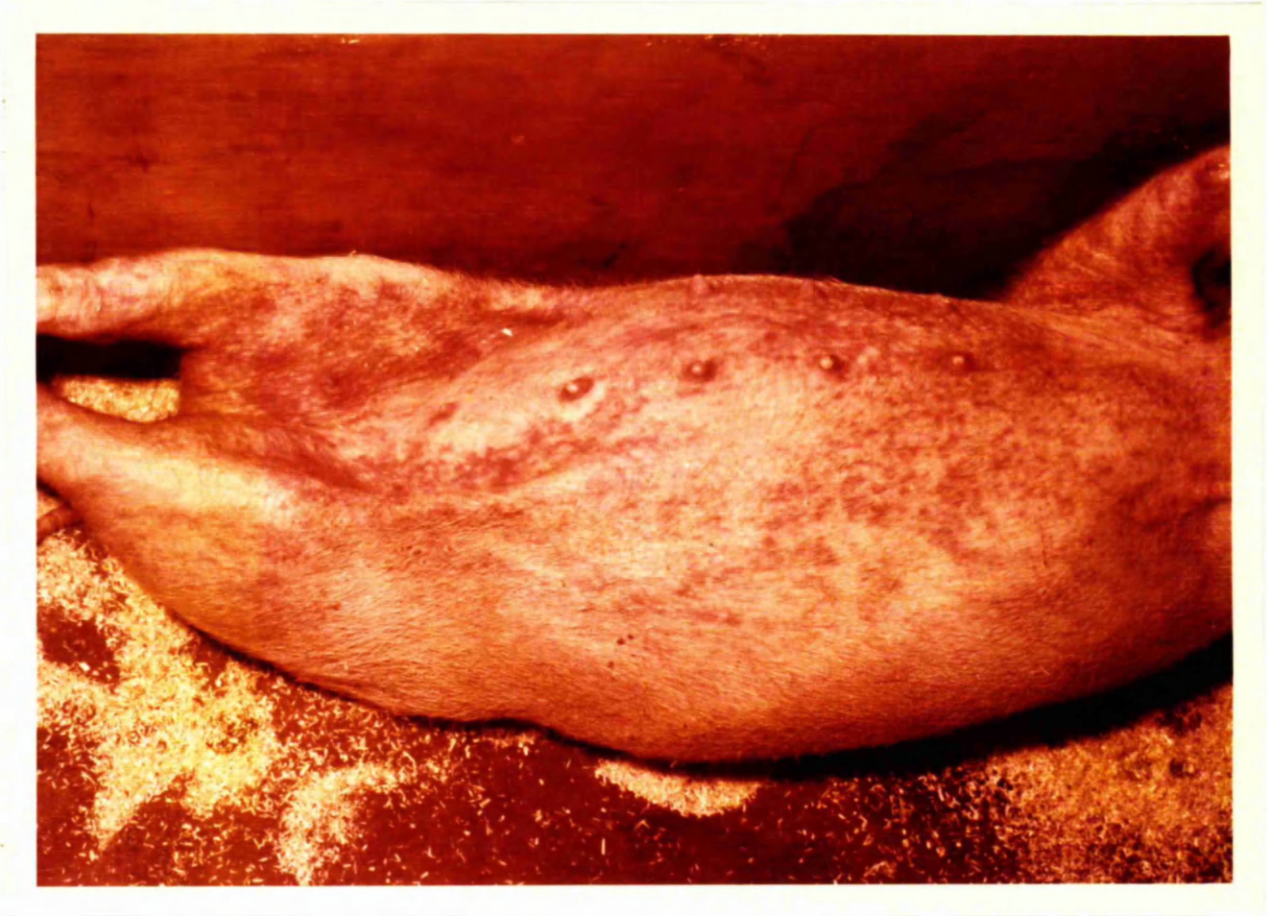


Fig. 31 Urticarial rash produced by a second injection of Cremophor given seven days after an uneventful first exposure

to Cremophor became unable to stand for a short period, salivated excessively and vomited. Rectal temperature did not change significantly during any of the reactions. No treatment or supportive therapy was given and all animals survived.

4.2 Sleeping times

Mean sleeping time on both exposures to each of the anaesthetic agents investigated are given in Table 49 and results obtained in individual animals are tabulated in Appendix 7.

Pigs receiving a second injection of 'Althesin' or alphaxalone/alphadolone in the alternative solvent slept for a longer period than that found on the first exposure to these agents, but the difference was not statistically significant.

4.3 Respiratory effects

In pigs showing an abnormal response a period of apnoea of 20-60s was seen frequently following the administration of a test agent. On other occasions a brief period of irregular respiration or coughing was noted. Respiratory depression appeared to be central in origin and not associated with bronchospasm. Changes in arterial PO_2 and PCO_2 in pigs showing abnormal responses are shown in Figure 31, in which it can be seen that PO_2 decreased slightly in the groups receiving the anaesthetic agents but was virtually unchanged at 2 min in the group responding to the second injection of Cremophor. In no case was the reduction in PO_2 values statistically significant. Later, when peripheral cyanosis

Table 49 Sleeping times produced by two separate administrations of a range of i.v. anaesthetic agents

Anaesthetic agent	No. in group	No. of responders on day 8	Mean ± S.D. sleeping time to head lift (min)	
			Day 1	Day 8
Thiopentone	6	0	10 ± 3.4	10.3 ± 3.7
'Althesin'	4	3	6.5 ± 1.2	13.5 ± 5.9
'Epontol'	6	5	2.9 ± 1.1	3.5 ± 3.3
*Alphaxalone/alphadolone	4	2	7.1 ± 2.3	10.6 ± 4.1
*Propanidid	4	0	4.0 ± 0.8	4.5 ± 0.57

*Prepared in non-Cremophor solvent

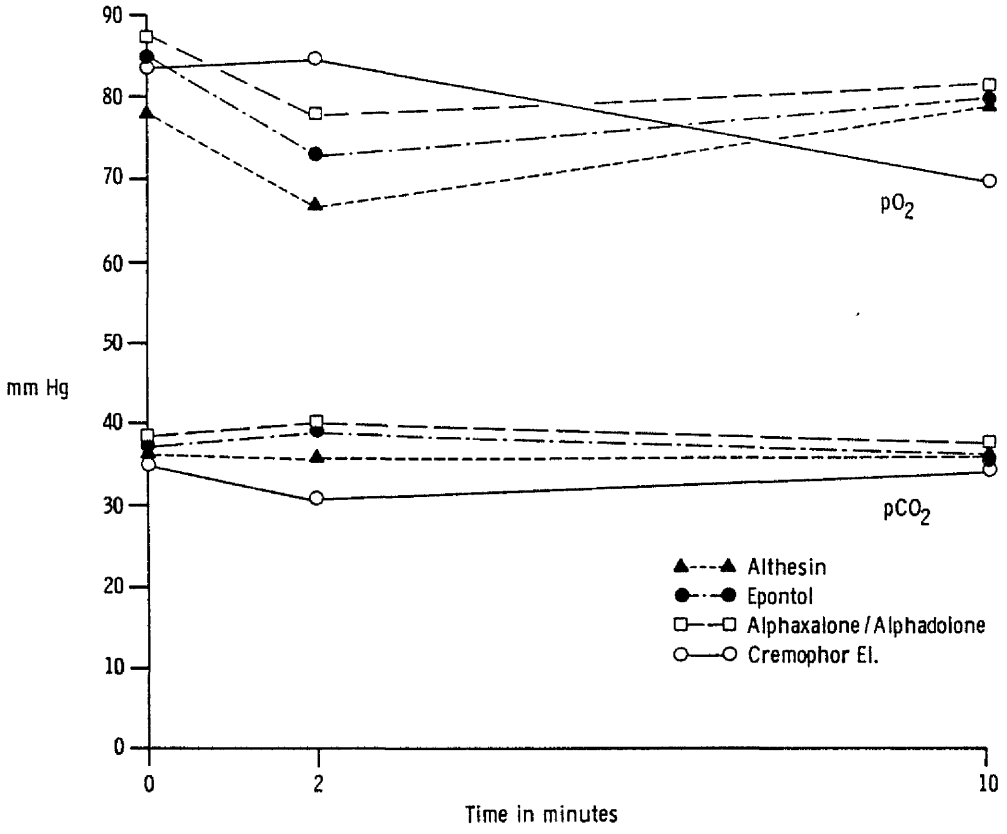


Fig. 31 Changes in arterial PO_2 and PCO_2 in pigs which showed an abnormal clinical response.

was frequently evident, arterial oxygenation had returned to normal in the groups which received the anaesthetic agents. Arterial PCO_2 values were not altered significantly and the changes in pH did not indicate any effect on metabolic acid-base status.

The decrease in PO_2 values in pigs given thiopentone, or propanidid in the alternative solvent, was similar to that seen in pigs showing an adverse response to one of the other anaesthetic formulations. It would appear, therefore, that adverse clinical responses are not associated with any significant change in blood gas values.

4.4 Plasma histamine concentration

Plasma histamine concentration was not invariably increased in pigs which exhibited an abnormal response (Table 48 and Appendix 6). Increases were found most consistently in pigs given Cremophor or 'Epontol'. Despite the finding of large concentrations of 106 ng ml^{-1} and 135 ng ml^{-1} at 2 min in individual pigs in the groups given Cremophor and 'Epontol' respectively, none of the groups showed a consistent increase which was statistically significant. The mean plasma histamine concentrations found in pigs which showed abnormal responses are given in Fig. 32. In one pig which showed an abnormal response to a second injection of alphaxalone and alphadolone in the alternative solvent, plasma

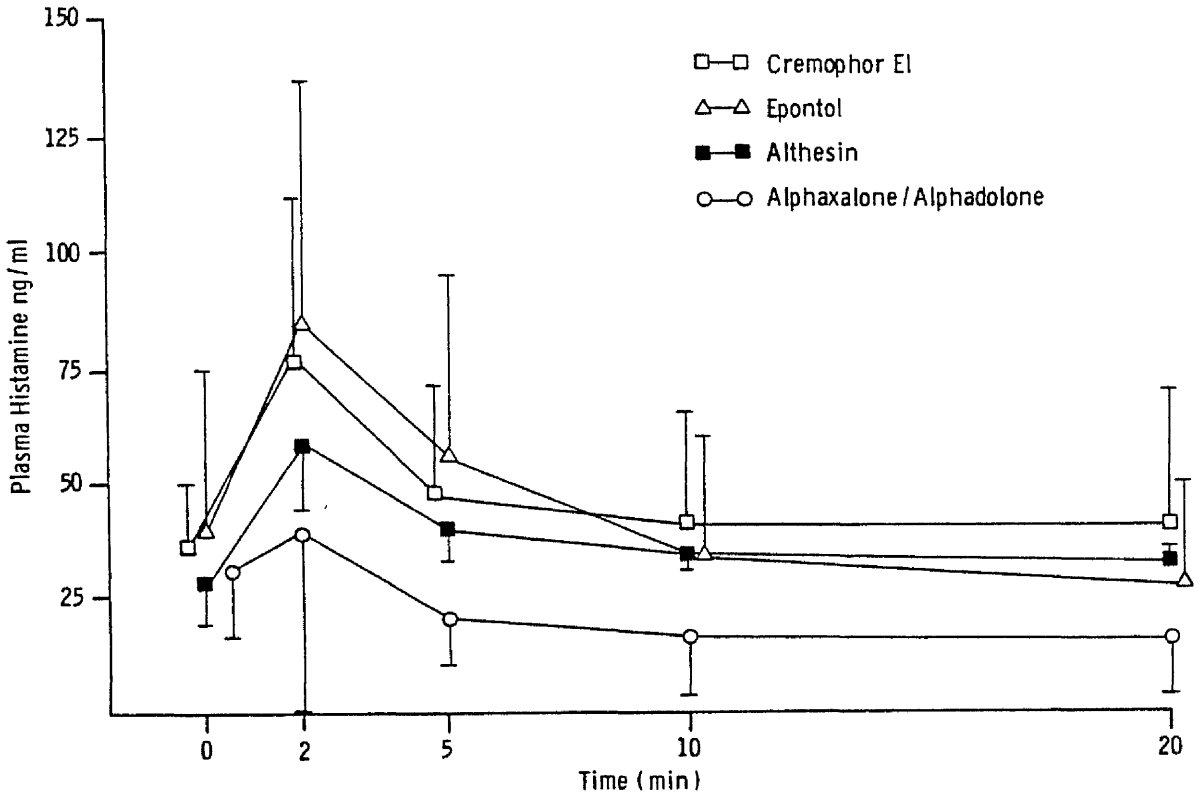


Fig. 32 Plasma histamine concentration in pigs which showed an abnormal clinical response.

histamine concentrations increased from 46 ng ml^{-1} to 84 ng ml^{-1} 2 min after injection. The increase noted in two pigs given a second injection of thiopentone was not associated with any other sign of an abnormal response.

4.5 Haemodynamic effects

In all animals in which an abnormal clinical response was noted, and on two occasions when no clinical signs indicating an abnormal response were seen, a marked hypertensive response developed a few seconds after the administration of the test agents. Maximum mean arterial pressures in the groups which showed abnormal reactions are given in Table 50.

Maximum increases in arterial pressure were achieved within 1 min and thereafter mean pressure returned to pre-dose values over the succeeding 5-10 min. The increase in arterial pressure usually preceded any overt sign of an adverse response and the maximum values reached were similar in all groups showing an anaphylactoid response. A typical record illustrating these changes is shown in Figure 33. In this animal the first administration of 'Epontol' produced only a brief depressor effect, whereas the second injection, given 7 days later, produced a marked pressor response. On only one occasion was a similar pressor response noted on the first administration of a test agent. The record obtained from this animal, which was given alphaxalone/alphadolone in alcohol and propylene glycol is shown in Figure 34.

Table 50 Hypertensive responses in pigs showing abnormal clinical responses

Anaesthetic or solubilizing agent	No. of responders on day 8	Arterial pressure (mean ± SD; mmHg) pre-dose	maximum recorded	p**
'Althesin'	3	103.7 ± 7.2	197.7 ± 7.9	< 0.01
'Epontol'	5	103.0 ± 18.4	180.8 ± 21.4	< 0.001
Alphaxalone/Alphadolone***	3*	106.3 ± 11.01	192.3 ± 21.5	< 0.05
Cremophor EL	4	107.2 ± 9.0	205.5 ± 10.4	< 0.01

* One animal which responded abnormally on day 1 included

** Student's paired t-test

*** Prepared in non-Cremophor solvent

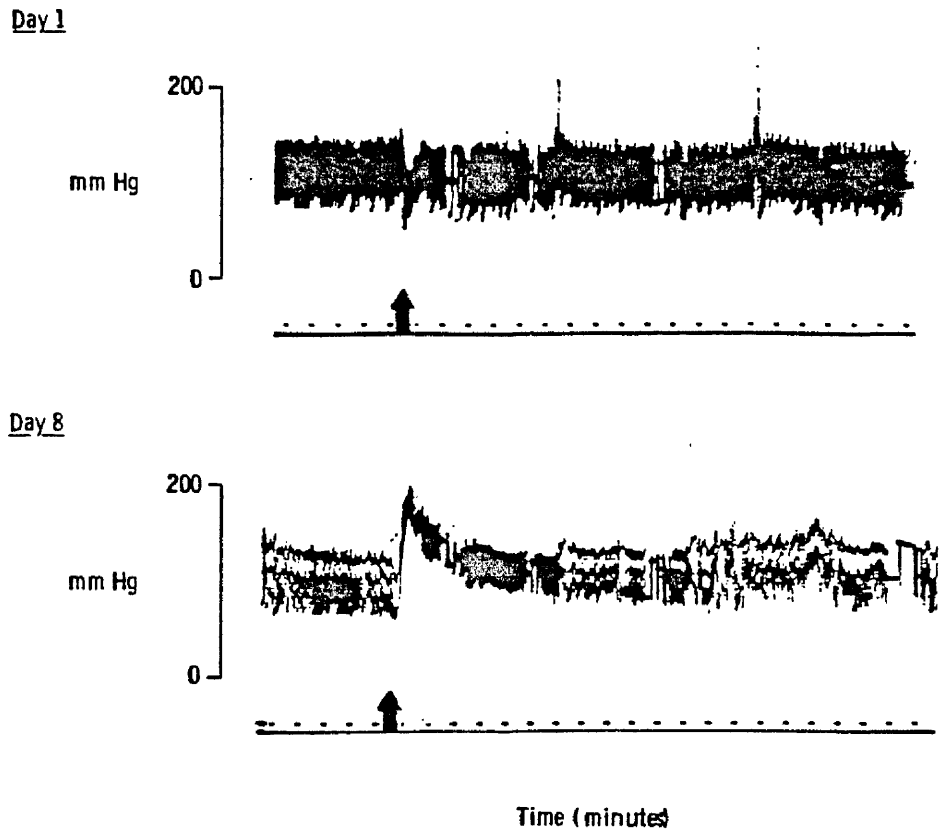


Fig. 33 The effect of two injections of 'Epontol' on arterial pressure in a pig.

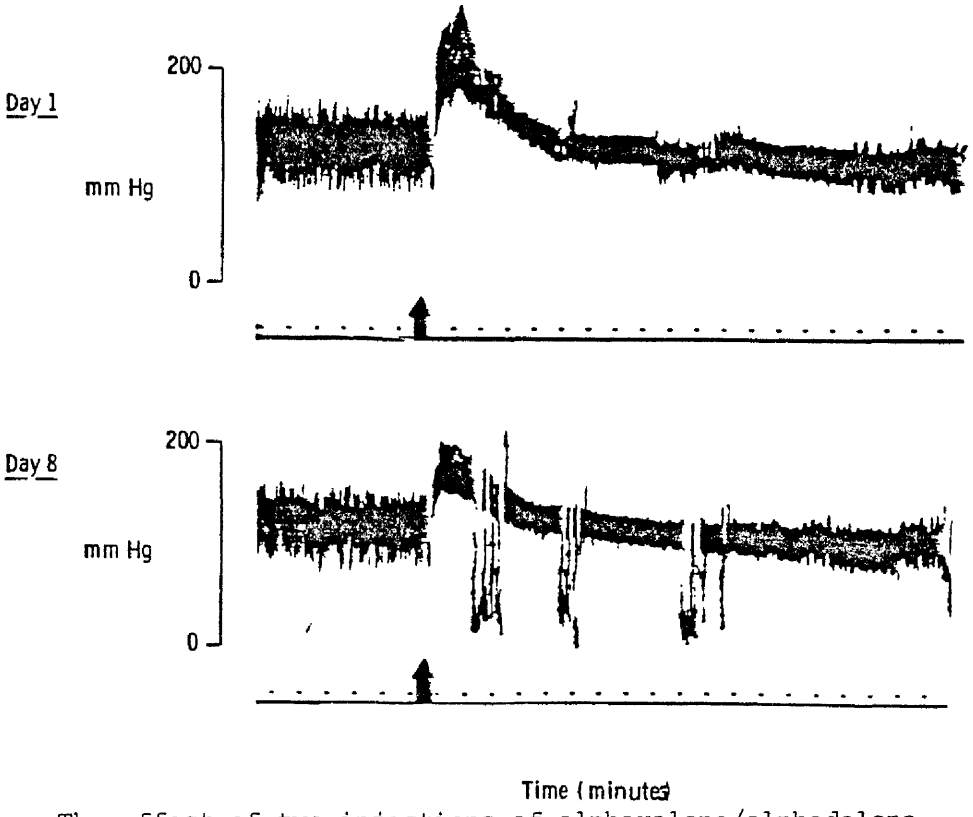


Fig. 34 The effect of two injections of alphaxalone/alphadolone on arterial pressure in a pig. Blood sampling artefacts are present on lower trace.

No marked pressor effects were seen on the second administration of thiopentone, or propanidid given in a non-Cremophor formulation, and it would appear that a hypertensive response is a consistent feature of the analphylactoid response seen with the other agents.

Heart rate increased as the pressor effect was initiated but in many instances reflex bradycardia occurred when the hypertensive response was fully developed. Pulse pressure was frequently reduced about 5 min after drug administration and at this time a peripheral pulse could not be readily detected.

4.6 Effects on white blood cells

In one pig in which an abnormal clinical response was noted following the first injection of alphaxalone/alphadolone in alcohol and propylene glycol, the polymorph count decreased from a value before injection of 9.03×10^9 litre^{-1} to 4.4×10^9 litre^{-1} 2 min after injection. No significant changes in total or differential white cell counts occurred in any other pig following the first administration of a test agent.

A typical finding in pigs showing an adverse response to a second administration of a test agent was the virtual disappearance of polymorphonuclear leucocytes in blood samples collected 2 and 5 min after injection. This decrease in polymorph count was reflected in the total white cell count. Lymphocyte numbers were not significantly altered (Figure 35). The mean changes in polymorph count 2 min after the second administration of each test substance are given in Table 51. 'Althesin', 'Epontol', alphaxalone/alphadolone in alcohol and propylene glycol, and Cremophor all produced marked changes at

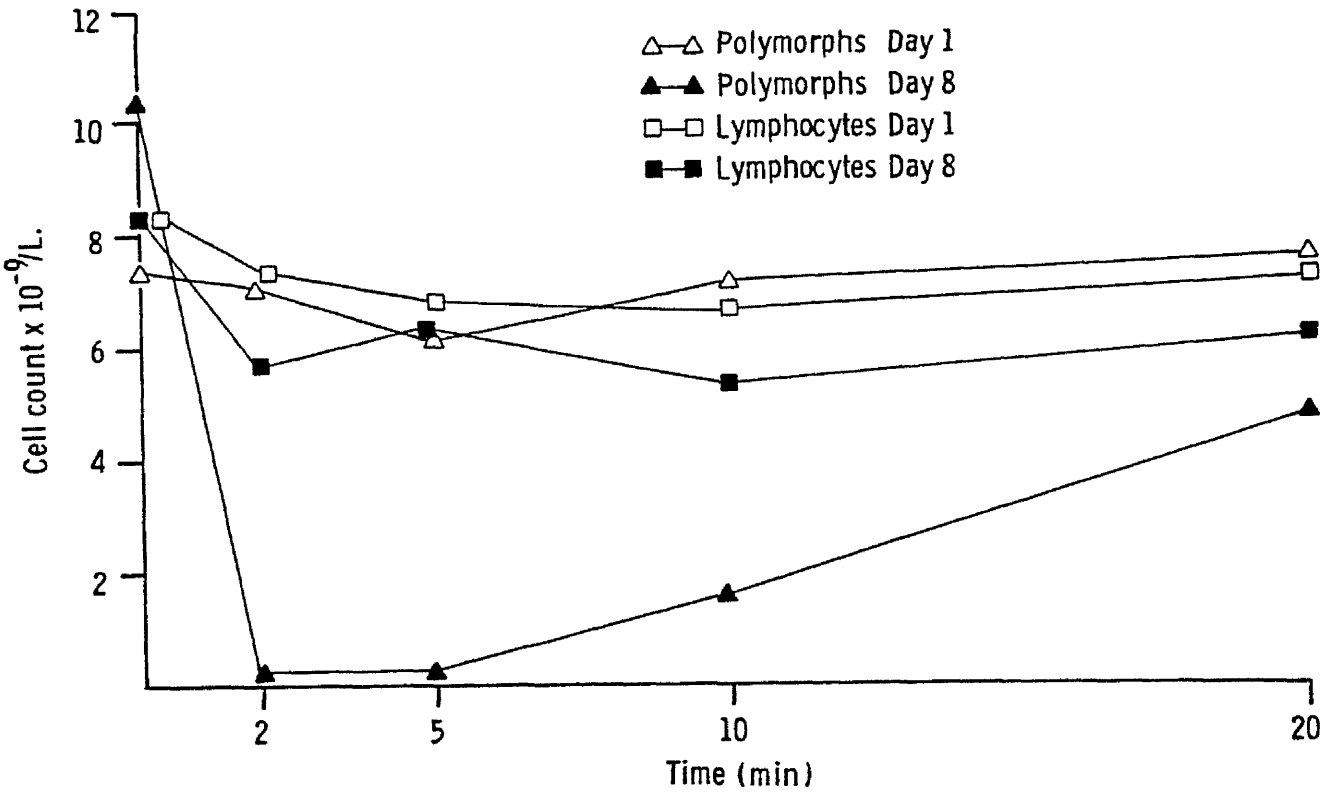


Fig. 35 Polymorph and lymphocyte counts in pigs which responded to a second injection of Cremophor EL given 7 days after an initial uneventful exposure.

TABLE 51 CHANGES IN POLYMORPH COUNT IN MINI-PIGS ON THE REPEATED ADMINISTRATION OF ANAESTHETIC AGENTS AND SOLVENTS, 7 DAYS AFTER AN INITIAL EXPOSURE

Agent	No. of pigs	No. showing abnormal clinical response	Polymorph count x 10^4 $litre^{-1}$ (mean ± SD)		P
			Before injection	2 min after injection	**
Thiopentone	6	0	9.06 ± 2.55	8.96 ± 3.72	ns
'Althesin'	4	3	6.90 ± 3.32	1.41 ± 1.33	<0.05
'Epontol'	6	5	7.49 ± 3.76	0.49 ± 0.61	<0.01
Alphaxalone/Alphadolone*	4	2	11.6 ± 7.33	6.53 ± 4.87	<0.05
Propanidid*	4	0	6.92 ± 2.22	6.66 ± 2.20	ns
Cremophor EL	5	4	10.94 ± 4.01	0.76 ± 1.41	<0.01
Ethyl alcohol/ propylene glycol	3	0	7.51 ± 2.05	8.77 ± 4.26	ns

*Solubilized in ethyl alcohol and propylene glycol

**Students paired 't' test

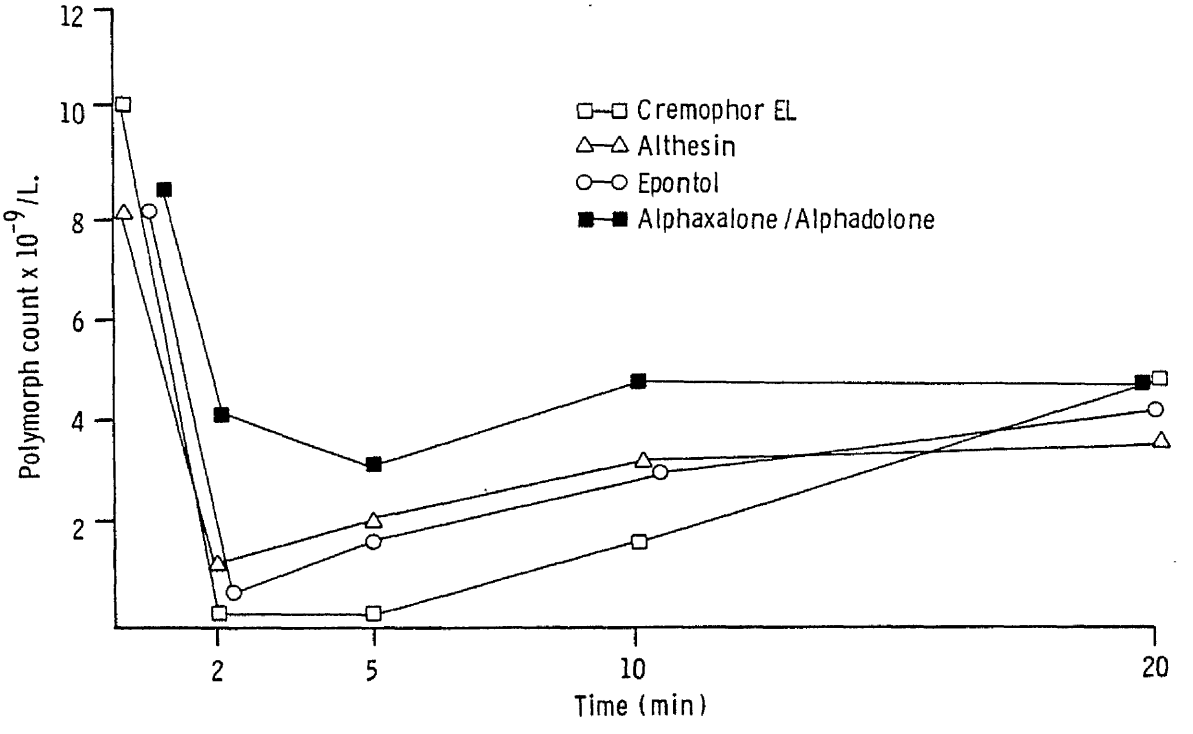


Fig. 36. Polymorph counts in pigs which showed an abnormal clinical response.

this time whereas thiopentone, propanidid in alcohol and propylene glycol, and this latter solvent mixture on its own, produced only minor effects. The time course of these changes in polymorph count, in animals in which adverse clinical responses were noted, is depicted in Figure 36.

4.7 Effect of varying the interval between injections

The results obtained in experiments in which the interval between two injections of Cremophor or 'Althesin' was varied between 4 and 21 days are summarized in Table 52. A high frequency of adverse responses to 'Althesin' occurred when a second injection was given after 1 or 2 weeks, whereas only one mild reaction was produced when the interval between two injections was 4 days or 3 weeks. Adverse responses to the second administration of Cremophor were noted less frequently when the interval between two injections was extended from 1 to 2 weeks. The changes in polymorph count, at the different times of challenge (Figures 37 and 38) reflected the changes in clinical responses. This result suggests that sensitivity to Cremophor may possibly be lost more rapidly than that to 'Althesin'.

In conclusion Cremophor and the Cremophor-containing agents 'Althesin' and 'Epontol' all produced similar analphylactoid responses when a second administration was given after an uneventful first exposure. In addition to clinical signs, a marked increase in arterial pressure and an immediate but transient decrease in the number of circulating polymorphonuclear leucocytes were the most

TABLE 52 ADVERSE RESPONSES TO 'ALTHESIN' AND CREMOPHOR WITH VARIOUS INTERVALS BETWEEN INJECTIONS

	Interval between injections (days)	No. of pigs	Abnormal clinical response	Hypertensive response > 50mmHg	Decrease in polymorph count > 50%	Increase in plasma histamine > 50%
'Althesin'	4	4	1	0	0	0
	7	4	3	3	3	2
	14	5	3	3	3	2
	21	4	0	0	0	0
Cremophor EL	7	5	4	5	5	4
	14	4	1	1	0	0

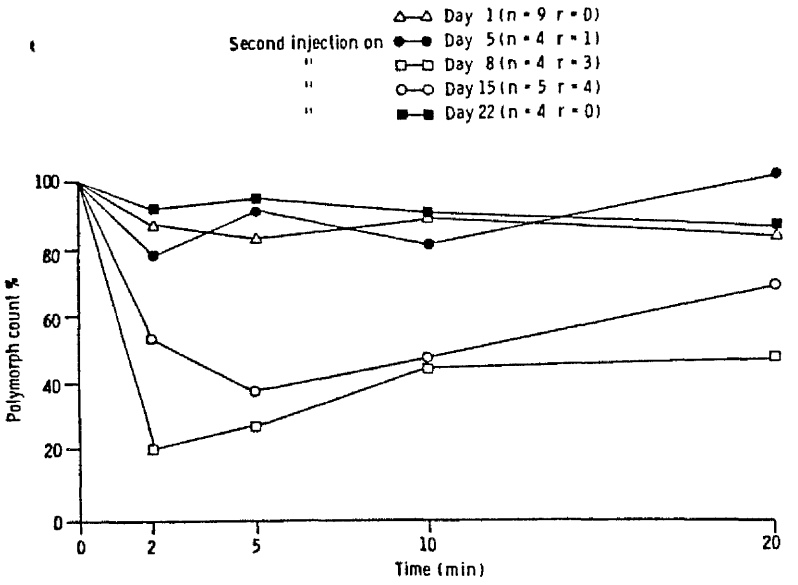


Fig. 37 Polymorph count (as % of pre-dose value) in pigs given 'Althesin' on Day 1 and after intervals of 4, 7, 14, or 21 days. n = number examined. r = number of adverse responses.

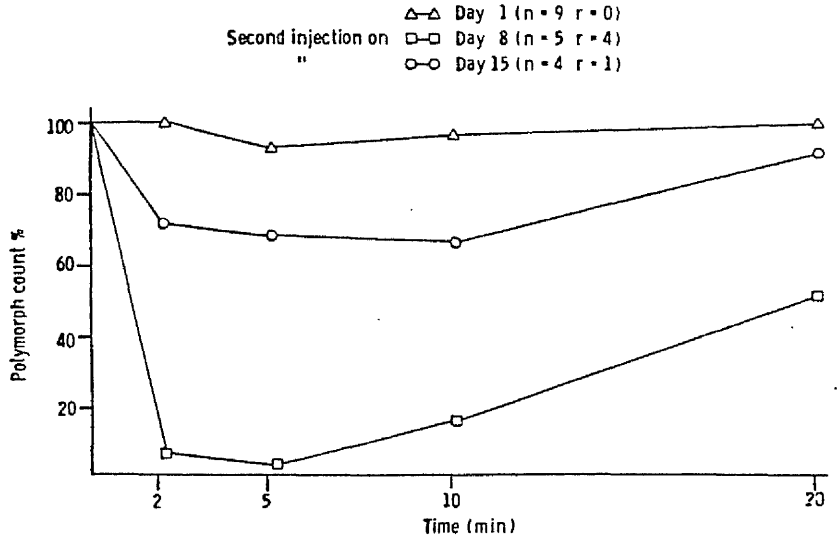


Fig. 38 Polymorph count (as % of pre-dose value) in pigs given Cremophor on Day 1 and after intervals of 7 or 14 days.

consistent features of the response in the pig.

No similar responses were induced by the repeated injection of thiopentone, or propanidid in a non-Cremophor formulation. The replacement of Cremophor by alcohol and propylene glycol as the solubilizing agent for alphaxalone and alphadolone failed to prevent the production of anaphylactoid responses with this mixture of steroids.

5. DISCUSSION

An examination of the results obtained with experimental anaesthetics in chapter 4 and a comparison of published results with the lipophilic anaesthetic 'Althesin' with those obtained with the related water soluble steroid, minaxolone, (Punchihewa, Morgan, Lumley and Whitwam, 1980; Prys-Roberts, Sear and Adam, 1981; Prys-Roberts and Sear, 1980; McNeill, Clarke, Dundee and Briggs, 1981) indicates that desirable pharmacokinetic properties and freedom from excitatory effects may be more readily achieved with lipophilic agents.

The new agent, disoprofol, examined in chapter 4, is a highly lipophilic anaesthetic and is currently solubilized in Cremophor EL. The use of this solubilizing agent has two main disadvantages. Firstly, the ability of Cremophor to produce histamine release and hypotension in the dog (Lorenz et al, 1971b) precludes the clinical or experimental use of disoprofol in this species. Secondly, the advisability of using Cremophor in the formulation of a new agent for use in man must be questioned as doubt still exists about the involvement of Cremophor in the production of anaphylactoid reactions in man following the administration of the Cremophor-containing anaesthetics 'Althesin' and 'Epontol'.

Lorenz (1975) has speculated that the role of Cremophor in producing reactions in man may be related to the combined effects of a surfactant with an anaesthetic agent, with mast cell membranes being altered by the anaesthetic drug to facilitate binding of the wetting agent and the release of histamine. This conclusion was reached, and Cremophor *per se* was exonerated, as previous studies had shown that the administration of Cremophor alone produced no effect in patients who had previously reacted adversely to the Cremophor containing agent 'Epontol' (Lorenz *et al*, 1972; Doenicke *et al*, 1973). An alternative explanation, proposed by Watkins *et al* (1976), was that Cremophor could possibly act as an adjuvant such that the allergenicity of the anaesthetic agent would be increased.

Previous attempts to produce hypersensitivity responses in laboratory animals by the repeated injection of 'Althesin' or Cremophor proved unsuccessful (G.E. Davies and A.V. Thompson, personal communication). It has also been shown, in man, that the injection of Cremophor alone produces no significant change in heart rate or arterial blood pressure (Savege *et al*, (1973b).

On the other hand, circumstantial evidence which tends to incriminate Cremophor, continues to accumulate. The introduction of 'Althesin' in a Cremophor formulation was followed by a greater number of reports of anaphylactoid reactions than had previously been noted with the water soluble barbiturates (Clarke et al, 1975). The features of the adverse responses described were similar to those which had been observed previously with another Cremophor-containing agent, 'Epontol' (Bradburn, 1970; Johns, 1970; Larard, 1970). In addition, reactions to 'Althesin' have been reported in patients previously anaesthetised with 'Epontol' (Notcutt, 1973) and adverse reactions to 'Epontol' have also been noted after an earlier uneventful exposure to 'Althesin' (Dye and Watkins, 1980). The presence of Cremophor as a common factor in reactions of this type has also been noted by Thornton (1978). Further circumstantial evidence pointing to the involvement of Cremophor has been provided by Huttel, Shou Olesen and Stoffersen (1980). These authors found that when the solvent in diazepam was changed from propylene glycol to Cremophor the frequency of hypersensitivity reactions increased from virtually zero to 5 in 5,200.

The results described in this chapter were obtained in an animal model in which it was possible to produce anaphylactoid reactions with Cremophor and Cremophor-containing anaesthetics. These results indicate that, in the mini pig, Cremophor is responsible for the reactions produced by 'Epontol'. No adverse responses were encountered when propanidid, the active agent in 'Epontol', was given in an alternative non-Cremophor solvent. Cremophor on its own produced typical reactions but the results obtained with 'Althesin' were less clear. While 3 out of 4 pigs showed an adverse response when a second administration of 'Althesin' was given, the steroid constituents of 'Althesin', alphaxalone and alphadolone acetate, also produced reactions when given in a non-Cremophor formulation. This indicates that, while Cremophor may play a part in some of the reactions to 'Althesin', hypersensitivity to the steroid constituents may also be important.

Reactions in man have been classed as 'anaphylactoid' as the clinical features of a response are insufficient to distinguish between antibody-mediated reactions, complement-mediated reactions, and reactions due to the release of histamine from mast cells by a direct action of the injected substance (Watkins and Clarke, 1978; Watkins, 1979). The time course and appearance of the response in the pig suggests that these reactions too should be called

'anaphylactoid'. Sensitivity was induced within 7 days of a previous exposure and disappeared if the interval between two injections was extended to three weeks. An additional feature of the response in the pig is that, in animals which have responded to a second injection of 'Althesin', the subsequent injection of Cremophor produces no further response (Glen, Davies, Thomson, Scarth and Thompson, 1978). Should a similar phenomenon occur in man, this mechanism could explain the failure of Lorenz et al (1972) to precipitate a response with Cremophor in patients who had already reacted to 'Epontol'. True analphylactic responses can be induced in the pig by the repeated injection of ovalbumin (Glen and Hunter, unpublished observations) and in this case an interval of 3 weeks between injections is required. A challenge dose of ovalbumin produces a response similar to that seen in pigs given Cremophor, but in contrast to Cremophor-sensitized animals subsequent challenges with ovalbumin produce a similar or greater response.

In assessing the relevance of results obtained in the pig it is necessary to examine the mechanisms thought to be responsible for reactions in man. The time course of the response to Cremophor in the pig indicates that the mechanism involved is unlikely to mimic the mechanism responsible for reactions which occur on the first exposure to a Cremophor-containing agent in man. Alternate pathway activation of complement C3 or the direct release of histamine from mast cells appear

to be responsible for most of these reactions (Watkins, Milford Ward and Appleyard, 1977; Watkins, 1979). The pig model is also unlikely to mimic true IgE mediated responses where sensitivity may persist for a much longer time (Lilly and Hoy, 1980; Fisher, 1980). Although reactions to barbiturate anaesthetics occur more rarely, a greater proportion of these reactions involve IgE mediated responses (Wyatt and Watkins, 1975).

Many of the adverse reactions to 'Althesin' do, however, follow a previous exposure to this drug or another Cremophor-containing agent. An analysis of the figures of Clark et al, (1975) indicates that of 65 patients who reacted to 'Althesin', 35 had received a Cremophor-containing anaesthetic previously. In more than 50 per cent of these patients the second administration had been given within 1 month of the first. A similar frequency of previous exposure to 'Althesin' was noted by Beamish and Brown (1981) while Radford, Lockyer, Sear and Simpson (1980) found that of 22 patients with clinical signs of hypersensitivity to 'Althesin', 17 had received the drug on at least one previous occasion. Watkins, Allen and Milford Ward (1978) have suggested that a short term 'memory' phenomenon, involving lymphocyte receptors and IgD, may be responsible for these reactions and the time course of the response in the pig supports this theory. Results obtained in the pig may therefore be of predictive value in detecting compounds likely to produce this latter effect in man.

In two important aspects the clinical response in the pig differs from the response in human patients. While hypotension and bronchospasm are frequently encountered in man, hypertension is produced in the pig and bronchospasm has not been observed. These differences are probably due to species differences in responsiveness to the biogenic amines or kinins released during a reaction. The histamine content of pig plasma is about 100 times greater than that of man (Lorenz et al, 1971a) and elevation of plasma histamine concentration was a minor and inconsistent finding in the present investigation. Bronchial and vascular tissue in the pig may therefore be relatively insensitive to small increases in plasma histamine concentration. It has also been shown by Lorenz et al (1971a) that a pressor response is produced in the pig by the injection of the histamine releaser, compound 48/80.

The possible involvement of complement in reactions in man has been mentioned. When complement is activated the anaphylatoxins C_{3a} and C_{5a} are released and leucocyte chemotaxis and aggregation is induced (Gigli, 1979; Williams, 1979). This results in a reduction in circulating polymorphonuclear leucocytes (Craddock,

Fehr, Dalmasso, Brigham and Jacob, 1977). The marked reduction in circulating leucocytes noted in the present investigation suggests that complement activation and the release of C_{5a} may also occur in the pig. As C_{5a} produces arteriolar constriction in the perfused porcine kidney and on topical application to rabbit omentum (Hugli, 1979) release of this anaphylatoxin could have contributed to the hypertensive response seen in the pig. In this respect species differences may also be important as, unlike animal sera, complement activated human serum contains little spasmogenically active C_{5a} (Hugli, 1979).

Aggregation of polymorphonuclear leucocytes, induced by C_{5a} release, may also cause pulmonary dysfunction due to aggregates becoming lodged in pulmonary capillaries (Hammerschmidt, Weaver, Hudson, Craddock and Jacob, 1980). Although only minor changes in blood gas tensions were found in pigs the transient presence of leucoemboli could have triggered a sympathoadrenal response. This may be considered as a further possible explanation for the hypertensive response noted in pigs. Further work would be required to examine fully the role of complement in the pig reaction.

At present no explanation can be given for the marked susceptibility shown by the pig to Cremophor containing agents. The complement system contains a number of interacting activating, inhibiting and inactivating

systems (Gigli, 1979). It is possible that a relative deficiency of an inactivator or inhibitor in the pig could cause the complement system to be more readily activated in this species. Whatever mechanism is involved it is apparent that a reaction is triggered only when an animal has been sensitized with an earlier injection of Cremophor.

Apart from the known direct histamine releasing properties of Cremophor on first exposure in dogs (Lorenz et al, 1971b), reactions to 'Althesin' have been reported in cats (Stogdale, 1978; Dodman, 1980). As most of these reactions were histaminoid in type and occurred on the first exposure to the drug direct histamine release by Cremophor was probably responsible. However, the possible involvement of complement activation in some of these reactions cannot be excluded.

GENERAL DISCUSSION AND CONCLUSIONS

The experiments described in chapters 1 and 2 were designed to investigate the accuracy of indirect methods for the measurement of systolic blood pressure and the estimation of arterial PCO_2 in dogs. This work was initiated as non-invasive techniques for the measurement of these parameters have not been widely used in routine veterinary anaesthesia or in the clinical evaluation of new anaesthetic agents in dogs. In earlier studies these indirect methods have been evaluated in relatively small numbers of animals and the accuracy of the indirect methods has not been systematically investigated in animals of widely differing size. In this study the indirect methods were examined in dogs, with body weights ranging from 5-47kg and anaesthetised with a range of different anaesthetic agents and techniques.

The accuracy of the indirect method of blood pressure measurement was found to depend on the selection of the correct width of occluding cuff for a given size of animal. On the basis of the results obtained it can be recommended that a cuff 2.5cm wide should be used in animals weighing less than 19kg and a 3.75cm cuff in animals of greater weight. The accuracy of the technique was also dependent on foreleg circumference; but the correlation obtained with the simpler measurement of body weight was equally good. The results obtained with the correct size of cuff for a given animal were deemed sufficiently accurate for the applications envisaged. The technique was readily applied

to anaesthetised dogs and problems with movement artefacts were rarely encountered.

Values of end-tidal PCO_2 were found to correlate closely with simultaneous direct measurements of arterial PCO_2, with the arterial value being on average 2.1mmHg greater than the end-tidal tension. While this mean value agrees well with results obtained in previous studies in dogs (Suskind and Rahn, 1954; Severinghaus and Stupfel, 1957) the range of differences between arterial and end-tidal tension was greater in this study. The accuracy of the method in estimating arterial PCO_2 was adequate for clinical studies as arterial and end-tidal values correlated closely in individual animals. Further work would be required to investigate possible differences in arterial-end-tidal PCO_2 gradients caused by different anaesthetic agents.

These indirect methods were used in the investigation of neuroleptanalgesic drugs described in chapter 3. Little difference could be detected in the hypotensive effects produced by the combinations examined; but measurement of end-tidal PCO_2 confirmed the presence of significant respiratory depression in dogs given fluanisone:fentanyl or methotrimeprazine:etorphine. In the examination of the respiratory effects of the fentanyl:droperidol mixture in anaesthetised dogs, end-tidal PCO_2 measurement proved to be a sensitive technique. With this method it was possible to show that droperidol anatagonised the respiratory

depressant effect of fentanyl to a significant extent. A similar effect could not be established by noting effects on respiratory rate due to the marked variation observed in the control values. Further evidence of the ability of neuroleptic drugs to antagonise respiratory depression was obtained in mice anaesthetised with disoprofol. This effect appears to be manifested only in the presence of respiratory depression as droperidol has been shown to produce little effect on ventilation in normal mice and rabbits (Greene, 1972; Khanna and Pleuvry, 1978). Although Janssen et al (1963) have postulated that droperidol may increase the sensitivity of the respiratory centre to carbon dioxide, the mechanism involved in this interaction requires further study.

Of the neuroleptanalgesic techniques investigated, the intravenous administration of the droperidol:fentanyl mixture produced the best overall results. However, a significant frequency of troublesome side effects was noted with all the drugs examined and the technique was not considered suitable for major surgery as advocated by Yelnosky and Field (1964) and Marsboom et al (1964). The neuroleptanalgesic technique could probably be improved by administering the neuroleptic component before the analgesic, but the delay involved would reduce the convenience of the technique in out-patients. While the ability to reverse immobilization with a narcotic-antagonist drug is an advantage it must be remembered that analgesic effects will also be terminated.

The experiments in laboratory animals, described in chapter 4 were done to identify a potential new intravenous anaesthetic and to compare the properties of disoprofol with those of currently used injectable anaesthetics. The search for new agents has continued because none of the available drugs can be considered to be completely satisfactory. Thiopentone, although widely used as an induction agent, has a long elimination half life (Ghoneim and Van Hamme, 1978; Christensen et al, 1980) and cannot be considered as a suitable agent for use in continuous infusion techniques. This agent may also cause extensive tissue damage if injected perivenously or intra-arterially (Kinmonth and Shepherd, 1959). Involuntary muscle movements and hiccough have been noted in a number of patients receiving methohexitone; and pain on injection, excitatory effects and emetic sequelae have been observed with etomidate (Holdcroft et al, 1976; Hendry, Miller and Lees, 1977; Zacharias et al, 1979). Propanidid is associated with a high incidence of post-anaesthetic nausea, vomiting (Clarke, Montgomery, Dundee and Bovill, 1971) and venous sequelae such as thrombosis or thrombophlebitis (Carson et al, 1972); and techniques of neuroleptanalgesia and dissociative anaesthesia have limited applications (Dundee and Wyant, 1974). 'Althesin' remains as a drug which produces relatively few excitatory effects (Clarke, Dundee and Carson, 1972), little venous damage (Carson et al, 1972) and has a short elimination half life (Simpson, 1978). The use of 'Althesin' has however decreased

due to the relatively high frequency of anaphylactoid reactions encountered with this agent (Clarke et al, 1975).

In the investigation of the anaesthetic properties of disoprofol in animals, new techniques were developed to examine the interrelationship between speed of injection, anaesthetic potency and speed of induction of anaesthesia. Anaesthetic potency and speed of induction increased when a faster rate of injection was used and it was concluded that disoprofol was a rapidly acting agent with a short duration of action and an anaesthetic potency 1.8 times greater than that of thiopentone.

An examination of recent trends in anaesthesia reveals renewed interest in techniques of total intravenous anaesthesia (Savege et al, 1975; Dundee, 1978). This trend coincides with current concern about theatre contamination by anaesthetic gases and vapours (Spence and Knill-Jones, 1978; Smith and Shirley, 1978). A major requirement in any agent used to maintain anaesthesia by intravenous infusion is that the injected compound should be rapidly metabolised, such that minimal tissue accumulation occurs and rapid recovery can be obtained. In experiments in which disoprofol was given by repeated injection to mice, and by infusion to rats, little evidence of cumulation was seen. Disoprofol may therefore have pharmacokinetic properties which would make it a suitable agent for maintenance of anaesthesia with an infusion technique.

In other studies with disoprofol cardiovascular and respiratory effects were found to be generally similar to those of thiopentone and 'Althesin'. No unexpected interactions were encountered when disoprofol was given in combination with drugs used in preanaesthetic medication, inhalation anaesthetics and neuromuscular blocking drugs. The combination of disoprofol and suxamethonium was examined in a new model, using mice, in which it was possible to demonstrate the known potentiating effect of propanidid on suxamethonium induced respiratory depression (Clarke, Dundee and Daw, 1964). No similar interaction of disoprofol with suxamethonium was found. This suggests that plasma cholinesterase is not involved in the metabolism of disoprofol and that potentiation of suxamethonium apnoea is unlikely to be produced in man with this agent.

Results obtained in preliminary clinical studies in human patients (Rogers, Dewar, McCubbin and Spence, 1980; Kay and Stevenson, 1980; Briggs, Clarke, Dundee, Moore, Baker and Wright, 1981) have confirmed that disoprofol produces anaesthetic effects in man similar to those predicted from these animal studies. Reports of pain on injection in a proportion of patients were however unexpected as this side-effect had not been predicted from the animal studies. Further work will be required to investigate the species differences responsible for this discrepancy.

Disoprofol is a lipophilic compound and, being poorly soluble in water, is currently formulated with the solubilizing agent Cremophor EL. As this agent is also present in 'Althesin', and 'Epontol' and as conflicting views have been expressed about the contribution of Cremophor to the relatively high frequency of anaphylactoid reactions encountered with these agents (Clarke et al, 1975; Lorenz, 1975; Watkins et al, 1976) it was necessary to examine further the role of Cremophor in these reactions.

The experiments described in chapter 5 were done in a new animal model in which it was possible to produce characteristic anaphylactoid reactions when a second administration of Cremophor or a Cremophor-containing agent was given 1-2 weeks after an uneventful first exposure to the same agent. Although it is unlikely that the mini-pig model reflects all of the mechanisms responsible for anaphylactoid reactions to injectable anaesthetics, the features of the model suggest that it may simulate those reactions in which a short term 'memory' phenomenon has been implicated in man (Watkins, Allen and Milford-Ward, 1978). The results obtained in pigs indicate that Cremophor alone can produce a response and any new agent formulated in Cremophor would be expected to induce the same response. The pig is apparently a very sensitive species in which to demonstrate this response and the model should prove useful in the search for more suitable non-Cremophor solubilizing agents for lipophilic injectable anaesthetics.

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APPENDICES

Appendix 1

Simultaneously measured values of indirect (Id) and direct (D) systolic pressure in a dog (32 kg) obtained with occluding cuffs of three different widths

Cuff width	2.5 cm			3.75 cm		
	Id	D (mmHg)	Id-D	Id	D (mmHg)	Id-D
	225	180	+45	170	180	-10
	195	188	+7	175	180	-5
	195	180	+15	175	180	-5
	170	160	+10	120	120	0
	190	176	+14	145	150	-5
	145	140	+5	154	160	-6
	130	130	0	140	148	-8
	145	142	+3	115	122	-7
	170	160	+10	120	130	-10
	145	139	+6	135	140	-5
	160	150	+10	135	140	-5
	160	150	+10	132	148	-16
	150	138	+12	180	210	-30
	155	160	-5	140	146	-6
	155	163	-8	140	160	-20
				145	158	-13
				165	174	-9
				170	171	-1
				135	150	-15
Mean ± SD	166 ± 25.3	157 ± 17.9	10.7 ± 10.3	147 ± 20.3	156 ± 22.5	9.3 ± 7.1

Appendix 1 (Cont'd.)

5.0 cm cuff

	Id	D (mmHg)	Id-D
	145	180	-35
	150	180	-30
	145	180	-35
	155	185	-30
	155	190	-35
	145	180	-35
	100	126	-26
	125	150	-25
	145	165	-20
	125	150	-25
	130	152	-22
	144	158	-14
	110	125	-15
	115	133	-18
	110	144	-34
	125	140	-15
	100	150	-40
	110	150	-40
	100	147	-47
	120	148	-28
	130	158	-28
	125	160	-35
	135	170	-35
Mean	128 ± 17.8	157.4 ± 18.6	29 ± 8.8

Appendix 2

Simultaneously measured values of indirect (Id) and direct (D) systolic blood pressure (mmHg) in dogs

Dog No.	2.5 cm cuff		3.75 cm cuff	
	Id	D	Id	D
1	151	160		
2	103	107	100	114
3	103	111		
4	152	163	135	153
5	152	199		
6	108	103	104	103
7	151	160	159	162
8	163	167	160	172
9	114	105	95	100
10	106	91		
11	138	150	125	160
12	81	95	76	95
13			110	148
14			118	120
15	133	125	128	126
16	168	164	150	163
17	96	93	86	97
18	128	106	113	107
19	120	132	96	120
20	183	166	155	171
21			160	175
22			111	132
23	127	112	109	112
24			148	160
25			83	96
26	161	150	143	150

Appendix 2 (Cont'd.)

Dog No.	2.5 cm cuff		3.75 cm cuff		5.0 cm cuff	
	Id	D	Id	D	Id	D
27			112	105		
28	210	220	190	225	179	220
29	210	210	179	198	168	168
30			100	102		
31			198	193		
32	170	160	165	170		
33	188	164	156	141	150	160
34			210	210	190	215
35			90	109		
36			75	78	71	82
37	160	138	152	158	130	143
38			140	129	128	141
39			155	155	153	159
40	161	122	148	137		
41			167	158		
42	145	114	117	115		
43			160	164		
44			154	171	143	171
45			135	128		
46					202	205
47			173	156		
48			127	114	105	103
49			213	177	162	173
50			119	98		
51			173	168		

Appendix 3

Breed, weight, foreleg circumference and the difference between indirect and direct measurements (Id-D) of systolic pressure in dogs

Dog. No.	Breed	Wt. (kg)	Foreleg circumference (cm)	2.5cm cuff Id-D (mmHg)	3.75 cm cuff Id-D (mmHg)
1	Terrier X	6.5	10.5	-9	
2	Collie X	8.0	14.5	-4	-14
3	Poodle	8.5	13.5	-8	
4	Spaniel	9.5	14.4	-11	-18
5	West Highland	9.75	15	-47	
6	Labrador	10	15.5	+5	+1
7	Terrier X	10.5	16	-9	-3
8	Whippet	11.25	16	-4	-12
9	Collie X	11.5	17.5	+9	-5
10	Spaniel	12.5	16	+15	
11	Collie X	13.0	15.5	-12	-35
12	Corgi	13.5	18.5	-14	-19
13	West Highland	13.5	18.5		-38
14	Beagle	13.5	17		-2
15	Collie X	14	16	+8	+2
16	Terrier X	14	17.25	+4	-13
17	Terrier X	15.5	16.5	+3	-11
18	Wire Haired Terrier	15.5	16.5	+22	+6
19	Collie	16	18.5	-12	-24
20	Collie	17.25	17	+17	-16
21	Terrier X	18	18.5		-15
22	Bull Terrier	18	21		-21
23	Spaniel	18.5	19	+15	-3
24	Labrador	18.5	17		-12
25	Terrier X	19	18		-13
26	Dalmation	19	18	+11	-7

Appendix 3 (Cont'd.)

Dog. No.	Breed	Wt. (kg)	Foreleg circum-ference (cm)	2.5cm cuff	3.75cm cuff	5.0cm cuff
27	Boxer	23	19.2		+7	
28	Greyhound	23	17.2	-10	-35	-41
29	Greyhound	24	19	0	-19	0
30	Setter	24	21		-2	
31	Collie X	24.5	20.5		+5	
32	Greyhound	27	19.5	+10	-5	
33	Greyhound	27	20.4	+24	+15	-10
34	Labrador	27	19.5		0	-25
35	Labrador	27	20.5		-19	
36	Alsatian	27	23		-3	-11
37	Greyhound	28	19.6	+22	-6	-13
38	Alsatian	28	22.8		+11	-13
39	Labrador	28	19		0	-6
40	Greyhound	28	20	+39	+11	
41	Alsatian	29	22.4		+9	
42	Alsatian	29	22	+31	+2	
43	Alsatian	30	22		-4	
44	Retriever	32.5	25		-17	-28
45	Doberman	33	22.5		+7	
46	Boxer	33	24			-3
47	Labrador	34	22		+17	
48	Alsatian	34	23		+13	+2
49	Labrador	39	25.2		+36	-11
50	Bull Mastiff	44.5	25.5		+21	
51	Labrador	47	22		+5	

Appendix 4

Effects of intravenous anaesthetics on mean arterial pressure in pigs

Thiopentone 10mg kg^{-1} — Mean arterial pressure (mmHg)

Pig No.	Pre-dose	Minutes after injection 1	2	3	4	5	20
74	83	65	93	128	121	115	80
12	84	62	89	85	84	83	
55	86	70	92	88	92	95	
43	119	87	126	125	123	120	129
3	84	70	87	87	87	87	82
4	94	80	118	108	91	91	91
5	87	64	88	89	90	86	90
6	95	74	93	94	93	95	93
Mean	92	72	98	101	98	97	94
± S.D.	±12	±8.6	±15	±17.6	±15.3	±13.7	±17.8

Thiopentone 20mg kg^{-1}

74	87	62	113	130	108	108	97
12	95	57	103	98	98	93	100
43	89	86	107	103	98	97	107
3	90	72	97	114	112	112	95
4	90	50	85	93	93	93	91
5	92	53	98	118	118	118	99
Mean	91	63	101	109	105	104	98
± S.D.	±2.7	±13.5	±9.6	±13.9	±9.7	±10.6	±5.4

Appendix 4 (cont'd)

'Althesin' 1.8mg kg^{-1} Mean arterial pressure (mmHg)

Pig. No.	Pre-dose	Minutes after injection 1	2	3	4	5	20
43	88	83	97	93	97	103	
12	86	76	89	89	91	97	
74	95	70	94	96	95	95	
55	97	84	113	103	105	102	
3	86	93	103	102	102	109	98
4	91	67	79	77	76	82	98
5	93	73	90	90	85	80	89
Mean ± S.D.	91 ±4.4	78 ±9.1	95 ±10.9	93 ±8.9	93 ±10.0	95 ±10.8	95 ±5.2

'Althesin' 3.6mg kg^{-1}

1	78	65	95	104	104	102	91
55	93	77	109	115	105	97	88
43	92	82	150	125	120	101	89
74	87	78	85	96	91	88	97
12	92	57	79	100	108	105	91
3	88	67	81	92	91	91	86
4	102	81	98	92	85	87	87
5	82	81	96	95	93	91	84
Mean ± S.D.	89 ±7.3	74 ±9.3	99 ±22.8	102 ±11.9	100 ±11.6	95 ±6.9	89 ±4.0

Appendix 4 (cont'd)

Disoprofol 2.5 mg kg^{-1} Mean arterial pressure (mmHg)

		Minutes after injection					
Pig No.	Pre-dose	1	2	3	4	5	20
55	91	87	86	82	81	83	85
43	102	77	97	90	91	96	99
12	86	61	145	142	98	87	70
74	89	68	83	83	83	83	94
3	93	77	86	87	88	89	88
4	88	55	77	80	81	80	
5	84	75	71	73	70	70	80
Mean ± S.D.	90 ±5.9	71 ±10.9	92 ±24.7	91 ±23.1	85 ±8.9	84 ±8.1	86 ±10.3

Disoprofol 5.0 mg kg^{-1}

55	85	75	96	99	93	90	92
74	87	53	67	85	87	87	86
12	87	58	67	93	97	96	80
43	97	80	81	89	92	93	107
3	89	53	85	90	91	91	85
4	101	66	71	82	91	94	86
5	89	65	73	75	80	82	83
Mean ± S.D.	91 ±5.9	64 ±10.5	77 ±10.8	88 ±7.8	90 ±5.4	90 ±4.7	88 ±9.0

Appendix 5

Effects of intravenous anaesthetics on heart rate in pigs

Thiopentone 10mg kg^{-1} Heart rate min^{-1}

		Minutes after injection					
Pig No.	Pre-dose	1	2	3	4	5	20
74	96	164	120	136	132	130	104
12	116	172	146	136	136	136	
55	124	196	180	196	200	184	
43	88	180	116	112	106	106	88
3	110	200	160	164	160	160	132
4	160	180	192	188	188	188	172
5	124	186	140	144	144	144	126
6	120	200	140	140	132	128	124
Mean	117	185	149	152	150	147	124
± S.D.	±21.7	±13.3	±26.8	±28.5	±31.3	±28.5	±28.5

Thiopentone 20mg kg^{-1}

74	104	160	180	138	138	140	132
12	98	162	184	172	164	150	114
43	116	180	148	144	140	138	116
3	148	200	216	212	208	208	172
4	140	188	184	184	180	180	164
5	136	186	170	198	196	196	168
Mean	124	179	180	175	171	169	144
± S.D.	±20.6	±15.6	±22.1	±29.4	±28.9	±30.1	±26.8

Appendix 5 (cont'd)

'Althesin' 1.8mg kg^{-1} Heart rate min^{-1}

		Minutes after injection					
Pig No.	Pre-dose	1	2	3	4	5	20
43	130	224	224	220	232	224	
12	144	200	220	220	220	200	
74	160	180	212	220	224	224	
55	148	224	212	208	212	220	
3	104	170	160	160	160	160	150
4	140	208	196	200	200	188	176
5	132	148	200				138
Mean ± S.D.	136 ±17.7	193 ±28.6	203 ±21.6	205 ±23.4	208 ±25.9	202 ±25.5	155 ±19.4

'Althesin' 3.6mg kg^{-1}

1	104	180	148	148		136	112
55	84	188	212	212	198	168	124
43	92	156	176	160	156	152	114
74	90	160	120	130	160	160	136
12	82	126	124	136	138	138	100
3	110	200	200	192	172	164	156
4	124	160	180	170	170	166	140
5	156	200	200	208	200	188	164
Mean ± S.D.	105 ±24.9	171 ±25.5	170 ±35.5	170 ±31.7	171 ±22.4	159 ±17.0	131 ±22.3

Appendix 5 (cont'd)

Disoprofol 2.5mg kg^{-1} Heart rate min^{-1}

		Minutes after injection					
Pig No.	Pre-dose	1	2	3	4	5	20
82	120			168		130	120
55	116	156	148	146	144	136	124
43	108	156	140	136	132	128	112
12	124	148	200	190	180	144	128
74	84	120	116	116	108	104	84
3	90	172	152	136	124	116	100
4	128	156	164	164	162	160	
5	134	164	154	156	152	144	132
Mean ± S.D.	112 ±19.1	153 ±16.4	153 ±25.5	151 ±23	143 ±24.2	133 ±17.5	114 ±17.1
Disoprofol 5.0mg kg^{-1}							
82	100	188	170	160	140	138	110
2	112	186	168	144	120	136	118
1	84		100	100	100	92	80
55	108	200	200	184	176	172	128
74	84	310	124	112	108	104	86
12	114	180	180	190	170	152	116
43						132	112
3	116	192	184	172	168	160	126
4	136	172	168	180	172	164	138
5	136	188	170	174	170	166	130
Mean ± S.D.	113 ±17.3	180 ±21.6	163 ±29.4	157 ±32.2	147 ±30.6	143 ±28.2	115 ±19.8

Appendix 6

Responses obtained in mini-pigs given a second administration of anaesthetic agents and their solvents 7 days after an initial exposure

Pig No.	Agent	Adverse clinical response (a)	Hypertensive response >50mmHg	Decrease in polymorphs >50%	Increase in plasma histamine >50%
312	Thiopentone	-	-	-	-
372	"	-	-	-	+
351	"	-	-	-	+
400	"	-	-	-	-
408	"	-	-	-	-
301	"	-	-	-	-
162	'Althesin'(b)	-	-	-	-
167	"	A,C,R	+	+	+
383	"	F,C,R	+	+	-
426	"	F,C,R	+	+	+
364	'Epontol'(c)	A,F,E,C	+	+	+
386	"	F,E,C	+	+	+
352	"	F,E,C	+	+	+
375	"	A,F,E,C	+	+	-
474	"	E,C	+	+	-
453	"	-	-	+	-

(a) Adverse clinical responses: A=Apnoea >30s; C=Cyanosis; R=Erythematous rash; F=Flush; E=Excitatory effects

(b) 'Althesin' contains alphaxalone/alphadolone in 20% Cremophor EL

(c) 'Epontol' contains propanidid in 20% Micellophor, a butanol extract of Cremophor EL

Appendix 6 (cont'd)

Pig No.	Agent	Adverse clinical response (a)	Hypertensive response 50mmHg	Decrease in polymorphs 50%	Increase in plasma histamine 50%
394	Alphaxalone/ alphadolone(c)	F,R	+	-	-
379	"	F	+	-	-
362	"	-	-	-	-
349	"	-	+	+	+
452	Propanidid(c)	-	-	-	-
482	"	-	-	-	-
522	"	-	-	-	-
543	"	-	-	-	-
250	Cremophor EL	-	+	+	-
254	"	V,E,C	+	+	+
318	"	V,E,C	+	+	+
329	"	V,E,C	+	+	+
366	"	E	+	+	+
397	Ethyl alcohol/ propylene glcyol	-	-	-	-
365	"	-	-	-	-
545	"	-	-	-	-

(a) Adverse clinical responses: F=Flush; R=Erythematous rash; V=Vomiting; E=Excitatory effects; C=Cyanosis

(c) Solubilized in ethyl alcohol and propylene glycol

Appendix 7

Sleeping times in pigs produced by two doses of anaesthetic agents given at an interval of 7 days

Thiopentone 10mg kg^{-1}	Sleeping time (min)	
Pig No.	Day 1	Day 8
312	14	14
372	8	14
351	12	11
400	7	4
408	13	10
301	6	9
Mean ± S.D.	10.0 ± 3.4	10.3 ± 3.7
'Althesin' 1.8mg kg^{-1}		
162	5	10
167	8	18
383	6	7
426	7	19
Mean ± S.D.	6.5 ± 1.2	13.5 ± 5.9
'Eptontol' 10mg kg^{-1}		
364	1.5	2.5
386	3.5	10
352	2.5	1.0
375	4	3.5
474	4	2
453	2	2
Mean ± S.D.	2.9 ± 1.1	3.5 ± 3.3

Appendix 7 (cont'd)

	Sleeping time (min)	
	Day 1	Day 8
Alphaxalone/Alphadolone 1.8mg kg^{-1}		
Pig No.		
394	8	12
379	10	13
362	5.5	4.5
349	5	13
Mean ± S.D.	7.1 ± 2.3	10.6 ± 4.1
Propanidid 10mg kg^{-1}		
Pig No.		
452	4	5
482	4	5
522	5	4
543	3	4
Mean ± S.D.	4.0 ± 0.81	4.5 ± 0.57