CLINICAL AND THEORETICAL STUDIES WITH THE OPIOID ANALGESIC FENTANYL

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CONTENTS

continued...

Compiled versions of the fentanyl and pethidine programs are enclosed in the back cover.

ERRATA

The symbol μ has been incorrectly used in place of μm as an abbreviation for micrometers throughout the text.

The words mucus (noun) and mucous (adjective) have been transposed in various places in chapter 12.

A formal summary is appended to the back of the thesis after page 284.

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PROLOGUE

This thesis concerns two investigations into the opioid analgesic fentanyl. The first is a clinical investigation into the safety and efficacy of fentanyl administered by inhalation. The second is a theoretical assessment of the feasibility and merits of applying an alternative form of pharmacokinetic model to the drug. The second project is linked chronologically and conceptually to the first in that it grew out of a general interest in the pharmacology of fentanyl developed during the first study and in that it provides a useful theoretical framework for considering the comparative pharmacokinetics of drug delivered intravenously and drug delivered for absorbtion from other sites.

The thesis is in two related but essentially self-contained parts preceded by a brief review of fentanyl pharmacology.

CHAPTER 1

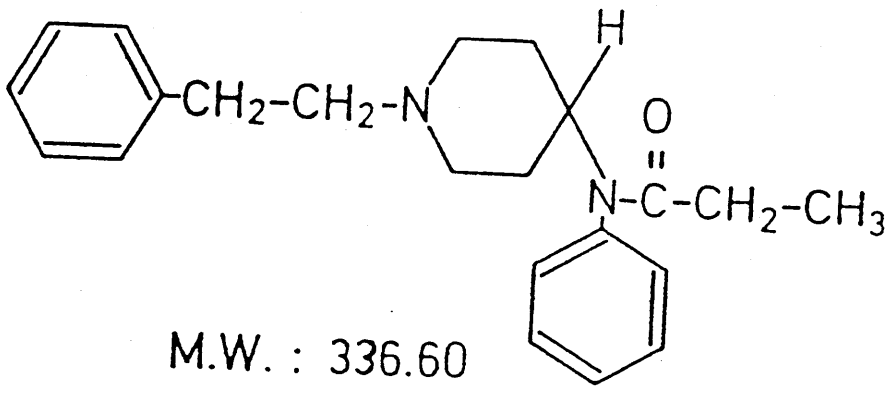
Introduction: Relevant Fentanyl Pharmacology

Fentanyl (figure 1.1) is a potent synthetic opioid which was introduced into clinical practice in the early 1960's. It is the chemical congener of the reversed ester of pethidine, and is the prototype of the 4-anilinopiperidine series of narcotic analgesics which include alfentanil, sufentanil, lofentanil and carfentanil. Until recently it has mainly been used by intravenous administration either as an adjuvant or main agent during anaesthesia where it has advantages of extreme antinociceptive potency (probably through agonist action at the mu opioid receptor), relative lack of cardiovascular side effects compared to older opioids and of not causing histamine release [35, 37]. These properties in addition to its high lipid solubility, which should enable it to equilibrate quickly across biological membranes, and lack of local tissue toxicity suggested to us that it might be a suitable drug for administration by nasal or pulmonary deposition. Other current, though not yet common, uses for the drug include the provision of post-operative pain relief by intravenous administration from patient controlled analgesia machines or by percutaneous absorption from specially prepared skin patches and the production of profound analgesia by administration into the subarachnoid and epidural spaces.

This chapter presents some relevant aspects of fentanyl pharmacology.

The pharmacology of fentanyl has been reviewed by Mather (pharmacokinetics only) [1], Bovill (pharmacodynamics only) [37], Andrews and Prys-Roberts [35] and Hug [36].

Figure 1.1



Fentanyl: N-(1-phenethyl-4-piperidyl) propionanilide

PHARMACOKINETICS

Oral Absorption

The bioavailability of oral Fentanyl is about one third that of intravenous Fentanyl reflecting its relatively high hepatic clearance [2].

Distribution in the Blood

This is discussed in more detail in chapter 13. At pH 7.4 fentanyl is about 80% bound to plasma proteins and this proportion is unaffected by plasma fentanyl concentrations within the clinical range [34, 155]. The blood : plasma concentration ratio is approximately 1 and is independent of pH and haematocrit [34, 157, 158]. Protein binding is decreased and comparative partitioning into red cells increased at low plasma protein concentrations [21, 34, 155, 158].

Distribution in the Body

Fentanyl is extremely lipid soluble, although there is considerable disagreement as to the exact value of standard solubility coefficients (see table 13.2 for comparison with morphine and methadone). On account of its high lipid solubility it equilibrates rapidly across biological membranes and across the blood brain barrier [26] and has a large volume of distribution (table 13.3) reflecting the relatively large mass of drug taken up by the tissues. More than 90% of an intravenous dose is lost from the blood within 2 minutes [38, 180].

The distribution of fentanyl in the body at any time will reflect both the relative blood flow to the various organs and

the avidity with which they take up fentanyl. There are no detailed studies in man. Matejczyk [43] reports fentanyl concentrations in liver and kidney tissue were 2.4 and 2.1 times the plasma concentration respectively at postmortem examination of a corpse following a fatal fentanyl overdose. The distribution of fentanyl to tissues and organs has been examined in rats and rabbits.

In the rabbit Hess and his colleagues [23] describe three groups of tissues. In the "central" group of tissues, containing lung, heart, brain, kidney and spleen, the maximum concentration of fentanyl occurred within half a minute of intravenous injection and then declined in parallel with the plasma concentration. In the second group; skeletal muscle, intestinal wall and liver, the maximum concentration of fentanyl occurred five minutes after injection. Finally,in fat the maximum concentration of fentanyl occurred half an hour after an intravenous bolus. (See figure 15.10). All tissue concentrations were greater than the plasma concentration after the first few minutes. The highest concentration of fentanyl at any time was in the lungs (100 times plasma concentration during the first hour). Brain concentration was approximately ten times plasma concentration.

In the rat Hug and Murphy [33] found a slightly different relative distribution although maximum drug concentrations occurred in the three tissue groups at the same times as the rabbit. Concentration maxima in kidney and liver occurred at 5 and 15 minutes respectively but this was ascribed to the artifactual measurement of metabolites as unchanged fentanyl and both organs should probably be grouped with the "central" group of tissues along with lung etc. Initially the highest

concentration was in lung but after the first 30 minutes or so the highest concentration was to be found in fat. Concentration in brain was 2 to 3 times plasma concentration, lung 10 times plasma concentration and fat 35 times plasma concentration.

Lung Sequestration

Of particular relevance to the present investigation is the observation that most of an intravenous injection of fentanyl is sequestered in the lungs after the first pulmonary pass. This fentanyl diffuses back into the circulation over the next few minutes (There is no evidence for any significant pulmonary metabolism of fentanyl). The phenomenon has been investigated in rabbits [23] and man [24, 25]. Roerig suggests that the mechanism of uptake by the lung is likely to be diffusion into non specific sites. The phenomenon will increase the concentration differences between arterial and mixed venous blood in the first few minutes after fentanyl injection and also smooth out the sharp high peak that would otherwise occur in the concentration of fentanyl in blood entering the brain immediately after intravenous injection. The subject is discussed at greater length in chapter 15.

Pharmacokinetic Constants

There are several published pharmacokinetic studies of intravenously administered fentanyl in healthy volunteers and in assorted patients undergoing surgery and anaesthesia. Most investigators have described the kinetics in terms of standard two or three compartment models. Experimental values of the pharmacokinetic constants for fentanyl are given in table 13.3. There is considerable inconsistency between the values reported

in different studies. The reasons for this are not clear. Similar studies on newer derivatives such as alfentanil have apparently [1] produced much more consistent results which suggests a particular problem with fentanyl rather than a general problem with study design. Mather [1] has suggested that the difficulty may be caused by inaccuracies or lack of specificity in fentanyl analysis at the low concentrations involved but this seems unlikely to be the sole cause. The subject is discussed further in relation to plasma clearance in chapter 13.

When fentanyl was first used clinically it was thought to have a short duration of action and this is indeed the case with single small (up to say 300 µg per adult man) doses of fentanyl. However when tissue distribution (see above) and pharmacokinetic studies became available it was clear that the initial fall in plasma concentration and consequent termination of pharmacological effect was due to redistribution of drug to the tissues. When fentanyl is administered by large dose, repeated doses or continuously, the plasma concentration may still be in the effective range after redistribution and further decrease is dependent on the slower process of metabolic clearance. This pharmacokinetic profile makes intuitively judging the consequences of a given dose regimen difficult and unfortunately the lack of agreement on the values of standard pharmacokinetic parameters diminishes the predictive power of pharmacokinetic models. Reilly and colleagues [20] say in this context "The best that pharmacokinetics can do ...is to say that following a 500 µg bolus the patient's plasma fentanyl concentration will fall below analgesic concentrations somewhere between 13 minutes and

6 hours later."

Secondary Peaks

After the administration of a large dose of fentanyl, during the period of falling plasma concentrations, the plasma concentration may spontaneously rise again before continuing to fall [16, 21, 27]. The cause of this fluctuation is not known. Release of fentanyl from muscle, stomach or lungs have been suggested as possible causes. These secondary rises can be associated with respiratory depression appropriate to the higher concentrations of fentanyl [16].

Clearance and Elimination

Clearance is mainly by metabolism in the liver to hydrophilic metabolites which are excreted by the kidney. Small amounts of fentanyl are excreted unchanged in the urine.

Man: McClain and Hug [21] used tritiated fentanyl to study fentanyl kinetics in normal male volunteers. They recovered 6.4% of the total dose unchanged in the urine over 72 h and a further 70% of the dose in the urine as metabolites. 1.2% of the total dose was excreted unchanged in the faeces with a further 8% present as metabolites (total recovery 85%).

There are few other studies which specifically examine the urinary excretion of fentanyl. In five patients with severe cardiovascular disease undergoing cardiac surgery on by-pass Bovill and Sebel [38] found only 2.1% of a large dose of fentanyl administered at the start of the anaesthetic was excreted unchanged in the urine by 24 hours. Fentanyl excretion increased and decreased with the rate of urine production. Large volumes of urine were produced by these patients but kidney

function may well have been abnormal as a result of the invasive procedures. Schleimer and his colleagues [179] found about 17% of a fentanyl dose excreted unchanged in the urine of three non-cardiac surgical patients by 50 h. However they used an early fentanyl radioimmunoassay (see p 163) which might well have had cross sensitivity to metabolites. Hess and colleagues [40] estimated unchanged fentanyl as a proportion of unchanged fentanyl plus metabolites in samples of urine from three subjects at various times after an intravenous dose (all samples less than 10% unchanged fentanyl in two subjects, 12 to 25% in the third) but they made no cumulative measurements. Corall and colleagues [39] studied plasma fentanyl concentrations in ten patients with end stage renal disease. Plasma levels of fentanyl decreased faster in the renal patients than normal controls in the first 4 h after an intravenous bolus dose suggesting that renal function is not necessary for clearance. There are no studies which examine the effect of urine pH on urinary fentanyl excretion.

Animals: In the dog Murphy and colleagues [22] using tritiated fentanyl, found 4% of an intravenous dose unchanged in the urine after 6 h compared with 32% as metabolites. The clearance of fentanyl in hepatectomised dogs was markedly reduced [41] but small amounts of metabolites were still excreted in the urine suggesting an extrahepatic site for biotransformation.

In the rabbit [23] the appearance of metabolites in the plasma within minutes after injection is prevented by evisceration (functional hepatectomy).

Bullingham and colleagues [42] studied fentanyl clearance directly in the chronically cannulated cow and found the hepatic

extraction ratio to be 1 (i.e. clearance equal to liver blood flow) at fentanyl concentrations in portal vein plasma above about 7 ng ml^{-1} although oddly it decreased with concentration at concentrations less than this.

PHARMACODYNAMICS

Anti-Nociception

The distinction sometimes drawn between analgesic and anaesthetic effects of opioids is rather arbitrary and vague depending on the context of the study and the parameters chosen for measurement as much as the underlying phenomena. The concepts of analgesia and anaesthesia themselves are difficult to define in objective physiological terms. Strictly, analgesia might refer to a diminution of pain in the absence of other cognitive effects. There are difficulties with this however when the complexities of pain perception and the relations of associated phenomena such as autonomic or hormonal responses are considered. In practice opioids, including fentanyl, manifest a range of effects from , at low doses, euphoria, perceptual detachment, and diminished pain awareness in conscious patients to, at high doses, a profound "anaesthetic" unresponsiveness and amnesia. The term analgesia is used below with its everyday meaning of pain reduction in a conscious patient.

Studies During Anaesthesia: Fentanyl is widely used during anaesthesia, usually with other drugs but sometimes alone. Doses vary from under 1 µg kg^{-1} to over 150 µg kg^{-1}. However, despite many studies, there is not enough information available to draw a clear pharmacodynamic picture. One problem in interpreting the

data is the fact that plasma concentrations are often rapidly changing during the investigations. Thus plasma concentrations do not have time to equilibrate with concentrations at receptor sites in the central nervous system. Scott and colleagues [31] suggested a mean half-time of 6.4 minutes for equilibration between plasma and an effect compartment when effect was measured by EEG changes. There are no systematic studies where plasma concentrations have been held steady above about 30 ng ml^{-1}.

Another problem is the difficulty, worsened by the poorly characterised pharmacokinetics, of devising dosage regimens which will produce a given plasma concentration. There is a wide scatter of plasma fentanyl concentrations within each dose group in most studies. This compounds a situation in which stimulation is variable and unquantifiable, subjects have usually received several drugs and there may be other physiological perturbations such as those introduced by cardiopulmonary by-pass. Drug effects and study events are sometimes poorly defined in physiological terms.

Despite this, there is evidence for various effects occurring in a concentration-related manner over a range of plasma fentanyl concentrations from about 5 ng ml^{-1} to over 20 ng ml^{-1}. Thus, "awakening" after anaesthesia is reported at mean concentrations of 6.4 ng ml^{-1} [6] and 8.6 ng ml^{-1} [32]. (In another study [3] onset of unconsciousness occurred at a mean concentration of 34 ng ml^{-1} but plasma concentration had been rising rapidly at this time). Several investigations are consistent with better cardiovascular stability during surgery (i.e. with better "anaesthesia") as plasma fentanyl concentrations rise from below

10 ng ml^{-1} to over 20 ng ml^{-1} [e.g. 4, 28, 29]. Hug and Moldenhauer [5] reported eye opening and movement during surgery in all of their 18 patients whose plasma fentanyl concentrations fell below 10 ng ml^{-1} during coronary artery by-pass surgery, whereas concentrations above 17ng ml^{-1} suppressed responsiveness in all patients.

It is not clear whether the antinociceptive or cognitive effects of fentanyl increase at plasma concentrations much above 20 ng ml^{-1} or whether indeed there is a concentration which will completely prevent the effects of noxious stimulation in all patients. Cardiovascular responses to skin incision and tracheal intubation are seen in some patients at plasma concentrations of over 30 ng ml^{-1} [4, 28]. There is evidence in dogs that fentanyl receptors become saturated at plasma concentrations of about 30 ng ml^{-1} [7, 8]. It is tempting to speculate that a similar thing happens in man but there is no clear evidence for this.

Analgesia: Difficulties of establishing an "analgesic blood concentration" of fentanyl or any other drug in a clinical setting include the variability of the pain stimulus, the variability of the pain response to that stimulus and the virtual impossibility of measuring either. Nevertheless attempts have been made to relate plasma concentrations of fentanyl to the extent of post-operative pain relief.

One approach is give the fentanyl according to fixed dosage regimens and to estimate the analgesia obtained either from additional opiate requirement or by subjective scoring systems or both [9, 10, 11]. Fairly constant plasma fentanyl concentrations of between 1 and 3 ng ml^{-1} were associated with a

reduction in expected morphine requirements in the first 24 hours following major orthopaedic, upper abdominal, prolonged or cardiac surgery (45 patients) [10]. Similarly, a mean concentration of 1.79 ng ml^{-1} (s.d. 0.67) in 8 post hysterectomy patients was associated with a diminished requirement for morphine and lower subjective pain ratings than a control group. A lower dose giving a mean fentanyl concentration of 0.56 ng ml^{-1} (s.d. 0.25) had a lesser but still demonstrable effect [11].

A second, perhaps more elegant, approach is to measure the concentrations obtained when the subject uses an intravenous patient controlled analgesia system. These allow, within certain preset limits, each patient to self-administer a small intravenous bolus of drug whenever they wish, thereby continually adjusting drug effect to the required level [12, 13, 14]. The limitations of this approach reflect the intrinsic limitations of the patient controlled analgesia systems. Thus, for instance, some changes in pain stimulus intensity, as during coughing or movement, may be too rapid to follow, or the end point of drug titration may represent a balance between analgesia and unwanted effects such as nausea, dysphoria or sleepiness. Minimal analgesic concentrations (the plasma concentration at the time the subject feels it necessary to command another bolus) have been reported as: median 1.2 ng ml^{-1} (range 0.2 to 8 ng ml^{-1}) [13], range 0.44 to 3.44ng ml^{-1} [14]) and mean 0.63ng ml^{-1} (range 0.23 to 1.18ng ml^{-1}) [12]. There is considerable variation between these values in different patients but much less variation between the values obtained at different times in any one patient.

In a variation of this approach, Cartwright and his colleagues

[15] titrated intravenous fentanyl into patients immediately they had woken from a fentanyl and nitrous oxide anaesthetic. Mean plasma concentrations from 2.3ng ml^{-1} to 3.2ng ml^{-1} were obtained for four small groups of patients but a description of the precise endpoint used to determine satisfactory analgesia was not given.

Respiratory Depression:

The ventilatory depressant effect of fentanyl is related to the plasma concentration [15, 16, 17, 18]. Slight respiratory depression, demonstrable by changes in the ventilation-carbon dioxide response curve, occurs in volunteers at plasma concentrations below 1 ng ml^{-1} [16], well within the range required for post-operative analgesia. A 50% decrease in the slope of the carbon dioxide response curve has been reported at plasma concentrations of 4.6 ng ml^{-1} in volunteers [17] and between 2.0 and 3.1 ng ml^{-1} in post operative patients [15]. Arterial carbon dioxide tension and minute ventilation can be expected to remain normal until the gradient of the carbon dioxide response curve has decreased by more than 50% [9, 15]. The presence of additional drugs may decrease respiratory drive still further as may other factors such as previous hyperventilation during anaesthesia [15]. The phenomenon of recurrent respiratory depression following apparent recovery from the effects of a single bolus of fentanyl may be associated with an increase in plasma concentrations of fentanyl occurring at the time [16].

PART 1

FENTANYL BY INHALATION

CHAPTER 2

Rationale and Aims

The body may be regarded as an organised mass of tissues bounded by various surfaces, including the invaginated or "internal" surfaces such as the alveolar membranes or intestinal mucosa. With the exceptions of injection and implantation all drug access to the body must take place by absorption across these surfaces, usually by concentration-driven passive diffusion. Problems of drug administration concern the relationship between the drug (lipid solubility, concentration etc.) and the local environment (pH, mucous barrier, epithelial type, blood supply, local metabolism etc.) Drug must be delivered to the absorptive surface and remain in contact with it long enough for the required absorption to take place.

Historically means of getting drugs into the body have grown from tradition and usage. Cultural and psychological ties have often bound a particular drug preparation to a method of delivery as strongly as considerations of efficacy. However, with the rise of modern pharmacology a more rational approach to the manipulation of drug administration has emerged.

Alongside interest in improved methods of drug delivery through the gut mucosa such as slow release preparations, pro-drugs etc. and the utilisation of the skin as a route of absorption for systemic drugs, there is growing interest in the respiratory tract, although of course the idea of using this region for drug delivery is not new. There are several considerations favourable to the idea of drug administration by this route. The local environment in the respiratory tract is biochemically less hostile towards drugs than that in the gut. Confounding factors such as the presence of food or drink and variations in gut motility are avoided. There is no first pass liver metabolism.

The blood supply to the lower respiratory tract comprises the whole cardiac output and is separated from the vast external surface by a fine barrier in an anatomical arrangement specialised for the transfer of small molecules. The mucosa of the upper respiratory tract (mouth, nose and pharynx) has a rich and reliable blood supply from the systemic circulation. Together these points suggest the possibility of rapid and predictable entry of drugs.

The upper membranes of the respiratory tract, those of the nose, mouth and pharynx, are readily accessible but have a relatively small area and it may be difficult to maintain drug in contact with the mucosa to provide a gradient for absorption, especially in the mouth. Several modern and traditional drug preparations are designed for absorption via the buccal or nasal routes. The popularity of the nasal route is growing rapidly [122]. Drugs targeted on the respiratory epithelium will also be partly deposited on and absorbed from the upper respiratory tract because of imperfections in present delivery techniques.

One of the main difficulties in using the epithelium of the lower respiratory tract is delivery of drug to the terminal airways. This is easiest for volatile substances and gas es. The method of quantifiable, controlled administration of sytemically active gas es and vapours has been highly refined in the practice of modern anaesthesia. Delivery is more difficult for non volatile drugs. The traditional answer has been to heat or burn them and breath the resultant smoke as in the smoking of tobacco, opium or cannabis. This method has been ignored by mainstream medicine in modern times, although it has been applied by social drug users to highly refined pharmacological

products such as cocaine base and diacetyl morphine. The drug smoke contains an aerosol of the active ingredients and the pharmacokinetic effects of its inhalation may resemble intravenous injection [120, 123]. A second method is mechanical aerosolisation, usually of a drug-containing solution but sometimes of a dry powder. This technique was originally developed for the local application of respiratory drugs but has recently been applied as a means of systemic administration [e.g. 121].

The Present Investigation

Intravenous administration remains the "gold standard" for the systemic administration of opioid analgesic drugs in that there is 100% bioavailability of the administered dose and almost instant control over the direction in which plasma concentrations are changing. Many patients dislike injections but the main practical disadvantage is the expertise which is required either for each separate administration or for the insertion of an indwelling cannula. Patient controlled analgesia devices allow patients to administer their own intravenous analgesia. These are becoming more portable but must nevertheless be linked to the patient by an indwelling intravenous catheter.

The respiratory route may possibly permit some of these problems to be overcome in that respiratory administration might approximate the pharmacokinetic advantages of intravenous administration while being potentially pleasant and having no requirement for technical expertise or for an invasive physical connection to the patient. A patient controlled analgesia device based on an inhaler for example would not have to accompany a

post-operative patient to the bathroom or could be carried in a handbag to allow a mobile patient with cancer pain instant analgesia for breakthrough pain.

There has been recent interest in the absorption of opioids from the upper respiratory tract [e.g. 107, 108] but very few studies on the clinical administration of opioid analgesic drugs by absorption from the lungs: Chrubashick and colleagues [121, 124] have investigated the effects and bioavailability of inhaled nebulised morphine.

We wanted to further investigate the use of the pulmonary route for the clinical administration of opioid analgesics.

As outlined in chapter 5 we chose fentanyl as it is very potent (and therefore needs only a small mass of drug to be delivered), it is rapidly acting in that there is only a small lag between the time course between changes in plasma concentration and effect (compared with morphine for example), it does not cause histamine release, there is no evidence it causes local tissue irritation and it is relatively free of cardiovascular side effects. Nebulisation of an aqueous solution was chosen as the method of delivery as it is at present the only available method of targeting the respiratory epithelium in a clinical setting.

The work described in part one of this thesis thus concerns an investigation into the safety and efficacy of inhaled nebulised fentanyl solution. The following two chapter review some theoretical aspects of aerosol formation and deposition and of pain measurement. The remainder is a report of the clinical study.

CHAPTER 3

Aspects of Nebulisation and Aerosol Deposition

Therapeutics aerosols are primarily used to deliver drug to the respiratory tract. They are the only practical means of delivering some drugs to the distal airways but also provide an effective and convenient means of distributing drug over the surface of the upper respiratory tract, particularly the nasal membranes. The physical characteristics of the aerosol in combination with structure and physiology of the respiratory tract will determine where the drug is deposited. This chapter comprises a review of aspects of aerosol characterisation, formation by nebulisation of solutions and deposition, with a particular emphasis on the functioning of jet nebulisers.

CHARACTERISATION AND DEPOSITION OF AEROSOLS

Definition:

An aerosol is an airborne dispersion of particles.

Characterisation

The following account has been taken largely from the reviews by Brain and Valberg [59], Morrow [66] and Lipmann [62].

One convenient way to characterise an aerosol is by the frequency distribution of the aerodynamic diameters of its particles. The aerodynamic diameter is the diameter of a unit density sphere having the same settling velocity as the particle in question. It is a device to allow easy description of the behaviour of particles of different shapes. Many therapeutic aerosols are made up of spherical liquid droplets so the actual diameters of their particles bear a simple relationship to the aerodynamic diameters. Other variables such as mass can be described in terms of aerodynamic diameter. For instance, the mass median aerodynamic diameter is that aerodynamic diameter

for which half the mass of the aerosol lies in smaller particles.

Depending on the variation of particle size and shape, aerosols may be described as monodisperse or heterodisperse. Strictly speaking the particles of a monodisperse aerosol should all be of the same size and shape but in fact the difference is one of degree and monodisperse aerosols are defined to arbitrary tolerance [e.g. 61].

Aerosols of biological interest, including therapeutic aerosols are almost always heterodisperse and often show a log-normal distribution of their aerodynamic diameters. That is a plot of the number of particles of given size interval versus the logarithm of size is a bell-shaped normal distribution curve. A given example of such a distribution can be described by two numbers, the count median diameter and the geometric standard deviation (which corresponds to the standard deviation of a normal distribution).

Other properties, such as mass, which are a function of particle aerodynamic diameter will also be log normally distributed and will have the same geometric standard deviation. The median diameters for these variables will be related to the count median diameter in a predictable way for example:

$$\ln(MMD) = \ln(CMD) + 3(\ln GSD)^2 \qquad (3.1)$$

where MMD is the mass median diameter, CMD is the count median diameter, ln is the natural logarithm and GSD is the geometric standard deviation. (Notice the mass median diameter is larger than the count median diameter).

Deposition

There is a large body of work, experimental and theoretical, on the deposition of inhaled aerosols [see 59, 62, 66, 122]. Particles are deposited when their motion brings them into contact with a surface. Any forces on a particle which give it a velocity relative to the gas stream in which it is suspended may do this. Brain and Valberg [59] describe four effects of importance in respiratory deposition: inertial impaction which occurs when there is a sudden change in direction or speed of the gas stream , sedimentation owing to gravity, diffusion or Brownian motion, and electrical attraction. In addition particles with the same velocity as the surrounding gas stream may be deposited should it happen that this gas becomes adjacent to a surface (interception). This last effect is small in the lungs since the volume of gas within a small (2 μ) particle size of any surface is small (140 ml) compared with the total lung volume (5 l). The role of electrical attraction may be to cause deposition of particles that are brought very close to a surface.

The main factor determining whether and where particles will be deposited is aerodynamic diameter. Because of differences in airflow in its different parts the respiratory system acts as a filter with larger particles being deposited more proximally.

In the nasopharynx airflow velocities are high, there are frequent changes of airstream direction and larger particles are deposited by inertial impaction. Small particles are less susceptible to inertial impaction. In the distal airways flow velocities are progressively smaller and deposition occurs because of sedimentation or for very small particles diffusion.

Most particles bigger than 5 μ are deposited in the nasopharynx. Particles of less than 0.1 μ are mainly deposited in the terminal airways and alveoli. About half the deposition of particles around 1 to 2 μ occurs in the respiratory bronchi and alveoli. Total deposition of particles (i.e. the ratio particles deposited:particles inhaled) is almost 100% for particles larger than 10 μ, reaches a minimum of 20 to 40 % for particles of 0.5 μ and increases markedly for particles less than 0.1 μ. The minimum occurs because particles with an aerodynamic diameter of around 0.5 μ are too small to be greatly affected by inertial impaction and sedimentation but too large for Brownian motion to be important.

Breathing pattern has a marked effect on deposition [see 59] and accounts for an important amount of inter-subject variation. Mouth breathing increases the penetration of larger particles distally. Deposition of 3 μ particles in the alveoli and respiratory bronchioles may reach 50% of the inhaled load during quiet mouthbreathing [62].

Much of the basic work on the deposition of aerosols in the human respiratory tract has been carried out using non-hygroscopic aerosols in relation to toxicity studies and the methods and results extended to cover therapeutic aerosols. Non-hygroscopic aerosols are easier to work with than hygroscopic aerosols as once produced the particles do not normally change size. Therapeutic aerosols however, being aqueous solutions or water soluble solids, are hygroscopic and the particles will change size by the absorption or evaporation of water depending on the relative humidity [60, 66]. This means that, in addition to the initial aerodynamic diameter, the osmotic composition of

the particles will affect the pattern of deposition since it is a major determinant of how particles change size in the respiratory tract. Despite work addressing these problems [e.g. 63, 66, 111], there are still difficulties in describing how a given aerosol will behave, for instance, the exact relative humidities of the different regions of the respiratory tract are not known [66]. The size profile of a hygroscopic aerosol will change between generation and inhalation making specification difficult. There is a relative lack of the kind of careful quantitative experimental studies that have been performed with nonhygroscopic aerosols. The general principles of aerosol deposition still apply however and other things being equal smaller particles will be deposited more distally in the respiratory tract [e.g. 64].

NEBULISERS

Definitions

A nebuliser is a device for converting a liquid into an aerosol. There are two types of nebuliser in clinical use, ultrasonic nebulisers, and jet nebulisers.

Strictly speaking, metered-dose inhalers are also nebulisers (although they sometimes aerosolise a dry powder) but in every day usage they are usually called "inhalers".

Another term occasionally encountered is "atomiser". An atomiser is a simple spray device distinguished from a nebuliser by the large size (>10 μ) of the particles it produces.

Small plastic disposable jet nebulisers are the most commonly used nebulisers in the United Kingdom and are freely available in most hospitals. Jet nebulisers are discussed in detail below.

Ultrasonic nebulisers are less commonly used, mainly because of the difficulties they pose with regard to cleaning and sterilisation. The mechanism of aerosol production and the factors governing output differ from those operating in jet nebulisers. For the purposes of the present study ultrasound nebulisers do not offer any clear advantages in terms of aerosol output or character that would justify overcoming the practical difficulties associated with their use [49, 52] and they will not be discussed further.

Jet Nebulisers

Jet nebulisers employ the Bernoulli effect whereby a flow of gas through a nozzle creates a region of subatmospheric pressure. This causes liquid to flow along a capillary tube from a reservoir into the gas stream. Shearing forces at the interface between the gas and the liquid break the liquid into droplets. The turbulence of the gas flow disperses the droplets throughout the gas stream. A small amount of energy is required for the processes of deforming the liquid and forming new surface, (overcoming surface tension and viscous forces). A larger amount is required to accelerate the particles to the velocity of the gas. Energy is supplied from the kinetic energy of the gas. The diameter of a droplet of average mass in such an aerosol is proportional to

$$D_L.(2T/(Pa.\ V^2.\ D_L))^{4.5}$$

where D_L is the diameter of the liquid flow tube, V is the relative velocity of the gas and the liquid, T is the liquid surface tension, Pa is the density of the gas [54].

Droplet size decreases as the gas velocity increases up to sonic

velocity where the Venturi effect fails. Droplet size increases with increasing viscosity or surface tension.

The main resistance to gas flow through the nebuliser occurs at the gas nozzle so the diameter of the nozzle largely determines the pressure difference that must be applied across the nebuliser to produce a given gas flow through it. For a given gas flow the smaller the nozzle the greater the velocity of the gas flow through it and the smaller the droplet size [53, 55].

This process produces an aerosol with droplets of relatively large average size compared to that of the final aerosol emitted from the nebuliser, however a baffle (see figure 3.1) is sited in the gas stream close to the nozzle, and larger droplets (most of the primary aerosol) deposit on this because of inertial impaction, coagulate and run back into the reservoir. Smaller droplets are carried out of the nebuliser without impacting.

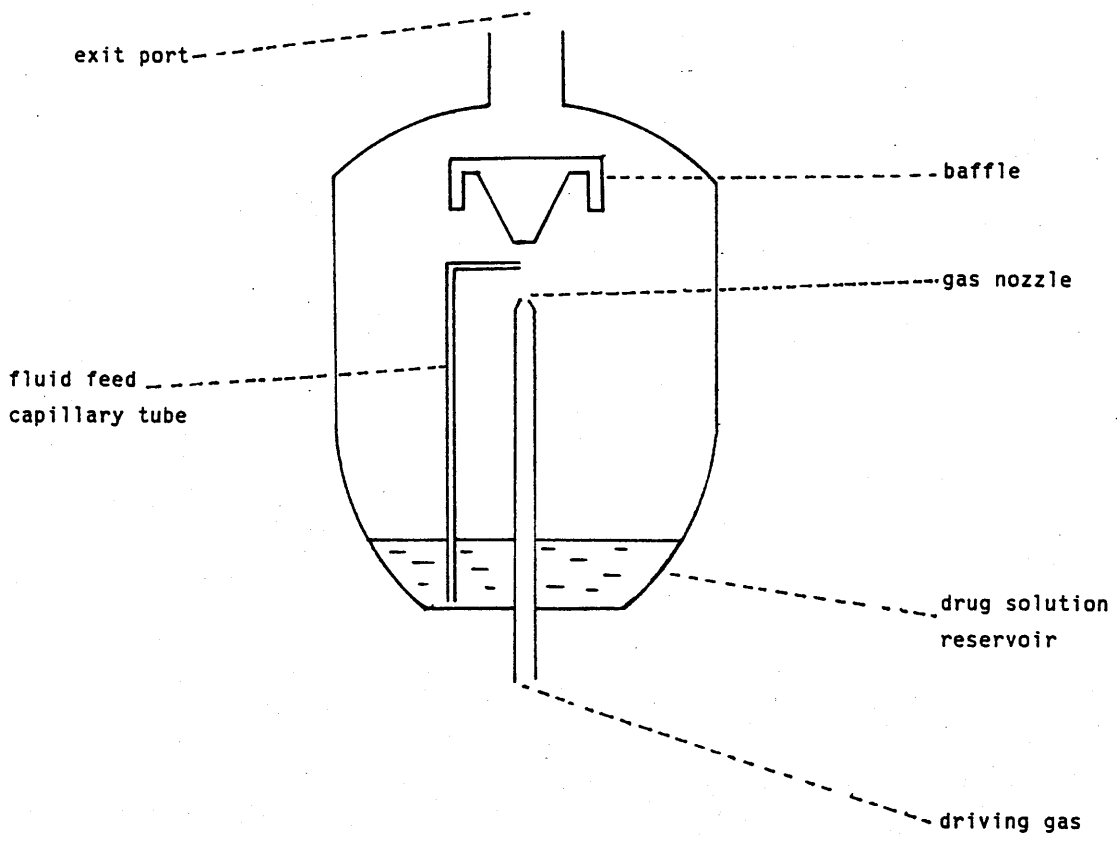
Nebulisers are usually made of glass or plastic and may be sterilisable or disposable. Some models have an adjustable vent drawing in room air by venturi effect to dilute the aerosol output. In practice aerosol production by jet nebulisers is affected by the following:

Make: The design of the nozzle, baffle, capillary tube and outflow path are important in determining the amount and size distribution of the output. There are differences between models [44, 46, 49] and between examples of the same model [49]

Filling Volume: A typical disposable nebuliser reservoir capacity is 10 ml, however, during nebulisation, when the volume of liquid falls to a certain low amount, which varies with the model but may be 2 ml or more, the reservoir end of the

Figure 3.1

Stylised Diagram of a Jet Nebuliser



In many common models, including the Lifecare Micro-Neb, the driving gas tube and fluid feed tube are concentric.

capillary tube is no longer continuously submerged but alternately exposed and submerged as large coagulated droplets from the baffle and inner surfaces run back into the bottom of the reservoir. The tube thus intermittently entrains gas rather than liquid and nebulisation becomes increasingly sporadic as time goes on. With some models the onset of this effect may occur at a higher residual volume if the nebuliser is not completely vertical. The efficiency of nebulisation during this stage may be improved by agitating or tapping the device in order to hasten the run back of drops to the reservoir [45]. Nebulisation of a given dose of drug is ended arbitrarily at a point when the process has become too inefficient to be worth continuing [44, 45]. At this point there will still be some liquid remaining in the nebuliser mostly clinging as large drops to the inside surface. This may be almost as much as 2 ml [44].

Driving Gas Flow: When there are several ml of fluid in the nebuliser nebulisation occurs continuously at a rate which increases with driving gas flow [44, 49]. Increasing the driving gas flow decreases the mass median diameter of the aerosol, a typical figure being a reduction in mass median diameter of 50% for a doubling of flow from 4 to 8 l min^{-1} [46]. Aerosols produced at these higher flows may not have a log normal distribution owing to droplet disintegration and coagulation. However they still have a larger proportion of smaller particles than aerosols produced at lower flows. Because they may not be strictly log normal therapeutic aerosols are sometimes described in terms of the proportion of particles below 5 μ rather than by log normal parameters [46]. Using a high flow may result in a smaller mass of solute (see below) being left in the nebuliser

at the end of nebulisation [52].

As flow increases above about 10 l min^{-1}, which is the upper end of the range for most studies and for present clinical use, the graph of aerosol output against driving gas flow rate appears to flatten [110]. The phenomenon has not been studied in detail but it implies that as gas flows increase above (roughly) this value the aerosol output becomes less concentrated (total aerosol mass production rate is still increased). This in turn has implications for therapeutic aerosol delivery since 10 l min^{-1} is less than normal peak inspiratory flow and mixing of aerosol with room air will occur during inhalation. There will be an interrelationship between respiratory pattern, aerosol flow rate and inhaled aerosol concentration which will determine how much aerosol is taken in by the subject. Despite work on the optimal respiratory pattern for pulmonary deposition this particular aspect seems not to have been examined.

Variable orifice rotameters are often used to measure and control flow through nebulisers, especially in clinical work. Errors in flow measurement occur when non pressure-compensated rotameters (i.e. those with the needle valve upstream of the flow measuring chamber) are used in conjunction with high resistance devices like nebulisers since the pressure in the rotameter chamber is raised to a value which depends on the size of the down stream resistance [55, 56]. If the space around the bobbin behaves like an orifice indicated flow (Fi) may be corrected to actual flow at atmospheric pressure (Fa) by:

$$Fa = Fi. \sqrt{(Pf/Pa)} \qquad (3.2)$$

where Pf is the pressure at the rotameter bobbin and Pa is

atmospheric pressure [57].

Unfortunately one cannot make this assumption when the bobbin is at the lower end of the tube [56, 58]. To give accurate flows throughout its range the rotameter scale must be individually calibrated to the working pressure.

Many published studies on the effects of gas flow through nebulisers fail to specify the exact method used to measure flow [e.g. 44, 46, 48].

Driving gas Pressure: The size of the internal nozzle varies between models and sometimes between nebulisers of the same model [53, 65]. This is reflected in the driving pressure required to produce a given gas flow and in droplet size.

In one study [49] driving pressures of 47 kPa and 75.5 kPa were required to provide a flow of 6 l min^{-1} through two different nebulisers. These were associated with aerosol volume mean aerodynamic diameters of 3.7 μ in the low pressure nebuliser and 2.6 μ in the higher.

The nature of the Solution: Different solutions may have different surface tensions and viscosities which may contribute to measurable differences in aerosol output. For instance changing the concentration of histamine acid phosphate from 0.5 to 32.0 mg ml^{-1} resulted in a fall in estimated primary (i.e. at the moment of formation) volume median aerodynamic diameter from 3.8 to 2.3 μ [50]. The differences between most commonly used clinical solutions are slight [46, 110], but relatively viscous solutions such as antibiotics may differ markedly from the rest [48].

Evaporation Effects: Gas leaving the nebuliser is saturated with water (or other solvent) vapour derived partly from the nebulised particles but mainly from the large area of liquid spread as films and droplets over the inner surface. Water therefore leaves the nebuliser both in the aerosolised particles and as vapour. Since solute only leaves in the nebules this means the concentration of the residual solution is continually increasing [50, 51, 52, 65]. Output of solution as determined by weighing the nebuliser or measuring residual volume is thus not equivalent to solute output and the rate of solute output changes as nebulisation progresses. The discrepancy between solute and solvent output increases as nebulisation goes on [51, 52] because evaporation continues unabated when aerosol output becomes intermittent. This is reflected in the data from studies designed to quantify the phenomenon. One group [50] found an increase in saline concentration in the residuum of a 4 ml fill from 8.8 to 9.2 mg l^{-1} after 2 min nebulisation at a gas flow of 8 l min^{-1}. Another group [52] using a different nebuliser found an increase in saline concentration from 70 mg ml^{-1} to 185 mg ml^{-1} when 4 ml of solution were nebulised over a much longer period to a residual volume of less than 1 ml by a gas flow of 6 l min^{-1}.

The proportional increase in concentration (concentration at time t / initial concentration) after a given time of nebulisation will depend on the filling volume, other things being equal. It is greater for low filling volumes [51] because the amount of water vapour leaving the nebuliser per unit time is independent of filling volume, being dependent only on the flow rate and saturation of the incoming gas.

Humidifying the driving gas lessens the effects of evaporation [51].

Nebulisers cool down over the first few minutes of use until an equilibrium is reached whereby heat entering the device down the temperature gradient from the surroundings is equal to the latent heat of vaporisation of the evaporating solvent. There is an initial fall in aerosol output corresponding to this cooling phase. This has been attributed to a temperature dependent increase in surface tension [44, 53].

Extension devices: Any extension device added to the nebuliser outlet will collect some droplets depending on its shape and size. These will mainly be larger droplets deposited by inertial impaction or sedimentation. This will shift the size distribution of the escaping aerosol towards the smaller end of the scale. The total mass of aerosol will be reduced. The longer the extension the more aerosol deposited [49].

In conclusion it must be said that interpretation of published work on the effects of such interrelated factors is often difficult because of varying experimental design and because the complete set of relevant information is often not given. For experimental work such as drug dose comparisons the problems of intermittent nebulisation can be avoided and the inaccuracies owing to evaporation lessened if nebulisers are filled with several ml of liquid and the last two ml not used.

CHAPTER 4

A Digression on the Measurement of Pain by Linear Visual Analogue

Pain like any other subjective psychological process may be regarded as a cognitive behaviour, that is a process of information transformation firmly dependent on underlying patterns of neuronal activity. As such it is perfectly objective and, in theory, as open to investigation, modelling and quantification as any other physical process. In practice lack of knowledge makes the difficulties immense.

The demonstration of an analgesic response in this project relies heavily on the use of Linear Visual Analogue (LVA) scores to measure pain changes. The apparent extreme simplicity of this method conceals some conceptual complexity and it is worth discussing the subject in detail.

Description

LVA scores are a method of allowing quantification of internal states such as feelings and emotions on a continuous scale. [74, 75, 76]. They are finding an increasing role in pain measurement. The ends of the scale are defined by verbal descriptions of states such as "no pain at all" and "the worst pain I could imagine". The rest of the scale is represented by an unmarked line of standard length assumed to represent a continuum between these two extremes. Subjects must decide where on this continuum their own pain lies and make a corresponding mark on the line. The score is converted into a number by measuring the distance between the mark and the "zero" end of the line.

The Concept of Measurement: Levels of Measurement

To begin examining the properties of LVAs in particular it is helpful to consider the concept of measurement more generally.

Measurement was defined by a committee of the British Association for the Advancement of Science in 1946 as "the assignment of numerals to things so as to represent facts and conventions about them" [67]. The numbers form symbolic systems or scales whose mathematical structures model these "facts and conventions". Stevens [67] classifies scales into a hierarchy of four types: Nominal, Ordinal, Interval, and Ratio. Each higher type of scale uses additional types of mathematical operation to model information. The scales thus have a progressively higher information content. The type of scale formed by a given measuring operation (i.e. the level of measurement) will depend on the rules used to assign numbers to the property being measured i.e. on the physical nature of the measurement process. For instance an ordinal scale of hardness for rock samples might be constructed by breaking different combinations of sample pairs against each other. A ratio scale could be constructed by ascertaining the height of drop required to shatter each sample against a standard surface. In the physical sciences the nature of the measurement process is usually apparent. In the behavioural sciences the measurement process often involves psychological mechanisms whose physical basis is not known. This may make it difficult to know what level of measurement one has achieved. However, psychological processes are simply a way of describing physical interactions between neurones. It is important to stress that the level of measurement remains an objective consequence of these interactions and is open to experimental verification even though we remain ignorant of the neuronal interactions themselves.

The level of measurement is important since it determines what

the numbers mean. One must know this in order to know how to interpret and manipulate the data since statistical tests and mathematical manipulations appropriate to ratio or interval scales of measurement may rely for their validity on information not contained in ordinal scales where the corresponding mathematical relationships are "empty".

Effects of Data Processing

After making a measurement, during any subsequent processing of data, it is possible to loose information and degrade data to a lower level. When the data refer to psychological measurements which have been made by a subject on his own mental state this may occur as a result of mental processes which occur before the subject reports the data (i.e. during the process of transforming the data into an output signal). For instance answering the question "Is your pain greater or lesser than before?" may involve processes which generate data at an interval or ratio level. The final output however is only ordinal.

Other systematic distortions may occur during the transformation of data to an output signal. For instance ratio level data may be subjected to a non linear transformation which renders the final numerical scale on which it is expressed logarithmic.

The difficulty of designing experimental techniques to measure pain or other such variables is thus twofold. Firstly there is the problem of getting the nervous system to measure an aspect of its own function as accurately, as precisely, and on as high a level of measurement as possible. Secondly there is the problem of making the resultant information available without loss or distortion.

Level of LVA Measurement

There is debate as to the level of measurement achieved with LVA scores i.e. whether they give ordinal or ratio information. If they represent an ordinal level measurement they can be used to show the direction but not the magnitude of pain changes within a given subject. Apparent information as to the magnitude of pain differences must be regarded as spurious and only very limited types of comparisons are possible between subjects. If on the other hand they represent a ratio level measurement they are a much more useful and flexible tool, particularly for making comparisons between subjects.

Stevens's categories are of ideal scales which reflect the ideal properties of the number system. All actual measurement systems are imperfect to some degree. (Since it is impossible to abstract pure reference attributes from the generality of material interactions this will be so even in the measurement of fundamental properties in physics). It is important to distinguish between a measurement technique which is inherently ordinal and one that is imprecise and inaccurate but still contains, albeit distorted, interval or ratio information.

It would seem intuitively that one can judge pain on a continuous scale and make judgements as to the relative size of pain differences (to comprehend, for instance, that a pain is a lot worse rather than just worse). The scale has an absolute zero (which distinguishes a ratio from an interval scale). Since there seems no reason to doubt the apparent ability of people to use this process to generate an output signal it seems likely that an LVA pain measurement is an inaccurate ratio measurement. The question then becomes how accurate and reliable a ratio

scale it is. The technique may be so invalid as to be useless. On the other hand there may be systematic distortions in parts of the scale either present always or appearing under certain circumstances which can be identified and to an extent corrected. There may be large variation in the measurement of similar pain within and between subjects. In measurement generally, random differences can be cancelled by repeated measurements of the same quantity so the mean value of many measurements may be very accurate even though individual measurements are unreliable. Although one cannot cancel the effect of **interindividual** variation in the measurement of a given pain such a technique may distinguish accurately between the average pain of different populations. The size of the intersubject variation will determine the discrimination which can be obtained with a given number of subjects.

Accuracy and Reproducibility of LVAs: Validation

LVAs are not accurate or reproducible enough between subjects for absolute individual measurements to be of value (see for instance [90]). That is one cannot measure an individual's pain as one can measure his blood pressure. Accuracy, reproducibility and practicality of LVA pain measurement have been investigated by various workers. In the absence of an accepted standard method of pain measurement most of this work has involved the following techniques.

1) Comparison with other methods of measuring pain such as rating scales, or pain behaviour.
2) Evaluation as a measure of experimental pain where it is assumed that the intensity of the pain experience is

related to the (measurable) stimulus intensity. This method allows fairly sophisticated investigation of their sensitivity and variability within and between individuals.

3) Use to detect known changes in clinical or experimental pain such as those induced by analgesics.

4) Evaluation of their consistency in repeated measurements of the same clinical or experimental pain.

Comparison with other methods: A number of studies [79, 82, 84, 85, 86, 87, 88, 100, 101, 102, 103] directly compare LVA scores with other methods of pain measurement, particularly with categorical descriptive scales in the measurement of clinical pain. A problem with this work is that results are often given as correlation coefficients between the various methods of pain measurement. Unfortunately the meaning of correlation coefficients in this context is not clear. Coefficients calculated by different methods may give different results. They may be influenced by factors such as the suitability of the numbers chosen to represent a categorical scale or the greater resolution (and hence variation) of a ratio scale. Depending on what is being compared, other analyses may be more informative. For instance where the pain can be measured in the same units, as in a comparison between an LVA and a numerical categorical scale, measures of the bias and the spread of the differences should be given throughout the length of the scales [80]. When a ratio scale is compared with a categorical scale a useful presentation is the average ratio value along with the distribution of individual ratio values associated with each category [see e.g.84].

Other important difficulties are that both the names and number

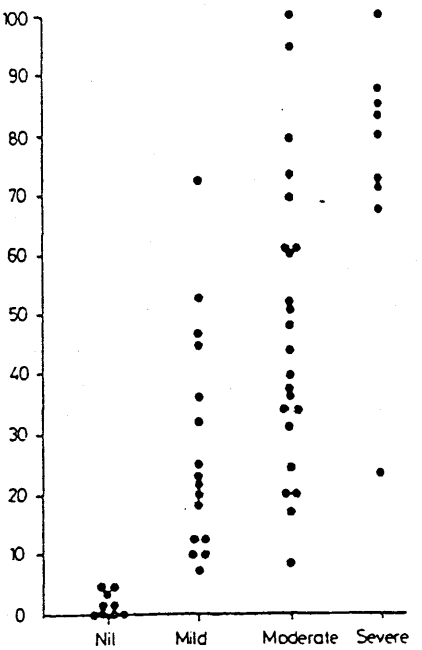
of the rating categories and the anchor phrases of the LVA may differ between studies, and finally, when there is a poor agreement between two or more methods there is no way of knowing which method is closer to the true value.

LVAs however are clearly used with enough consistency by different individuals to demonstrate at least rough agreement with other methods of pain measurement using groups of around 15 to 100 subjects. Thus, when LVA measurements are compared with simple verbal categorical scales, average LVA values rise with words denoting increasing pain severity. There is a marked overlap in the distribution of individual LVA values associated with each word (figure 4.1). Unfortunately there are not enough comparable data available to examine usefully the association between average numerical scores and specific words for different groups of subjects. (Wallenstein and colleagues [100] make an interesting attempt for two populations).

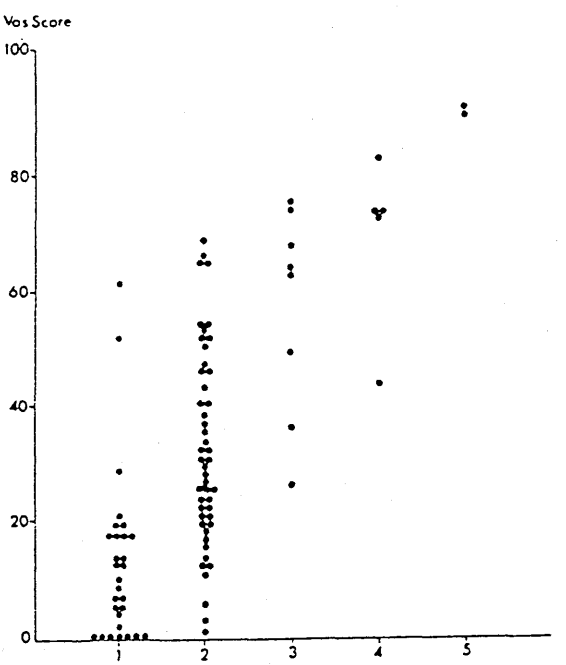
Experimental pain: Price and colleagues [72] conducted a detailed investigation of pain scores as a measure of heat induced experimental pain and concluded that they behaved as ratio scales. The relationship between pain score and stimulus intensity could be described by a power function. This function was then predictive of the temperature required to produce a doubling of pain intensity or of the proportional increase in pain associated with a given temperature increase. In chronic pain patients there was good agreement between measurement of pain by LVA and by matching to heat stimulus. Conclusions were drawn from pooled data of between 20 and 60 observations. Unfortunately the investigators did not provide an analysis of

Figure 4.1

Relationship between Linear Visual Analogue Score and Verbal Rating of Pain



i Reproduced from [79]. The extremes of the analogue scale were marked "no pain" and "unbearable pain".



1. mild
2. discomforting
3. distressing
4. horrible
5. excruciating

ii. Reproduced from [82]. The extremes of the analogue scale were marked "no pain" and "the worst pain imaginable".

the data from individual subjects as opposed to pooled results or of the variability between subjects. The related question of what resolution could be obtained in discriminating between stimuli was not addressed.

Detection of expected pain changes: Various workers [81, 84, 91, 92, 93, 94, 95, 96, 97, 100, 102] have successfully demonstrated the ability of LVA measurements to reveal expected drug effects such as superior analgesia from an active drug compared with a placebo or dose response relationships. These studies provide strong circumstantial evidence that LVAs measure pain on a ratio scale since interval or ratio information from the data is often implicitly used in the analysis of the results. With crossover designs to minimise the effects of inter-subject measurement variation and very carefully controlled experimental conditions it is possible to detect slight drug effects with a surprisingly low number (6 to 12) of subjects [81, 93, 97]. Clinical studies which used different subjects for each treatment [84, 94, 95, 96] used larger numbers (10 to 20 subjects per treatment) and results were not always as unequivocal. Different experimental designs and data analysis techniques were used in each case.
Six of the above studies have compared LVAs with simple categorical scales (a widely used alternative). In four of these [84, 95, 97, 100] LVAs are the more sensitive method, achieving higher probability values for expected changes. One study [94] is equivocal with the performance of the two methods depending on how the results are analysed. One small study of six patients [102] (cross-over design) failed to show any significant differences with either method but the expected trends were more pronounced (higher F-ratio values) using the categorical scale.

Repeatability: The repeatability of LVA measurement was addressed by Revill and colleagues [77] who found the LVA very consistent in repeated measurements of a clinical pain that had occurred weeks or months in the past. They found an average absolute difference of 2.5% between ratings 24 hours apart compared with 4.8% in duplicating from memory a randomly placed mark. Scott and Huskisson [70] found a high correlation between successive pain measurements of ongoing pain which they interpreted as an indication of consistency of measurement. Ekblom and Hansson [85] found good agreement in the pain scores for an ongoing pain that was reported as unchanged.

Output Distortion and Data Processing:

LVAs may be particularly susceptible to output distortion into a non linear scale because the total range of the scale is fixed. Thus a subject who indicates near maximum pain on a pain scale but then finds his pain markedly increased can only increase his score by a small amount. This might make the scale approximately logarithmic particularly towards the upper end. This would only happen during increasing pain when the subject was caused to revise his conception of maximum possible pain and the occurrence of the effect would thus be influenced by previous pain experience (see below). Such distortions should be amenable to experimental analysis. For example during decreasing pain it might be possible to assess non linearity of a pain LVA by comparing pain score differences with pain relief scores marked "no pain relief" / "complete pain relief", assuming, of course, that the fundamental measuring process is the same in both cases and the only difference is in the data output (see below).

Practical Aspects

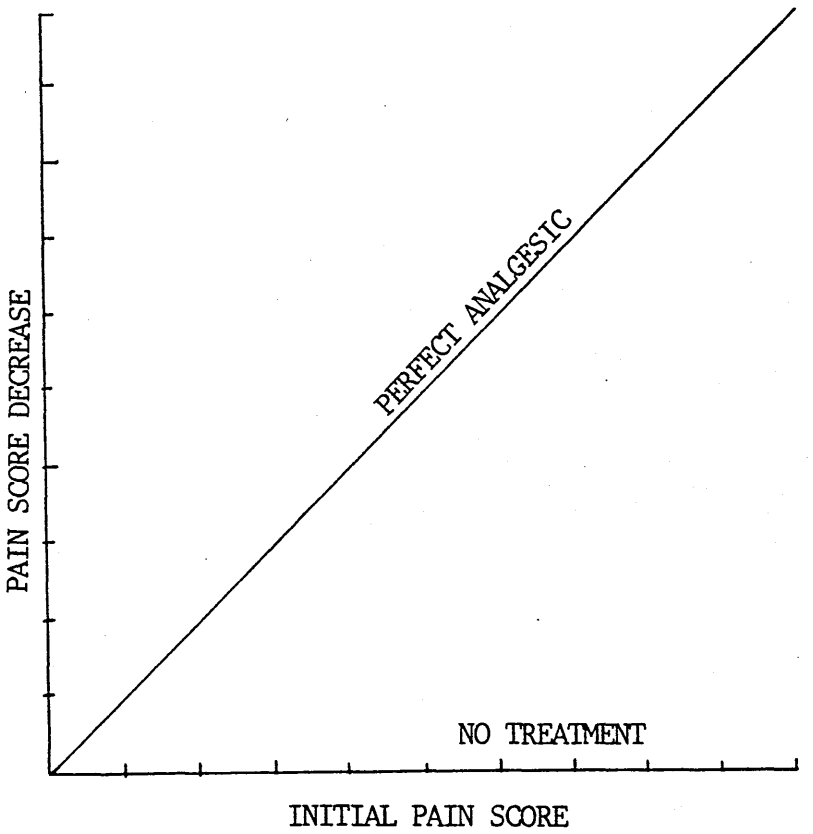
Serial measurements: Should previous scores be available for comparison when new measurements are being made during serial measurements of ongoing pain? It seems that using previous readings for comparison does influence the values obtained, particularly when making readings over a long period (weeks) [68, 69, 70]. It is not clear in which situation readings are more likely to be most accurate. The case against allowing comparison with previous scores is that it may introduce a bias. (This constraint applies to all measurements where there is a necessity to exercise observer judgement and there is a danger of bias towards an expected value created by knowledge of the previous reading. When measurements are truly independent this systematic error is removed). The argument for allowing direct comparison is that it decreases the error due to inaccuracy in memory. The scale becomes calibrated at more fixed reference points and any tendency to a "calibration drift" is opposed.
The premise that pain score readings can be completely independent is false because the measuring device (neuronal circuitry) remembers, i.e. is changed by, successive pain states. Moreover, it is possible that adjustment and recalibration of the scale in the light of experience is a beneficial process leading to greater accuracy. Maxwell [71] showed that when LVAs were used to measure the loudness of a series of sounds a more evenly distributed set of corresponding LVA values was obtained when the same sounds were presented on a second occasion 2 days later. (Unfortunately the elegance of the experiment was diminished by a lack of sophistication in the production and objective measurement of the sounds).

Essentially however, the arguments on both sides are speculative and there is no conclusive evidence. It is important to be consistent whichever method is chosen.

Pain relief scores It has been suggested [73], that pain relief scores rather than the subtraction of pain scores should be used to measure analgesic effect. This has three proposed advantages. Firstly, patients most naturally express the efficacy of a treatment in terms of pain relief. Secondly the problem of possible non-linearity of pain scales is apparently circumvented. Thirdly the phenomenon is avoided whereby pain score decrement tends to be related to the initial pain score. This phenomenon has two causes. Firstly, people may calibrate their scales differently such that some subjects give a similar degree of pain a higher rating. Since the magnitude of any changes will also be exaggerated there is a tendency for the size of the pain relief to be related to the initial pain. Secondly smaller initial pains have less scope for decrease (figure 4.2).

There are several good arguments against reliance on analgesia scores. Analgesia scores by themselves give no indication as to the initial distribution of pain between groups and may thus disguise an inadequacy of trial structure. Pain scores should show up a difference in initial pain between groups and (correctly measured) differences in pain decrease between groups could then be rightly attributed to the differing ability of the groups to respond rather than the relative efficacies of the treatments. Analgesia scores (or calculated fractional pain score changes) abolish real differences of absolute magnitude

figure 4.2



Theoretical Relationship between initial pain score and pain score decrease: all treatments must lie between the lines representing no treatment and the perfect analgesic. Adapted from [73].

between subjects. A small decrease in a small pain is made equivalent to a large decrease in a large pain. This again may lead to erroneous conclusions when groups are not perfectly matched in terms of initial pain. Analgesia scores are unable to show increases in pain. In some circumstances this will result in a waste of useful information, for instance an analgesic treatment may prevent or attenuate a pain increase that would otherwise occur. Some investigators [83, 85, 90] have attempted to avoid this last problem by using a pain change scale with the midpoint labelled "initial pain" or "present pain" and the ends variously anchored to indicate increasing pain in one direction, decreasing pain in the other. This introduces further difficulties of interpretation which will vary with the individual scales. For instance it is possible for the scaling on both arms to be very different depending on the magnitude of the initial pain.

The assumption that the problems of non-linearity are avoided by using an analgesia scale, although plausible, may be unsound. If pain scores are not linear, numerically equal differences derived from different parts of the scale will not represent equal differences in pain. With analgesia scores the difficulty should not arise since all differences on an analgesia scale are measured from the same starting point of "no analgesia". The analgesia scale is anyway more likely to be linear since there it is no possibility of resetting its extremities. (By the same token interindividual differences in scaling may be minimised). However, if analgesia scores are **not** linear the pattern of non-linearity may differ with the absolute amount of pain. For instance the position of the mark may be unduly influenced by

the absolute amount of residual pain rather than corresponding strictly to the proportional decrement and this influence may be more or less pronounced depending on the size of this residual pain. Thus numerically equal analgesia scores would represent different proportional pain decreases, an exactly analogous difficulty to that with pain score differences.

It is not clear whether the use of pain and analgesia scores represent different outputs from the same psychological process (i.e. that analgesia is measured by manipulating estimates of pain generated in the same way as the estimates used to give pain scores) or whether a different psychological process is involved.

Line length, orientation and visiospacial factors To determine the error involved in marking an LVA in a given place Reville and colleagues [77] asked subjects to make several attempts at placing a mark one fifth of the way along the line. The 95% confidence limits for the error were ± 7% for an individual mark and ± 2% for the group mean (20 subjects 10 marks each). This visio-spacial judgement error may vary depending on the position along the line [78]. The length of the analogue line [77, 82] and its orientation [98, 99] may influence the results obtained with LVA scales. These effects are probably small compared with other sources of inter-subject variation. LVAs should be 10cm or more long and should be presented consistently in the same orientation.

End Phrases In the present study LVAs have been used to make a single compound measure of pain magnitude. However pain is better characterised as a multidimensional phenomenon whose

aspects can be quantified separately. LVAs can be used to measure any aspect of pain by means of careful instructions and choice of end phrases. Most commonly a distinction has been made between unpleasantness and intensity [e.g. 81, 92]. Within a given dimension the choice of end phrases will influence the magnitude and distribution of the measurements [82].

Statistical treatment of LVA data Investigators have employed a wide variety of statistical methods in the treatment of LVA data. In considering the broad question of whether to use parametric or non parametric methods the arguments from principle centre around the measurement level and the distribution of the data. I have argued here that LVAs represent inaccurate ratio level data. The distribution of scores is variable [73, 74, 82]. This is as one would expect since it will depend both on the real distribution of the pain and the distribution of any measuring error. One should not assume the scores will be normally distributed and there is no transformation that can be reliably applied to make them so. On these grounds operations like the subtraction of scores or the calculation of areas under painscore/time curves are justified (providing any conclusions are based on a large enough number of subjects) but it is logical to use non parametric tests of significance. In practice [71, 96], as with the present study, parametric and non parametric significance tests are unlikely to lead to very different conclusions.

Conclusion

LVAs are probably an inaccurate ratio measure. In addition to random error they may be subject to systematic distortions.

These distortions may vary with the circumstances of use. Despite this they can be used to distinguish clinically useful differences in pain between groups of subjects and they are probably more sensitive than simple categorical scales for this purpose. Unfortunately despite a reasonable body of published experimental work there is still much that is unclear about their behaviour in common situations. In particular, firstly, it would be useful to have a better qualitative and quantitative understanding of inter-subject measurement variation. This might allow trials to be designed so that such variation was minimised but it would also allow better estimation of the power of various sizes of trial to demonstrate given pain differences. Secondly, (and this is different from the application of transformations for statistical analysis) it would be useful to have information on the feasibility of applying corrections to the raw scores in various conditions.

CHAPTER 5

Method

Route

The purpose of the study was to investigate the effectiveness and safety of fentanyl delivery by the pulmonary route. We wanted to deliver drug to the terminal airways for absorption into the pulmonary circulation as we expected absorption here would be more rapidly complete than absorption across the upper respiratory tract i.e. more likely to mimic intravenous administration. Streisland and his colleagues have administered fentanyl by buccal absorption to good effect [107] but with apparently slower absorption than would be achieved intravenously (Time to peak sedation between 25 and 45 minutes). This may have reflected a slow release effect from their fentanyl lollipops. Another group of investigators [108] demonstrated the onset of effects within 10 minutes of nasally administered sufentanil but they used a comparatively large dose and the timing of the full response was masked by induction of anaesthesia and institution of controlled ventilation.

Nebuliser and Delivery System

Currently the only practical way of delivering fentanyl to the respiratory epithelium is by nebulising fentanyl solution. We chose the simplest possible way of doing this using a commercially available disposable jet nebuliser, driven by the hospital piped oxygen supply and attached directly to a disposable plastic face mask. This simple arrangement is effective for other drugs such as salbutamol and is commonly used in clinical practice. It is not an efficient way of delivering drug however. Drug nebulised outwith inspiration is wasted. Furthermore the nebuliser flow rate is unlikely to approximate the maximum inspiratory flow of even a quietly

respiring patient. During part of each inspiration therefore the aerosol will be diluted with room air, decreasing the rate of drug administration from its potential maximum. Various improvements to this basic system have been proposed such as the addition of chambers and valves [e.g. 109]. However such devices are not yet in widespread clinical use and their effects on aerosol delivery and deposition have not been clearly evaluated. The results of a given modification to a nebuliser breathing system are difficult to predict because of the many factors involved.

The nebuliser used in the study was the model used routinely in the study hospital; the Lifecare Micro-Neb. This has good output characteristics in terms of percentage of nebulised droplets below 5 μ (about 75% at a driving gas flow of 8 l min^{-1}) and rate of aerosolisation [110]. (The relationship between initial particle size and pulmonary deposition is more complex for therapeutic than nonhygroscopic aerosols. Nevertheless, aerosols of smaller particles tend to deposit more peripherally in the respiratory tract than aerosols of larger particles (chapter 3)). Preliminary tests showed that nebulisation was continuous down to residual volumes of about 1.5 ml. The design of the supplied facemask connector allows the nebuliser to remain upright when the subject is lying on her side. This is an important consideration in the present study as it is difficult to sit some patients upright immediately after their operation. Experiments showed that, when the nebuliser was filled to 5 ml, it would nebulise 3 ml of normal saline (determined by weighing the nebuliser before and after nebulisation) in 9 minutes using a driving gas flow rate of of 8 l min^{-1} (measured uncorrected on

the oxygen pipeline system wall rotameters upstream of the nebuliser: the actual oxygen flow at atmospheric pressure would be greater than this. (See page 31). The flow rate, which fell at the upper end of the manufacturer's recommended range, was chosen to maximise the rate of aerosol delivery and minimise particle size. The effects of using higher flows than this are unpredictable since they are outwith the range for which performance data are available. At higher flow rates connecting tubing tended to blow off at the nebuliser or at the wall rotameter. The filling volume was chosen to minimise the effects of evaporation and intermittent nebulisation thereby increasing the standardisation of the drug dose.

There was variation in the rate of nebulisation on different occasions even when a standardised regimen was attempted. Some of this was no doubt due to differences between the nebulisers themselves and some to factors such as differences in calibration between different oxygen flow meters (see chapter 3). In order to know what amount of solution had been nebulised we decided to weigh the nebulisers in the study before and after each aerosolisation.

It is possible that delivering the aerosol by a mouthpiece rather than a face mask increases pulmonary deposition by avoiding nasal filtration of particles [59]. However, a face mask was chosen for this study as it does not require any special co-ordination or co-operation from the subjects at a time when they are likely to be still influenced by residual anaesthetic.

Persons and her colleagues [111] suggest that slow deep continuous breathing maximises the amount of pulmonary

deposition during a given period of aerosol exposure.

The Fentanyl Solution

Prior to the present study a pilot study [117] was carried out by Worsley and colleagues using standard commercial fentanyl citrate solution (50 μg ml^{-1} fentanyl base) in a maximum dose of 6 ml nebulised "to dryness" over 12 to 17 minutes. This would correspond to a nebulised dose of about 200 to 250 μg fentanyl base. The results of this study were interesting in that they suggested fentanyl by this route was providing substantially greater analgesia than would be expected from the estimated absorbed dose or the plasma concentrations. Unfortunately early attempts to replicate these promising results were unsuccessful. We concluded the doses nebulised had been too small and the pilot results had been largely artifactual.

A longer period of nebulisation using a larger volume of fentanyl solution would result in a larger total dose of fentanyl. However the slow rate of drug delivery would not be increased. For a very lipid soluble drug like fentanyl with a short redistribution half life it is unlikely that we would achieve a substantial increase in plasma concentration with even quite a marked increase in aerosol exposure time. Furthermore this would be unacceptable for two other reasons; firstly patients become increasingly intolerant of longer periods of nebuliser therapy and secondly, pain relief has to be achieved within a time at least comparable to that for standard intramuscular analgesia. For patients who failed to get pain relief from the nebulised fentanyl this time must include an allowance for the assessment of analgesic adequacy and the administration of intravenous escape therapy. For these reasons

we considered nebulisation time should be limited to a maximum of ten minutes. In order to increase the dose without prolonging the nebulisation time it is necessary to use a more concentrated solution. For the present study therefore fentanyl citrate crystals were obtained and made up to order in the hospital pharmacy.

Fentanyl doses were chosen on the basis that about 10 to 20 % of the nebulised dose would enter the patient [118, 119]. A maximum strength solution of 318 µg ml^{-1} fentanyl base (500 µg fentanyl citrate ml^{-1}) was prepared to provide a nebulised dose of 954 µg (3ml), corresponding to an estimated delivered dose of between 100 and 200 µg fentanyl base, to be given over 9 minutes. One would expect to detect the analgesic effect of an intravenous dose of 100 to 200 µg given over the same time period. Larger doses than this given intravenously would be expected to produce clinical respiratory depression in increasing numbers of patients. Two lower doses, one corresponding to the dose used in the pilot study and one intermediate betwen that and the high dose were also administered. These were 477 µg fentanyl base (given as 3 ml of 159 µg ml^{-1} solution) corresponding to a delivered dose of between 50 and 100 µg, and 192 µg (given as 3 ml of 64 µg ml^{-1} solution) corresponding to a delivered dose of between 20 and 40 µg. A saline placebo was not necessary since the dose groups could be compared against each other.

Persons and her colleagues [111] have considered the effects of solution tonicity on pulmonary deposition of hygroscopic aerosols. They suggest that better peripheral deposition can be obtained by using a hypertonic saline solution. However hypertonic saline is more likely to cause bronchospasm or

coughing than normal saline and we felt that normal saline was more appropriate as a vehicle in this trial. Hence the three strengths of fentanyl citrate solution were made up with normal saline and sealed in coded glass ampoules of 5 ml each.

General Design, Study Population and Environment

The method of drug administration as it stood was untried and relatively cumbersome. It would have been difficult to incorporate it in a repetitive-dose analgesic regimen. As with the pilot study we thus designed a trial to examine the effectiveness of a single dose of nebulised fentanyl in immediately-postoperative patients. The advantages of this were: Firstly, the study took place in the operating theatre recovery rooms where the patients were constantly under the observation of trained nursing staff and full resuscitation facilities were to hand. This was particularly important in planning to deal with dangerous respiratory depression or bronchospasm, both of which were possible, though unlikely, effects of the treatment. Secondly, the timing of subjects' need for analgesia was relatively predictable. Thirdly, we were able to avoid confounding effects resulting from variable amounts of previously administered intra muscular opiates. Fourthly, we avoided confounding effects resulting from the subjects undertaking different degrees of activity (sitting out of bed, washing etc.) which might change the pattern of their pain.
The main disadvantage of this situation was the residual effect of anaesthesia which would be expected to interfere with the ability to carry out the assessment tasks and might affect the patients' experience of and reaction to their pain.
Approval for the trial was obtained from the local ethical

committee. Potential subjects were selected from patients undergoing a broad range of elective general surgical and gynaecological procedures. Suitable candidates were in good general health and about to undergo what was expected to be uncomplicated surgery. They were all patients who would have normally been offered opioids for postoperative pain relief. Specific reasons for exclusion are detailed in the summary below. Eligible patients from the lists of two operating theatres (general surgical, and gynaecological) were interviewed the day before surgery. They were told to request analgesia as soon after waking up as they felt the need for it. It was emphasised that this was normal procedure and that inclusion in the study should have no influence on whether or not, or when they requested pain relief. Formal written consent was sought. For logistic reasons not all suitable patients were actually included in the study. Decisions as to which patients to include were made on the grounds of convenience. Subjects entered the study when they spontaneously requested analgesia in the recovery room. Patients who were too distressed by pain at this stage to be rational and co-operative were automatically excluded. We intended that the selection procedure would thus impose a degree of standardisation on the initial pain levels. In retrospect not choosing patients undergoing a single standard operation was a design fault (chapter 7).

Randomisation and Drug Accounting

The trial was double blind. This is of paramount importance since the important measurements in the trial such as pain scores and time to escape analgesia are highly susceptible to subconscious bias and manipulation. Eligible subjects were

randomised to one of three groups at the time they made their first request for postoperative analgesia. Each group received a different dose of fentanyl. The solutions were made up in advance in ampoules labelled only with the group code. Accounting was in a special drug book.

Anaesthetic Standardisation

All patients were given a routine balanced anaesthetic, i.e. they were premedicated, anaesthesia was introduced with an intravenous agent and maintained with nitrous oxide, a volatile agent and morphine.

Intraoperative analgesia is a normal part of balanced anaesthesia. It allows the use of lower doses of the other anaesthetic agents and prevents the patient waking in agony at the end of the operation. Morphine was chosen because it is commonly used by the anaesthetists in the study hospital and because it does not interfere with estimations of plasma fentanyl concentration. All patients were given intravenous morphine approximately 0.07 mg kg^{-1} at induction followed by increments of 2 mg as required. We chose this flexible dose regimen because there is no predictable relationship between dose of opioid and the effect it will have in a given individual and because subjects were undergoing a range of different operations.

Premedication was with an oral benzodiazepine. No patient received intramuscular drugs, thereby avoiding the possibility of rising plasma opioid concentrations in the postoperative period as a result of previously administered opioids.

We did not think it was desirable to further standardise the anaesthetic technique in view of the range of operations. Choice

of ventilatory method, induction agent, muscle relaxant (if any) and volatile agent were left to the individual anaesthetist (see chapter 7).

The Measurements

Respiratory Rate: Fentanyl causes a decrease in respiratory rate when the dose is high enough. It is a relatively crude sign of opiate action in that there may be a varying degree of central respiratory depression before any changes in respiratory pattern are evident (chapter 1). Many other factors apart from opioids also change respiratory pattern. Deep breathing during nebuliser therapy could cause a lowering of blood carbon dioxide tension leading to a slower respiratory rate afterwards. In some post operative patients the pattern of respiration is irregular and the rate is difficult to determine. Nevertheless a more marked decrease in respiratory rate in the group receiving high dose fentanyl would be confirmatory evidence of an opiate effect. Monitoring the respiratory rate is also an important safety precaution against the onset of dangerous respiratory depression. Respiratory rate was measured by inspection and palpation.

Pulse and Blood pressure: Blood pressure was measured manually using a mercury sphygmomanometer. Pressures corresponding to the first and fourth Korotkoff sounds were recorded. Pulse was recorded by palpation.

Arterial Oxygen Saturation: It was possible that subjects' oxygenation might be compromised after nebulised fentanyl should either central respiratory depression or bronchospasm occur. In either case it would be important to detect it quickly so that

relevant treatment could be instituted. Arterial oxygen saturation was measured with a BTI BIOX III pulse oximeter. Pulse oximetry gives moment to moment information on the percentage saturation of the subjects haemoglobin. It is rapidly becoming a routine anaesthetic monitoring technique. The Biox III has a digital display of the current arterial oxygen saturation and is equipped with an alarm which will alert the observers attention should it fall below a preset value. Saturation is measured by probes which attach either to a subject's finger or his earlobe. If the subject has marked peripheral vasoconstriction the pulse signal may be too weak for the machine to make a reliable measurement. This occasionally happens with otherwise "normal" postoperative patients and is indicated on the display . The situation can usually be rectified by either repositioning the probe or increasing local blood flow by gentle rubbing. Pulse oximetry is not of any use for following changes in arterial oxygen tension around and above the normal range because the shape of the oxygen dissociation curve is such that changes here are not reflected in changes of haemoglobin saturation.

Airway Resistance: Bronchospasm is a possible hazard of inhaling any foreign substance. We felt that our fentanyl preparation was unlikely to cause bronchospasm as the solution was isotonic, the molecular concentration of fentanyl was relatively low and fentanyl is not known to be irritant to tissue. Fentanyl does not cause tissue histamine release [114]. (Histamine release is one mechanism whereby drugs may cause bronchospasm). Morphine causes tissue histamine release and is thus more likely to cause bronchospasm than fentanyl. Inhaled morphine causes a slight

transient increase in respiratory resistance in normal subjects [115] but this effect does not appear to be marked enough to be clinically important [121]. Despite its unlikelihood we felt it was important to look for any change in airway resistance caused by fentanyl inhalation. We measured the ratio Forced Expiratory Volume in One Second to Forced Vital Capacity (FEV1/FVC) in each subject before and after aerosol therapy using a Micro Medical Spirometer. The FEV1/FVC is widely used as a measure of small airways resistance. A decrease in the ratio is a measure of bronchoconstriction. We expected this would both allow us to diagnose bronchoconstriction in individual patients and, by looking at the inter group differences, to discover any tendency for the fentanyl preparation to cause small airways obstruction. We also expected that anaesthesia and surgery themselves would change the ratios from their preoperative control values. In fact the effects of anaesthesia and surgery were so marked that further interpretation was difficult (chapter 7). The Micro Medical Spirometer is a small hand-held battery-operated spirometer. Its performance has been favourably evaluated in this department.

Fentanyl Concentrations in Plasma: In four patients peripheral venous blood samples were taken for fentanyl assay. The samples were centrifuged and the plasma separated within four hours of collection. The plasma was frozen to await analysis. Fentanyl analysis was carried out using a commercially available radioimmunoassay kit (Medgenix FEN - RIA - 100) (chapter 7).

Pain and analgesia Pain relief was mainly assessed by changes in linear visual analogue (LVA) pain scores. We chose LVAs to

measure pain because of their simplicity and speed of use. The subject is discussed in some detail in chapter 4. The LVAs were constructed as horizontal 10cm. lines marked "no pain" and "agonising pain". The ends of the lines were marked clearly with vertical bars. The text was separated from the ends of the lines by a 1cm space and was typed in thin clear script so that it was unlikely to be mistaken for a continuation of the line (figure 5.1). Subjects who normally wore reading glasses had their glasses available in the recovery room. The LVAs were arranged serially on a separate single sheet of paper for each patient so that at each assessment the patient has his previous scores for comparison. This was to maximise the accuracy of the scores as a record of the change of each patient's pain with time.

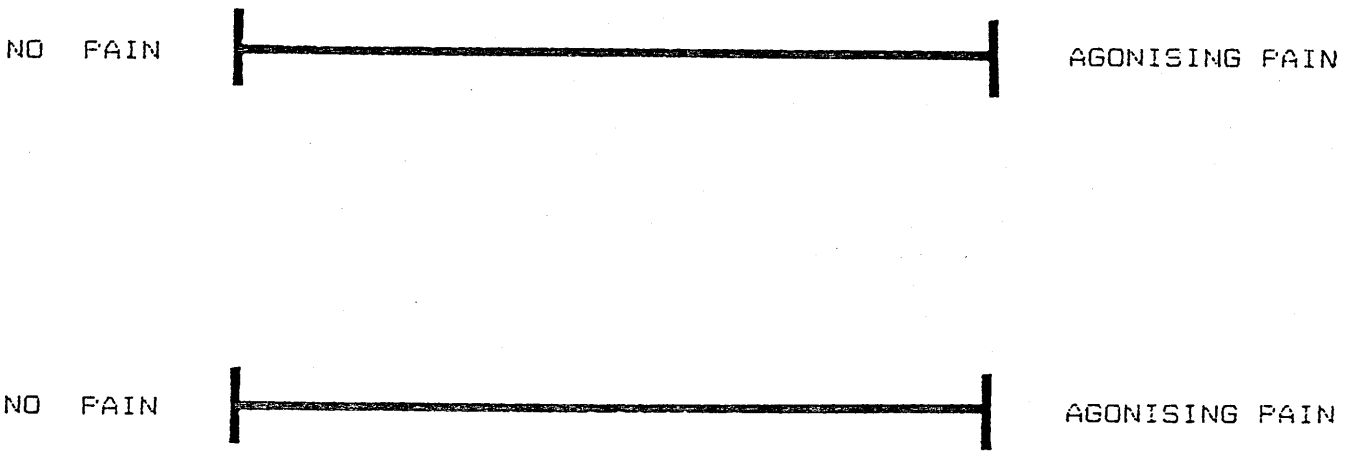
In addition to the LVA pain scores we also used a simple categorical analgesia score although we expected LVA scores to be a more sensitive indicator of drug effect:

no pain relief (0).
a little pain relief (1).
moderate pain relief (2).
almost complete pain relief (3).
complete pain relief (4).

There were no numbers on the scale as presented to the subjects. It would have been interesting to design the analgesia score as a LVA. Direct comparisons could then have been made between the pain score changes and the analgesia scores. However we felt there was a danger of confusing the subjects between two such similar scales.

Figure 5.1

Linear Visual Analogue Scales



Five scales were arranged on each sheet of A4.

We carried out a preliminary experiment to test subjects' ability to use pain scores on waking after anaesthesia. Ten patients were given, double blind, a small intravenous dose (100 µg) of fentanyl or saline placebo when they first asked for analgesia in the recovery room (followed if necessary by intravenous morphine after assessment of analgesia). They completed pain scores and analgesia scores. Other observations were made as in the main study. Lack of physical co-ordination, a residual effect of anaesthesia, proved to be an obstacle to the completion of pain scores. Subjects appeared to know where they wanted to mark the line but were unable to manipulate a pen. Other workers have designed mechanical or computer controlled analogues to overcome this problem (85, 105, 106). We adopted a simpler solution whereby subjects pointed to where they wished the mark to be. The mark was then made by the observer after he had checked the pen was correctly positioned. It is likely that some precision in the placing of the marks was lost by this method. Any errors should have been randomly distributed. Patterns in the pain score results showed wide inter subject variation. It was not possible to predict from the pain scores which individuals had received fentanyl and which had received placebo. There was however a trend for pain score decreases to be greater in the fentanyl group than the placebo group ($p = 0.1$ Mann Whitney U test). We accepted this as evidence that the pain scores could be used to distinguish an analgesic effect of similar magnitude between groups in the main study using a larger number of subjects.

Analgesia scores only showed a slight tendency to be be greater in the treatment group.

Sedation: This was scored by one observer according to the following scale

0. No obvious sedation. Patient interested in surroundings. Eyes open most of the time.
1. Slight sedation. Eyes closed most of the time. Responds to speech alone.
2. Moderate sedation. Requires gentle shaking and speech to rouse.
3. Difficult to rouse. Requires vigorous shaking or painful stimulus. Falls immediately back to sleep.
4. Unconscious. No response beyond localisation of or incoherent vocalisation in response to painful stimulus.

Follow-up Questionnaire: We did not expect that, even if it proved to be effective, this particular method of administering fentanyl from a nebuliser over nine minutes would be useful without further development. The purpose of the study was to investigate the feasibility of the respiratory route. However we were interested to discover the impressions of the subjects as to its acceptability or otherwise and to find out any unforeseen subjective effects. We therefore administered the following short questionnaire the next day:

Do you remember your treatment with the nebuliser in the recovery room after your operation yesterday?

Analgesia score as above.

Was there anything about the treatment or its effects that you found unpleasant?

Was their anything about the treatment or its effects that you particularly liked?

Would you like to have this form of pain relief again if you had another operation?

Side effects: Signs were noted by the observer as and when they occurred. Symptoms, if not volunteered, were sought at the end of the session by first of all a general and then specific questions.

Collection and Recording of Data

Data were recorded manually on a standard form.

Number of Patients

Thirty patients were studied, ten in each group.

Data Analysis

Initial comparisons between groups were made for each variable using the Kruskal-Wallis Test (nul hypothesis: no difference between the three groups). If this showed the groups differed significantly ($p < 0.05$) the groups were compared in pairs using the Mann-Whitney U test. Pulse and blood pressure were compared within each group before and after drug administration using the Wilcoxon matched pairs test.

Pain score changes, time to further analgesia, respiratory rate change and possibly analgesia score can be regarded as separate measures of drug effect. To give an overall measure of response the variables were put on a common scale by ranking, and the arithmetic mean of each subject's four rank values used as a single combined index of relative drug effect. The groups were then compared using the Kruskal-Wallis and Mann-Whitney U tests on these averaged ranks. Demonstration of a dose response relationship was to be regarded as an indication of efficacy.

Summary of Method

Eligible patients were between the ages of 18 and 65 years of age and ASA grade I or II. The following patients were excluded:

1 Patients with liver disease, renal disease, severe hypertension, symptomatic ischaemic heart disease, bronchial asthma or severe respiratory disease of any sort.

2 Patients who are allergic to opiates.

3. Patients with a history of opiate addiction

4 Patients taking drugs known to interfere with the action or metabolism of opiates.

Potential subjects were interviewed the night before theatre and invited to sign the consent form. The relevant tests were explained and demonstrated and pre-operative values obtained as part of the familiarisation process. All patients received routine balanced anaesthesia. An oral benzodiazepine was used for premedication and intravenous morphine was the only analgesic used pre or intra operatively. This was given in a dose of 0.07mg per kg at the beginning of anaesthesia with further incremental doses of 2mg as required for anaesthetic stability. In three patients an intravenous cannula was inserted and heparinised for post operative blood sampling.

After the operation the patients were returned to the recovery room where they breathed 28 per cent oxygen from a Ventimask and had regular heart rate, blood pressure and respiratory rate measurements according to normal practice.

Subjects were assessed as normal at 5 minute intervals to determine their level of consciousness. When each subject was awake and asked for pain relief the following procedures were undertaken:

1 Each patient was allocated to a dosage group 1,2 or3.

2 Patients were invited to perform an FEV/FVC manoeuvre using the pocket Spirometer. A pulse oximeter was used to assess oxygen saturation. Sedation scores were noted.

3 Patients were asked to indicate the extent of their pain using a 10cm linear visual analogue scale (LVA).

4 In four patients, including one from each group, a peripheral intravenous sample was taken for baseline fentanyl analysis. The samples were centrifuged and separated soon after collection and the serum frozen for storage.

5 Each patient was positioned on his side with his head at the edge of a pillow. A nebulised solution containing 64 $\mu g\ ml^{-1}$ fentanyl base (group 1), 159 $\mu g\ ml^{-1}$ (group 2) or 318 $\mu g\ ml^{-1}$ fentanyl base (group 3) was administered. In each case the drug was dissolved in normal saline. Subjects were asked to breath slowly and deeply during nebulisation. The drug was nebulised such that 3 ml of solution were administered. (Nebulisation over 9 minutes by an oxygen flow of 8 $l\ min^{-1}$). The nebulisers were filled with 5 ml of solution. The filled nebulisers were weighed before and after nebulisation. To prevent evaporation whilst awaiting weighing they were sealed in small airtight polythene bags.

6 In the four patients from whom samples had been taken previously, peripheral intravenous blood samples were taken at the following times from the start of nebulisation: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 24, 29, 39, 49, 59, 69,

129, and 189 minutes. The samples were centrifuged and separated soon after collection and the serum frozen for later fentanyl analysis.

7 At five minutes after nebulisation was complete measurements were taken of pain (using the LVA), FEV/FVC, respiratory rate, pulse and blood pressure. Analgesia was assessed by the patient on a five point rating scale.

8 If pain relief was inadequate at this point or at any point thereafter, the patient was offered the escape therapy. The escape therapy consisted of intravenous morphine titrated until the patient was comfortable. After receiving escape therapy the patient was considered to have left the trial.

9 Measurements of pain, sedation score FEV/FVC, respiratory rate, pulse and blood pressure were made at 15 minutes, 30 minutes, 45 minutes, 60 minutes and thereafter at 30 minute intervals until three hours after nebulisation or until the patient received escape analgesia, whichever was the sooner. The time to escape analgesia was noted.

10 In addition the following were noted if they occurred at any time: cough, bronchospasm, laryngospasm, flush/rash, headache, nausea, vomiting, euphoria, dysphoria, confusion, restlessness, taste in mouth, rhinorrhea, itch.

After the three hour study period or after their escape analgesia patients received routine analgesia as prescribed by the anaesthetist who gave their anaesthetic.
The next day the patients were invited to complete a brief questionnaire about side effects and acceptability.

CHAPTER 6

Results

Table 6.1: Concentration of Solution: μg ml^{-1} Fentanyl Base

Group 1	Group 2	Group 3
64	159	318

Table 6.2: Mass of Fentanyl Solution Delivered (g): mean (s.d.)

Group 1	Group 2	Group 3	
3.01 (0.31)	3.10 (0.23)	2.99 (0.42)	*

* Groups not significantly different at p = 0.05 Kruskal-Wallis test (null hypothesis: no difference between the three groups)

Tables 6.1 and 6.2

TABLE 6.3: Patient Data

Group	1	2	3	
Age years mean (s.d.)	42.3 (14.6)	35.8 (7.2)	35.1 (11.9)	*
Sex m	3m	1m	1m	*
f	7f	9f	9f	
Height cm mean (s.d.)	167 (8)	154 (7)	160 (7)	**
Weight Kg mean (s.d.)	66.9 (11.7)	64.7 (12.4)	62.4 (8.3)	*
Operation	Laparoscopy 2	Laparoscopy 3	Laparoscopy 8	
	(Sterilisation 1)	(Sterilisation 3)	(Sterilisation 7)	
	(Diagnostic 1)		(Diagnostic 1)	
	Hysterectomy 3	Hysterectomy 6	Hysterectomy 1	
	Herniorrhaphy 2	Herniorrhaphy 1	Herniorrhaphy 1	
	Other 3			

* Groups not significantly different at p = 0.05

** Groups significantly different ($p < 0.01$)

Kruskal-Wallis test (null hypothesis: no difference between the three groups)

Table 6.4: Times to Escape Analgesia in Minutes

	Group 1	Group 2	Group 3
Subject no. 1	80	180+	90
2	15	5	24
3	5	5	80
4	10	5	5
5	5	12	180+
6	5	5	5
7	5	120	10
8	5	5	180+
9	5	5	30
10	30	5	180+

Groups significantly different at p = 0.05 Kruskal-Wallis test (null hypothesis: no difference between the three groups)

Comparison between pairs of groups. Mann-Whitney U test (null hypothesis: no difference between groups):

1 and 2 p = 0.91

3 and 2 p = 0.08

3 and 1 p = 0.04

Table 6.4

Table 6.5: Analgesia Scores at Five Minutes after the End of Nebulisation

	Group 1	Group 2	Group 3
Subject no. 1	3	1	3
2	2	1	2
3	1	1	2
4	1	0	0
5	1	2	2
6	1	0	1
7	0	2	2
8	1	2	2
9	0	0	3
10	3	0	2

Groups not significantly different at p = 0.05 Kruskal-Wallis test (null hypothesis: no difference between the three groups)

TABLE 6.6: Pain Scores before Start of Nebulisation and Change by Five Minutes after End of Nebulisation (Maximum Pain Score = 10)

	Group 1	Group 2	Group 3
Subject no. 1	7.5 (-4.5)	5.8 (-0.7)	6.9 (-5.4)
2	6.4 (-3.3)	6.0 (-1.5)	7.7 (-2.0)
3	2.9 (-2.1)	7.3 (0.0)	4.7 (-2.1)
4	3.8 (-1.0)	8.2 (-1.3)	10.0 (0.0)
5	8.9 (-2.7)	3.6 (-0.2)	5.0 (-4.0)
6	8.2 (-0.8)	7.0 (+1.3)	10.0 (-2.4
7	8.9 (+0.2)	7.7 (-4.4)	6.4 (-3.7)
8	5.3 (0.0)	8.2 (-1.5)	5.9 (-5.5)
9	8.7 (+0.2)	7.3 (0.0)	7.2 (-5.4)
10	3.9 (-3.0)	8.7 (+0.6)	6.1 (-3.6)

Kruskal-Wallis test (null hypothesis: no difference between the three groups):

Initial pain scores: Groups not significantly different ($p = 0.95$)

Pain score changes: Groups significantly different at $p = 0.05$

Comparison of pain score changes between pairs of groups. Mann-Whitney U test (null hypothesis: no difference between groups):

1 and 2 $p = 0.28$

3 and 2 $p = 0.004$

3 and 1 $p = 0.05$

Table 6.6

TABLE 6.7: Respiratory Rates (Breaths per Minute) before Start of Nebulisation and Change by Five Minutes After End of Nebulisation

	Group 1	Group 2	Group 3
Subject no. 1	12 (+1)	16 (+1)	16 (-4)
2	26 (+2)	31 (-9)	18 (-8)
3	22 (-2)	22 (0)	26 (-12
4	16 (0)	26 (-2)	24 (-4)
5	16 (-6)	28 (-12)	16 (-4)
6	24 (+2)	20 (0)	24 (-8)
7	28 (-8)	14 (+2)	30 (-4)
8	26 (-2)	24 (-4)	16 (0)
9	20 (-2)	17 (-1)	26 (-6)
10	13 (+3)	28 (-4)	16 (+2)

Kruskal-Wallis test (null hypothesis: no difference between the three groups):

Initial respiratory rates: groups not significantly different ($p = 0.62$)

Respiratory rate changes: groups not significantly different at $p = 0.05$

TABLE 6.8: Spirometry Average (s.d.) Before Surgery, Before Nebulisation, and Five Minutes after Nebulisation

	Before Surgery	Before Nebulisation	After Nebulisation
FEV1. Litres			
group 1	2.72 (0.52)	0.94 (0.48)	1.17 (0.67)
group 2	2.36 (0.64)	0.88 (0.28)	1.15 (0.19)
group 3	2.48 (0.43)	1.18 (0.59)	1.55 (0.67)
FVC Litres			
group 1	2.95 (0.61)	1.13 (0.55)	1.44 (0.84)
group 2	2.58 (0.74)	1.07 (0.33)	1.28 (0.26)
group 3	2.71 (0.58)	1.41 (0.56)	1.82 (0.52)
FEV/FVC			
group 1	0.92 (0.06)	0.83 (0.18)	0.85 (0.11)
group 2	0.92 (0.05)	0.83 (0.14)	0.91 (0.09)
group 3	0.93 (0.07)	0.82 (0.14)	0.82 (0.19)

Figure 6.1

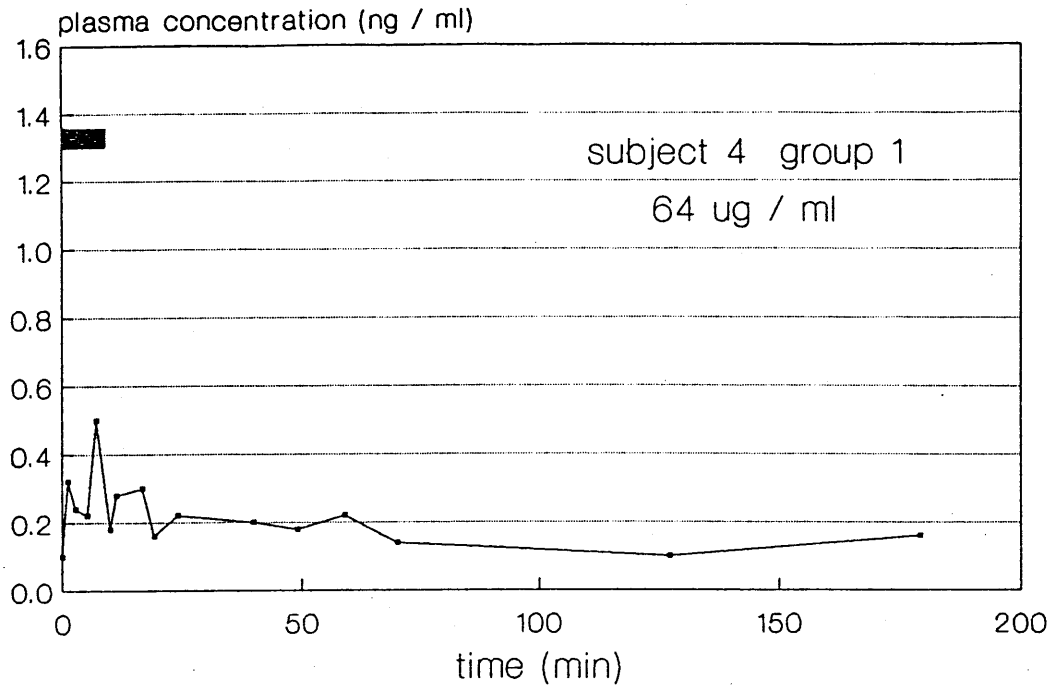
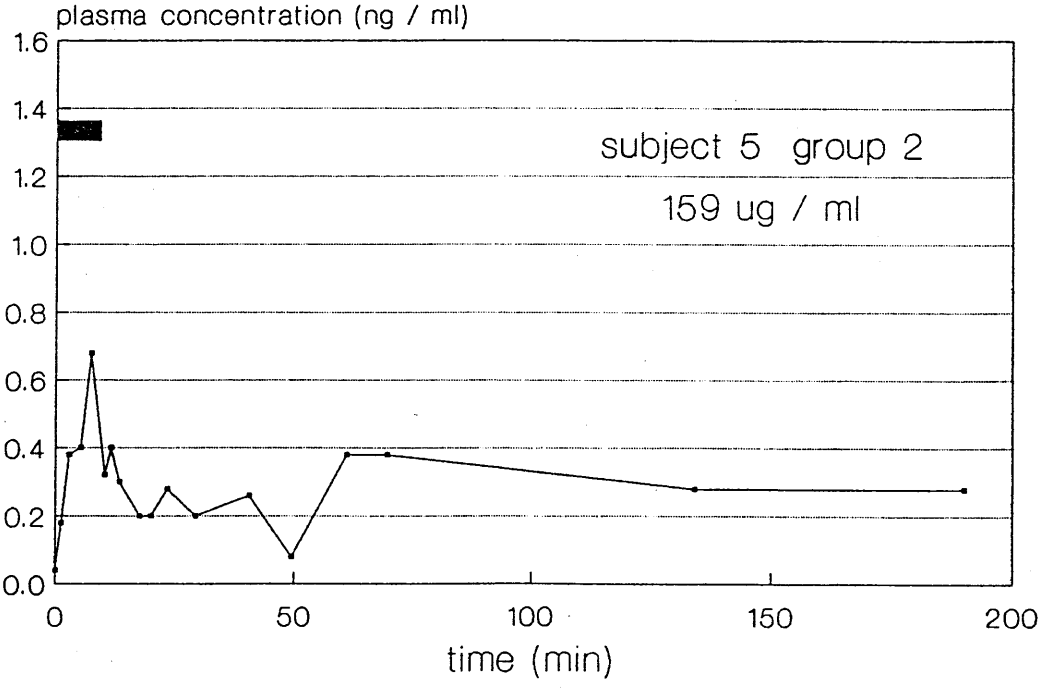


Figure 6.2



Fentanyl concentrations in peripheral venous plasma. The bar shows the period of neblisation.

Figure 6.3

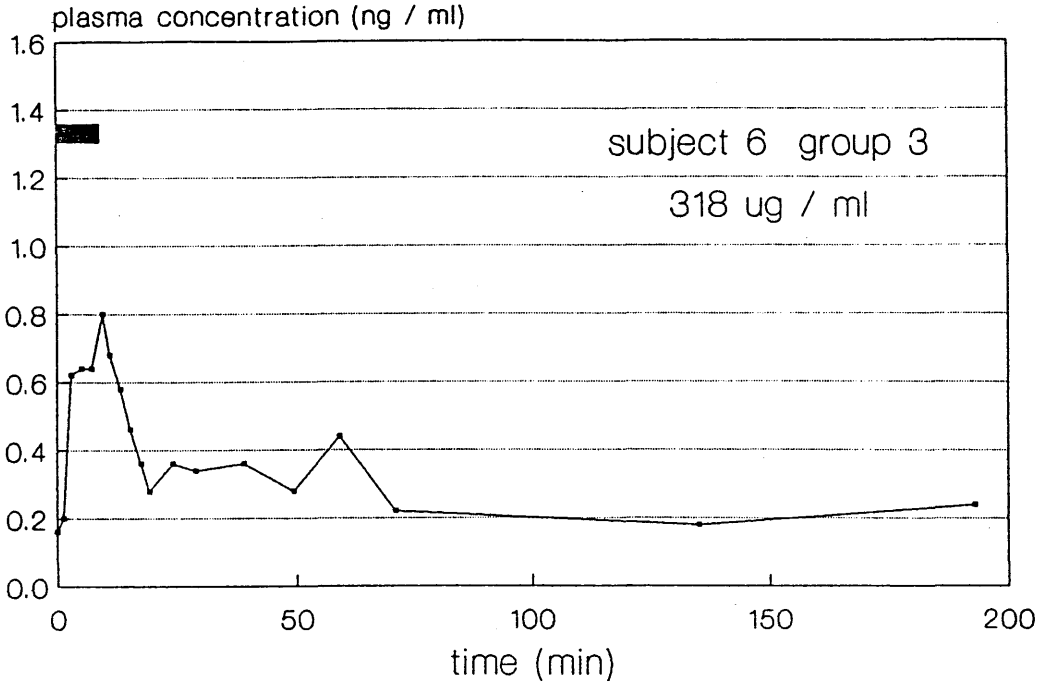
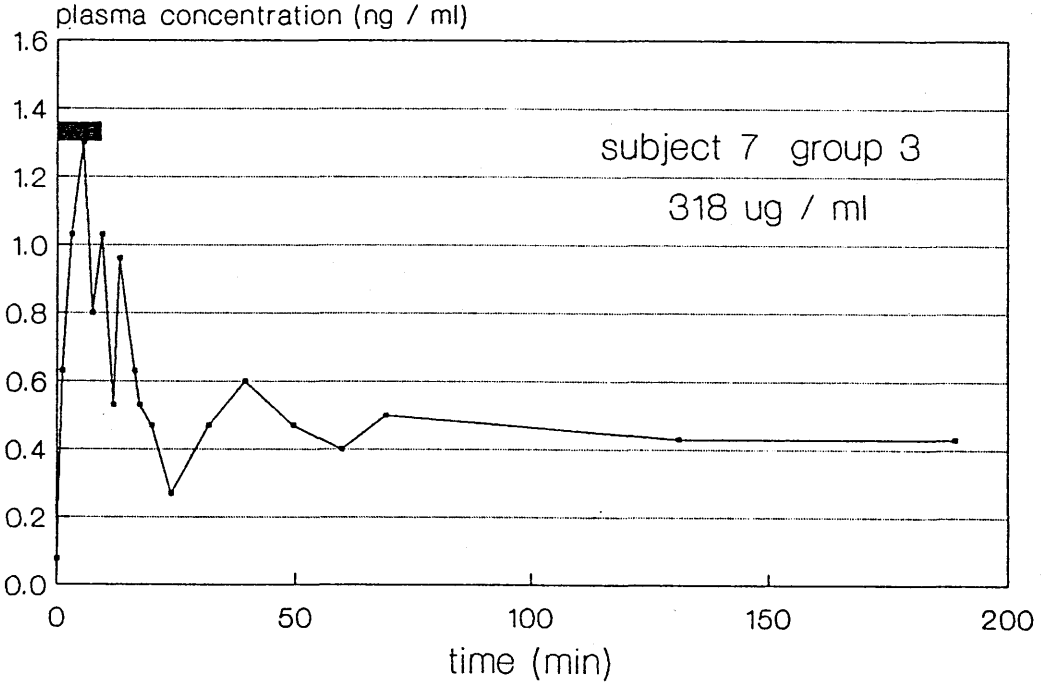


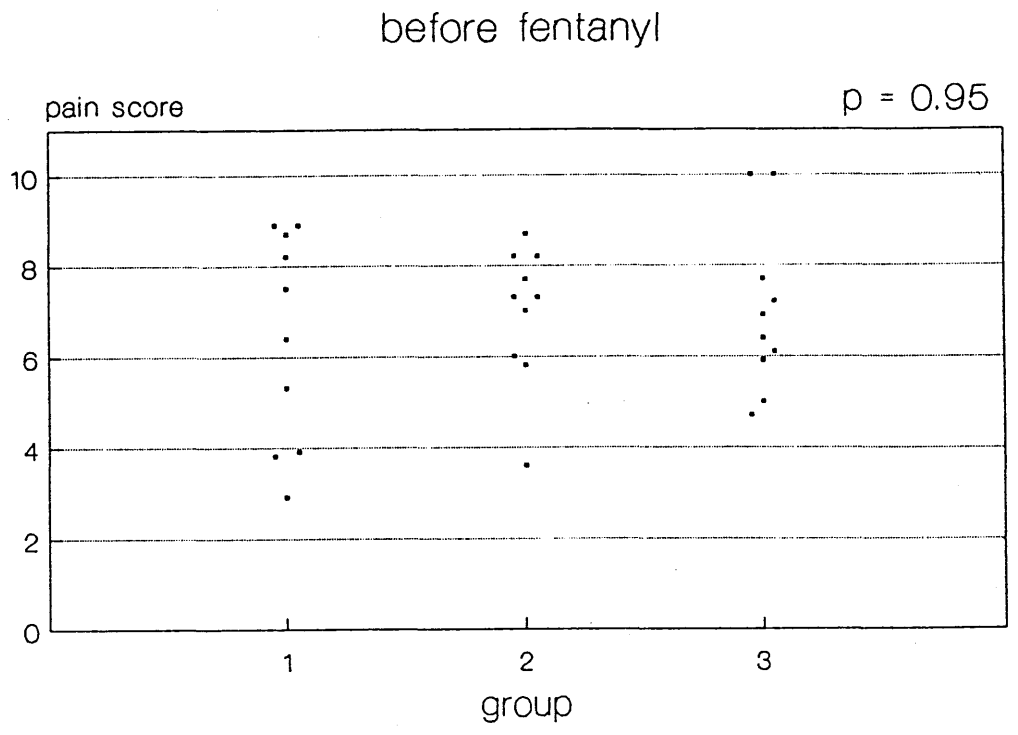
Figure 6.4

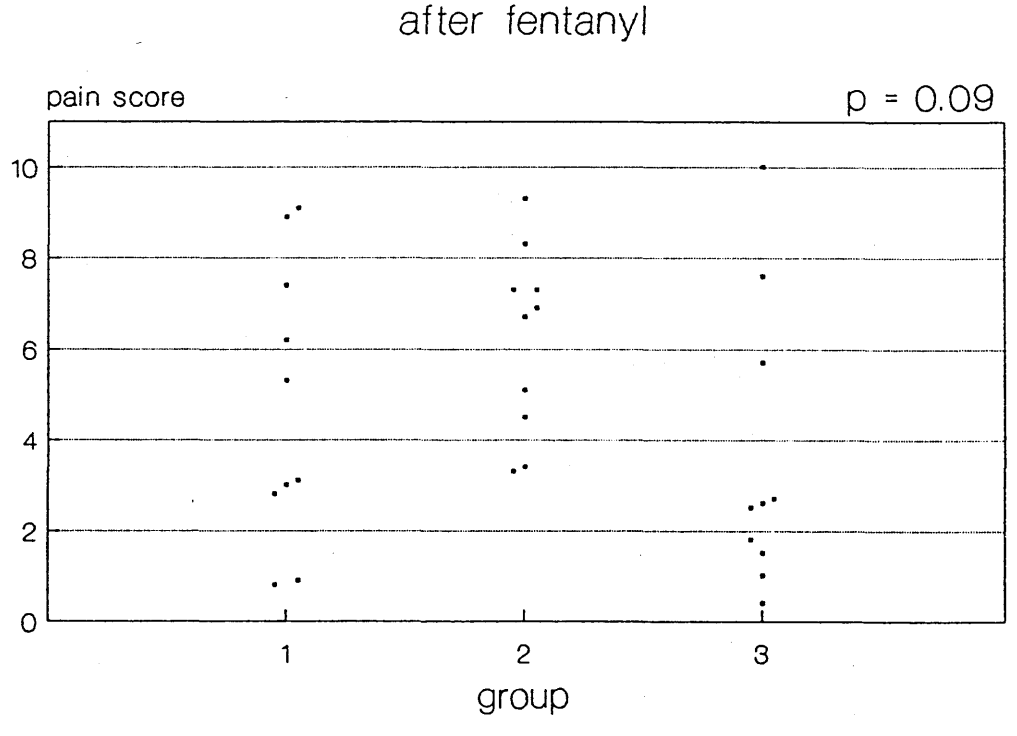


Fentanyl concentrations in peripheral venous plasma. The bar shows the period of neblisation.

Figure 6.5

Pain scores Before and Five Minutes After Fentanyl

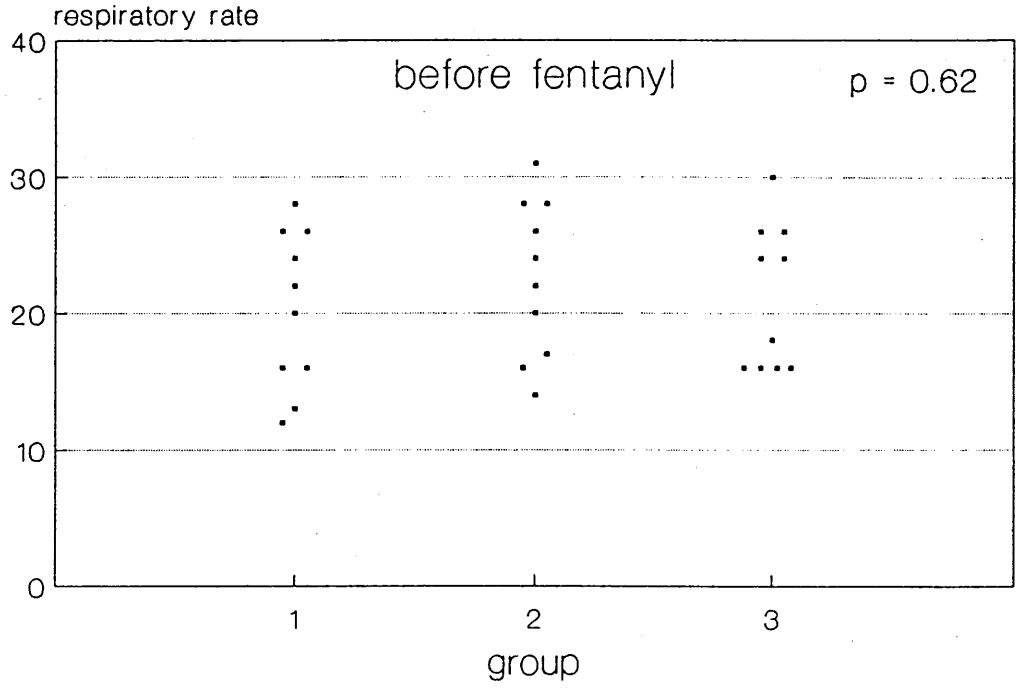


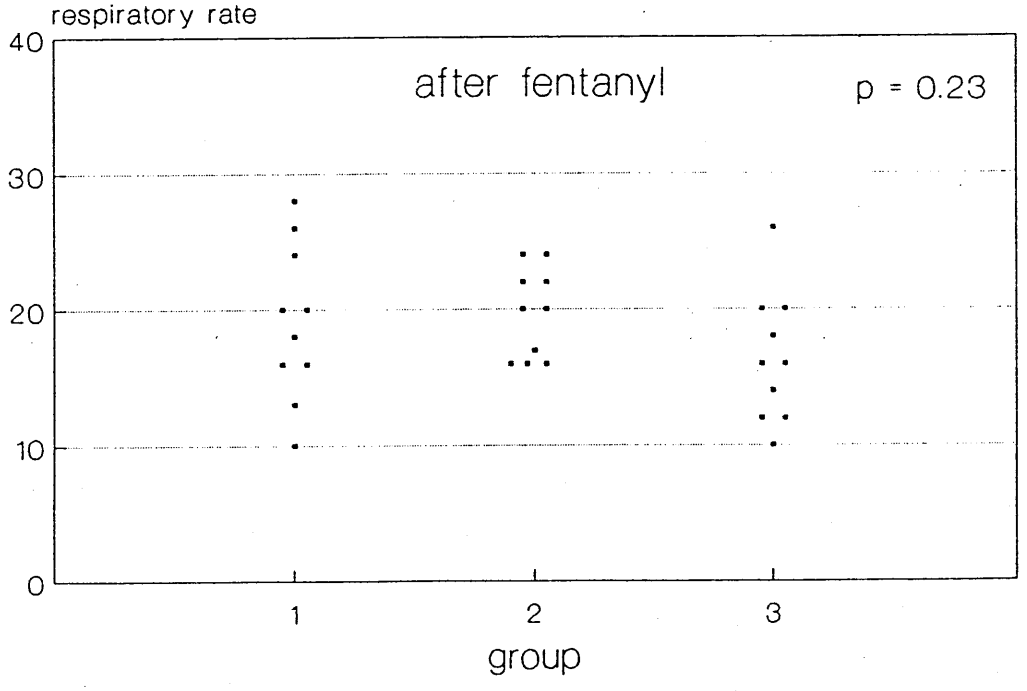


p values refer to the Kruskal-Wallis test (nul hypothesis: no difference between the three groups).

Figure 6.6

Respiratory Rates Before and Five Minutes After Fentanyl

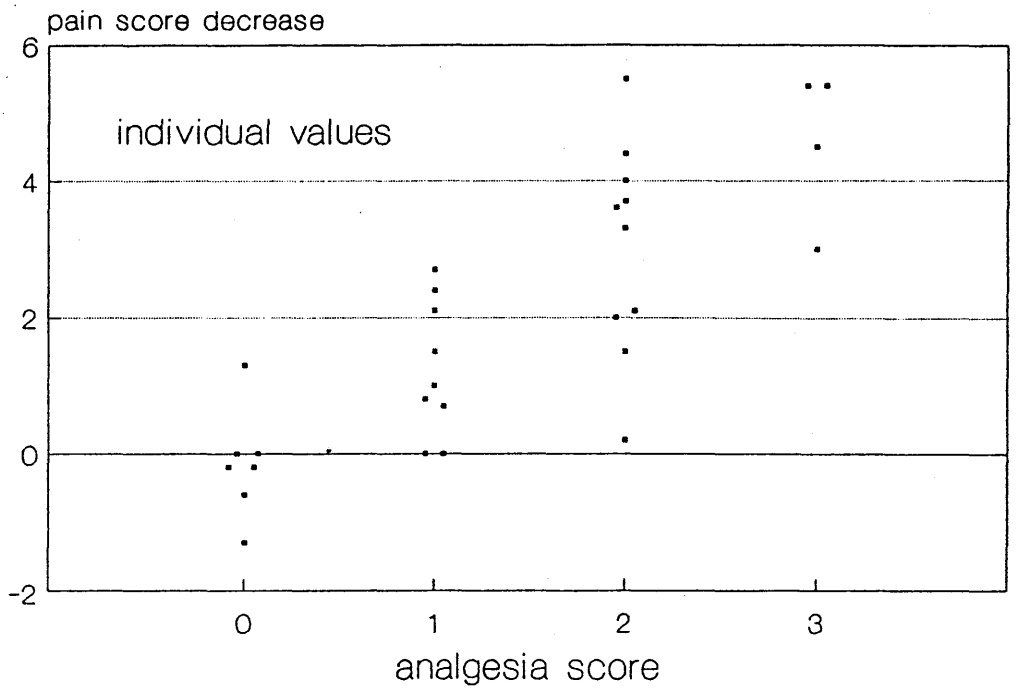


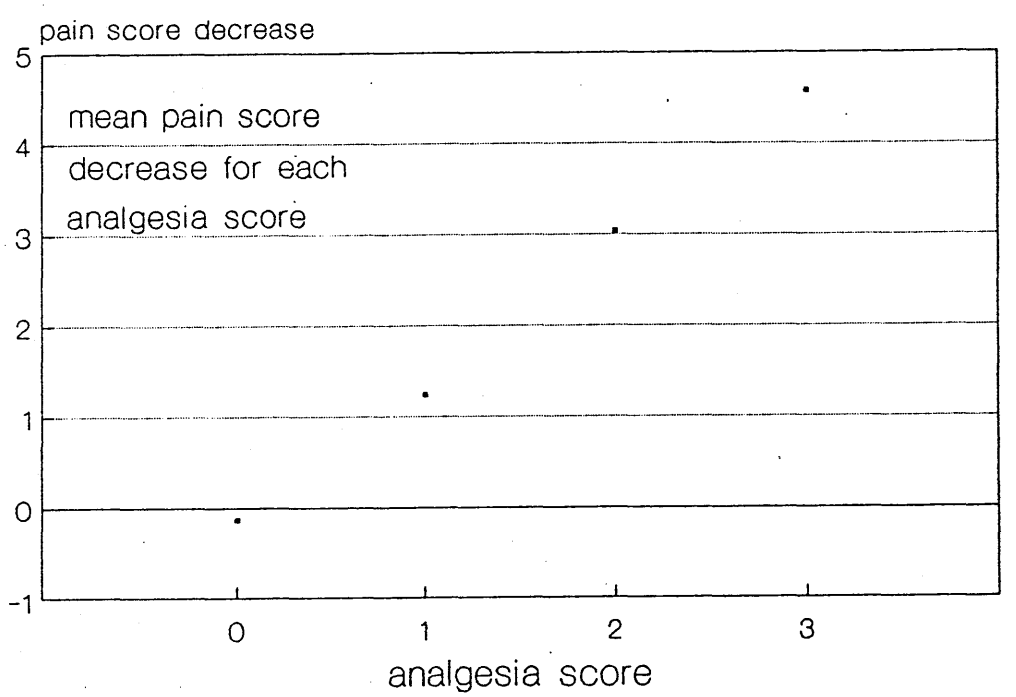


p values refer to the Kruskal-Wallis test (nul hypothesis: no difference between the three groups).

Figure 6.7

Relationship Between Absolute Pain Score Decrease and Analgesia Score





0 no pain relief
1 a little pain relief
2 moderate pain relief
3 almost complete pain relief

Figure 6.8

Relationship Between Pain Score Change and Percentage Pain Score Change

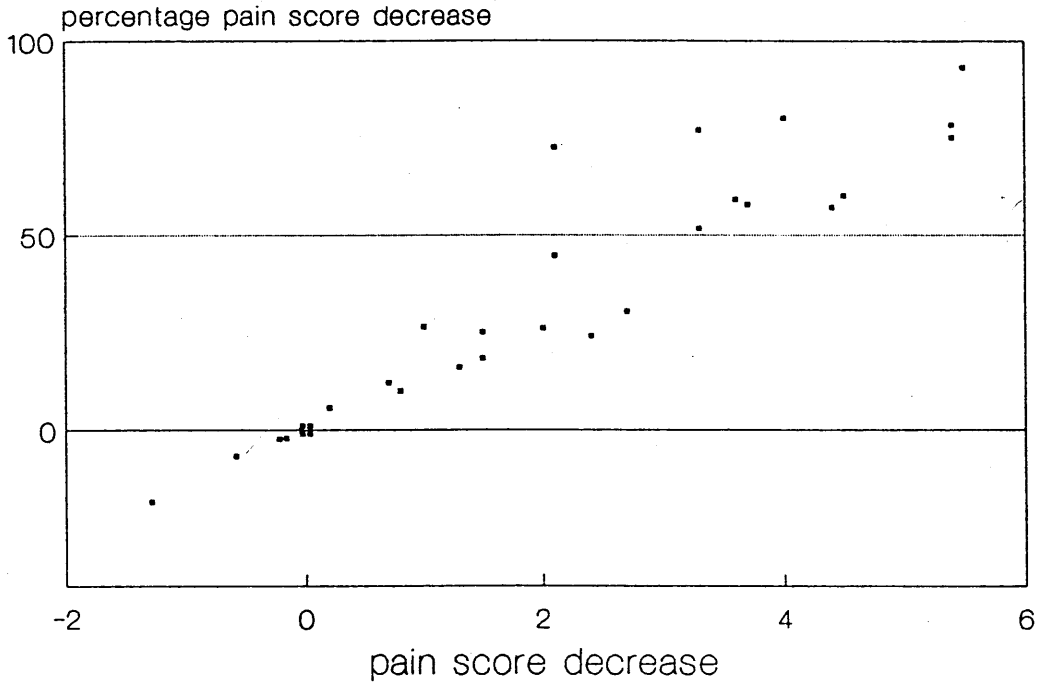
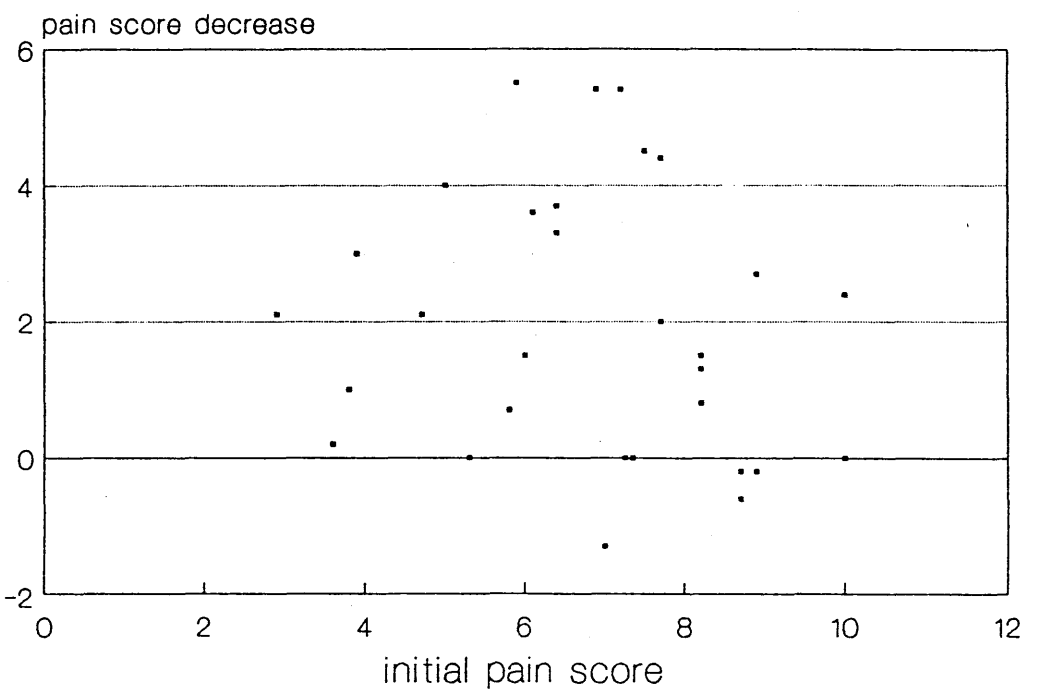


Figure 6.9

Relationship Between Initial Pain Score and Pain Score Decrease



Combined Analysis of Drug Effect

Each subject was ranked separately in terms of the analgesia scores, the pain score decreases, the respiratory rate changes and the time to further analgesia. A combined score then was calculated for each subject by taking the arithmetic mean of these four rank values (see page 69). The groups differed significantly in terms of these combined scores ($p = 0.003$ Kruskal-Wallis test). Using the combined scores to compare the groups against each other with the Mann-Whitney U test showed no significant difference between the two lower dose groups 1 and 2 ($p > 0.5$) but a significant ($p < 0.01$) difference between group 3 (318 $\mu g\ ml^{-1}$) and each of the other two groups. It could be argued that the analgesia scores and the pain score changes should not both be included in the combined score calculation on the grounds that they carry the same information. However the analysis is essentially unchanged when it is repeated using combined scores calculated without analgesia scores.

Sedation Scores

All patients had a sedation score of 1 (Slight sedation responds to speech alone. Eyes closed most of the time) at the time they first requested analgesia. No subjects increased their sedation score after fentanyl.

PLasma Fentanyl Concentrations

Plasma samples from four subjects were analysed for fentanyl concentration. Two of the subjects were in group 3 (318 $\mu g\ ml^{-1}$) and one in each of the two lower dose groups. The results are shown in figures 6.1 to 6.4. Unfortunately (see chapter 7) these assay results are not felt to be reliable.

Side Effects

There were no serious side effects. In particular there was no clinically important respiratory depression (arterial oxygen saturation < 90% or respiratory rate < 10). No patient suffered a fall in arterial oxygen saturation as a result of treatment. No patient became wheezy or complained of difficulty breathing. The results obtained from micro-spirometry are difficult to interpret since all the early post operative values around the time of aerosol therapy are markedly reduced compared to the preoperative values (see table 6.8). Major factors contributing to this were a lack of co-ordination and motivation owing to the residual effects of anaesthesia. Both FEV and FVC values showed a gradual increase back towards pre operative values whilst the subjects were in the recovery room. Most of the changes in FEV/FVC ratios about nebulisation occurred as a result of disproportionate increases in either FEV or FVC values. In this situation changes in the FEV/FVC ratio are probably not a valid measure of changes in airway resistance.

None of the groups showed significant ($p < 0.05$ Wilcoxon matched pairs) changes in pulse rate or systolic blood pressure five minutes after fentanyl compared with immediately before.

Two subjects had a slight dry cough intermittently during inhalation, one in group 1, one in group 2. The subject in group 1 had been coughing before aerosol therapy.

Eight subjects complained of nausea during the study period, Four in group 3, two in group 1, and two in group 2. All except three of these (all in group three) had also been nauseated before fentanyl. No subjects complained of unpleasant taste.

Questionnaires

There was generally poor recall of the treatment the next day. Six patients (four in group one, and two in group three) were either completely unable to remember having the treatment or remembered virtually nothing about it. In addition many of the remainder who did complete the questionnaire stated that their memories were vague or hazy. Some were clearly mixing up the nebuliser therapy with the spirometer measurements or the effect of the injected escape therapy. No new side effects were discovered but four patients commented that the process of nebulisation (noise, wetness, mask on face etc.) was unpleasant. Two found it soothing. The most common favourable comment was on the absence of injections (five patients).

CHAPTER 7

Discussion

Efficacy of the Fentanyl

Group 3, receiving the highest concentration of fentanyl (318 $\mu g\ ml^{-1}$ fentanyl base), produced larger decreases in pain score and respiratory rates, higher analgesia scores and longer times to further analgesia than both the lower dose groups (tables 6.4 to 6.7, figures 6.5 and 6.6). The differences in analgesia scores and respiratory rate changes between the groups were not significant statistically but there were significant differences between group 3 and the lower dose groups in terms of the pain score changes, times to further analgesia and in particular, the combined rank scores (p 69) which were intended to give an overall measure of effectiveness.

None of the differences between the two lower dose groups approached statistical significance and it was not possible to distinguish between the efficacy of the two lower concentrations or to demonstrate a dose response relationship across the three groups. The 64 $\mu g\ ml^{-1}$ solution produced greater pain score decreases and analgesia scores than the 159 $\mu g\ ml^{-1}$ solution but the 159 $\mu g\ ml^{-1}$ solution gave longer times to further analgesia and caused greater slowing of respiratory rate (none of these changes statistically significant).

Thus patients receiving the highest fentanyl dose (group 3) showed greater evidence of opioid effect than patients in the two lower dose groups which were indistinguishable.

Unlike Worsley and colleagues' pilot study [117] which suggested that fentanyl might be efficacious at surprisingly low doses there was no evidence on this occasion that the effects were greater than might be expected from the plasma concentrations. Indeed it is hard to see any reason why the

pharmacodynamics of fentanyl absorbed from the lung should differ from those of intravenously administered fentanyl which enters the lungs at first circulation [24, 25]. There is, however, some evidence that drugs absorbed through the nasal mucosa are able to gain direct entry to the brain and cerebrospinal fluid via the olfactory mucosa and via communications between the subarachnoid space and nasal cavities [122]. It seems unlikely that this could have a significant effect on dynamics in the case of a rapidly equilibrating drug like fentanyl but the possibility is intriguing and could be tested with an experiment that simultaneously made a precise examination of pharmacodynamics and pharmacokinetics (as for example in the work of Scott, Poganis and Stanski [31] who related opioid-induced EEG changes to plasma concentrations in the study of intravenous fentanyl and alfentanil).

In a given patient the plasma concentration range, and hence usually the dose range, over which fentanyl causes useful analgesia without marked respiratory depression is quite small (chapter 1). This will be particularly so in the immediate postoperative period in the presence of other respiratory depressants. If, as appears, the strongest solution here was giving measurable analgesia without marked respiratory depression it is likely that both the weaker solutions were resulting in doses that were only marginally or not at all effective. The trial was unlikely to show up a marginal difference in effectiveness between solutions because of the many sources of variability. The present experiment was designed with three groups to cover a wide range of doses since we had little idea of what fentanyl concentration would give

doses in the correct range. Given this knowledge, efficacy might have been more elegantly demonstrated using two dose groups only.

Pain and Analgesia Scores

The agreement between pain score decreases and analgesia scores is shown in figure 6.7. There is a large scatter in the individual matching of pain score decreases to analgesia scores but the agreement between analgesia scores and average pain score changes is surprisingly good. Intergroup differences in pain score decreases and analgesia scores are in the same direction (tables 6.5 and 6.6) but the pain scores are a more sensitive measure in that the p values are lower. This is in agreement with the findings of other investigators (chapter 4) and it increases confidence in the validity of pain scores in this difficult situation where cognitive and motor functions are impaired by residual anaesthetic drugs. Interestingly, there was nothing to choose on this occasion between using absolute and proportional changes in pain score (chapter 4) since pain score changes and proportional pain score changes were roughly linearly related (figure 6.8). Neither was there an obvious relationship between initial pain scores and pain score decreases (figure 6.9).

Subject Characteristics

Subjects were not limited to those undergoing a standardised procedure. We thought it preferable to have a range of common postoperative patients in each dose group thereby making the findings more widely applicable. Unfortunately the trial was not large enough for the distribution of operations to even out and the groups are not matched in terms of surgery. In particular

there is a marked preponderance of laparoscopies in the high dose group as opposed to the other groups. The severity and natural history of postoperative pain will depend to some extent on the nature of the surgery so this chance imbalance is unfortunate since it weakens the argument for attributing the apparently better analgesia in the high dose group to fentanyl. However, the pre-analgesia pain scores in all three groups are well matched. Furthermore even in the high dose group all but three subjects requested further opiate analgesia within three hours of receiving fentanyl. This would indicate that the analgesia attributed to fentanyl was not purely an artefact of post laparoscopy pain being mild or fleeting. The effect (trend) on respiratory rates is unlikely to be influenced by the nature of the surgical procedures.

Blinding

The blinding of the experiment can be criticised as all the ampoules of the same concentration were marked with the same code. This arrangement was made in order that the collection of blood samples could be arranged efficiently to include the various dose groups in equal proportion. At the time the trial was started it was hoped that it would be possible to assay the blood from more patients than proved the case. With hindsight it would have been a better arrangement to have made the blinding watertight and to have used separate additional subjects for the few analyses we were able to carry out.

Measurement of Respiratory Rates

Changes in respiratory rate are a useful objective sign of opioid effect, particularly in a controlled comparative trial

(although only a suggestive trend was seen on this occasion). It was surprisingly difficult to measure respiratory rates without disturbing the patients, a combination of listening, inspection and sometimes palpation being required. Measurement often took over a minute. The patients consequent awareness of the process may have altered respiratory pattern. An automated method of measuring respiratory rate unobtrusively, for instance by sensing exhaled carbon dioxide and movement of the chest and abdomen [116] would be of great benefit in a similar trial.

Plasma Concentrations

The fentanyl radioimmunoassay proved disappointingly inaccurate in my hands to the extent that the results cannot be regarded as reliable. The procedure is very susceptible to laboratory technique [112] and is therefore best performed by someone who has practice in its use. Unfortunately this was not possible. The assay was performed in duplicate and the overall coefficient of variation between the paired samples was high at 16.3%. The instruction booklet gives typical examples around 5%. (Woestenborghs and colleagues [113] however found a coefficient of variation of 14.2% at a fentanyl concentration of 0.25 ng ml^{-1} though it was around 5% at higher concentrations). Furthermore for all four patients there was a difference between the counts obtained from the standard blank (no fentanyl) and the first patient samples (which were drawn before the start of analgesia and should also have been free of fentanyl). The effect of this was to indicate a small amount of fentanyl in these base line samples (around 0.1 ng ml^{-1} where the claimed detection threshold of 0.02 ng per tube corresponds to 0.04 ng ml^{-1}). Although in theory this effect could have been caused by cross

reaction between antibody and another constituent in the plasma samples it most likely reflects unknown technical errors. One specific error should be noted. In the preparation of the standard samples for the calibration curve an incorrect amount of buffer solution was pipetted into the tubes. This was noticed and corrected at a later stage but meant that the fentanyl / tritiated fentanyl / antibody reaction took place in a smaller absolute volume in the standards compared with the samples although relative concentrations of the three reactants were not altered. For financial and logistic reasons it was not possible to repeat the assay.

Because of the uncertain accuracy of the plasma concentrations detailed analysis is not justifiable although the following general points may be noted.

Peak plasma concentrations of around 1 ng ml^{-1} were found in the two patients who received the 318 ng ml^{-1} fentanyl solution. Concentrations in the other two subjects were lower. Such concentrations are just compatible with a clinical picture of analgesia with slight respiratory depression especially since arterial concentrations would have almost certainly been higher, and the subjects had also received morphine and anaesthetic drugs (see chapter 2). The plasma concentration rose sharply within a minute or so of starting the nebulisation and fell again as soon as nebulisation ceased but, in the case of two subjects, remain at around a third and a half of peak value for the three hours of the study.

The Method of Delivery: Jet nebulisation, as expected, is a very inefficient way to deliver fentanyl. A comparatively concentrated (and commercially unavailable) solution is

required. On this occasion in order to maximise the efficiency of the nebulisation the last 2 ml (636 µg in group 3) were discarded. Even if the solutions were nebulised to the point where aerosol production ceased there would still be a considerable wasted residual volume (chapter 3). Of the nebulised 3 ml (954 µg in group 3) it is likely that only a relatively small proportion, perhaps in the region of 200 µg or so, is absorbed by the patient. The actual dose received will vary greatly between patients depending on the pattern of respiration.

The method is also awkward. Constant supervision was required to keep the mask in position, the nebuliser upright and the tubing attached. Drug administration is relatively slow. Several patients disliked the experience. In order to exploit the benefits of the respiratory route a more efficient, and convenient means of delivery is required. We are hoping that the continuation of this work will involve the design and testing of a suitable hand-held pressurised fentanyl aerosol dispenser.

Summary

The trial would have been improved if only two groups had been used corresponding to the highest and the lowest dose groups in the present trial,if the blinding had been unimpeachable and if one class of patients had been chosen, all undergoing the same operation with a completely standardised anaesthetic. Nevertheless the evidence suggests that it is possible to deliver fentanyl effectively and safely via the respiratory route. In order to do this with a standard jet nebuliser a high concentration of fentanyl citrate solution (around 318 µg ml fentanyl base) is required. The method tested is very

inefficient and awkward but these problems could be overcome with better techniques of delivery such as the use of pressurised inhalers.

CHAPTER 8

Summary and Conclusions

Summary and Conclusions

1) The study examines the efficacy of nebulised inhaled fentanyl for post-operative pain relief.

2) There were no major side effects following administration to 30 patients.

3) The particular regimen we employed has major disadvantages and would not be suitable for general use. It might be profitable to develop and evaluate more convenient methods of delivering intrapulmonary fentanyl such as small pressurised inhalers.

4) Improvements in trial design were discussed.

5) Within the limits of the trial design the study suggests possible efficacy of nebulised inhaled fentanyl for postoperative pain relief.

PART 2

A PHYSIOLOGICAL MODEL OF FENTANYL PHARMACOKINETICS

CHAPTER 9

Rationale and Aims

In conventional pharmacokinetics drug concentration changes in plasma are usually described in terms of empirical compartmental models. The models correspond to reality in that the physical processes responsible for these changes, redistribution of drug from the plasma to the rest of the body and elimination of drug from the body, have explicit mathematical representation. However the models have no representation of the **mechanisms** whereby elimination, distribution or relevant physiological processes occur and the model compartments between which drug moves are mathematical devices with no direct relation to physical entities. Empirical compartmental models cannot therefore predict the effect of alterations in the real structure and function of the system.

Once a model has been fitted to a set of plasma concentration data and the appropriate constants determined the model can be used to calculate the plasma concentration at any time during or after any pattern of drug administration providing the rate of entry of drug into the plasma can be constantly specified and providing the physical system under study (i.e. the subject(s) and their physiological state) remains the same as the one in which the model was originally determined. In real life the quantitative predictive abilities of such models are much diminished by biological variation and constantly changing physiological conditions. Even so they provide a powerful conceptual framework for the qualitative understanding of some common pharmacokinetic phenomena such as the marked prolongation of effect that may emerge with large or repeated doses.

The predictive ability of these models is best for intravenous administration where entry of drug into the plasma can be

directly measured. For other routes e.g. intramuscular, oral or nasal an empirical relationship must be determined between the pattern of drug administration and the entry of drug into the plasma. Where data do not exist to determine this relationship, as for instance in the intranasal or intrapulmonary administration of fentanyl, conventional models are of little help either quantitatively or conceptually.

Whilst developing the theoretical background to the clinical trial of inhaled fentanyl I became aware of alternative pharmacokinetic approaches and in particular the work of Mapleson [127] in developing realistic physiological models of drug kinetics. In this approach explicit representation is made of the mechanisms (diffusional equilibration, mass transport, metabolism and excretion) that determine drug entry into, disposition within and exit from the body. This type of model has the theoretical advantage that if it were fully valid and provided it were possible to measure all the relevant anatomical and physiological variables, it would be possible not only to adjust the model to any individual but to incorporate the effects of ongoing physiological changes. In these terms the models remain at an early stage of development. Nevertheless they are extremely useful for exploring the interrelations of factors governing drug concentrations in different parts of the body (including locations where concentrations could normally not be measured). The basic elements of which the models are built can be rearranged or extended to make predictions about new situations such as new routes of absorption.

A physiological model of fentanyl kinetics able to encompass all methods of administration would thus be particularly useful

during the development of new routes. For instance it might help answer the questions of the possible mechanisms and likely rate of absorption following deposition on the nasal mucosa. Again, there have been some tantalising suggestions that administering drug through the respiratory tract might produce greater effect than one would expect at the same plasma concentrations following intravenous injection [117, 120, 121]. A suitable physiological model could be used to explore possible differences between intrapulmonary and intravenous administration in terms of initial drug concentration changes in arterial plasma and brain tissue. Similarly, a comparison between methods of the time course of arterio-venous concentration differences and the role played by tissue binding in lung would be of interest. In addition there are particular problems with the conventional approach to fentanyl pharmacokinetics. The inconsistency between different estimations of pharmacokinetic constants and the consequent poor predictive power of conventional kinetic models has been discussed in chapter 1. Conventional pharmacokinetic models are not able to throw any light on phenomena such as the occurrence of secondary peaks in fentanyl concentration as a result of changes in drug distribution.

Ideally one would be able to use a physiological model to make accurate predictions of specific measurements. However an equally valid, and probably at the moment more realistic, role is in the qualitative exploration of alternative situations. At the very least such a model should provide a single conceptual framework within which to address disparate questions.

Mapleson's models [127, 128] were originally developed for

inhaled volatile anaesthetic agents, however Davis [125, 126, 129] has extended Model P to encompass injected pethidine. As a step towards developing a comprehensive physiological model for use as described, I felt it would be worthwhile to attempt the adaptation of Davis's pethidine version to cover the respiratory administration of fentanyl.

The remainder of part 2 concerns this project. The next chapter, chapter 10, describes Davis's existing model for injected pethidine. Chapter 11 summarises the method of the present project and discusses rewriting the original pethidine program. Chapters 12 and 13 describe adding a nasal compartment and quantifying the model for fentanyl. A summary of the new fentanyl program is presented in chapter 14 and evaluation is discussed and conclusions presented in chapters 15 and 16. The complete fully commented listing of the fentanyl program is given as appendix 1 to this thesis and compiled versions of both programs are enclosed in the back cover on a 5.25" floppy disk.

CHAPTER 10

Davis's Physiological Model of Pethidine Pharmacokinetics

Davis's physiological model of injected (intramuscular or intravenous), pethidine kinetics exists in the form of a Fortran computer program suitable for running on a mainframe computer. Both the program and detailed descriptions of the model, its derivation and quantification have been published [125, 126, 129]. However a brief outline of the structure of the model is necessary in order make the following chapters intelligible. Some of the mathematical symbols below differ from those used by Davis but otherwise this chapter summarises the account given in his thesis [125].

General Description

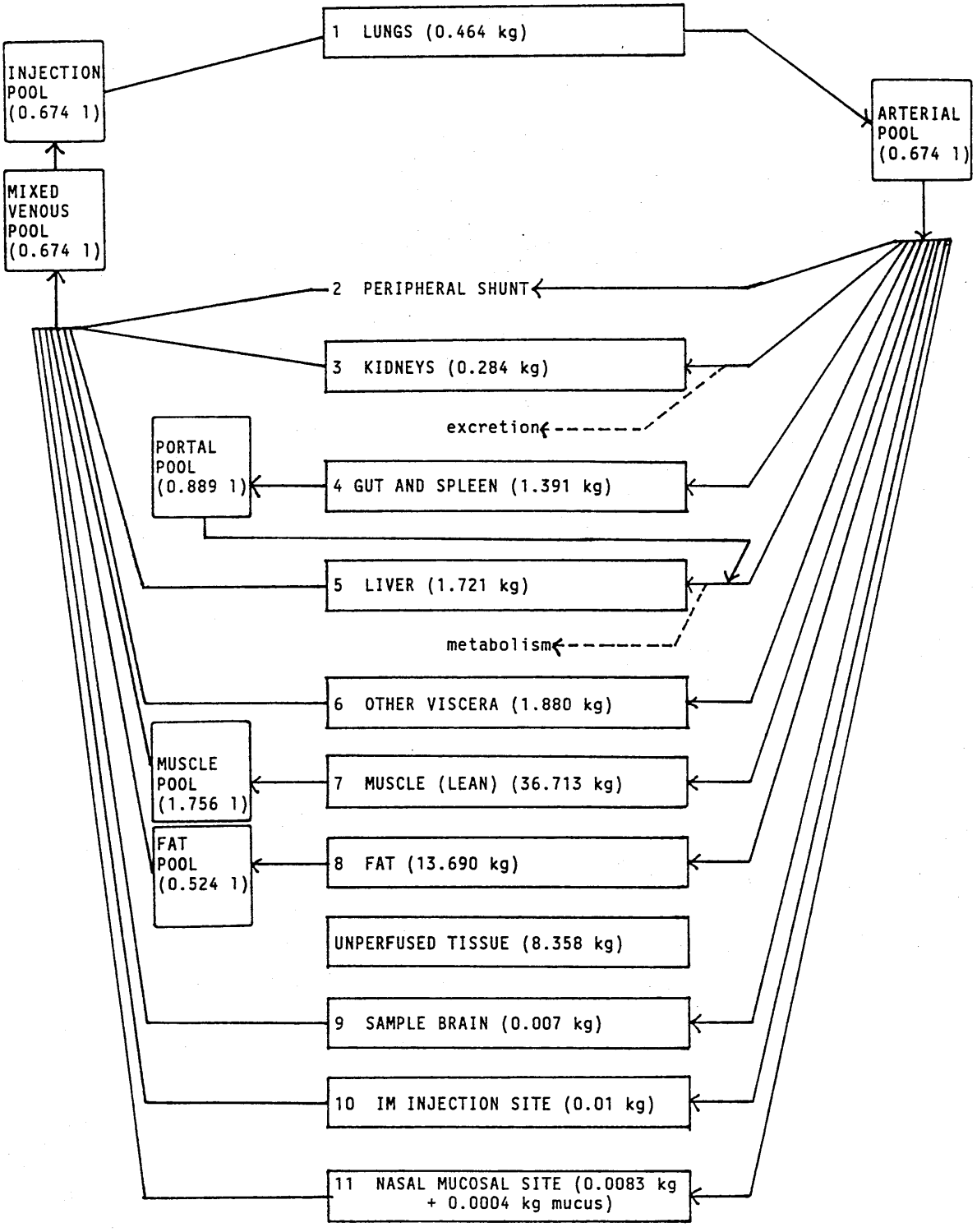
The model is based on an ideal man. Its central features (figure 10.1) are a division of the bloodless body mass into various discrete blocks and a representation of the blood circulation (including the hepatic portal circulation) which simulates both the rates of blood volume flow and the mean transit time (i.e. volume of blood between two points divided by flow) through these blocks. This allows a realistic representation of the distribution of drug (given as the local concentrations) between the tissues and different regions of the circulation at any time.

The gut and spleen, kidney, liver, and lung blocks correspond to their anatomical counterparts, the rest of the body mass is divided between four blocks; unperfused tissue, other viscera, lean and fat on the basis of relative blood flow. There are, in addition, (see below) two other blocks; sample brain and intramuscular injection site which have been given almost negligible mass.

The total volume of the circulation is realistic. The volume

Figure 10.1

The Physiological Model



The numbers on the tissue blocks are the labels used in the computer program. The mass of each tissue block and the resting volumes (between calculation cycles) of the blood pools are given in brackets. Density of blood = 1.06 kg l^{-1}. Total mass of model man (excluding blocks 9 to 11) = 70.001 Kg.

Adapted from Davis [125]

distribution of the circulation and hence the blood transit times, are modelled by placing blood pools in series with and downstream of each tissue block.

Blood is circulated in discrete aliquots or "stroke volumes". The size of each "stroke volume" is given by:

(10.1) cardiac output (litres s^{-1}) x cycle time (s)

where cycle time is the elapsed time represented by each calculation cycle. Divergent blood flow from the arterial pool to the tissues is achieved by splitting the stroke volume into fractional stroke volumes in proportion to the cardiac output to each tissue block. The sum of the fractional stroke volumes is equal to one stroke volume.

At the start of a calculation cycle all the blood is in the pools. One calculation cycle involves computing the local concentration changes consequent on the simultaneous removal of a stroke volume or fractional stroke volume of blood from each of the pools followed by its addition to and instantaneous mixing with the next pool downstream. If a tissue block separates the two pools, drug is distributed between the blood and the tissue block as described below before the blood is mixed with the downstream pool. The two fractional stroke volumes which converge on the liver from the portal pool and from the arterial pool are added together before drug is distributed between the combined blood and the liver tissue.

Drug may be added directly into the blood (venous injection pool) or into the tissues (intramuscular injection block). Drug leaves the system by excretion from blood entering the renal block or metabolism from the blood entering the liver block

after mixing of the arterial and portal fractions. Otherwise the total mass of drug in the system is conserved.

If the mass of drug which moves from each (fractional) stroke volume into the corresponding tissue block (or vice versa) at each cycle is known, together with the amounts of drug administered and eliminated then the concentration changes which result from the circulation of the blood can be calculated by keeping a tally of drug movement. For example to calculate the change in drug concentration over one cycle in the injection pool:

(10.2) $babi_{old} = api_{old} / wpi$

(10.3) $afi_{out} = api_{old} \times (svw / wpi)$

(10.4) $afv_{out} = apv_{old} \times (svw / wpv)$

(10.5) $afi_{in} = afv_{out}$

(10.6) $api_{new} = api_{old} + afi_{in} - afi_{out} + dose$

(10.7) $babi_{new} = api_{new} / wpi$

where

$babi_{old}$ = concentration drug injection pool: start of cycle

$babi_{new}$ = concentration drug injection pool: end of cycle

afi_{out} = mass drug in blood leaving injection pool

afv_{out} = mass drug in blood leaving mixed venous pool

afi_{in} = mass drug in blood entering injection pool

apv_{old} = mass drug mixed venous pool: start of cycle

api_{old} = mass drug injection pool: start of cycle

api_{new} = mass drug injection pool: end of cycle

dose = mass drug administered in that cycle

wpi = mass blood in injection pool

wpv = mass blood in mixed venous pool

svw = mass blood in stroke volume

Drug Distribution Within and Between Blood and Tissue

Each tissue block is characterised in terms of the proportion of cells and extracellular fluid (ecf) it contains. Each of these subdivisions is further characterised in terms of the proportion of its mass made up of water, fat and protein. The four non-anatomical blocks are given the average characteristics of their constituents. Blood also is divided into cells and plasma which are in turn divided into water, fat and protein.

In life, as blood passes through the tissues, drug will diffuse from plasma in the capillaries into the ecf and cells of the tissues (or vice versa if plasma drug concentration is low compared to tissue concentration). As the concentration of drug in plasma changes there will be a simultaneous redistribution of drug between red cells and plasma. The lipid membranes of the tissue cells, red cells and capillaries act as diffusion barriers which slow the passage of drug. However, providing the drug is lipid soluble enough, diffusion equilibrium is reached during the blood's passage through the tissue and drug distribution is said to be perfusion as opposed to diffusion limited. This is the case assumed in the model.

In aqueous solution drugs such as pethidine or fentanyl are present in two forms, ionised and unionised whose relative proportions depend on the pH. The model contains equations which give the pH in each compartment from the plasma pH.

Drug distribution between each tissue block and its corresponding fractional stroke volume (or whole stroke volume in the case of the lungs) of blood is thus modelled by calculating the equilibrium distribution of both drug species (ionised and unionised) between the three constituents

(water,fat and protein) of each of the four compartments (tissue cells, blood cells, plasma and ecf).

Derivation of equilibrium calculation: Equation 10.8 relates the total amount of drug present in a compartment to the concentrations of each species in the different constituents.

(10.8) $Aa_x = Wt_x.(Cb_x.gp_x + Cl_x.fl_x + Cs_x.fw_x + Ci_x.fw_x)$

where

Aa_x = Total amount drug (mmol) in compartment x

Ci = aqueous concentration ionised drug (mmol drug / g water)

Cs = aqueous concentration unionised drug (mmol drug / g water)

Cl = concentration of drug in lipid (mmol drug / g lipid)

Cb = ratio (mmol bound drug / mmol protein)

gp = concentration protein (mmol protein / g compartment mass)

fl = concentration lipid (g lipid / g compartment mass)

fw = concentration water (g water / g compartment mass)

Wt = Mass of compartment (g)

At equilibrium the aqueous concentration of unionised drug is assumed to be the same in all four compartments since unionised drug, unlike the ionised species, diffuses freely across membranous lipid barriers. The other concentrations in each compartment may be related to the unionised aqueous concentration as follows:

The aqueous concentration of ionised drug will reach an equilibrium with the aqueous concentration of unionised drug according to the Henderson-Haselbach equation (shown here for a basic drug and rearranged conveniently).

(10.9) $Ci = Cs.10^{(pKa - pH)}$

where

pKa = negative logarithm of the acid dissociation constant

pH = pH in the compartment concerned

The unionised drug equilibrates between aqueous and lipid phases according to an appropriate solubility coefficient. The ionised form is lipid insoluble.

(10.10) $Cl = Cs.lambda$

where

lambda = lipid solubility coefficient

Finally, binding to protein is characterised according to the Scatchard equation which is derived directly from the law of mass action [164, 165] and describes the relationship between the equilibrium concentrations of free drug, bound drug and protein in aqueous solution. Two situations are considered (see chapter 13) in alternative forms of the model: one in which only unionised drug binds to protein and one in which both unionised and ionised forms bind with equal avidity. The Scatchard equation for binding of unionised drug only is shown here.

(10.11) $$Cb = \frac{n.k.Cs}{(1 + k.Cs)}$$

where

n = a constant (number of binding sites per protein molecule)

k = binding constant

The total amount of drug present in a tissue block and its associated (fractional) stroke volume of blood is simply given by the sum of the amounts in the constituent compartments:

(10.12) $Aa_{bt} = Aa_p + Aa_r + Aa_c + Aa_e$

where

bt = tissue block and (fractional) stroke volume of blood

c = tissue cells

p = plasma

e = ecf

r = red blood cells

Equations 10.9 to 10.11 may be substituted in equations 10.8 and 10.12 to give equation 10.13 which relates the total amount of drug present in the blood and tissue mass (Aa_{bt}) to the (same in all compartments) unionised aqueous concentration at equilibrium (Cs).

(10.13)

$$Aa_{bt} =$$

$$Wt_p \cdot \left[\frac{n_p.k_p.Cs.gp_p}{1 + k.Cs} + Cs.lambda.fl_p + Cs.fw_p + Cs.10^{(pKa - pHp)}.fw_p \right]$$

$$+$$

$$Wt_r \cdot \left[\frac{n_r.k_r.Cs.gp_r}{1 + k.Cs} + Cs.lambda.fl_r + Cs.fw_r + Cs.10^{(pKa - pHr)}.fw_r \right]$$

$$+$$

$$Wt_c \cdot \left[\frac{n_c.k_c.Cs.gp_c}{1 + k.Cs} + Cs.lambda.fl_c + Cs.fw_c + Cs.10^{(pKa - pHc)}.fw_c \right]$$

$$+$$

$$Wt_e \cdot \left[\frac{n_e.k_e.Cs.gp_e}{1 + k.Cs} + Cs.lambda.fl_e + Cs.fw_e + Cs.10^{(pKa - pHe)}.fw_e \right]$$

Aa_{bt} is known from tally keeping. Equation 10.13 is solved by an

iterative method to give Cs and thence the total drug concentrations and amounts in each of the four compartments.

Note on sample brain and injection compartments: The sample brain tissue has the estimated characteristics of real brain including blood content and perfusion per unit mass but has a mass (7 g) only a small fraction the mass of the complete brain (which is included in the "other visceral" tissue block). The device of giving sampling compartments very small mass allows additional sampling compartments to be added to and removed from the model at will without having to consider the effect on the overall distribution of blood, tissue and drug. The concentrations of drug in the tissue calculated by equilibrium with arterial blood will be unaltered by the absolute size of the tissue block providing perfusion is correctly scaled. The intramuscular compartment and in the new version the nasal compartment are also so small as to have negligible effect on the overall performance of the model.

CHAPTER 11

Method.

Replication of the Existing Model

The immediate aim of the project was to quantify Davis's pethidine model [125] for fentanyl and extend it for the administration of drug into the respiratory tract. The model already has a lung compartment perfused by the pulmonary circulation and drug may be simply added into the lung tissue at the end of a relevant calculation cycle and distributed between the lung and the perfusing blood at the next cycle. This takes no account of the processes of drug inhalation, deposition and absorption. The model would lend itself to such extension but the task is too large for the present occasion. A first step in the construction of such a comprehensive model would probably be to incorporate an existing model of aerosol deposition [e.g. 63]. For each administered dose the prevalent breathing pattern, and the size distribution, chemical composition, inhaled particle density and method of administration of the aerosol would have to be specified.

The simpler arrangement of adding drug directly into the lung tissue is useful in that it represents an idealised "best case" for comparison with intravenous administration.

During aerosol inhalation an important proportion of the inhaled drug may be deposited in the upper respiratory tract which is perfused by the systemic circulation. Drug deposited in the mouth and pharynx is probably rapidly washed away by saliva and swallowed but there is likely to be significant absorption from the nasal mucosa. The nasal mucosa is increasingly often targeted as an area for drug administration in its own right. A nasal compartment would thus be a useful addition to the basic model.

Method

The following method was used:

1) The original program was rewritten in a form convenient for use by people with minimal computer expertise on a microcomputer of the kind available in this department.
2) A nasal compartment was added and the program modified to allow drug administration directly into the lung tissue.
3) A version of the program was quantified for fentanyl.
4) The model was evaluated with particular regard to the purposes suggested in chapter 9.

Rewriting the Original Model

In the following sections there are necessarily references to subroutines and variable names from the original program. For further details see Davis [125]. Rewriting was carried out using the quantifying data and detailed model description given by Davis [125]. The exercise had two main purposes. Firstly to familiarise myself with the model and to gain programming expertise. Secondly to allow the model to be checked for correctness at this intermediate stage against the published simulation data from the original. It would also result in a useful working version of the pethidine model.

Agreement with the original model: I was unable to get the simulation output to match that published by Davis [125]. A careful comparative scrutiny of the old and new programs in collaboration with Davis who provided a full list of input data, the listing of a working version of the program and detailed simulation results lead to the discovery of previously unnoticed faults but also to several errors in his original program. The

original program exists in several versions of which two are given in Davis's thesis [125]. Not all the errors are present in all versions.

The errors are:

1) In the subroutine lamset the variable rkar is used in the place of rkap in the calculation of mp.
2) In the subroutines pend and lamset in the common block statement pdlt the arrays phe(15) and phc(15) are transposed compared with the common block statement in the main program resulting in the transposition of their values.
3) In the subroutine tbrat in the calculation of baer an erratic + sign occurs so that 10.0^{**} becomes $1+0.0^{**}$ and the value of the calculation is changed.
4) In the subroutine blconc both in the calculation of bap and bar the sign for exponentiation ** is used in place of the sign for multiplication * changing the values of the calculations (not in the main listing given in the thesis)
5) In the main program in the calling routine for subroutine tbrat the variable bpratio is translocated from the end to the middle of the calling statement resulting in a complex transposition of variable values (not in the main listing given in the thesis).
6) In the main program time is incremented inbetween calling the subroutines blconc and tbrat which each calculate part of the results set. Thus results from the same calculation cycle are attributed to different simulation times (and vice versa).

In addition the value of one variable is incorrectly given in Davis's thesis [125]:

	thesis	correct
rkar	0.12287	0.012287
Kg tissue / kg total mass		
tissue 4	0.1990	0.0199

Once these discrepancies had been taken into account the new and the old versions of the program agreed exactly and later versions of the program incorporating slight modifications could be compared against this correct "standard". The existence of these errors may have implications for the published quantitative evaluation of the pethidine model and the choice of best alternative parameter values. (The version enclosed with this thesis although "correct" has not yet been re-evaluated).

Final version of pethidine program The final version of the pethidine program written for this project differed from the original in the presence of a nasal compartment, input routines to accept drug dose information and variable changes from a keyboard, and the program output (see note page 179). In addition two small changes were made to the mathematics:

1) The sum of the fractional stroke volumes was made equal to exactly unity. As previously written there was a slight tendency for drug to accumulate in the arterial pool since the combined stroke volume entering from the lungs was slightly larger than the sum of the fractional stroke volumes leaving for the tissues. Similarly there was a tendency for drug to be excessively removed from the mixed venous pool. (The weights of the tissue blocks as a proportion of total body mass were also

specified more precisely to give absolute weights which were correct to the nearest gram).

2) Originally two different iterative methods were used to solve the equation for equilibration of drug between blood and tissue in subroutine equil and the equation for equilibration between red cells and plasma in the subroutine blconc. The method used in equil (which uses the differential to provide estimates of the dependent variable) was adapted for blconc since it is superior in that it solves the equation to a given tolerance in much fewer iterations.

Note On the Programming Language

Both the new version of the pethidine program and the adaptation to fentanyl were written in Turbo Basic. Turbo Basic is a compiled language marketed by Borland International Inc. and is a development of Basic. It is relatively easy language for a programming novice. It lends itself to making programs which are "friendly" at the time of use, for instance it is easy to devise keyboard input routines which incorporate simple error checks and allow re-input of correct information if necessary. Turbo basic was already in use in this department when the project was undertaken.

Unfortunately, the language has a serious drawback which only became apparent once the project was well underway and which cost many hours of programming time. There is no obligation to declare variables in the start of the main program. New variables if not allocated a value are given the default value zero the first time they are used. Similarly the declaration of variables in subroutines is optional. If variables are not declared "common" to a subroutine and main program they are

given the default type "static" (i.e. recognised by that subroutine only but keep their value between callings of the subroutine) and if necessary given a default value of zero. This means that not only may accidental failure to declare a variable to a subroutine go unnoticed, with the result that a value is not passed, but misspellings of variables, either in the main program or in subroutines, may also go unremarked with consequent distortion of the calculations. As there is a large number of variables in these programs such errors were fairly common and time consuming to weed out. Furthermore it was difficult to recognise their presence unless they occasioned final numerical results which were obviously incongruous. In the case of the the fentanyl program there was no standard against which its output could be checked and the only solution was careful checking of the program listing. Thus a design presumably intended to simplify programming in fact detracts from the safety of the language and adds difficulty. I would not recommend Turbo Basic for future similar applications.

CHAPTER 12

Addition of a Nasal Compartment

To allow nasal administration of drug I had to add a nasal compartment to the model. The compartment consists of a mass of nasal mucosa, its associated mucus and perfusing blood. Its quantitative features were based on the following anatomy and physiology.

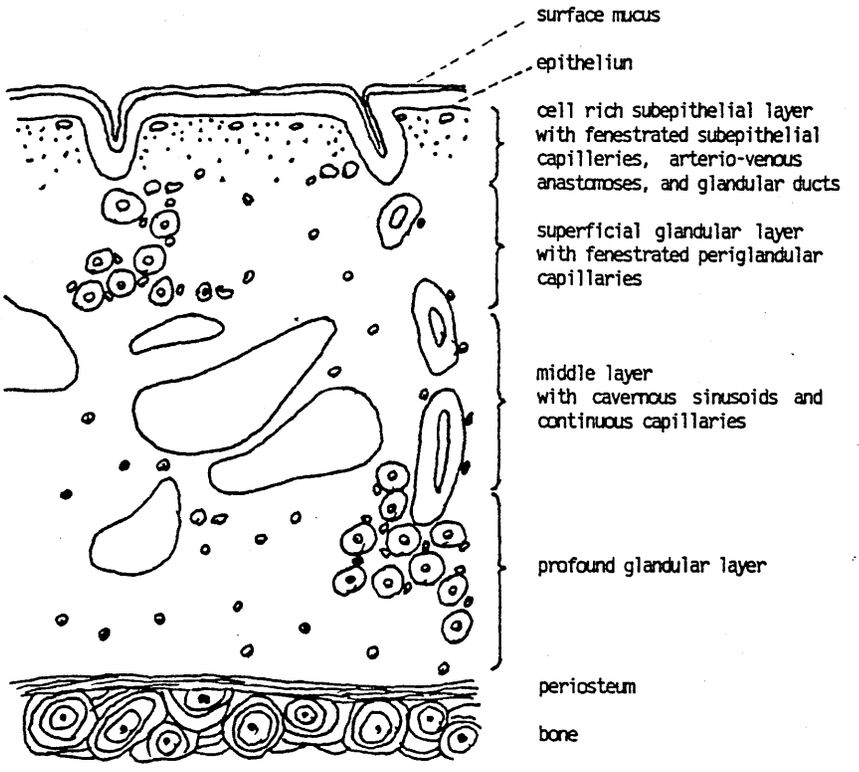
Anatomy and Physiology

Gross Anatomy: The paired nasal cavities are irregular, high, narrow slits separated by the nasal septum and extending from the nares anteriorly to the posterior choanae where they open into the naso-pharynx. Several large air-filled cavities lined with respiratory mucosa, the paranasal sinuses, open off the nasal cavities by small apertures. The distance from the nares to the posterior wall is around 12 to 14 cm. The combined surface area of both cavities excluding the paranasal sinuses is about 150 cm^2 and the combined volume about 15 cm^3 [136].
The nasal cavities are lined throughout by mucus membrane which is continuous with the periosteum and perichondrium of the bone and cartilage walls.

The mucus membrane: The mucus membrane consists of an epithelium separated from a lamina propria by a basement membrane [136]. The lamina propria merges with the underlying perichondrium or periosteum. Most of the epithelium consists of ciliated pseudostratified columnar epithelium, but anteriorly a mixture of types are found and the epithelium becomes squamous towards the nares. The olfactory epithelium is a patch of specialised epithelium of about 10 cm^2 each side on the upper posterior aspect of the cavities. The lamina propria may be divided into four indistinct layers (figure 12.1).

Figure 12.1

Mucus Membrane of the Nose



Redrawn from Myngind [136].

Mucosal blood supply: Immediately below the epithelial membrane is a layer rich in large capillaries. The capillaries are fenestrated and have (in common with the arterioles and venules of the mucus membrane) an unusually porous basement membrane. The fenestrations are described as openings in the epithelial lining where the endothelial cell consists of only a single membrane, this membrane along with the basement membrane constituting the barrier between blood plasma and tissue fluid. [135, 136]. This arrangement will obviously speed the equilibration of lipid soluble molecules. The implications for large ionised molecules (such as ionised fentanyl) are not clear.

Deeper in the mucosa is found the cavernous plexus of venous sinusoids. When the sinusoids are engorged the blood content and thickness of the mucosa are considerably increased. The sinusoids receive blood from capillaries draining the surface layer and surrounding glands but also direct by arteriovenous anastomoses. The filling of the sinusoids is probably regulated by a combination of functional sphincters controlling input from the anastomoses and sphincters controlling the outflow resistance [135]. In the cat Anggard [137] estimated 60% of the nasal mucosal blood flow went through the arteriovenous anastomoses. Capillary flow was 0.5 ml min^{-1} g mucosal $tissue^{-1}$. Capillary flow was less variable than shunt flow in response to sympathetic nervous stimulation.

In man, total nasal mucosal blood flow has repeatedly been estimated as about 0.4 ml^{-1} g^{-1} in the mucosa of the nasal passage by a technique based on the clearance of injected radioactive Xenon [138, 139, 140, 141].

Blood flow in the mucosa of the human maxillary paranasal sinus has been estimated as 1.25 ml min^{-1} cm^{-3} and 0.88 ml min^{-1} cm^{-3} by a plethysmographic technique and 0.93 ml min^{-1} cm^{-3} by clearance of xenon [142, 143]. However these estimates of sinus mucosal blood flow were calculated using an estimated mucosal thickness of 125 µ which is at the lower range of the likely values (see below). If the mucosa were thicker the blood flow per unit volume tissue would be reduced proportionately. The mucosa in the paranasal sinuses is very similar to that in the nasal cavity but there is no cavernous plexus [135].

Mucosal thickness: The thickness of the mucosa varies in different parts of the nose, mainly because of variation in the the amount of cavernous tissue present. In the nasal cavity proper it ranges from several hundred µ to about 5 mm [135, 144]. Drettner [145] gives the thickness of the mucosa in the paranasal sinuses as between 100 and 500 µ. There are no venous sinusoids or glands in the mucosa of the paranasal sinuses. In one of the micrographs of the mucosa of the nasal cavity presented by Cauna [135], the sinusoids of the cavernous plexus begin 200 to 400 µ below the epithelial surface. The IRCP report on reference man [151] estimates nasal mucosal thickness as 2 mm.

Nasal mucous: The ciliated epithelium of the nasal cavity is covered with a continuous layer of mucous. Widdicombe and Wells have reviewed the composition and physiology of nasal mucous [147]. Mucous is a mixture of secretions from various cells, (some of which are collected into glands) and plasma transudate. On the epithelium the mucus is in two layers, a watery layer surrounding the epithelial cilia and a superficial

viscous layer which is moved along by the beating cilia in the manner of a conveyer belt. The thick layer may be completely continuous or may exist in confluent plaques.

The viscous properties of mucous derive from its high content of glycoproteins which account for 70 to 80% of its dry weight. Glycoproteins consist of polypeptide chains linked by sugar side chains. The resulting polymers aggregate to form a continuous network. Mucus glycoproteins contain 50 to 80% carbohydrate (as opposed to the glycoproteins found in plasma which contain less than 25% and are associated with different sugar residues). In addition to glycoproteins and inorganic salts many other proteins are present, some of which, for example albumin, are also found in plasma. The total protein content of mucus has been measured as 6.36 mg ml^{-1} [146] which if mucus water content were 94% [122] would be an aqueous concentration of 6.77 mg ml^{-1}. (This figure refers to "whole mucus": mucus can be separated in the laboratory by centrifugation into watery gel and viscous sol layers which may or may not correspond to their in vivo counterparts). Techniques for estimating total protein content in mucus are likely to measure the polypeptide chains in the glycoproteins but exclude the associated carbohydrate residues [147].

Mucus from the lower airways contains some lipid, partly as a consequence of the presence of cellular debris. Lipid content of nasal secretions is unknown [147].

The pH of nasal secretions in health is between 5.5 and 6.5 [122].

Thickness of the mucus layer: The watery layer of mucus extends to the tips of the epithelial cilia which are 4 to 6 μ long

[136]. The depth of this layer appears to be closely regulated by absorption of fluid into the epithelial cells. I could find no estimates of the average thickness of the viscous layer in humans although the cilia are capable of moving very large loads [150]. The IRCP report on reference man [151] uses an estimate of 0.5 mm for the total thickness of the mucous layer but this seems a little large. No justification or reference is given. In a variety of unspecified laboratory animals Graziadei [149] gives the thickness of the mucous layer over the olfactory mucosa as 20 to 50 μ thick (presumably measured by direct microscopic observation though this is not stated).

Mucosal Clearance: As a result of the beating action of the epithelial there is a continuous conveyer belt-like flow of mucous over the nasal mucosa though the watery mucus surrounding the cilia and the superficial viscous layer may not flow together [150]. Most clearance is backwards into the pharynx where mucous and deposited particles are swallowed. The mucus from some small areas of mucosa flows anteriorly whence accumulated matter is removed by sneezing or grooming. Proctor [150] suggests that there is large interindividual variation in the flow rate of nasal mucus with 80% of individuals falling in the range of 2.3 to 23.6 mm min^{-1} (Av. 8.4 mm min^{-1}) and a group of slower clearers having rates less than this. Hardy and colleagues [148] discovered a half time of 6 to 9 min for the clearance of radioactive tracer from ciliated epithelium.

Nasal deposition: Material may be introduced into the nose in many different ways including the simple instillation of fluid or powder, the use of sprays and atomisers and the generation

and inhalation of aerosols.

Inhaled aerosols comprising particles larger than 5 μ are almost completely deposited in the nose. Most inhaled particles less than 1 to 2 μ will pass through the nose into the lower airways [122]. The paranasal sinuses can probably be safely ignored as there is very little bulk flow of air between them and the nasal cavity on account of their small openings [145].

The location of substances on the nasal mucosa and the area of mucosa over which deposition occurs will depend both on the nature of the material and the manner of its introduction. The area covered may range from a few square millimetres in the case of badly instilled drops [see 136] to most of the nasal mucosa [e.g. 148]. After deposition the distribution of substances will be changed by mucus clearance currents.

The Nasal Compartment

In life drug-containing solution will be deposited on the nasal mucosa and drug will diffuse from the administered solution into the nasal mucous, the tissue of the mucosa and the perfusing blood, drug presumably penetrating more deeply into the tissue with time. There is evidence in rats [152] that absorption is pH dependent, absorption being faster for unionised species and that substances which are more lipid soluble are absorbed more rapidly. However this work also suggested that there may be considerable absorption of lipid insoluble species. Furthermore, dissolution in nasal lymph and direct diffusion into nerve cells through the olfactory mucosa or into the subarachnoid space may be relevant [122]. For the model I have assumed a simpler situation in which drug is deposited into the nasal mucus and an immediate concentration equilibrium, based on equal

aqueous unionised fentanyl concentration, is reached with the more superficial part of the underlying mucosa and its perfusing blood.

Assuming that a large proportion of fentanyl absorption would take place into the superficial capillary plexus I have given the absorbing tissue a thickness of 1 mm. This should exclude most of the cavernous sinusoids. The absorptive area was set at 80 cm^{-2}. This is probably generous for most applications but the continuous inhalation of a heterodisperse aerosol, as in the nebulised inhaled fentanyl study, is likely to result in deposition of particles over a wide area of mucosa. This sort of coverage can probably also be achieved with properly applied nose drops [148].

The mass of the nasal tissue block assuming a tissue density of 1.04 gm ml^{-1} is thus 8.3×10^{-3} kg

The mucus layer was given a total thickness of 50 µ.

The mass of mucous associated with the tissue block is thus approximately 4.0×10^{-4} kg

In the absence of specific data on nasal mucosa the tissue block was given the same density, proportional division into cells and ecf, and proportional content of water, fat and protein as intestine, a tissue which contains a large amount of mucosa.

Mucosal perfusion was set at 0.5 ml min^{-1} g^{-1} and the tissue was given the fastest mean transit time of 18.7 s along with brain other viscera and peripheral shunt. (This might be inappropriate if there were a relatively large amount of blood stored in the sinusoids).

Mucus was given a protein content of 6.77×10^{-3} kg kg^{-1} and the proteins were assumed to have the same binding characteristics

and molecular weight as plasma proteins. (The device of giving these and other tissue proteins the same average molecular weight as plasma proteins, even when it is clearly not the case, is simply a convenient way of giving these proteins the same drug-binding ability gram for gram as plasma proteins. An alternative would be to express the binding constants in terms of g or kg rather than µmol of protein). No account was taken of any contribution to binding by the carbohydrate groups of the glycoproteins and mucus was supposed to be lipid free.

Mucus pH was set = 6.0.

Other assumptions were that absorption would be complete before drug was removed by mucus clearance and that the volume of water in which the drug was administered would be negligible. The justification for this last assumption is that the volume of the watery mucus phase appears to be regulated and any excess water added to the mucous layer would be rapidly removed by a mixture of absorption into the epithelial surface and evaporation into the nasal airstream.

CHAPTER 13

Adaptation of the Model to Fentanyl

Protein Binding pKa and Lipid Solubility

To quantify the original pethidine model Davis [125] performed in vitro binding experiments with three different groups of proteins, those from blood plasma, muscle cells and erythrocytes.

He considered two interpretations of the resulting data, firstly that only unionised pethidine bound to protein and secondly that binding of both ionised and unionised forms occurred. The data was fitted to alternative forms of the mass action or Scatchard binding equation. (Strictly the term Scatchard equation refers only to one particular arrangement of this equation but it is convenient to use it as a general label for equation 13.1 no matter how it is arranged [see 164, 165]).

$$(13.1) \quad C_p = \frac{n.k.C_{free}}{1 + k.C_{free}}$$

where C_p is moles drug bound per mol protein, C_{free} is the aqueous molar concentration of free drug and

a) $C_{free} = Cs$

or b) $C_{free} = Cs + Ci$

where Cs is the aqueous molar concentration of free unionised drug and Ci is the aqueous molar concentration of free ionised drug.

Rather than resolving these alternatives with in vitro experiment Davis included the two forms of the equation with their corresponding values of n and k in a set of alternative parameter values. He then examined these in the completed model to determine which gave the best fit between simulation results

and published experimental pharmacokinetic data.

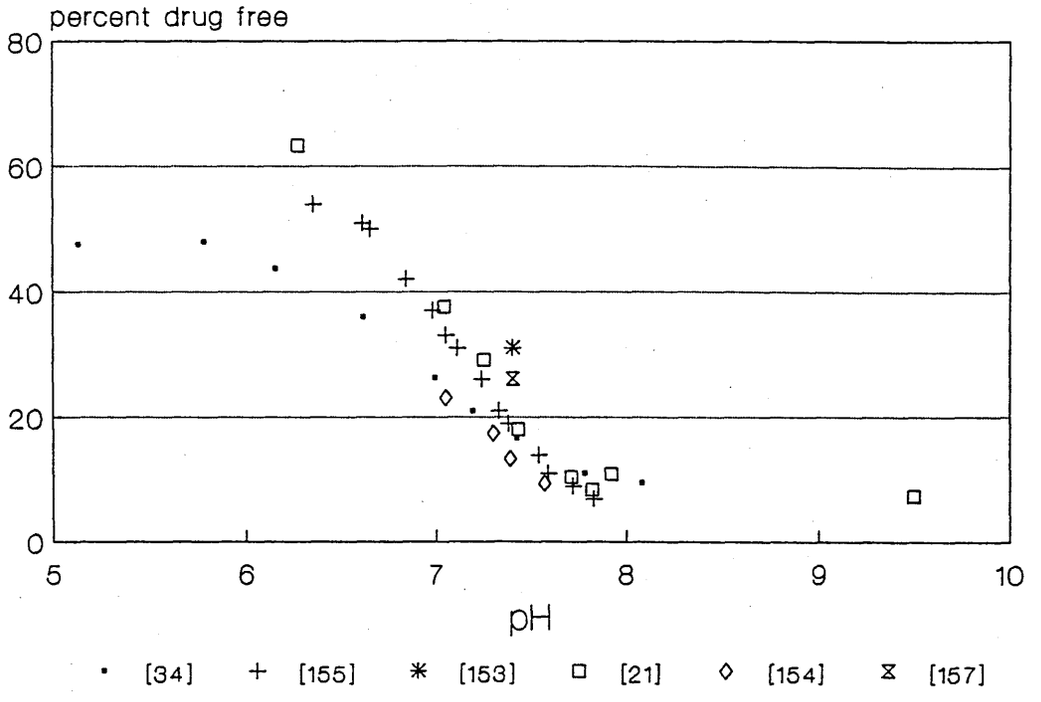
Protein binding of fentanyl. Available information: A comprehensive search yielded several investigations of binding to human plasma proteins, but no information on binding to other types of protein. I thus had to extrapolate plasma binding data to characterise all protein binding (except for binding to blood cell proteins, see below) in the fentanyl version of the model. The two most detailed studies are those of Bower [155] and of Meuldermans and colleagues [34] both of whom investigate the effects of pH, drug and protein concentration and other variables on binding. Holt and Teschemacher [153] carried out a detailed study but used rabbit plasma or albumin solution for several of the experiments. Hargrave [154] reports briefly on the effects of pH, as do Maclean and Hug [21] who also report on binding at two different protein concentrations though at an unphysiological pH. Lehman and colleagues [157] measured binding at pH 7.4 and 37°C. Schaer and Jenny ([163] quoted in [155]) performed binding experiments at 25°C but as binding is increased by temperature [155, 34] their results are not comparable with the other studies.

Estimates of binding to whole human plasma at various pH values are shown in figure 13.1 and the results of the investigations of Bower and of Meuldermans and colleagues into the effects of plasma dilution are shown in figure 13.2.

If β is the proportion of the total fentanyl concentration which is bound to protein, Holt and Teschemacher [153] and Lehmann and colleagues [157] report a smaller β under normal physiological conditions than other investigators. Apart from these two groups there is good agreement between laboratories on β in whole

Figure 13.1

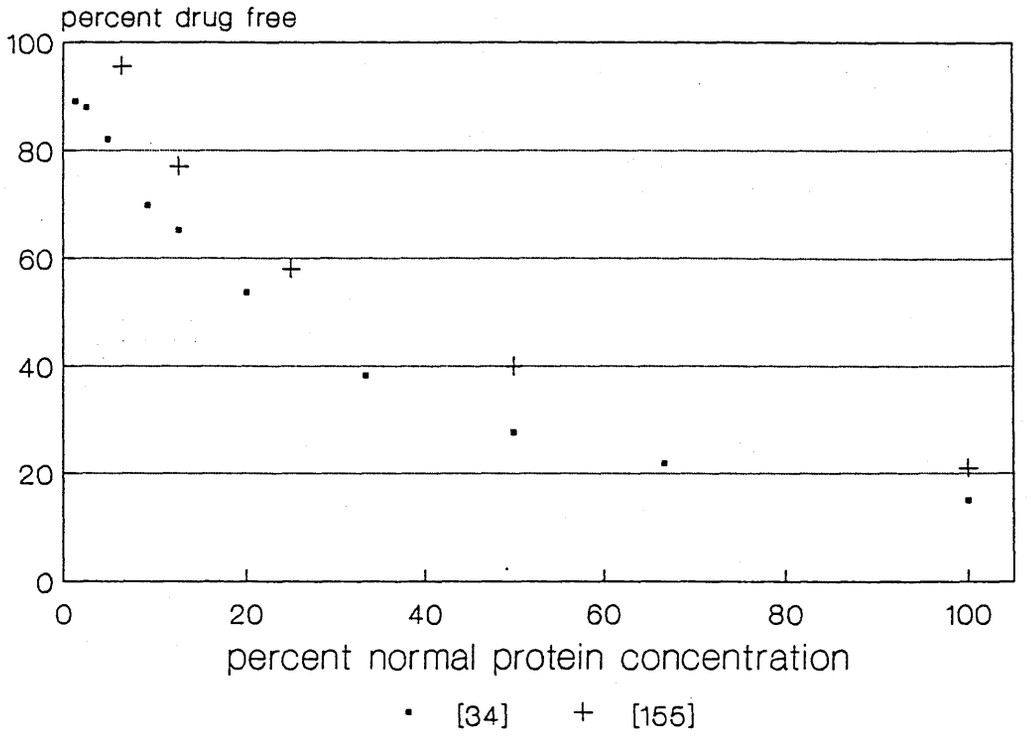
Binding of Fentanyl to Plasma Protein Showing Changes with pH



[Numbers] refer to the main reference list

Figure 13.2

Changes with Plasma Dilution in Binding of Fentanyl by Plasma Proteins



[Numbers] refer to the main reference list

undiluted plasma at pH 7.3 and above, but wide disagreement at the other end of the pH range. Lehmann and colleagues [157] found a very much greater variation in β between plasma from different individuals than did Meuldermans and colleagues [34] or Bower [155].

Four additional conclusions may be drawn from the available work.

i) Albumin probably accounts for about half plasma fentanyl binding with a number of different proteins responsible for the remainder.

ii) β is independent of fentanyl concentration, probably up to concentrations as high as about 6 mg ml^{-1} in undiluted plasma.

iii) β is dependent on pH increasing with higher pH

iv) β is dependent on protein concentration increasing with higher protein concentration.

Fitting the data to a model: Alternative (b) of the Scatchard equation 13.1 (above) is precluded as it does not account for the pH dependence of fentanyl binding. Since the ratio of ionised to unionised free drug changes with pH according to the Henderson Hasselbach equation (13.12), the pH dependence of binding may be explained by assuming that proportionately more unionised fentanyl binds than ionised fentanyl. i.e. n.k is larger for unionised than ionised fentanyl. Alternative (a) represents an extreme form of this situation where the value of n.k for ionised drug is 0. However n.k for ionised fentanyl may well be > 0.

Independence of β from drug concentration (conclusion ii above) is predicted by the Scatchard equation when drug concentration

is low compared with the concentration of available binding sites.

Thus:

$$(13.2) \quad Cp = \frac{C_{bound}}{P}$$

where

P = aqueous molar concentration of protein

C_{bound} = aqueous molar concentration of bound drug

Substituting equation 13.2 for Cp, the Scatchard equation (13.1) may be rearranged to give β

$$(13.3) \quad \beta = \frac{1}{1 + \frac{C_{free}}{n.P} + \frac{1}{n.k.P}}$$

where

$$(13.4) \quad \beta = \frac{C_{bound}}{C_{bound} + C_{free}}$$

When the concentration of binding sites in the system is high compared with the concentration of unbound drug the term $C_{free}/n.P$ approximates to zero and equation 2 simplifies to:

$$(13.5) \quad \beta = \frac{n.k.P}{1 + n.k.P}$$

In this situation β approaches a maximum which depends only on the concentration and affinity of binding sites and not on the concentration of drug. Note that n and k cannot be determined separately. Substituting for β from equation 13.4, equation 13.5 may be

rewritten:

(13.6) $\frac{C_{bound}}{P} = n.k.C_{free}$

or, substituting from equation 13.2

(13.7) $C_p = n.k.C_{free}$

This simplified Scatchard equation can be expanded to accommodate different binding constants for the ionised and unionised forms. Thus, from equation 13.6:

(13.8) $C_{boundi} = P.Ci.ki$

(13.9) $C_{bounds} = P.Cs.ks$

where ks = n.k for the ionised species

ki = n.k for the unionised species

C_{boundi} = aqueous concentration of unionised bound drug

C_{bounds} = aqueous concentration of ionised bound drug

but

(13.10) $C_{bound} = C_{boundi} + C_{bounds}$

substituting for C_{boundi} and C_{bounds} from equations 13.8 and 13.9

(13.11) $C_{bound} = P.(Ci.ki + Cs.ks)$

but from the Henderson Hasselbach equation

(13.12) $Ci = Cs.10^{(pKa - pH)}$

substituting in equation 13.11 for C_{freei} from equation 13.12 and rearranging gives

$$(13.13)\quad \frac{C_{bound}}{P} = Cs.(10^{(pKa - pH)}.ki + ks)$$

which substituting from equation 13.2 may be written:

$$(13.14)\quad C_p = Cs.(10^{(pKa - pH)}.ki + ks)$$

In order to calculate ks and ki from the available data equation 13.13 must be rearranged to give β. Thus:

$$(13.15)\quad C_{free} = Ci + Cs$$

substituting for Ci from equation 13.12

$$(13.16)\quad C_{free} = Cs.(1 + 10^{(pKa - pH)})$$

substituting equation 13.16 and equation 13.13 in equation 13.4 Cs cancels to give:

$$(13.17)\quad \beta = \frac{ki.P.10^{(pKa - pH)} + ks.P}{10^{(pKa - pH)}.(ki.P + 1) + 1 + ks.P}$$

The Scatchard equation is the simplest of many possible mathematical models of protein binding. It is derived directly from the law of mass action [see 164, 165] assuming:

a) There is only one class of binding site all members of which have equal affinity for the ligand.

b) All protein molecules have an equal number of binding sites

c) There is no cooperativity between binding sites i.e. that binding at one site does not affect the affinity of adjacent sites on the same molecule.

In addition, to apply the model to fentanyl as equation 13.12 (a) must be rewritten as:

d) Each species has only one class of binding site all members of which have equal affinity for their respective ligand.

and...

e) A logical consequence of (d), the ionised and unionised forms bind to separate sites with no cross binding.

f) The concentrations of both species of unbound drug are low compared to the concentration of available binding sites

When assumptions (a) to (c) are met a plot of Cb/C_{free} against C_{free} will yield a straight line with n and n.k as the intercepts on the abscissa and ordinate respectively. When in addition assumption (f) is met, i.e. when the value of $C_{free}/n.P$ is negligible, the line will be parallel to the abscissa intercepting the ordinate at n.k. When all the above assumptions are met for two separate species the line will intercept at an apparent n.k which is the concentration-weighted mean of the individual n.k values.

It might seem unlikely that these rigorous conditions will be met in life when binding occurs to a heterogeneous mix of plasma proteins. Meyer and Guttman [164] caution against attempting to apply Scatchard plots to whole plasma and a Scatchard plot for a ligand obeying equation 13.1 at several sites of different affinity is curved. If binding at all the sites obeys equation 13.7 however the plot is a straight line irrespective of how many different sites there are. Bower's experimental Scatchard plot of fentanyl binding to whole plasma approximates well to a straight line parallel to the abscissa [159].

Furthermore equation 13.7 is of the same form as an equation describing partition of a solute between two solvent phases (e.g. equation 13.19 below) where n.k is the partition coefficient. If the process of binding by unionised species involved non-specific partitioning into hydrophobic regions on the protein molecule it would not be necessary to postulate a large number of separate individual binding sites on each protein molecule in order that binding should obey this equation up to relatively high molar concentrations of free drug. n.k in this case could be conceptualised as a mean partition coefficient, weighted according to the relative concentrations of sites with different affinity, between the aqueous phase and available hydrophobic areas in the protein molecules.

Binding of ionised fentanyl accounts, with this model, for only a small proportion of the total binding at physiological pH. Thus though it may also obey the low-free-drug-concentration approximation it would be difficult to confirm or refute this from its proportionately small effect on the shape of a Scatchard plot at pH 7.4.

In order to determine ki and ks from equation 13.17 the pKa of fentanyl must be supplied. Meuldermans and colleagues [34] quote 8.43 determined by electrometric titration in the laboratories of the original manufacturers Jansen Pharmaceuticals Ltd. There are no experimental details. Apart from this I was able to discover only two other estimates both derived from the effects of pH on lipid / aqueous phase apparent partition coefficients. Bower [159] gives 6.9, Holt and Teschemacher [153] give 7.7 (see lipid solubility below).

The aqueous concentration of protein is also required. This

should be the concentration of the protein to which binding occurs. Since binding occurs to a range of plasma proteins but the precise pattern is unknown I have followed Davis's procedure for pethidine and used, for whole plasma, his estimate of total molar concentration of plasma proteins.

P = $7.47\ 10^{-2}$ µmol Kg^{-1} plasma = $8.16\ 10^{-2}$ µmol Kg^{-1} plasma water

The protein concentration in diluted plasma has been calculated accordingly. (In the published studies relevant plasma protein concentrations are mostly expressed on an arbitrary scale of plasma dilution. An absolute value of plasma protein concentration is required for determining values of n.k which can be applied to tissue cell proteins whose concentration is expressed in the same units but note that it has no influence on the fit of the experimental data to equation 13.17).

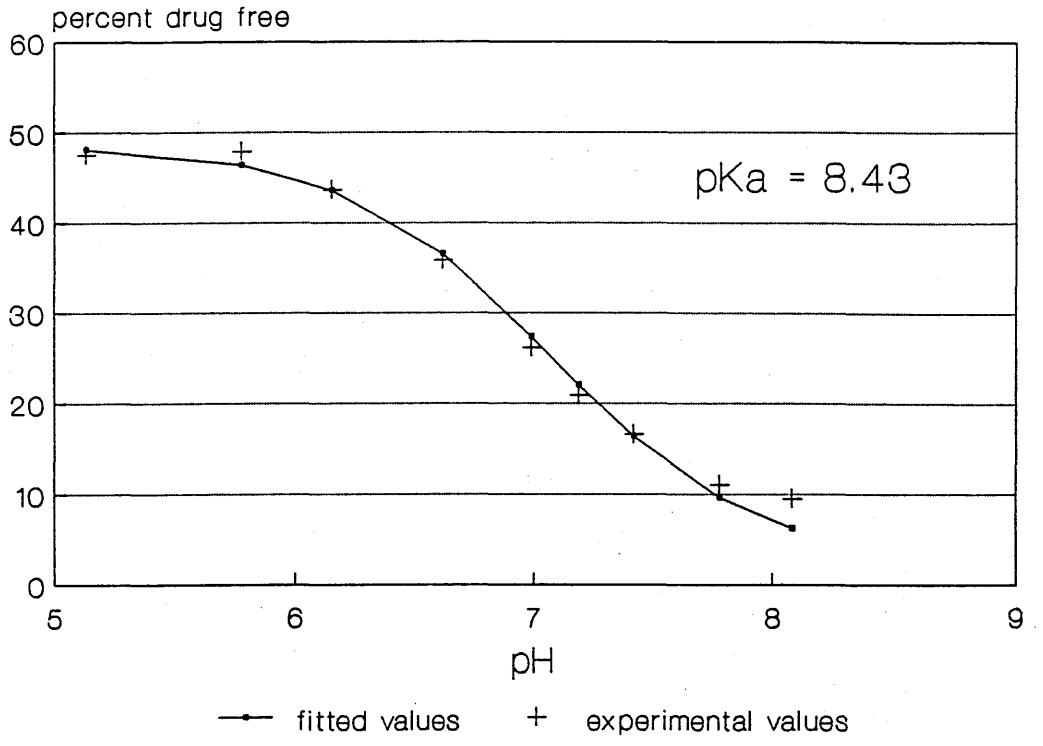
The data of Meuldermans and colleagues [34], Bower [155] and McLean and Hug [21] were fitted separately to equation 13.17 using a computer curve-fitting program based on the Levenberg-Marquardt routine [166] to give ki and ks. The results are shown in figs 13.3 to 13.5.

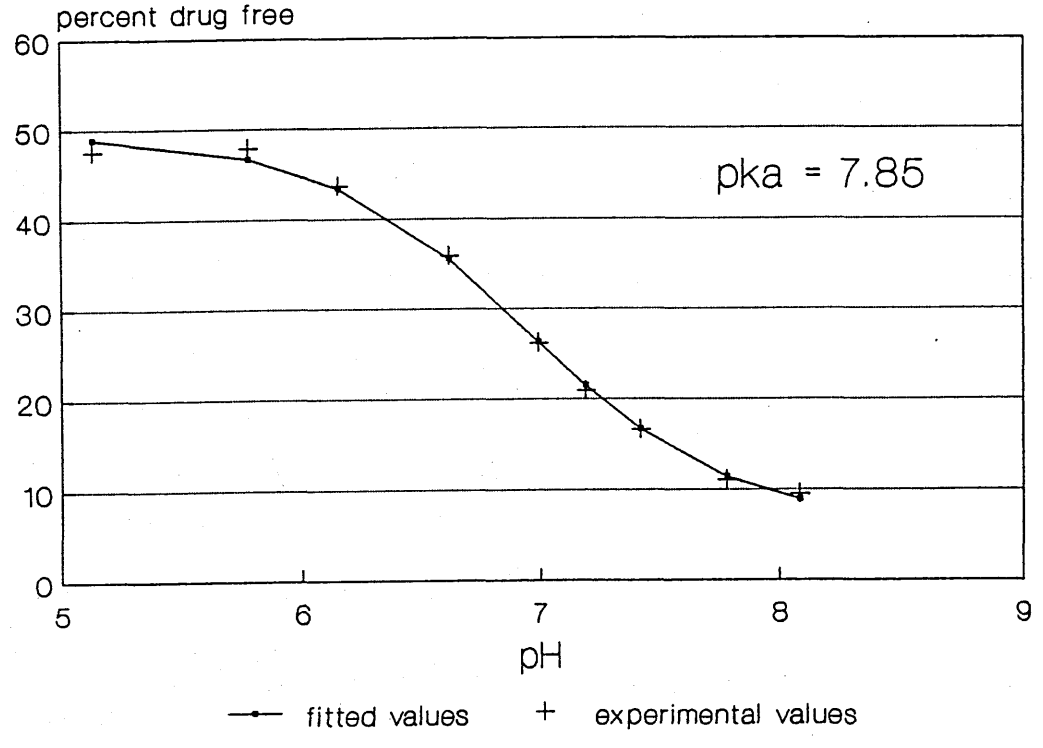
When the pKa is fixed at 8.43 (not shown for McLean and Hug's data) there is not a great deal to choose between the data sets. Meuldermans and colleagues' data are possibly a marginally better fit than that of Bower. Including the pKa as an unknown variable in the fitting process gives pKa = 9.59 (Bower), pKa = 7.85 (Meuldermans), and pKa = 8.18 (Mclean). There is only marginal improvement in the overall fit of Bowers' data but a noticeable improvement in that of Meuldermans which is now better than both others. (note Meuldermans' investigation spans a larger range of

Figure 13.3

Change with pH in Binding of Fentanyl by Plasma Proteins. Experimental and Predicted Values

Meuldermans and Colleagues [34]



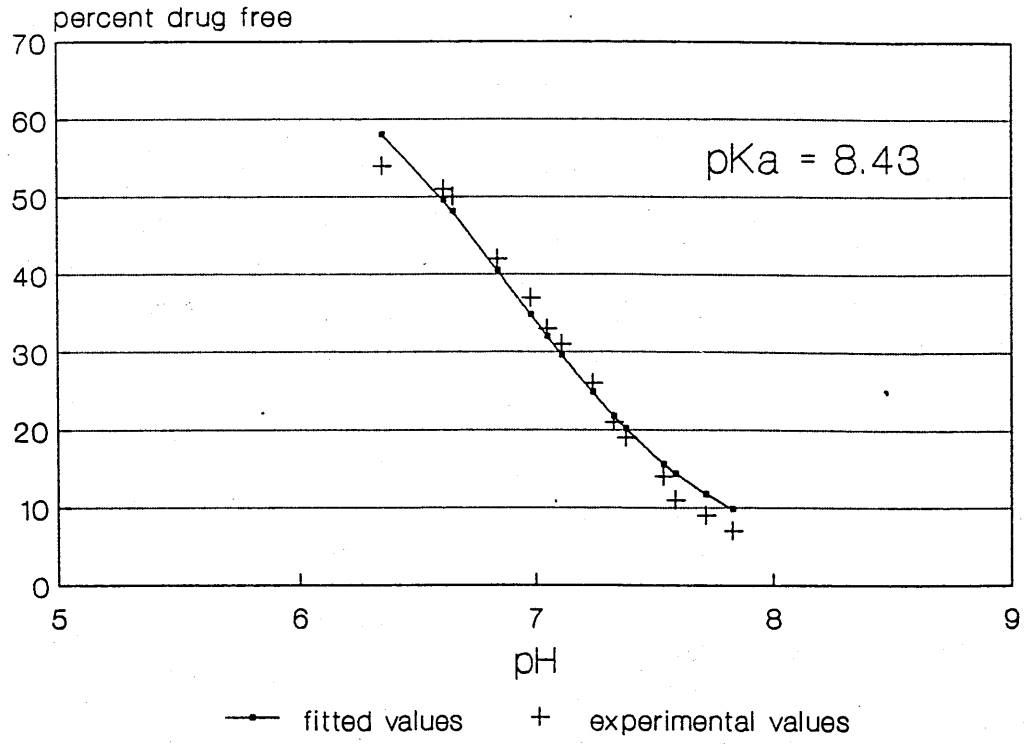


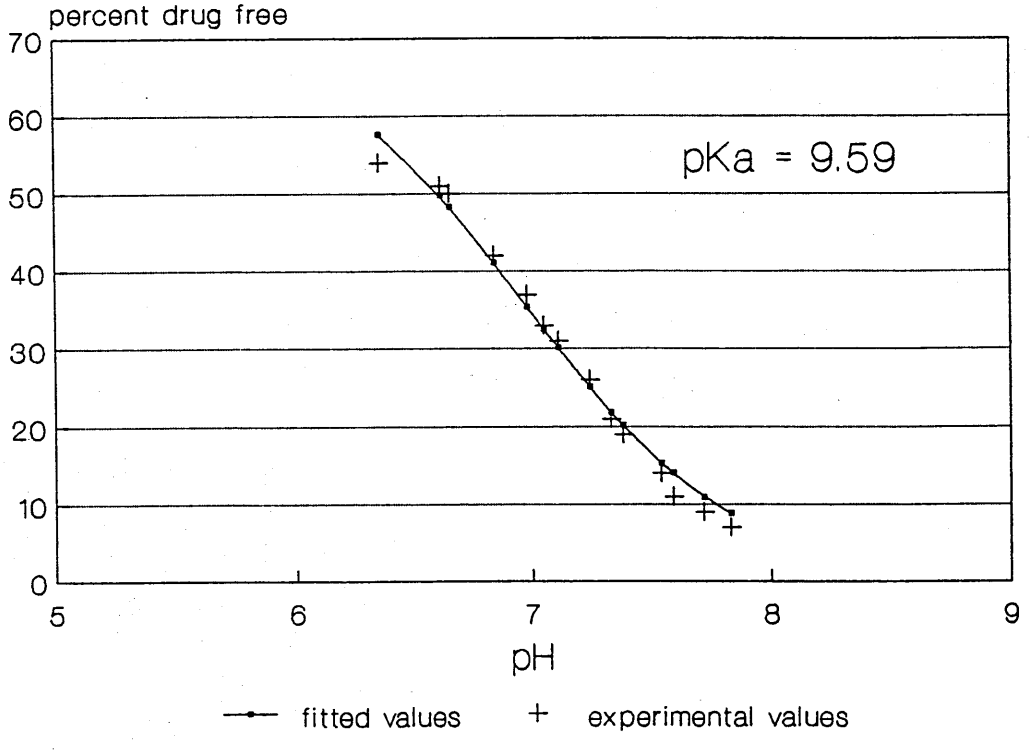
See text for details

Figure 13.4

Change with pH in Binding of Fentanyl by Plasma Proteins. Experimental and Predicted Values

Bower [155]



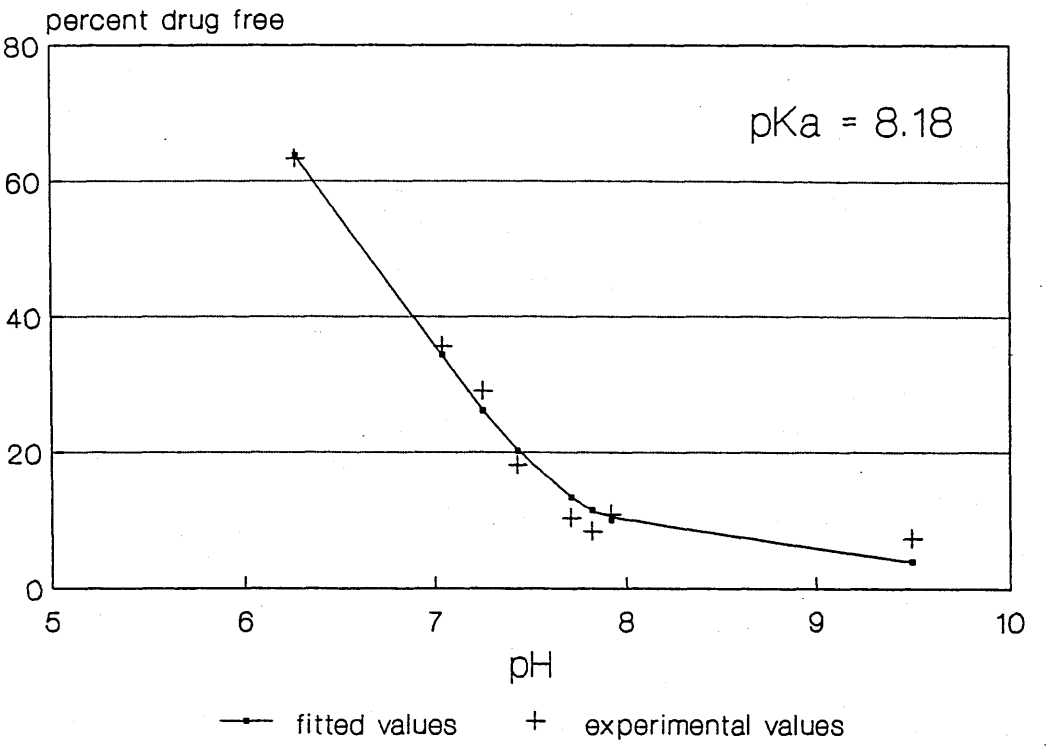


See text for details

Figure 13.5

Change with pH in Binding of Fentanyl by Plasma Proteins. Experimental and Predicted Values

McLain and Hug [21]



See text for details

pH than Bower's). 7.85 is within the range of possible pKa values suggested above though it is not possible to estimate how likely this really is compared with the manufacturer's value.
Experimental values of β may deviate from predicted values because of inadequacy of the binding model (binding is more complex, for example cooperative binding is involved, or the nature/number of binding sites is directly altered by changes in pH) or because of experimental error. In the present case it is not possible to say which of these factors are involved. No doubt better fitting of individual data sets could be obtained with more complex models but there is insufficient agreement between the results from different laboratories to justify this. Further experimental exploration of the problem would involve confirmation of the apparent pKa and studies of binding at different protein concentrations over a range of pH.
It seems reasonable then to accepted the model as quantified by the data which fits it best. This, from Meuldermans data, gives:

pKa = 7.85

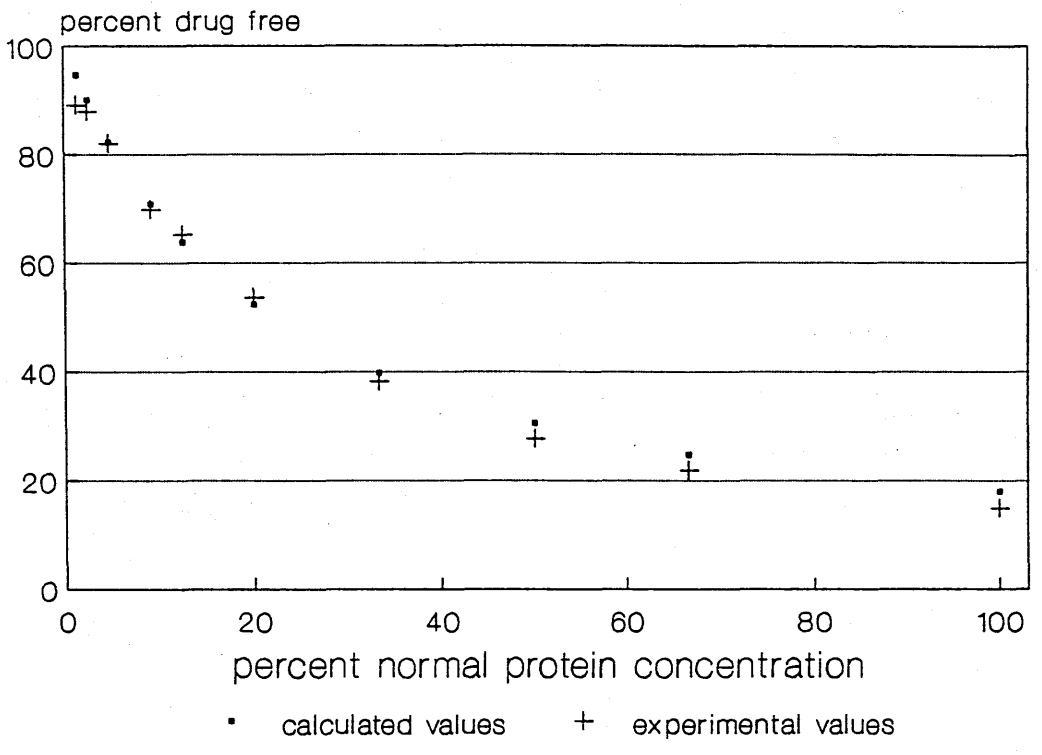
$ki = 1.246 \times 10^{-3}$

$ks = 1.9121 \times 10^{-2}$

Fig 13.6 compares Meuldermans and colleagues' experimental results for the variation in binding with plasma protein concentration at pH = 7.35 with values calculated using the values above in equation 13.17. Some of the disparity here between model and experiment is the consequence of an inconsistency in the experimental data: the proportion of bound drug in undiluted plasma reported in the plasma dilution

Figure 13.6

Change with Plasma Dilution in Binding of Fentanyl by Plasma Proteins. Experimental and Predicted Values for Meuldermans and Colleagues [34]



experiment, apparently performed at pH 7.35 is different from that reported at pH 7.35 in the separate experiment to examine the effects of pH on binding to undiluted plasma. This is reflected in a larger difference between calculated and experimental values at ph 7.35 in undiluted plasma in fig 13.6 compared with fig 13.3.

Note: As pKa and the binding constants are mutually dependent in equation 13.17, should the pKa used in the model be changed for any reason, ki and ks must also be recalculated from the in vitro data using the new pKa.

Lipid in plasma and ecf: The experimental studies of fentanyl plasma protein binding discussed above make the assumption that all plasma binding of drug is due to protein binding. No allowance is made for dissolution in plasma lipids such as the triglycerides present in chylomicra after fat absorption from the intestine. Bower found no increase in plasma fentanyl binding after a fatty meal associated with increased plasma triglyceride concentration [156] and no change in binding to artificially defatted plasma [155]. In contrast to the original pethidine program therefore no term was included in the fentanyl version for plasma lipid. Similarly no term was included for lipid in the extracellular fluid.

Lipid solubility

Ideally a set of solubility coefficients for the major types of lipid present in each tissue would be available. Failing that the average solubility of fentanyl in representative human or animal triglycerides (which comprise the largest proportion by mass of body fats) would be useful. In fact, as for pethidine,

neither of these levels of information are available and only solubilities in the industrial solvents heptane and octenol can be found. Even here the available data is not consistent. Table 13.1 shows the data I was able to find. Lambda has been calculated in each case from the measured apparent coefficient $lambda_x$ at pH x

(13.18) $lambda_x = Cl/Cw$

where Cl = concentration of drug in lipid phase

Cw = total concentration of drug in aqueous phase

The true partition coefficient, lambda is given by

(13.19) $lambda = Cl/Cs$

where Cs is the concentration of unionised drug in the aqueous phase and it is assumed that only the unionised form is soluble in lipid.

lambda was calculated using

(13.20) $lambda = lambda_x.(1 + 10^{(pKa - pH)})$

which is derived by combining the Henderson Hasselbach equation with equations 13.18 and 13.19.

There is an obvious difficulty in choosing which value of pKa to use. Internal consistency within the model would suggest 7.85 but this value is not the best fit to all the data. Bower [159] investigated the variation in $lambda_x$ with pH and her results give a very good fit to the dissociation curve of a base of pKa 6.9. Holt and Teschamacher [153] calculate a pKa of about 7.7 from their investigation of partitioning into heptane. Meuldermans and colleagues [34] quote the official pKa of 8.43

Table 13.1: Fentanyl Solubility in Heptane and Octenol

Heptane			Octenol				Study
L	$L_{8.64}$	$L_{7.4}$	L	$L_{9.8}$	$L_{8.34}$	$L_{7.4}$	
			*** 9957	**9550**			[34] #
			539 *		**520**		[159] ##
			11220				[162] ####
49 **	**43**		878 ** 2928 ***			**250**	[153] ##
68 ** 227 ***		**19.35**					[160] ###
11.8 * 105 ***		**9.0**					[161] ##

L = true oil / aqueous phase partition coefficient for unionised species (lambda)

L_x = apparent oil / aqueous phase partition coefficient at pH x ($lambda_x$)

measured values are bold underlined

* calculated assuming pKa= 6.9
** calculated assuming pKa= 7.8
*** calculated assuming pKa= 8.43

\# temperature not given. "double extraction" method
37°C. tritiated fentanyl
room temperature. Beckman spectrophotometer
no experimental details

Table 13.1

in their paper but solving equation 13.20 for pKa using the two values they give for $lambda_x$ results in pKa = 6.46. In addition to this problem there are considerable differences in absolute measured values of $lambda_x$. All in all the lack of agreement is remarkable for what appears at face value a fairly straightforward piece of physical chemistry.

The data in table 13.1 may be used as a pointer to the solubility of fentanyl in triglyceride in the form of olive oil (plant triglyceride) by following the same interpolation procedure Davis employed for pethidine. Table 13.2 shows the opioids fentanyl, morphine and methadone ordered in terms of their solubility in various solvents. The lambda for fentanyl in octenol is probably close to that for methadone. lambda for fentanyl in heptane lies in a fairly intermediate position between that for methadone and morphine. On the other hand lambda for methadone in olive oil is less than in both octenol and heptane. lambda for morphine in olive oil is less than in octenol but perhaps greater than in heptane though both values are small. On these grounds lambda for fentanyl in olive oil should lie between that of methadone and morphine but probably nearer to methadone. It should be less than lambda for fentanyl in octenol.

A value of 500 was chosen as a first approximation but given the variability in Table 13.1 this amounts to little more than a guess.

Table 13.2: Solubility of Fentanyl, Methadone, and Morphine in Heptane, Octenol, and Olive Oil. After Davis [125].

OLIVE OIL morphine	OLIVE OIL fentanyl	OLIVE OIL methadone
0.05 e	?	716 e
HEPTANE morphine	**HEPTANE fentanyl**	**HEPTANE methadone**
< 0.00004 b	12 to 227 a	3298 b
OCTENOL morphine	**OCTENOL fentanyl**	**OCTENOL methadone**
6.23 d 6.6 c	539 to 11220 a	8621 d

Values are true partitition coefficients of the unionised species (lambda) if necessary calculated from the measured apparent partition coefficient ($lambda_x$) using pKa values as in table 13.1 or as given by Kaufman and colleagues [133].

a see table 13.1
b [160] measured at room temperature pH 7.4.
c [153] measured at 37°C pH 7.4
d [133] measured at 37°C
e [183] $lamda_x$ measured at 37°C pH 7.55 to 7.58

Some of these data appear in more than one publication. Although the situation is not completely unambiguous, the values quoted by Davis [125] from Kutter and colleagues [184] and from Kosterlitz and colleagues [185] almost certainly derive from the experiments reported in [160].

Distribution in Blood

Three groups of investigators have examined the distribution of fentanyl between erythrocytes and plasma. All three determined very similar values for the ratio

(13.21)

$$\text{eprat} = \frac{\text{total fentanyl concentration in red cells (mg ml}^{-1})}{\text{total fentanyl concentration in plasma (mg ml}^{-1})}$$

under normal physiological conditions:

Lehman and colleagues [157]	1.01
Meuldermans and colleagues [34]	0.92
Bower [158]	0.98
Mean	0.97

Bower [158] carried out the most detailed investigation. As might be expected eprat is independent of haematocrit and of total blood fentanyl concentration up to at least 0.5 mg ml^{-1}. Plasma dilution experiments confirm that the concentration of fentanyl in the red cells is a linear function of the unbound fentanyl concentration in plasma with $\text{eprat}_{\text{free}} = 5.02$ where

(13.22)

$$\text{eprat}_{\text{free}} = \frac{\text{total fentanyl concentration in red cells (mg ml}^{-1})}{\text{unbound fentanyl concentration in plasma (mg ml}^{-1})}$$

Unexpectedly, eprat is also independent of plasma pH over at least the range 5.04 to 7.56 [157, 158] which implies that $\text{eprat}_{\text{free}}$ is dependent on plasma (not red cell) pH in exactly the same manner as $1 - \beta$, the ratio of free to total concentrations of drug in the plasma (see protein binding

above). As most erythrocyte binding of fentanyl is accounted for by binding to haemoglobin a more detailed investigation of this problem would involve examining fentanyl binding to haemoglobin over a range of pH.

The simplest and most robust way of using this information in the present model is to incorporate eprat directly to relate the total plasma concentration of fentanyl to the total red cell concentration. This will be valid in the face of the plasma pH changes already possible with the present version and will remain valid should the model be extended to allow changes of haematocrit. However it would not be valid should either the plasma protein concentration or the red cell haemoglobin concentration be changed.

As concentrations in the model are expressed per unit mass rather than per unit volume eprat must be corrected for the densities of blood and plasma:

$$(13.23)\quad eprat_{model} = eprat \times \frac{\text{density of plasma}}{\text{density of erythrocytes}}$$

$$= 0.908$$

Elimination

Davis models pethidine elimination by dividing it between hepatic metabolism and renal excretion. Hepatic clearance was calculated from

(13.24)

hepatic clearance = whole body clearance - renal clearance

Whole body clearance and renal clearance (or rather an expression which relates renal clearance to urine pH) were

derived from published pharmacokinetic studies. For convenience of modelling the clearances are expressed as the respective extraction fractions where the extraction fraction is the fraction of drug in the incoming blood removed by the organ. Thus

(13.25) extraction fraction = clearance / perfusion

This also has the advantage of relating elimination to organ blood flow such that decreased blood flow results in decreased elimination and vice versa. As both fentanyl and pethidine have relatively high extraction fractions and are likely to show perfusion-limited clearance [see 167] this should result in at least qualitatively correct effects should the model be extended to permit changes in the distribution of cardiac output.

Since fentanyl is mainly removed from the body by hepatic metabolism with a small amount excreted unchanged in the urine (chapter 1) broadly the same procedure may be applied here. However two potential inaccuracies must first be considered. The first source of potential error is the difference between blood and plasma clearance. Clearance is often given as plasma clearance:

$$(13.26)\quad \text{plasma clearance} = \frac{\text{rate of elimination}}{\text{plasma concentration}}$$

where rate of elimination is the rate of elimination of drug from the system. The units are volume time^{-1} where volume can be conceptualised as the volume of plasma that would be required to contain the mass of drug being eliminated per unit time at the

concentration concerned. Blood clearance is given by

$$(13.27)\quad \text{blood clearance} = \frac{\text{rate of elimination}}{\text{blood concentration}}$$

Hence

(13.28)

$$\text{blood clearance} = \text{plasma clearance} \times \frac{\text{plasma concentration}}{\text{blood concentration}}$$

Again the units are volume time^{-1} and the volume on this occasion is the volume of blood that contains the mass of drug being eliminated per unit time at the concentration concerned. Obviously the clearance required for equation 13.25 is blood clearance. Note also that plasma clearance and plasma perfusion cannot be substituted in equation 13.25 to give the observed hepatic plasma extraction fraction since this gives the plasma extraction fraction that would occur if all eliminated drug were to be accounted for from the plasma (rather than red cells and plasma) and may result in an extraction ratio > 1.

For fentanyl since the plasma / blood concentration ratio is approximately unity blood clearance may in practice be used interchangeably with plasma clearance though a correction factor has been used below for the sake of logical rigour. Davis apparently made no distinction between blood and plasma clearance in his quantification of the pethidine model. Since the blood / plasma concentration ratio of pethidine has been estimated between 0.68 and 1.43 [125] this is a possible source of error.

The second source of potential error is that whole body

clearance refers to clearance of arterial (or sometimes mixed venous) blood. Strictly speaking hepatic clearance for equation 13.25 should be calculated from the drug concentration in liver afferent blood. If this differs from the drug concentration in arterial blood because of portal mixing, then the clearances of liver afferent blood and arterial blood will differ and an extraction fraction based on arterial blood clearance will be in error.

In practice the error will only be important in the distributive phase during the first few minutes after administration when the part played by elimination in determining drug concentration is small anyway. After the distributive phase there should be little concentration difference between arterial and liver afferent blood and the calculated extraction fraction should provide the required observed arterial blood clearance.

There are analogous problems in comparing pharmacokinetic parameters calculated from arterial and venous blood concentrations.

Table 13.3 shows values for fentanyl plasma clearance obtained from conventional pharmacokinetic studies in volunteers and patients undergoing non-cardiac surgery. There is considerable variation between studies. Reilly and colleagues [20] have examined the variability of fentanyl kinetics. Variation may be due to experimental error, to differences in methods of parameters calculation or to measurement of real differences in the subjects under study. It is likely that several different factors are involved and that they will not be clearly delimited without further careful experimental work.

Most investigators have fitted their data to two or three-

Table 13.3 Pharmacokinetic Constants for Fentanyl in Volunteers and Non-Cardiac Surgical Patients.

study	year	n	sample	assay	duration (hours)	clearance (ml/min)	half lives (min) pi	alpha	beta	Vss (litres)	Vbeta	model	comments
Volunteers													
[21]	1980	7m	a	3H	8	882	1.7	13.4	255		285	3 comp	
[161]	1982	5m 2f	pv	RIA	6	1530			185	335		2 comp	
[174]	1980	9 m+f	a	RIA	5	160	3	25	853	182	202	3 comp	only higher dose group quoted
[180]	1977	6	pv	RIA	6	620		7	172			2 comp	values as calculated in [1]
Patients													
[171]	1982	5f	a	GLC	7	991	1.4	28	265		* 381	3 comp	controls for effect of age
[172]	1982	13	?	RIA	10	702		9	263	241	267	2 comp	controls for effect cirrhosis
[173]	1980	5f	pv	RIA	4	428		1.4	141		77	2 comp	
[10]	1986	33	pv	RIA	24	847 **			514	521			cardiac group excluded. infusion
[175]	1988	4m 3f	a	RIA	4	973			133		159		controls for effect of age
[176]	1988	39m	a	RIA	24	1060 ***			480				controls for transdermal. infusion
[177]	1986	9m 1f	a	GLC	24	769	2.1	31	522	378		3 comp	
[178]	1987	15m	a	RIA	24	574	1.0	18.5	475	339		3 comp	
[11]	1985	17f	?	RIA	24	986 ***							infusion
[170]	1981	6	a	RIA	6	829			198		224	2 comp	controls for effect cardiac bypass
[169]	1981	3	?	GLC	7	1116	1.8		230		* 390	3 comp	controls for effect of obesity
[179]	1978	7m 7f	a	RIA	6	280	1.6	21	347	118	141	3 comp	values as calculated in [174]

key: Vss volume of distribution at steady state Vbeta volume of distribution during elimination RIA radioimmunoassay
GLC gas-liquid chromatography 3H tritiated fentanyl a arterial pv peripheral venous 2 comp 2 compartment model
3 comp 3 compartment model

* "volume of distribution" no method given ** clearance from dose /area under concentration-time curve
*** clearance from steady state concentration

values originally per unit body mass given for 70 kg man
study [numbers] refer to main reference list

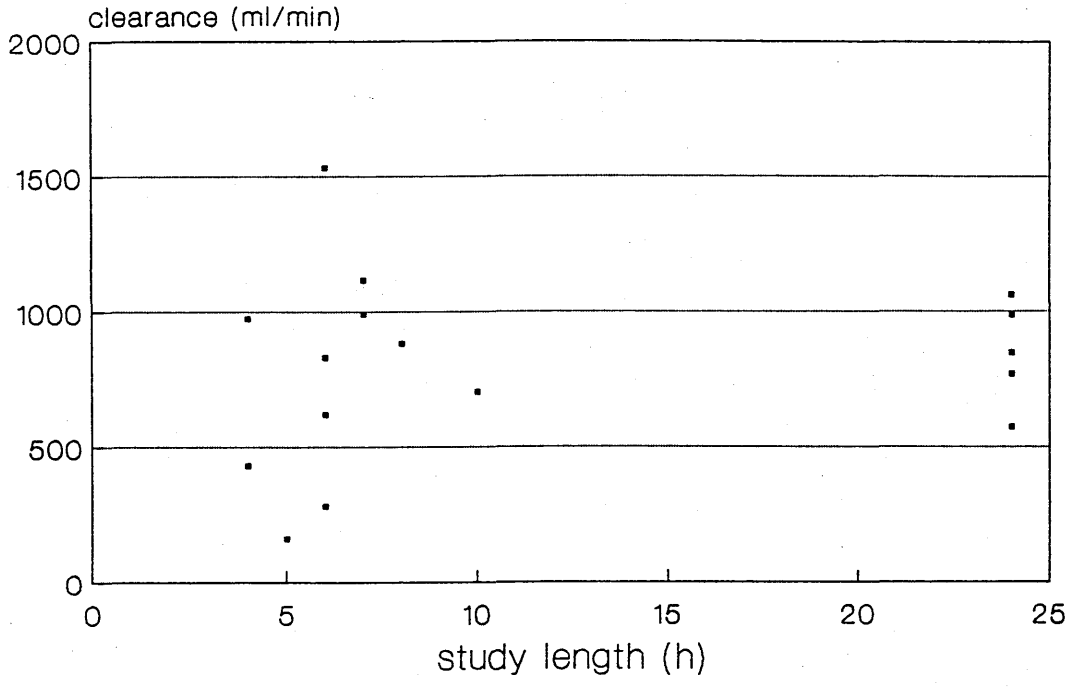
compartmental models. Duthie and colleagues [10] and Singleton and colleagues [175] both use non-compartmental methods of calculation. Holley and Van Steenis [176] and Nimmo and Todd [11] calculate clearance from steady state infusion concentrations. Fung and Eisele [174] present their data processed in three different ways. There are considerable differences: clearance was 440 ml min^{-1} calculated from a two-compartment model compared with 160 ml min^{-1} calculated from a three-compartment model which was a better fit to the combined group concentration curve but could not be fitted to the individual subject concentration curves.

Reilly and colleagues [20] point out that many existing studies are on small numbers of subjects and are of short duration compared with the elimination half life. Scott and Stanski [178] argue that short studies tend to overestimate clearance and underestimate terminal half life. However there is no obvious relationship between study length and clearance in these investigations (figure 13.7).

Assay inaccuracy is another possible contributing factor. In general insensitive assays produce uncertainty in estimates of kinetic parameters. However an assay that fails to discriminate between a drug and its metabolites or that over estimates drug concentration such that measured concentration is a constant multiple of the real concentration will underestimate clearance. Most studies have used radioimmunoassay to determine fentanyl concentrations in plasma. Schleimer and colleagues [179] and Fung and Eisele [174] both used the earliest available assay [181]. Their clearance values are by far the smallest published. Michiels and colleagues [180] developed a more sensitive assay

Figure 13.7

Relationship Between Study Length and Plasma Clearance of Fentanyl for the Studies Listed in Table 13.3



soon afterwards. This has become the commercially available standard and was used by all the other investigators who employed radioimmunoassay, with the exception of Bower and Hull [161] who used their own assay to determine the largest published clearance. In 1984 Schuttler and White [112] pointed out that the assay procedure then recommended for the commercial version of Michiels and colleagues' assay was very susceptible to small variations in experimental technique and prone to overestimation of fentanyl concentration. A more robust procedure was introduced and was used by subsequent investigators.

No unequivocal associations have been discovered between the size of pharmacokinetic parameters and the characteristics of healthy subjects. Three groups of investigators report no correlation between fentanyl pharmacokinetic parameters and age or body weight [161, 176, 178]. Bentley and colleagues [169] studied five obese and three non-obese patients and found no difference in volume of distribution, clearance or terminal half life. Singleton and colleagues [175] using non compartmental methods of calculation found a reduced volume of distribution in seven elderly (over 71 y) patients but no difference from younger controls in clearance or terminal half life. Bentley and colleagues [171] by contrast found a prolonged terminal half life, markedly reduced clearance and unchanged volumes of distribution in four elderly (over 60 y) patients. Anaesthesia and surgery might be expected to change fentanyl pharmacokinetics, clearance, for instance, might be smaller on account of reduced liver perfusion. There is some indirect evidence for this [182] but with the exception of Bower and Hull

[161] (seven subjects) pharmacokinetic parameters measured in unanaesthetised volunteers are not noticeably different from those measured in non-cardiac surgical patients.

The model requires a value for fentanyl clearance ideally derived from young fit adult male subjects. However the two studies that fit these requirements [21, 161] are both on small numbers of patients and show different results: 1537 ml min^{-1} and 882 ml min^{-1} respectively. An average plasma clearance for patients and volunteers combined can be calculated from the investigations in table 13.3. The two lowest values may be excluded since it is highly likely they reflect inadequacies in fentanyl assay. It also seems reasonable to use only the control groups from studies into the effects of age, weight, disease, or cardiac by-pass. Otherwise all data are included. The result, 879 ml min^{-1} (or 881 ml min^{-1} weighted for the square root of the number of subjects) is almost identical to that determined by McLean and Hug [21] in fit young male volunteers and I thus chose a plasma clearance of 880 ml min^{-1} as the basis for further calculations:

from equation 13.28

blood clearance = plasma clearance x 1.01

= 889 ml min^{-1}

but if 6% of the total elimination of fentanyl occurs through the kidney

renal blood clearance = 889 x 6/100

= 53 ml min^{-1}

metabolic blood clearance = total clearance - renal clearance

= 836 ml min^{-1}

From equation 13.25 if liver perfusion = 1554 ml min^{-1} [125]

hepatic extraction fraction = 0.538

Renal elimination: Renal clearance could be expressed as a renal extraction fraction and inserted directly into the model. However renal elimination would then show no dependence on urine pH. This is unfortunate as by analogy with similar drugs it is highly likely that fentanyl excretion is dependent on urine pH. and urine pH is readily measurable. The original model mimics this aspect of pethidine excretion. As there is no experimental evidence with which to define an empirical relationship between fentanyl excretion and urine pH a theoretical model must be used.

Existing models have been reviewed by Davis [125] and are based on renal clearance or on adding drug to and subtracting drug from the glomerular filtrate. I have adopted an alternative approach which avoids the problems of modelling the complex sequential changes in the glomerular filtrate.

On the premise that distribution of fentanyl within the kidney occurs as a result of passive diffusion (i.e. that fentanyl is not actively secreted into the kidney tubules) the starting point is to assume that fentanyl equilibrates between perfusing renal blood, kidney tissue (cells and ecf) and the final urine volume. However this is an oversimplification since the kidney could be divided into a large number of functional compartments and concentrations will often not be at equilibrium. A higher concentration of fentanyl in the urine than would occur by simple equilibrium partition may occur as a result of the faster diffusion of water than fentanyl from the filtrate. The

countercurrent mechanism in the renal medulla may possibly magnify such concentration differences [168]. To allow for this I have introduced a concentrating factor. This operates by temporarily decreasing the mass of the kidney and its perfusing fractional stroke volume after the total mass of drug in blood and kidney have been calculated thus avoiding any change in the total mass of drug in the body (see chapter 14).

To determine a suitable value the program was initially constructed with a concentrating factor (F) of 1 (i.e. simple partitioning of drug between blood, tissue and urine). This was then adjusted until renal elimination accounted for approximately 6% of total elimination with a normal urine output which gave F = 17.

The finished program allows urine flow to be specified for each simulation.

In life, urine is formed from the perfusing renal plasma. Strictly speaking therefore, urine volume should be subtracted from the plasma volume. However the mass of urine is very small compared with the mass of plasma and may be treated as a separate extra compartment without noticeably affecting the calculation, which simplifies the model considerably.

This sub-model of urinary excretion is compatible with rather than derived from the meagre quantitative information available and is hence speculative. Its merits are discussed further in chapter 15.

Conclusions

The numerical information corresponding to that used by Davis to quantify the model for pethidine is not all available for fentanyl and where information exists there is often poor

agreement between different investigators. However it has been possible to devise a fentanyl version of the model by estimating parameters for which there are no experimental values and by altering the method of calculating drug distribution within the blood and between blood and tissue to fit the available data. Evaluation of the model is discussed in chapter 15.

CHAPTER 14

The Fentanyl Model

The following is a summary of the physiological model of fentanyl pharmacokinetics. It assumes the description of the original pethidine model in chapter 10. The complete fully commented text version of the computer program is given as appendix 1 and includes definitions of all variables used therein and their initial values.

Calculation of Blood-Tissue Distribution

The total amount of fentanyl present in the blood and tissue of a tissue block and its (fractional) stroke volume (Aa_{bt}) is related to the aqueous concentration of unionised drug (Cs) by

(14.1)

$$Aa_{bt} = Wt_p.Cs.((10^{(pKa - pHp)}.ki + ks).gp_p + fw_p + 10^{(pKa - pHp)}.fw_p) + Wt_r.eprat.Cs.((10^{(pKa - pHp)}.ki + ks).gp_p + fw_p + 10^{(pKa - pHp)}.fw_p) + Wt_c.Cs.((10^{(pKa - pHc)}.ki + ks).gp_c + lamda.fl_c + fw_c + 10^{(pKa - pHc)}.fw_c) + Wt_e.Cs.((10^{(pKa - pHe)}.ki + ks).gp_e + fw_e + 10^{(pKa - pHe)}.fw_e)$$

where

eprat = erythrocyte : plasma concentration ratio

ki = protein binding constant ionised species

ks = protein binding constant unionised species

and other variables are as in chapter 10

Equation 14.1 is derived in the same way as equation 10.13 except that Cp is given by equation 13.14 rather than equation 10.11, there is no term for plasma lipid or ecf lipid (i.e. $Cl_p = Cl_e = 0$) and the total red cell concentration of fentanyl is calculated from the total plasma concentration using eprat (see

equation 13.21).

As in the original model Aa_{bt} is known from the amount of drug in the incoming blood fraction and the amount of drug left in the tissue at the end of the previous cycle. In all the tissue blocks except the nasal and kidney blocks 14.1 is solved to give the equilibrium Cs (note equation 14.1 is linear and can be solved directly whereas equation 10.13 is cubic and requires an iterative method). Cs is then used to calculate the equilibrium total drug concentrations and amounts in the individual compartments.

Nose: In the nasal block there is an extra compartment, the nasal mucous, to be included in the calculation. The total amount of drug in the nasal block tissue, fractional stroke volume and mucus is given by:

(14.2) $$Aa_{btm} = Aa_{bt} + Aa_m$$

where Aa_m the total amount of drug in the mucous is given by

(14.3) $$Aa_m = Wt_m.(Cp_m.gp_m + Cs.fw_m + Ci_m.fw_m)$$

Substitution for Cp_m from equation 13.14 and for Ci_m from equation 10.9 gives an expression in terms of Cs:

(14.4)

$$Aa_m = Wt_m.(Cs.(10^{(pKa - pHm)}.ki + ks).gp_m + Cs.fw_m.(1 + 10^{(pKa - pHm)}))$$

where pHm = pH mucus

Equations 14.4 and 14.1 are combined in equation 14.2 to calculate Cs and thence the total equilibrium concentrations and amounts in each compartment including the mucus.

Kidney: In the kidney block there is also an extra compartment, the urine. In addition the concentrating factor F operates to increase the effective equilibrium concentration governing partition of drug into the urine. The total amount of drug in blood, kidney tissue and urine (Aa_{btu}) is given by:

(14.5) $Aa_{btu} = Aa_{bt} + Aa_u$

where Aa_u, the total amount of drug in the urine is given by

(14.6) $Aa_u = Wt_u.(Cs + Ci_u)$

where Wt_u = mass of water in the urine produced during calculation cycle (kg)

Wt_u approximates to the urine flow (Urine is made up of water and solutes. In practice the quantitative distinction between urine water and urine is relatively unimportant but is made here for completeness).

Substitution for Ci_u from the Henderson Hasselbach equation (11.9) gives an expression in terms of Cs.

(14.7) $Aa_u = Wt_u.Cs.(1 + 10^{(pKa - pHu)})$

where pHu = urine pH

Aa_{bt} is calculated as for other tissue blocks. Aa_u before equilibration = 0. Equations 14.1 and 14.7 are combined in equation 14.5 . However further substitutions of Wt_p/F for Wt_p, Wt_r/F for Wt_r, Wt_e/F for Wt_e and Wt_c/F for Wt_c are made before the equation is used to calculate the apparent aqueous unionised drug concentration (Cs_{conc}) for this weighted equilibrium. The total amounts of drug (Aa_x) in each individual compartment at equilibrium are then calculated from Cs_{conc} still using the

terms Wt_x/F. Finally the equilibrium concentrations in all the compartments apart from the urine are calculated from the amounts Aa_x and the actual compartment masses Wt_x. The true aqueous unionised concentration (Cs) for all the compartments except the urine is calculated from:

(14.8) $Cs = Cs_{conc} / F$

Calculation Sequence

1) The amounts of drug in the (fractional) stroke volumes leaving each pool and the amount of drug left behind in each pool are calculated from the drug concentrations in the pools (see chapter 10).
2) The fractional stroke volumes for the liver from the arterial and portal pools are added together.
3) The amount of drug metabolised is removed from the combined liver fractional stroke volume according to the extraction fraction.
4) Drug is equilibrated between the blood fractions and the destination tissue blocks: The new amounts and concentrations of blood in the tissue blocks, the blood fractions leaving for the downstream pools, the nasal mucus and the urine are calculated (see above). All drug in the urine is excreted.
5) The equilibrated (fractional) stroke volumes are added to the downstream pools and the new amounts and concentrations of drug in the pools are calculated (see chapter 10).
6) If appropriate, increments of new drug are added to the injection pool, the intramuscular injection site , the lung or the nasal mucosa. Each increment is the amount of each

prescribed dose that would be administered during the current calculation cycle given the total duration of administration.

Program Input

The remarks in this and the following section, program output, apply both to the fentanyl program and to the new version of the pethidine program.

Input is from a standard keyboard. The program can be easily operated by anyone minimally familiar with computers. Particular attention was paid to preventing the accidental and unnoticed entry of erroneous information. The input routine contains simple error traps and there are opportunities to examine and correct all important entries. All simulation conditions must be specified in advance at the start of the simulation.

Simulation duration: Any simulation duration may be specified.

Drug administration: Drug may be administered intravenously, intramuscularly, intranasally or into the lung. Any number of doses may be given in any combination. For each dose the total amount of drug and the simulation time at which administration is to commence are required. For all apart from the intramuscular doses the duration over which the drug is administered must also be given. Bolus doses are thus allocated a short duration. Constant infusions are modelled by giving the total dose over a long administration period. Variable infusions must be modelled by making each change of infusion rate the start of a new dose.

Urine: The urine pH and the urine flow rate may be specified for

any time during the simulation. Default values are 6.2 and 100 g h^{-1}

Cardiac output: The cardiac output may be specified for any time in the simulation. The default value is 6.48 l min^{-1}.

Plasma pH: The plasma pH may be specified for any time in the simulation. The default value is 7.40.

Calculation time: The elapsed simulation time represented by one calculation cycle may be varied up to about 6 seconds at which point, with the default cardiac output, the circulating stroke volumes start to become larger than the pools. There is a checking routine in the program which notifies the user and automatically reduces the calculation time if this occurs. This is necessary because cardiac output can be varied independently but is also a useful safeguard in case the model should be extended in future to allow changes of blood distribution.
A large calculation time means that a simulation of given duration is completed more quickly. A small calculation time is necessary to follow the initial distribution changes with high temporal resolution. The size of the calculation cycle time also directly affects the distribution of drug in the body during the first seconds after drug administration (see chapter 15). Different calculation cycle times may be specified for any time in the simulation.

Program Output

The program has optional outputs to screen, data file and printer.

Screen: The program will plot the fentanyl concentration in up

to six body regions to a suitable visual display unit as the simulation progresses. Both time and drug concentration are plotted on a logarithmic scale to show the differences between concentrations in the first few minutes after drug administration. The following concentrations are available: peripheral arterial plasma (peripheral shunt compartment), central venous plasma (central venous pool), fat tissue, lean tissue, lung tissue and brain tissue.

Printer: Details of variable changes and administered doses are optionally printed as the simulation progresses. (This facility was provided as there is no indication of these events on the screen display).

Data file: The program outputs its main results to a data file whose name must be specified at the beginning of each simulation. The following results are given:

Total amounts of drug administered, in blood, in tissues, excreted, and metabolised.

Amount and concentration of drug in each tissue block.

Aqueous unionised concentration in each tissue block.

Tissue / arterial blood and tissue / arterial plasma concentration ratios for all the tissue blocks.

Concentration in blood and plasma and unionised aqueous concentration in all the blood pools, peripheral shunt and notional peripheral venous blood (as above).

The results are available according to one of two sampling schemes:

	time from dose or variable change	results
standard scheme:	0 to 1 hour	1 min 5 min every 5 min
	1 hour onwards	every 30 min
frequent scheme:	0 to 2 min	every few seconds
	2 min to 20 min	every 30 seconds
	20 min to 1 hour	every 2 min
	1 hour to 6 hours	every 10 min
	6 hours onwards	every 30 min

The frequent scheme is useful for following detailed changes immediately after drug administration or variable changes but generates a large amount of data. All the results are calculated at the end of a cycle (ie after equilibration of drug between tissue and blood and addition of equilibrated (fractional) stroke volumes to pools) with the exception of the tissue / arterial blood and plasma concentration ratios where peripheral shunt concentration is used as arterial concentration and tissue concentrations are those at the start of the cycle before equilibration.

The following information is also given at the end of each simulation:

Times of concentration maxima for tissue blocks 1 to 9.

The tissue / blood and tissue / plasma concentration ratios for each tissue block at diffusion equilibrium.

The erythrocyte / plasma and blood / plasma concentration ratios.

An indication of the cumulative calculational rounding error which also serves as a check on the calculation

integrity: amount of drug remaining in body + amount of drug eliminated - amount of drug administered.

All details of drug doses and variable changes are notified to the results file.

In addition the program will optionally list to a separate file the basic data used to quantify the model.

Note on the pethidine model: In the fentanyl model the tissue / blood and blood / plasma equilibrium concentration ratios are fixed (unlike the tissue / arterial blood concentration ratios). In the pethidine model these ratios may change with total drug concentration and in Davis's original program [125] these ratios were calculated for the results file every 100 cycles. However Davis found that for concentrations produced by clinically realistic doses of pethidine the changes were negligible. The new pethidine program thus uses the same results scheme as the fentanyl program and calculates the equilibrium and erythrocyte / plasma ratios only once from the concentrations at the end of the last cycle of the program.

CHAPTER 15

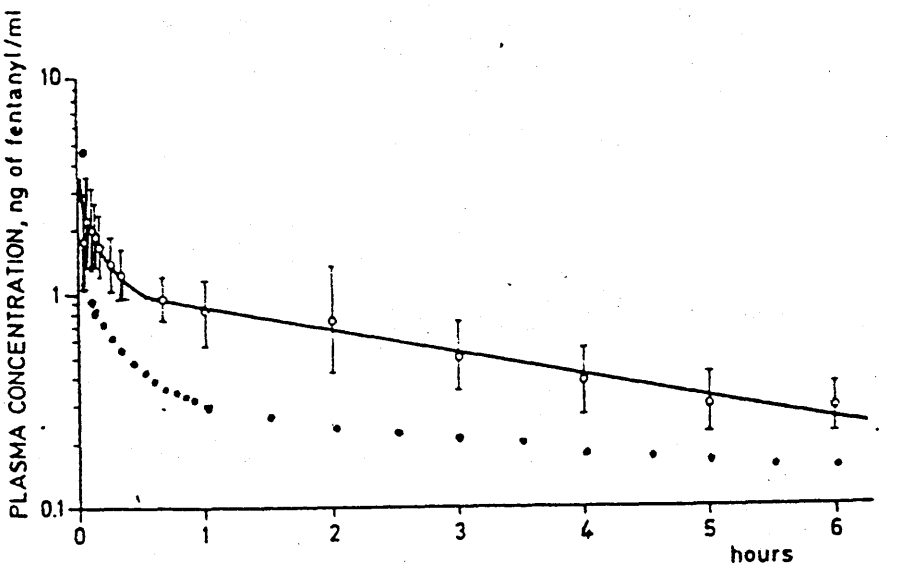
Evaluation and Discussion

The assessment and validation of models is a major topic in systems science. An excellent account is given by Leaning [130]. A formal validation of the model according to a scheme such as that proposed by Leaning has not been possible in the time available for this project. However I have made some attempt below not only to assess the overall performance of the model in terms of the likelihood of the computed plasma concentrations but also to examine the component parts with the aim of identifying areas where further development would be both practical and profitable.

Drug Concentrations in Plasma: Simulation of Existing Studies

Figures 15.1 to 15.3 show simulations of three studies from table 13.13 which examine fentanyl kinetics in young healthy volunteers (comparison is not made with the fourth volunteer study as Fung and Eisele [174] used an early and possibly inaccurate radioimmunoassay). Michiels and colleagues [180] gave an intravenous bolus of 200 µg each to six volunteers of unspecified weight and sex. Bower and Hull [161] gave 170 µg each as a short (2.5 min.) infusion to five males (weight range 65.1 to 83.8 kg) and two females (weights 54.9 and 61.2 kg). Both sets of investigators measured fentanyl concentrations in peripheral venous blood. Mclean and Hug [21] gave 6.4 µg kg^{-1} to five male volunteers the arterial plasma concentrations from four of whom (weight range 65.0 to 84.5 kg) are presented. The weight of the model man is 70 kg in all simulations. (The present model has the correct proportion of fat tissue for a non-obese 70 kg man. Adjusting the model weight without adjusting the relative proportion of different body tissues will not necessarily result in a closer fit to experimental subjects

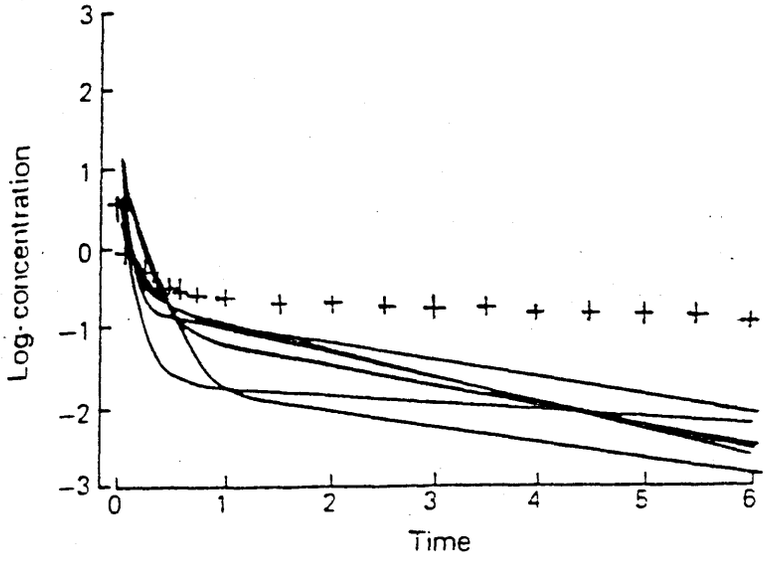
Figure 15.1



- ∘ experimental data
- • simulated data

Adapted from Michiels and colleagues [180]. Experimental points are logarithmic mean values from peripheral venous plasma (definition of error bars not given in original). See text for details.

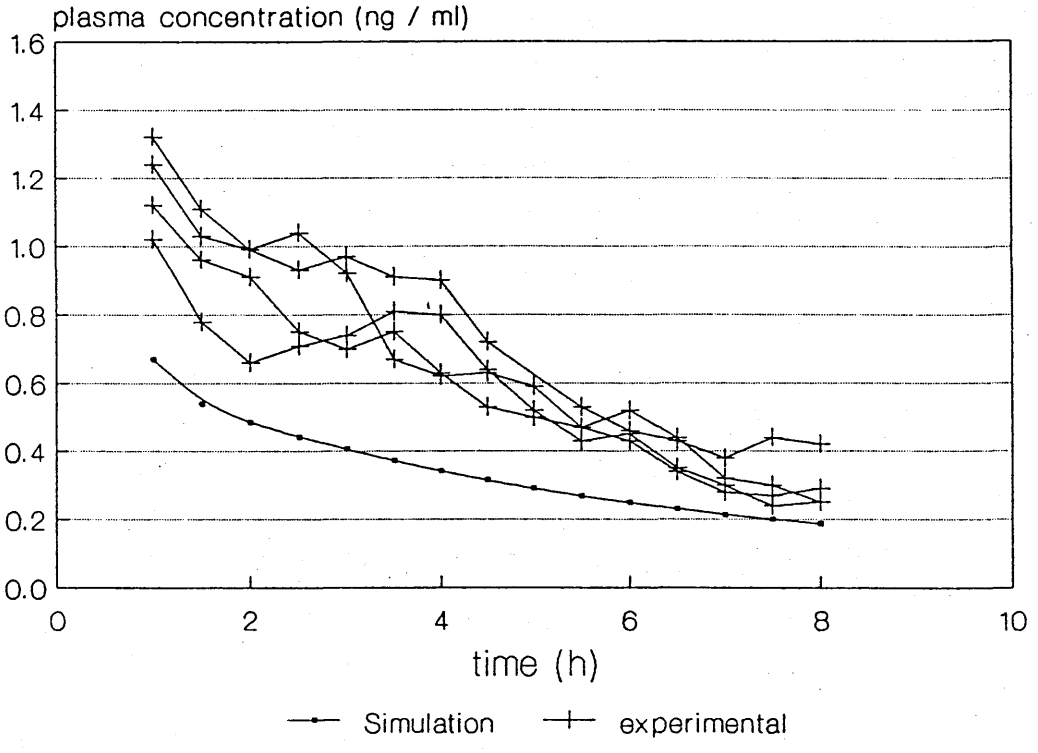
Figure 15.2



+ simulated data

Adapted from Bower and Hull [161]. Lines are the log-concentration (ng ml^{-1})-time (h) data given by the two compartment model fitted to the experimental data of each subject by Bower and Hull. See text for further details.

Figure 15.3

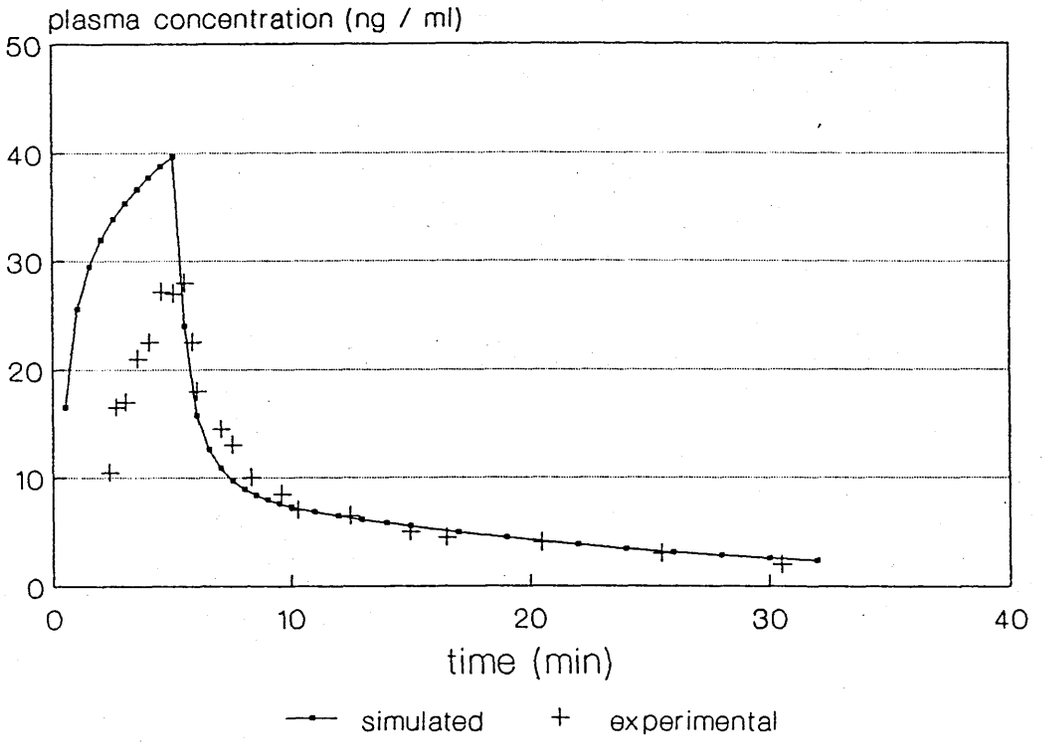


Experimental data from McLean and Hug [21] shows values from four individuals. See text for further details.

since no relationship has been determined between fentanyl kinetic variables or plasma concentrations and subject weight). After the first 30 minutes simulated concentrations are higher than the experimental concentrations reported by Bower and Hull. The model gives a better fit to the data of the other two groups, simulated concentrations being mostly slightly lower than experimental in both cases.

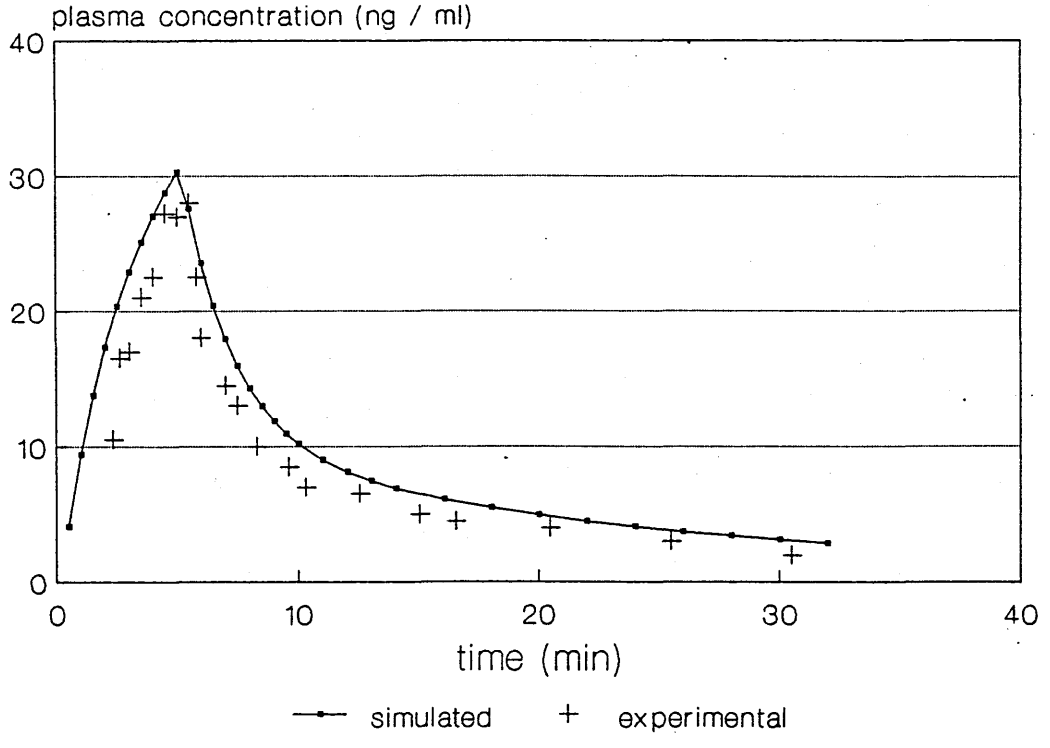
In order to make a simultaneous study of fentanyl pharmacodynamics and pharmacokinetics Scott and colleagues [31] administered a 5 minute intravenous infusion of fentanyl to 12 unpremedicated healthy male patients before anaesthesia. This study is useful because the investigators present a detailed plot of plasma concentrations during and for 30 minutes after the infusion i.e. at the time when the concentration profile is determined by drug distribution. Unfortunately it is not clear whether the plot represents the results from a representative subject or the whole group combined. There is no indication of scatter. The weight of subjects in the study ranged from 73 to 104 kg (weight of model subject 70 kg). A simulation of this study is shown in figure 15.4. It clearly approximates the experimental results moderately well both qualitatively and after the end of the infusion, quantitatively. During and immediately after the infusion both the initial rise in concentration and the terminal fall appear too steep and the peak concentration too great but this conclusion remains tentative without a knowledge of the experimental inter-subject variation. Figure 15.5 shows the same simulation repeated but in this case the model has been altered to increase partitioning into lung tissue by increasing the mass of the lung sevenfold

Figure 15.4



Experimental data from Scott and Colleagues [31]. Infusion of 150 μg min^{-1} for five minutes. Timing from start of Infusion. See text for further details.

Figure 15.5



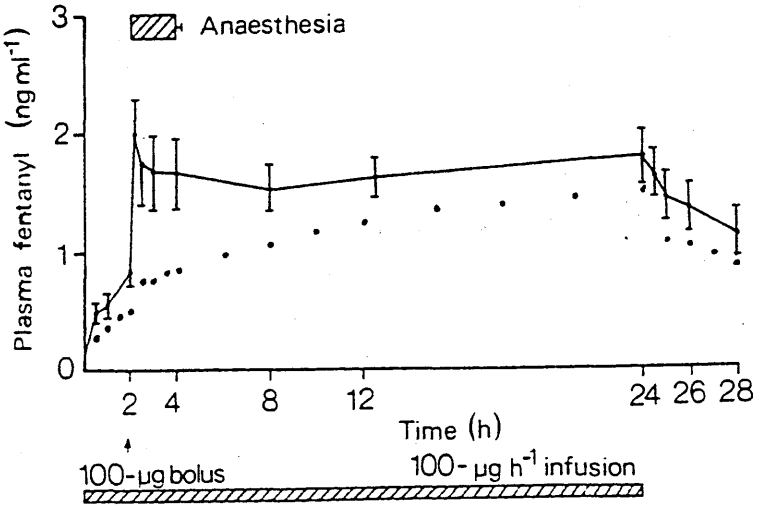
As figure 15.4 but with simulated lung affinity for fentanyl increased sevenfold.

leaving other variables, including the absolute lung perfusion, unchanged. (This is simply a quick and easy method of altering the overall equilibrium partition coefficient of a tissue block to assess the consequent effect. Within the present model framework any permanent alteration of distribution coefficients would have to be expressed in terms of drug distribution to lung water, fat and protein). The peak concentration during infusion is now a much better fit and the decline at the end of infusion less steep.

Figures 15.6 and 15.7 show results from two studies of prolonged intravenous infusions of fentanyl with the corresponding simulations. Both groups of investigators studied the kinetics in surgical patients of constant rate intravenous fentanyl infusions started before anaesthesia and continued for 24 hours. Duthie and colleagues [10] measured fentanyl concentrations in the peripheral venous plasma of a number of patients grouped according to the type of surgery. There were no significant differences in concentrations between the groups and figure 15.6 shows results from 10 orthopaedic patients of weight 69 (s.d. 12) kg and unspecified sex. Holley and Van Steenis [176] measured fentanyl concentration in the arterial plasma of patients undergoing various surgical procedures grouped according to infusion regimen. The results from two groups (10 men weight 76 (s.d. 7) kg and 9 men weight 77 (s.d. 5) kg) are shown in figure 15.7. The plasma fentanyl concentrations reported by both groups agree reasonably well with each other and with the simulation.

Figure 15.8 shows a simulation of the inhaled nebulised fentanyl study reported in part 1 of this thesis (see figures 6.1 to 6.4

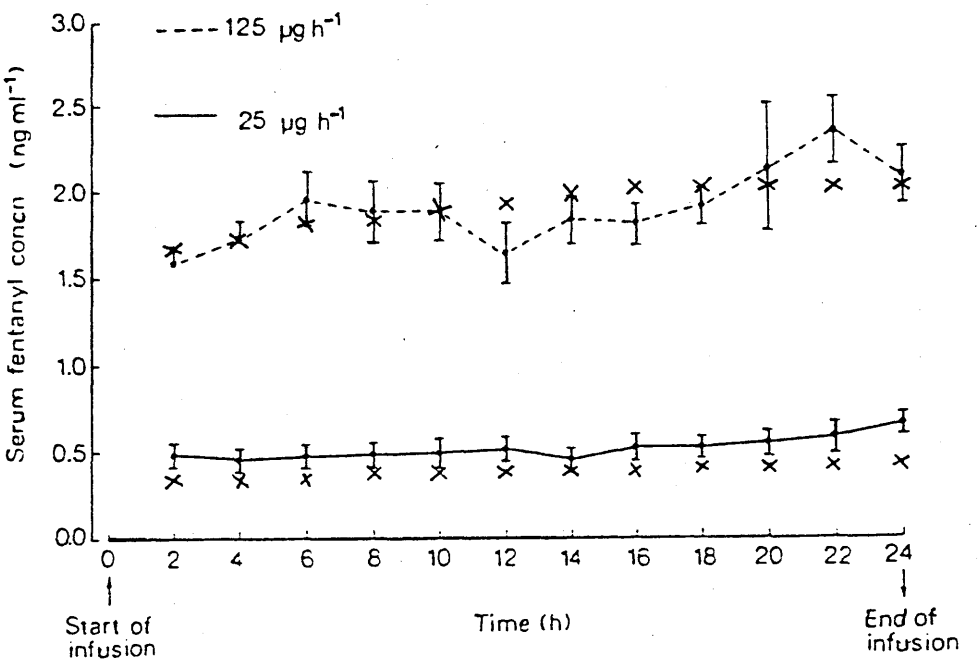
Figure 15.6



• simulated values

Adapted from Duthie and colleagues [10]. Infusion and bolus as shown. Experimental values are mean (SEM). See text for further details.

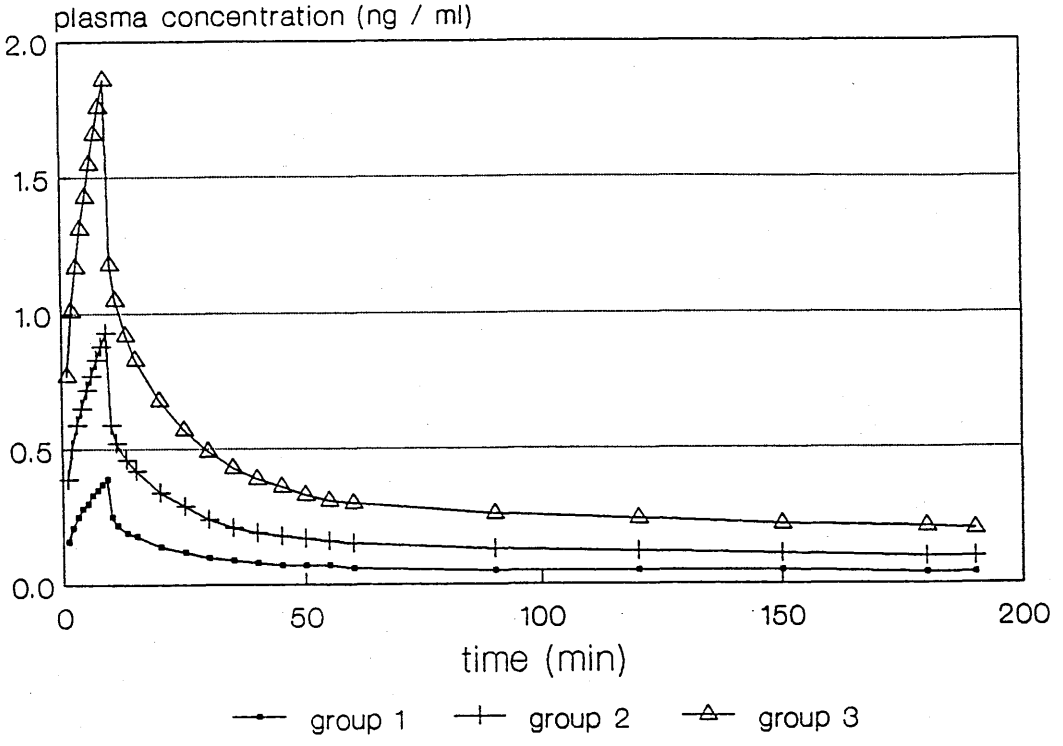
Figure 15.7



× simulated values

Adapted from Holley and Van Steenis [176]. Experimental values are mean (SEM). Infusions as shown. The high dose group received a loading bolus of 500 μg fentanyl at the start of the infusion and the low dose group a loading bolus of 100 μg.

Figure 15.8

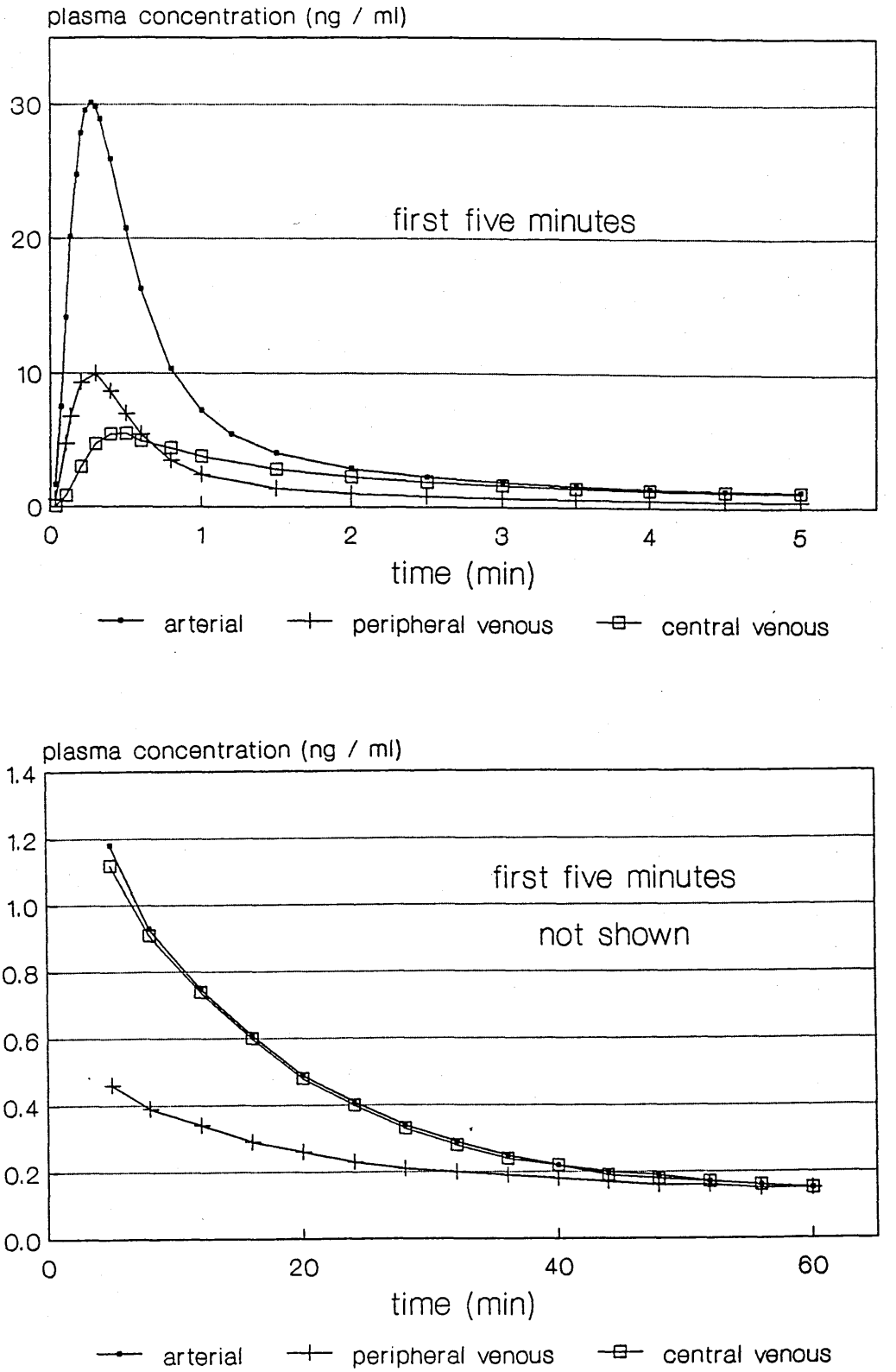


Simulation of the nebulised fentanyl study reported in part one of this thesis. See text for details.

for the experimental results). The simulation was set up assuming 12% of the fentanyl delivered from the nebuliser would be deposited in the patients' lungs and that a further 8% would be deposited nasally. (This is a fairly crude simplification as a variety of respiratory patterns including both nose and mouth breathing were seen). Again no attempt was made to adjust the model weight to that of the experimental subjects three of whom were female. As with the simulation of Scott and colleagues' work [31] there is a suggestion (even more tentative this time given the uncertainties) that the initial simulated concentrations during nebulisation may be too high.

Note on peripheral venous concentrations: Peripheral venous drug concentration is modelled, after Davis [125] by calculating the concentration in an equal mix of blood from the lean pool, fat pool and peripheral shunt. However this device is arbitrary and unvalidated in that the likely proportions for the mix are actually unknown and are likely to vary from person to person, with site of venous access and with circumstances. Whereas the concentration difference between arterial and central venous blood becomes negligible a few minutes after simulated drug administration (figure 15.9) the concentration difference between arterial and the notional peripheral venous blood persists since it reflects closely the slower uptake and release of drug in the lean and fat compartments (This pattern has also been described experimentally in sheep using thiopentone [131]). The inclusion of a greater proportion of lean at the expense of fat tissue blood (which might be appropriate in modelling forearm venous blood from a non-obese male) would lessen the difference slightly between arterial and peripheral venous

Figure 15.9



Simulated fentanyl concentrations in arterial, peripheral venous and central venous plasma following 100 µg given intravenously over 1 s.

blood. With the exception of the inhaled fentanyl study, changes in the modelling of venous plasma would not have an important effect on the fit of the above simulations to the experimental data. Blood concentrations during the first few minutes of the inhalation study however are governed mainly by the effects of drug distribution, simulated arterial concentrations are up to three times higher than simulated peripheral venous concentrations and changes in the modelling of mixed venous blood might well affect the apparent fit of the model.

Tissue Distribution

Only 7.4% of the administered dose remains in the blood 2 minutes after simulated intravenous injection (100 µg over 1 s) which agrees approximately with experimental findings in man of 5% 2 min after an intravenous bolus [180] and 6% (i.e. 3% in plasma) 2 min after the end of a 2 min infusion [39].

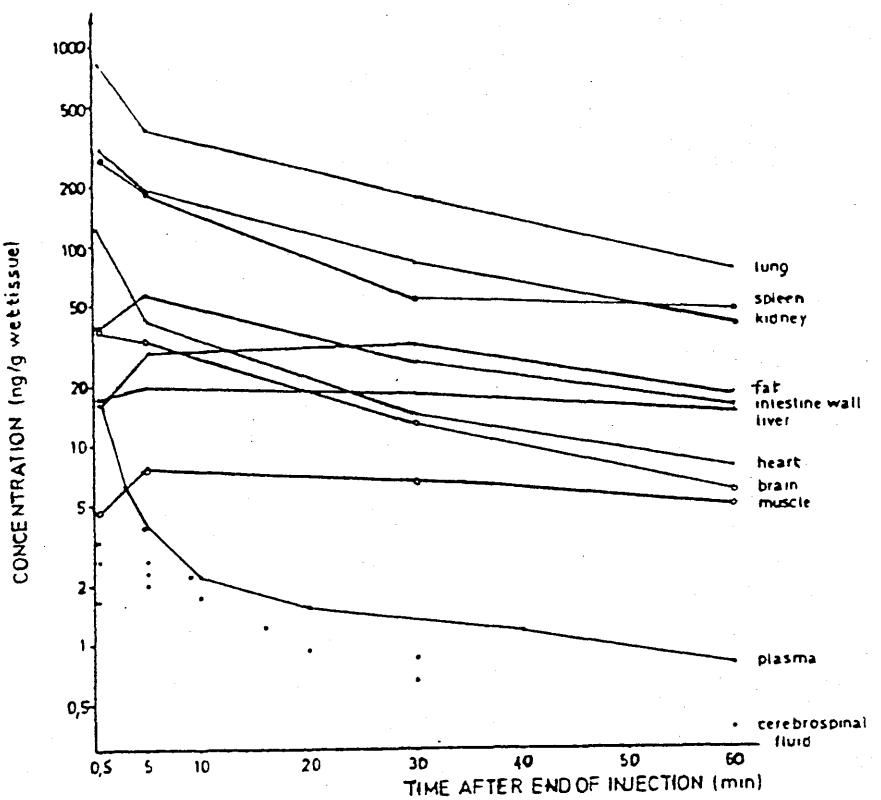
Studies of the tissue distribution of fentanyl in rat [33] and rabbit [23] are described briefly in chapter one. Data from these studies are presented for comparison with the model output in figures 15.10 to 15.13. There are differences between the species.

The **equilibrium** tissue / plasma concentration ratios for the fentanyl model are fairly similar for all the tissue blocks with the exception of fat tissue (table 15.1). (The equilibrium tissue / plasma concentration ratios for the pethidine model are less uniform). For the fentanyl model differences between the tissues in tissue / arterial plasma concentration ratios are thus mainly determined by differences in tissue perfusion.

After a single intravenous bolus (see figure 15.11), for all tissues apart from lung, fat, liver and kidney the

Figure 15.10

Tissue Concentrations of Fentanyl in the Rabbit Following Intravenous Injection (20 µg kg^{-1}).



Reproduced from [23]

Figure 15.11

Simulated Tissue Concentrations Following Intravenous Injection of 500 µg over 5 s

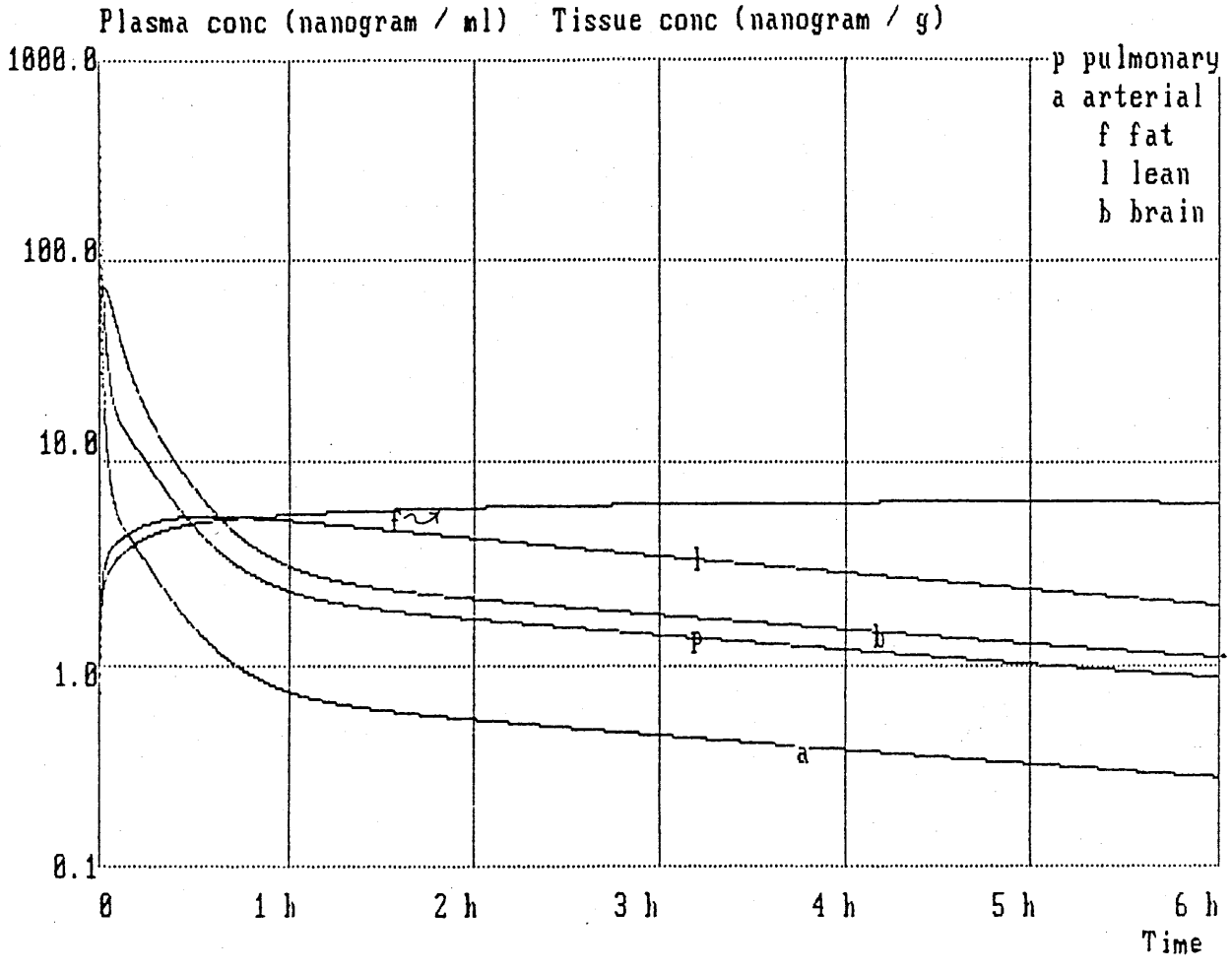
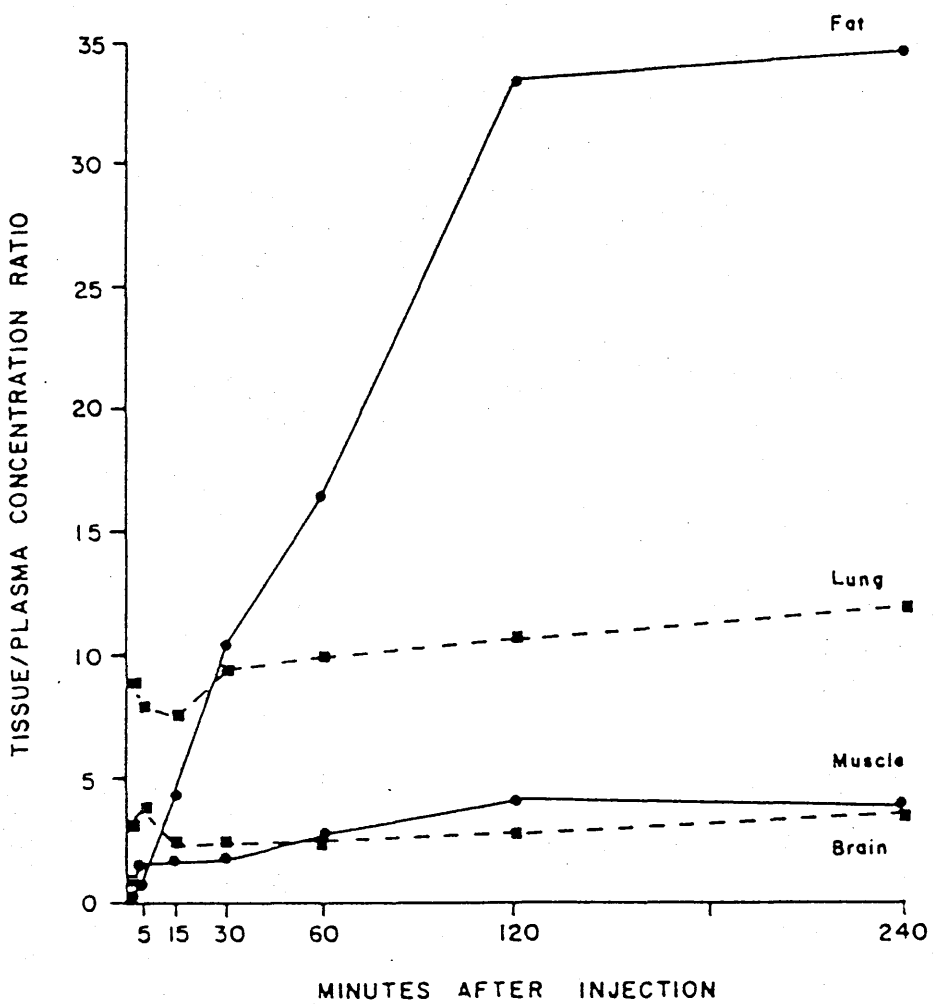


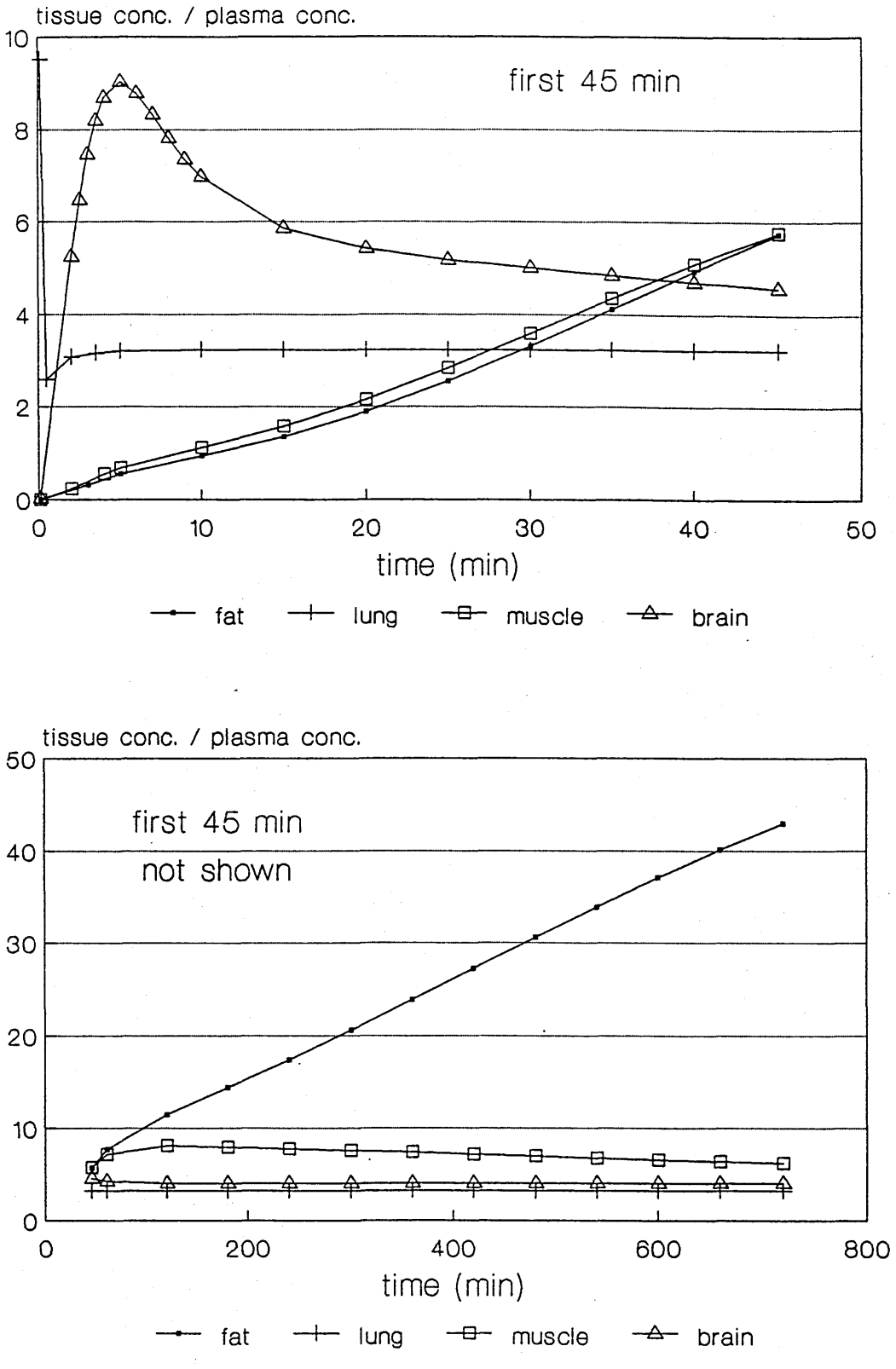
Figure 15.12

Tissue / Plasma Concentration Ratios Following Intravenous Injection (50 µg kg^{-1}) in the Rat



Reproduced from [33]

Figure 15.13



Simulated tissue\arterial plasma concentration ratios following intravenous injection of 500 µg over 5 s.

Table 15.1: tissue / plasma concentration ratios at distribution equilibrium for fentanyl and pethidine models

TISSUE	RATIO	
	FENTANYL	PETHIDINE
Lung	3.24	5.62
Kidney	3.88	4.30
Gut and Spleen	4.26	6.77
Liver	4.37	4.54
Other Viscera	4.65	4.72
Lean Tissue	4.37	64.98
Fat tissue	20.28	1.30
Brain	4.03	2.15
Intramuscular injection site	3.45	57.65
Nasal mucosa	3.60	3.40

Ratios for the pethidine model were calculated 30 min after simulating intravenous administration of 100 mg pethidine over 1 s

Table 15.1

tissue / arterial plasma concentration ratio is at first lower than the equilibrium ratio, becomes equal to the equilibrium ratio at the time of maximum tissue concentration, overshoots as tissue concentration initially declines more slowly than plasma concentration, then approaches the equilibrium concentration once more as tissue concentration declines faster than and finally in parallel with plasma concentration. The time course of these changes is very different in different tissues.

Liver and kidney follow roughly the same pattern but post equilibrium tissue concentrations and hence tissue / arterial plasma ratios are lowered as a result of removal of drug for elimination. The tissue / arterial plasma concentration ratio for fat tissue is still increasing 24 hours after drug administration as tissue concentration continues to decline more slowly than plasma concentration.

The tissue / arterial plasma ratio in lung reflects the dilution of pulmonary venous blood in the arterial pool. In this last case the marked changes of the first two minutes or so are very influenced by changes in calculation cycle time and must be regarded as artifactual since the circulatory model was not designed to reflect detailed flow patterns in the heart and large vessels (Mapleson's model M [127] simulates longitudinal mixing of blood along stylised vessels and in this respect an elaboration of model M might be more suitable than the adopted model P).

Times of maximum tissue concentrations after a simulated single intravenous injection are shown in table 15.2. The absolute times differ from those found in the animal studies (see chapter 1) but the pattern is similar with maximum concentration in fat

Table 15.2: Times of concentration maximima after a simulated single intravenous bolus of fentanyl (500 µg given over 5 s)

TISSUE	TIME
Lung	11 s
Kidney	47 s
Brain	1 min 35 s
Gut and Spleen	1 min 51 s
Other Viscera	2 min 15 s
Liver	6 min 39 s
Lean Tissue	35 min 8 s
Fat tissue	294 min 6 s

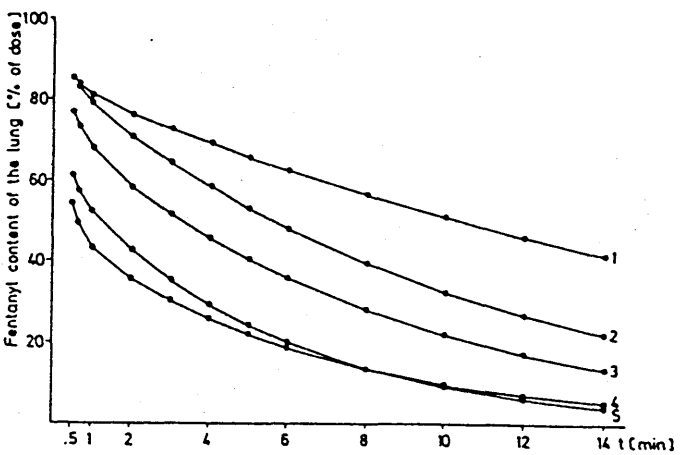
Table 15.2

occurring many times later than in other tissues, maximum concentration in lean tissue also delayed but to a much lesser extent than in fat tissue, and early maximum concentrations in a group of tissues including lung, brain and kidney.
It is noticeable that tissue / plasma concentration ratios in the lung are high compared to other tissues in both rat and rabbit but this is not mirrored in the model simulation.

Distribution to lung: Taeger and colleagues [24] examined the uptake into the lungs of a bolus of fentanyl administered simultaneously from a central venous catheter with a dye which would not diffuse out of the circulation. They found uptake to range from 55 to 85% in five patients over the first pass of the fentanyl bolus through the lung. Apparently, most of this fentanyl was released from the lung over the next 14 minutes (figure 15.14). Roerig and colleagues [25] also examined first pass uptake, but not subsequent washout, using a similar experimental arrangement. They found a mean of 75% (range 63 to 87%) of the fentanyl dose was absorbed by the lung in eight subjects. The model is based on the assumption that distribution of fentanyl between blood and tissue occurs according to a diffusion equilibrium. The data of Roerig and colleagues [25] is consistent with this proposition. They calculated the extraction ratio (proportion of fentanyl content removed from the blood) at each moment of the arterial first pass concentration-time curve. (They call this the "instantaneous extraction ratio" although for each volume element of blood it represents the cumulative extraction ratio for its passage through the entire lung). At the beginning of the first pass (i.e. for the first portion of the blood in which the bolus was distributed) it was greater

Figure 15.14

Distribution of Fentanyl to the Lung in Five Subjects Following a 300 µg Bolus into a Central Venous Catheter



Reproduced from [24]

than 90%. During the remainder of the first pass it decreased rapidly but was still positive at the end. Peak arterial fentanyl concentration also occurred later than that of the simultaneously injected dye. Together these facts suggest a flux of fentanyl was occurring in both directions (into and out of the lung). If fentanyl was binding to sites in the lung from which it had a long dissociation time constant or the diffusional process was so far from equilibrium that flux out of the lungs was negligible compared to flux into the lungs then the extraction ratio would remain constant and there would be no delay of the fentanyl peak.

Figure 15.15 shows a plot of the amount of drug in lung tissue as a percentage of a simulated rapid (1 s) intravenous dose for the first minute after administration. The proportion of the simulated dose partitioning into the lung in the first few seconds after administration is slightly too low and the washout is far too rapid. Figure 15.16 shows the simulation of figure 15.15 repeated with the model altered by increasing the mass of the lung tissue block 5, 10, and 15 fold leaving all other variables including absolute lung perfusion unchanged (note the change in timescale). An increase in lung "binding" of 10 or 15 times provides a reasonable approximation to the experimental washout data. A note of caution: Taeger and colleagues [24] present their data in an abstract. Fentanyl washout was calculated "from the arterial- mixed venous (a-v) concentration difference" using the initially measured cardiac output. No other details are given. Clearly the pulmonary release of fentanyl cannot be calculated from the instantaneous a-v difference since this will also depend on the flux of

Figure 15.15

Simulated Distribution of Fentanyl to the Lung Following Intravenous Injection of 100 µg over 1 s

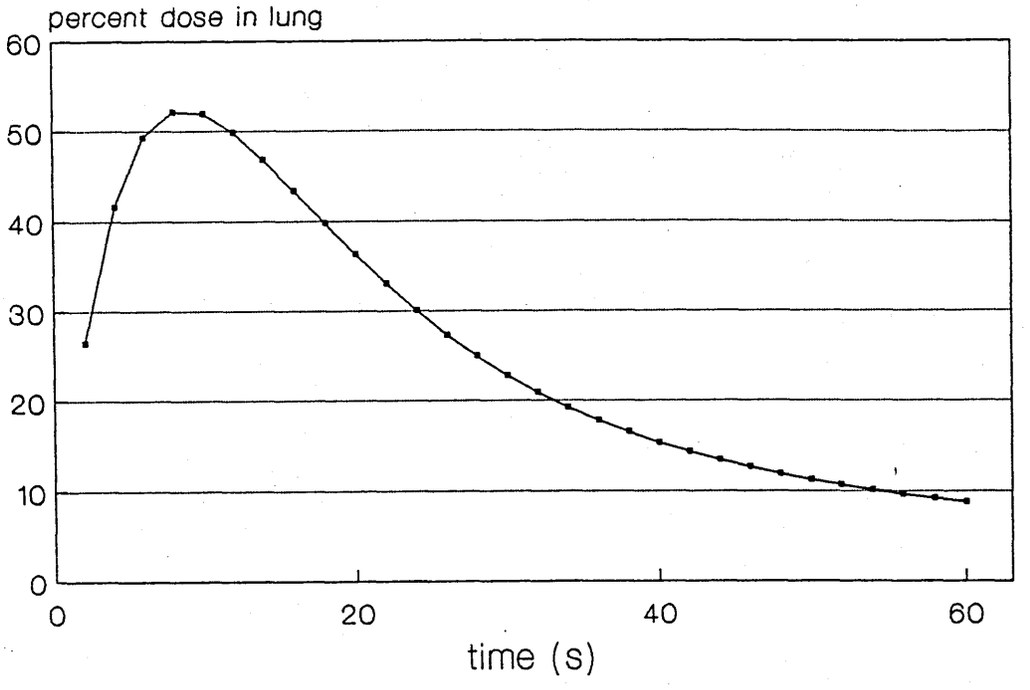
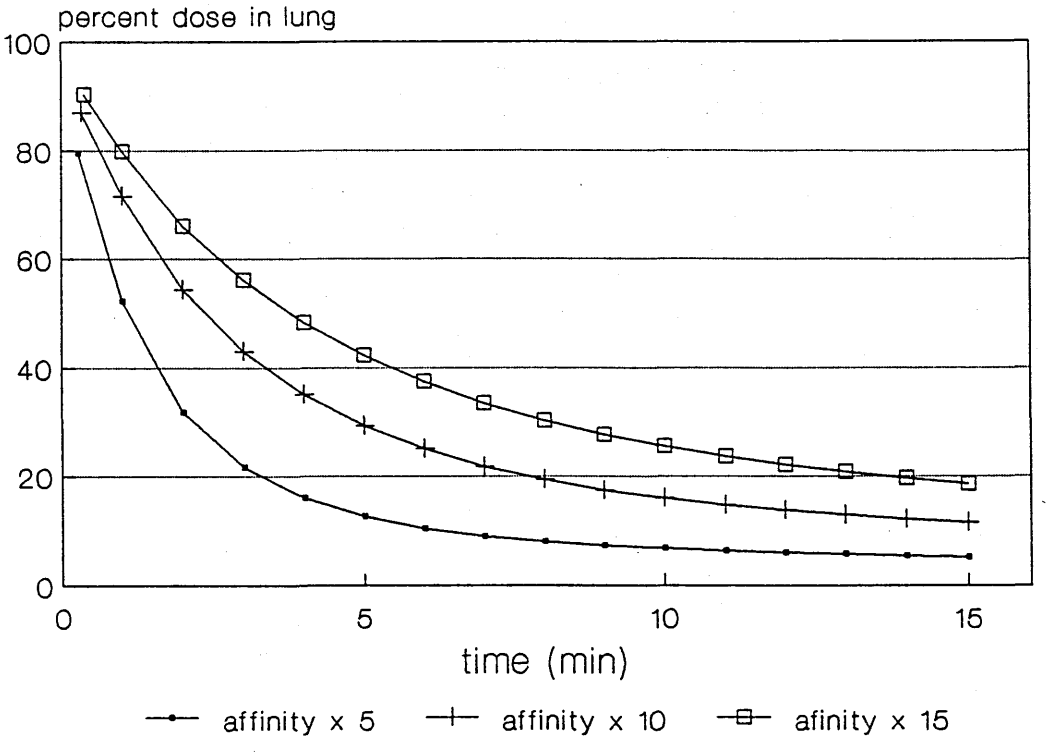


Figure 15.16



As figure 15.15 with increased lung affinity for fentanyl

fentanyl between blood and other body tissues. In theory it should be possible to estimate systemic and pulmonary fluxes separately by taking into account the respective transit times, but the likely accuracy of this method (if this indeed was the method used) cannot be judged without a more detailed presentation.

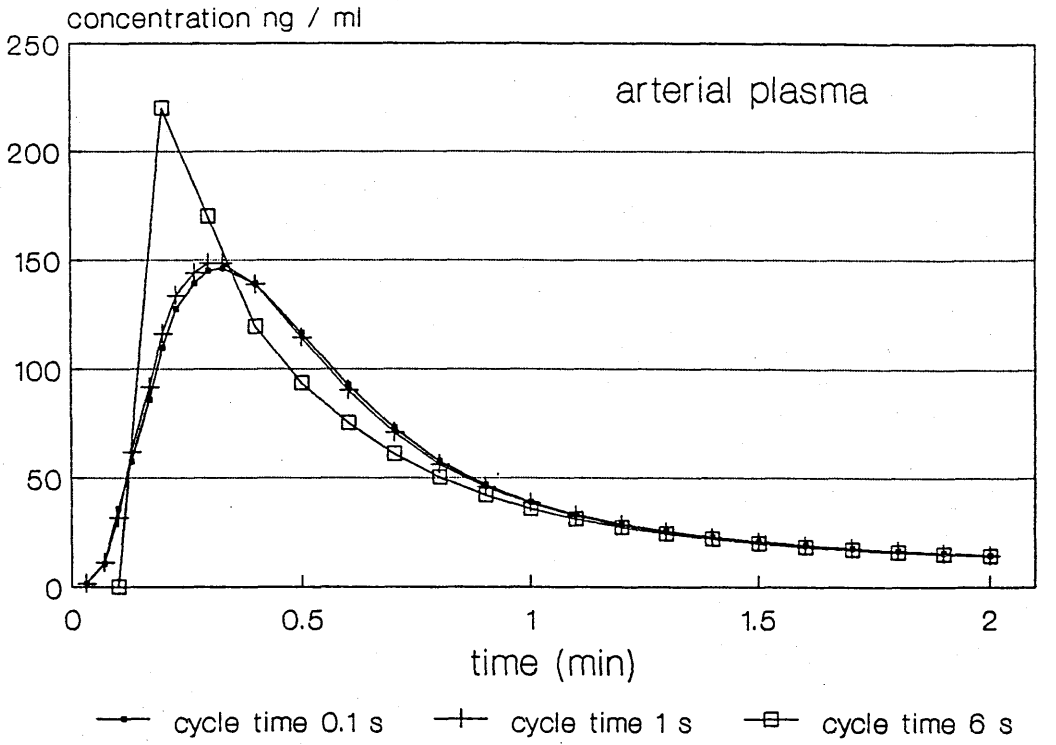
In all three simulations of figure 15.16 simulated maximum lung content of fentanyl is at or just above the upper end of the range for lung content at the end of first pass in the experimental subjects. (The experimental fentanyl lung content is calculated as total dose minus amount in first pass blood. This is not strictly accurate as by the time the last first pass blood leaves the lungs further exchange has already taken place with the capillary blood immediately upstream). Unfortunately the simulated data are not directly comparable to the experimental as the model does not allow the first passage of a bolus of drug to be simulated realistically. This would require capillary volume to be taken into account together with a more realistic representation of blood flow and longitudinal mixing. In the present case the maximum lung content is influenced by the calculation cycle time. For instance in the case of the middle simulation of figure 15.16 (lung binding x 10) when the cycle time is set to 1 s as shown, maximum lung content is 87% of the dose at 20 s whereas with a cycle time of 6 s maximum lung content is 92% at 6 s. Setting a small calculation cycle time gives, as in life, a rising then falling concentration in the peripheral arterial blood with the peak concentration occurring slightly earlier than life at around 16 s. However the concentration of blood entering the lung during this time is

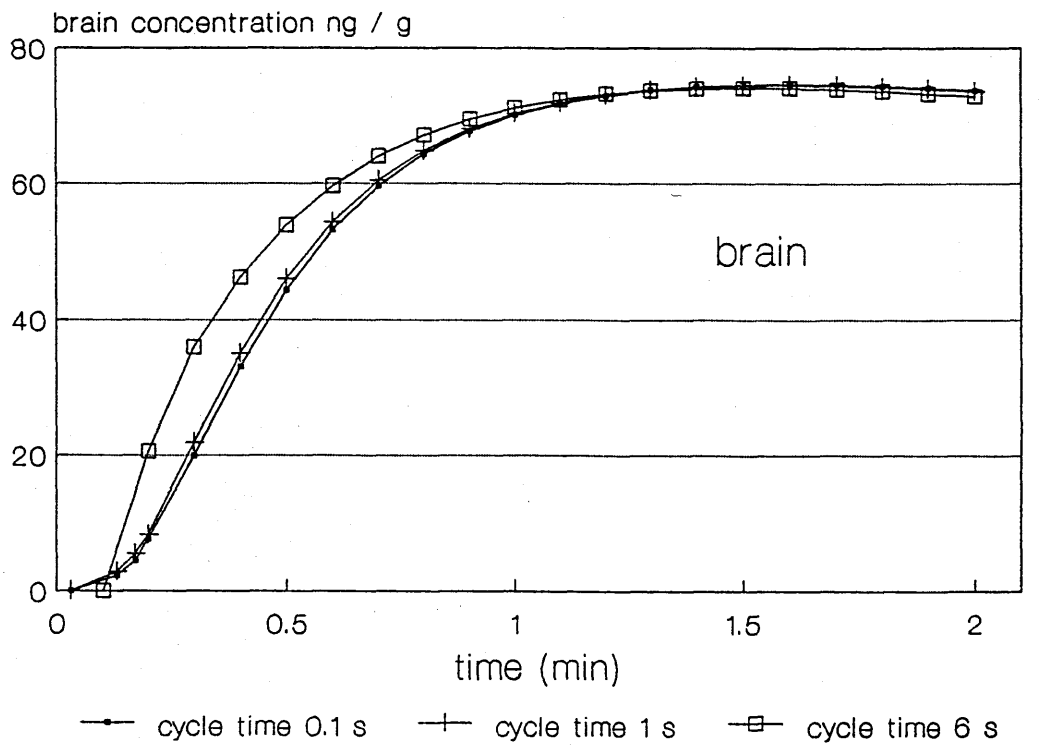
steadily decreasing as a result of venous blood entering and diluting the injection pool. In life the concentration in the pulmonary artery blood as the drug bolus passes through is increasing then decreasing. When the cycle time is set to six seconds virtually the whole of the drug bolus is in the blood which equilibrates with the lungs at the first calculation cycle after injection. The largest fentanyl concentration in arterial blood is the first calculated concentration and thereafter arterial concentration steadily decreases. There will be similar artefacts in the modelling of initial drug concentrations in other tissues, which may be of importance if these transient initial changes have any relevance for drug effect.

Note on calculation cycle time: The effect of calculation cycle time on the uptake of drug into the lung and on lung tissue arterial plasma concentration ratios has already been discussed. Figure 15.17 shows the effect on arterial plasma and brain tissue concentrations of simulating a brief (6 s) intravenous injection with cycle times of 6, 1, and 0.1 seconds. The influence of cycle time on the simulation is negligible at cycle times of less than one second and after the first few seconds of simulation. Where the effect of cycle time is noticeable it is not clear whether it is more realistic to set it large or a small. A cycle time of 1 s has been adopted as standard and was used for all simulations unless specified otherwise.

Figure 15.17

Effect of Calculation Cycle Time on Fentanyl Concentration in Brain and Arterial PLasma





Pharmacokinetic Constants

The volume of distribution at steady (V_{SS}) state may be calculated directly from the total mass of plasma, red cells and tissue blocks and the respective equilibrium distribution ratios. This gives

V_{SS} = 468 kg = 454 l plasma

which comparison with table 13.3 shows to be larger than all but one of the experimental values. Of this 454 l 270 l is accounted for by the fat-tissue block and 156 l by the lean..

Plasma clearance was explicitly set when the model was constructed (chapter 13).

Plasma Clearance = 880 ml min^{-1}

Plasma clearance will vary slightly depending on urine flow and pH.

The terminal half life may be calculated after fitting an exponential function to the simulated concentration time curve after intravenous administration, which also provides an opportunity to examine the model output in terms of a conventional compartmental pharmacokinetic model. (This is done to provide evidence of the realism or otherwise of the simulated results. Experimental arterial concentration profiles treated in this way yield certain values for the standard pharmacokinetic constants. Do the simulation results yield similar values? The results must be interpreted with caution. The concentrations in the physiological model are determined by a relatively small number of approximately exponential processes and there are bound to be systematic errors in fitting simulated concentration

time curves to bi or tri exponential functions. To assess the respective merits of the physiological and conventional models it would be necessary to compare the output of each separately against sets of actual measured concentration-time data).

Arterial plasma concentration-time data for the 24 hours following a single simulated bolus intravenous injection was fitted to bi and tri exponential functions of the form

$$(15.1) \qquad C = P.e^{-\pi t} + A.e^{-\alpha t} + B.e^{-\beta t}$$

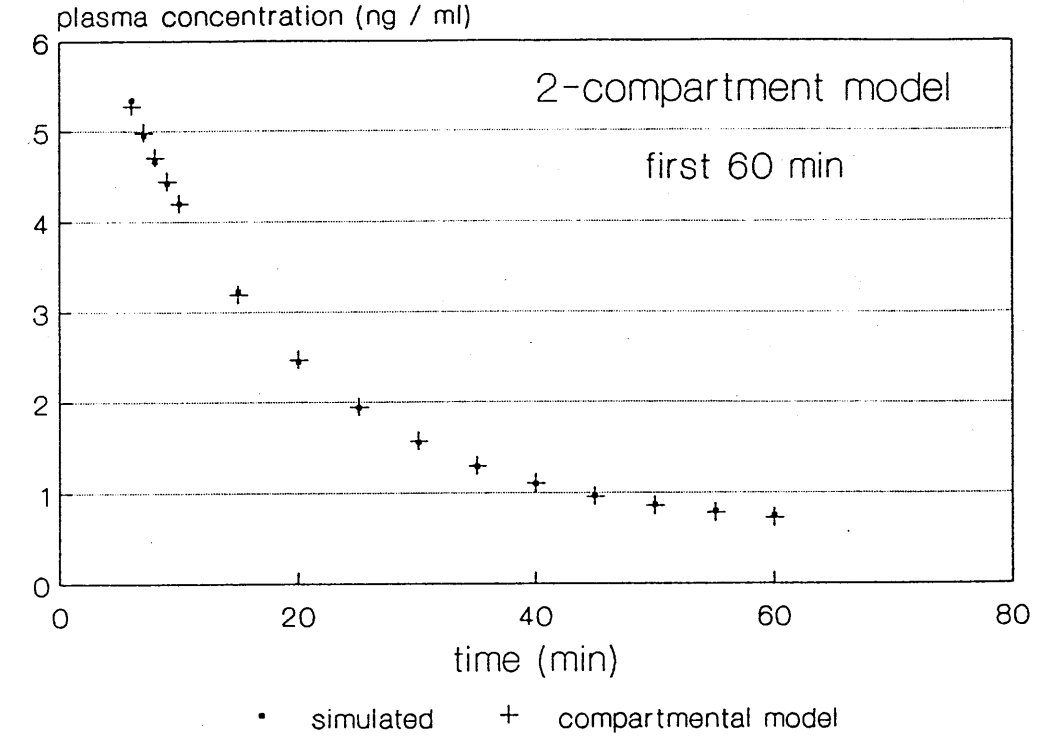
where C is concentration, t is time P, A and B are the zero time intercepts and α, β and π the rate constants for the exponential terms.

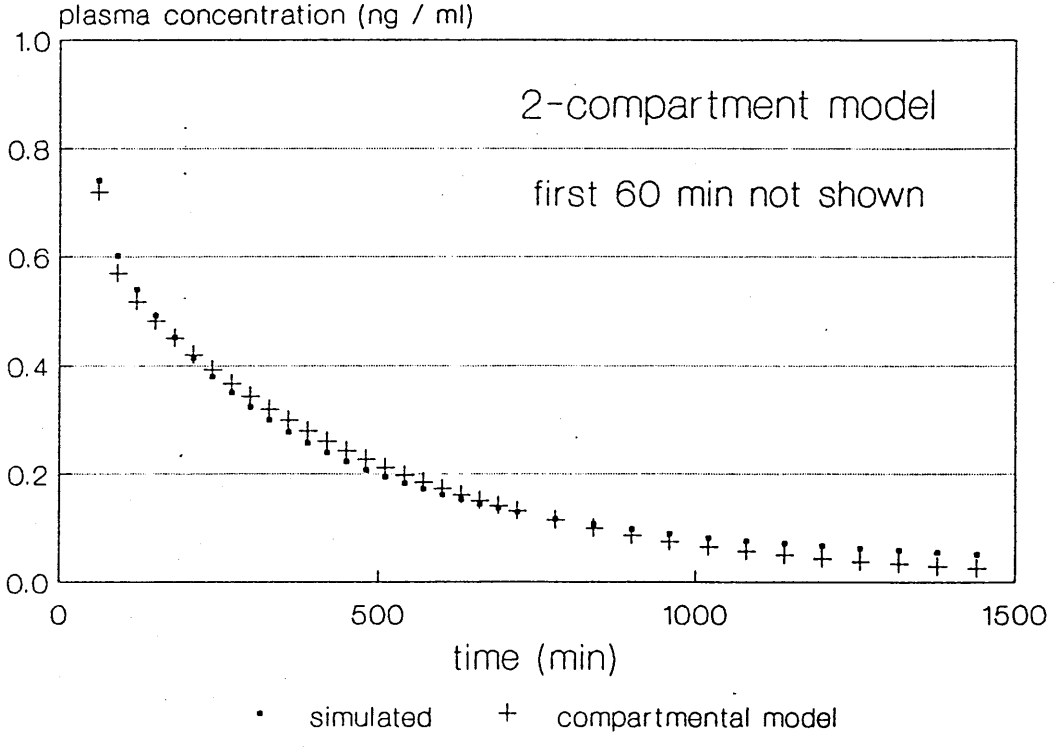
Fitting was carried out using a computer curve-fitting program based on the Levenberg-Marquardt routine [166]. To prevent the fitted values declining to zero after the first few hours it was necessary to exclude data from the first few minutes of the simulation. The bi-exponential curve is fitted to data from 6 minutes onwards and the triexponential curve to data from 2 minutes onwards. Figures 15.18 and 15.19 show the simulated and fitted curves. Intercompartmental rate constants for a standard two compartment and mamillary three compartment models and the corresponding pharmacokinetic variables were calculated from P, A, B, α, β, and π according to standard formulae [132, 134]. They are shown in table 15.3. Experimental values for comparison are found in table 13.3.

The terminal half lives are within the range of experimentally determined values. The actual model clearance is overestimated and the actual model V_{SS} underestimated by this technique but clearance and V_{SS} as calculated by this method are within the

Figure 15.18

Simulated Concentrations of Fentanyl in Arterial Plasma and Fitted Bi-exponential Curve

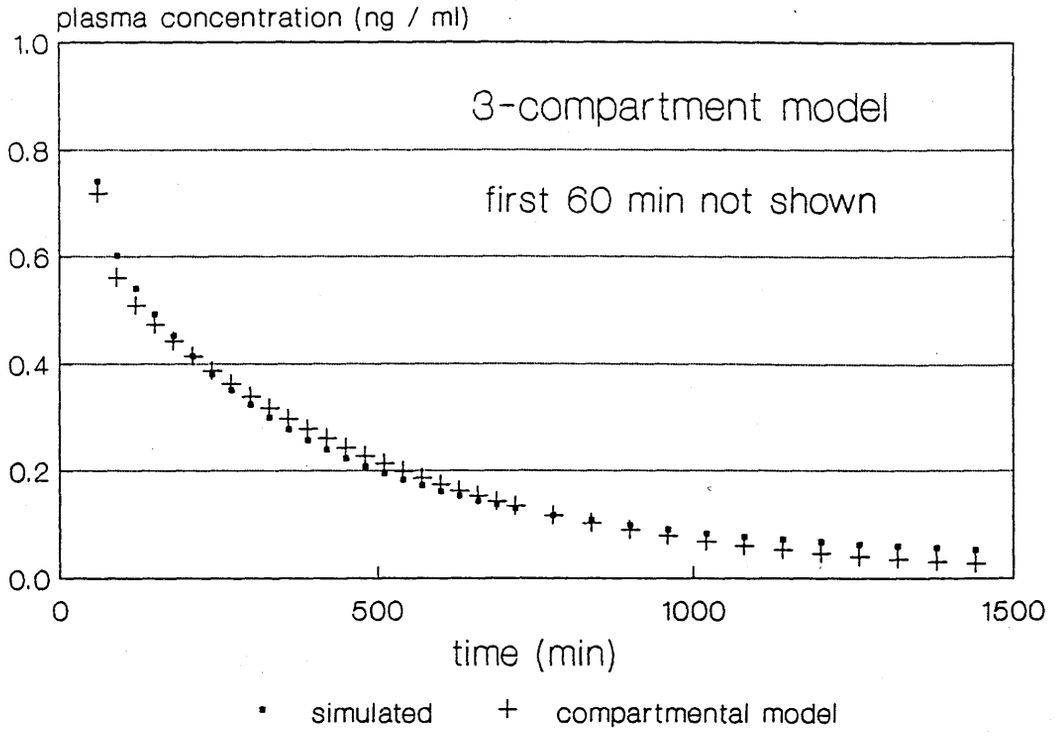


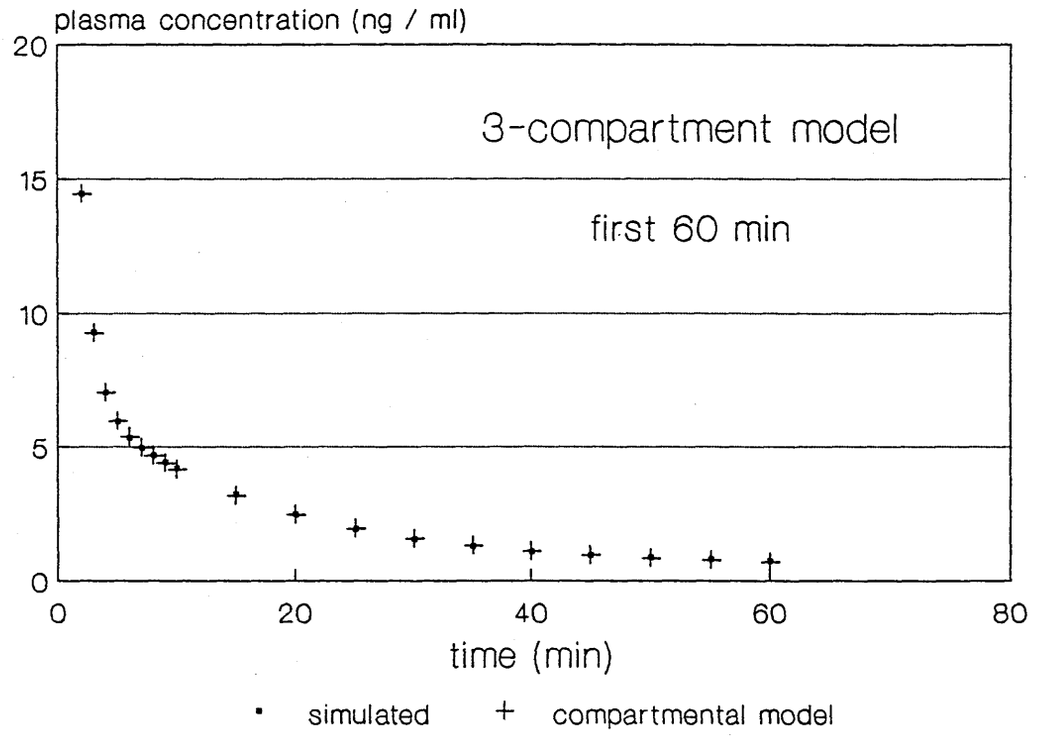


500 µg intravenously over 5 s

Figure 15.19

Simulated Concentrations of Fentanyl in Arterial Plasma and Fitted Tri-exponential Curve





500 µg intravenously over 5 s

Table 15.3: Pharmacokinetic Constants Calculated from Simulated Data According to Two and Three Compartment Models

	two compartment	three compartment
π half life		0.7 min
α half life	10.4 min	10.8 min
β half life	303 min	311 min
Clearance	1248 ml h^{-1}	1099 ml h^{-1}
V_{ss}	410 l	326 l
V_{β}	546 l	493 l

See text for details.

V_{ss} volume of distribution at a steady state
V_{β} volume of distribution during elimination

Table 15.3

upper end of the experimental range. V_β is greater than any of the experimental values.

Nasal Compartment

Figure 15.20 shows a plot of the percentage of the administered dose remaining in the nasal mucosa for the first 20 minutes after the simulation of a bolus administration to the nose. Less than five percent of the administered dose remains in the nasal mucous at the end of the first calculation cycle reflecting its small mass compared to the mass of the nasal mucosa. The effect of mucus clearance will thus be negligible. Peak arterial plasma concentration occurred at 2 min 27 s.

Intrapulmonary Administration

Figure 15.21 shows the arterial plasma and brain concentrations during the first minute after the administration of 100 µg of fentanyl over 1 s either into the lung or intravenously. The calculation cycle time is one second. Brain concentration is greater over the first 40 s or so after pulmonary than after intravenous administration. The pulmonary administration can be considered a "best case" in which drug is delivered uniformly throughout the lungs and thence diffuses freely into the circulation. Unfortunately, as discussed in relation to first pass lung uptake, the model does not have the resolution to simulate the transient local concentration changes consequent on a bolus of drug mixing with the blood, and equilibrating realistically across a capillary bed. The quantification of the difference between the two modes of administration must therefore be regarded as artifactual. As might be expected, when the calculation time is set to 6 s and the volume of the

Figure 15.20

Fentanyl Content of Nasal Mucosa Following Simulated Nasal Administration of 100 µg over 1 s

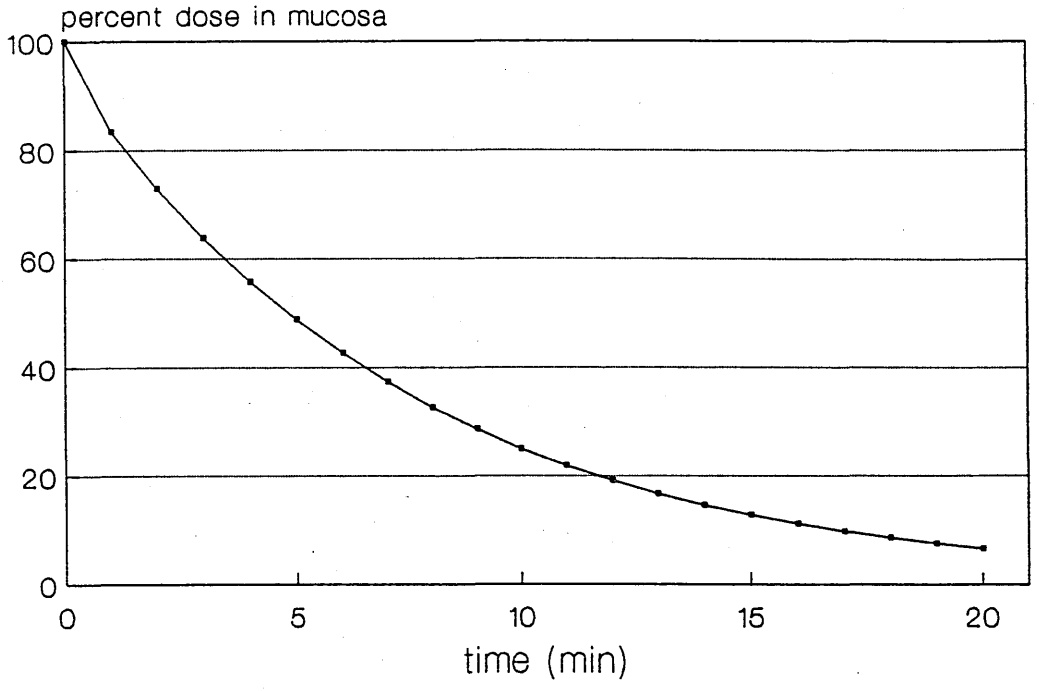
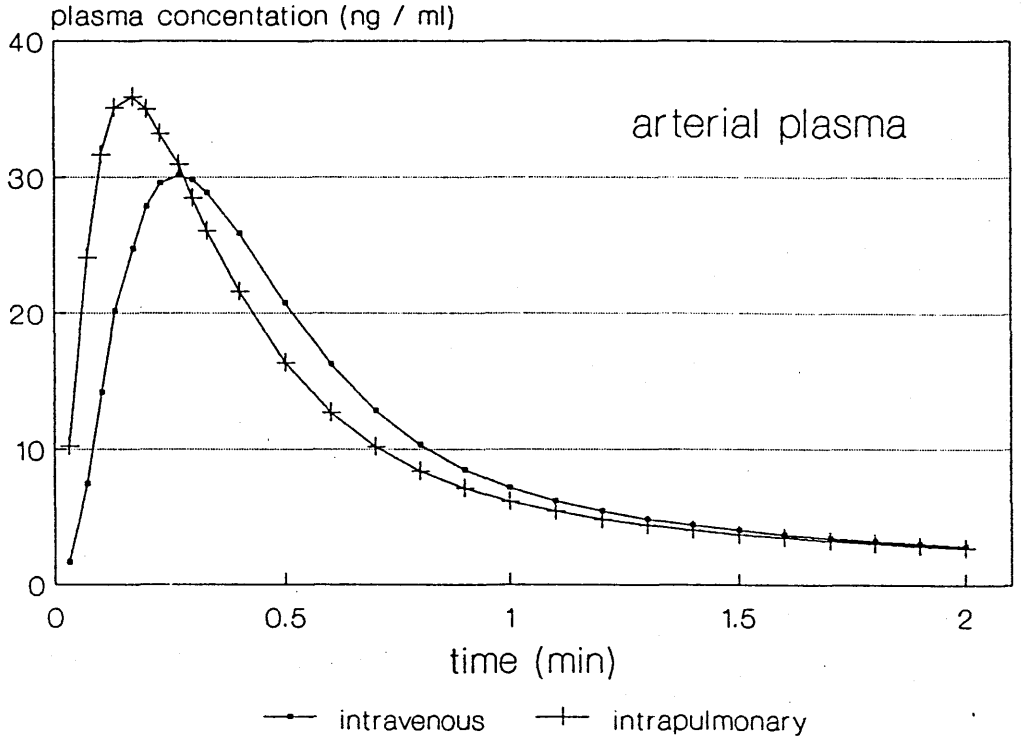
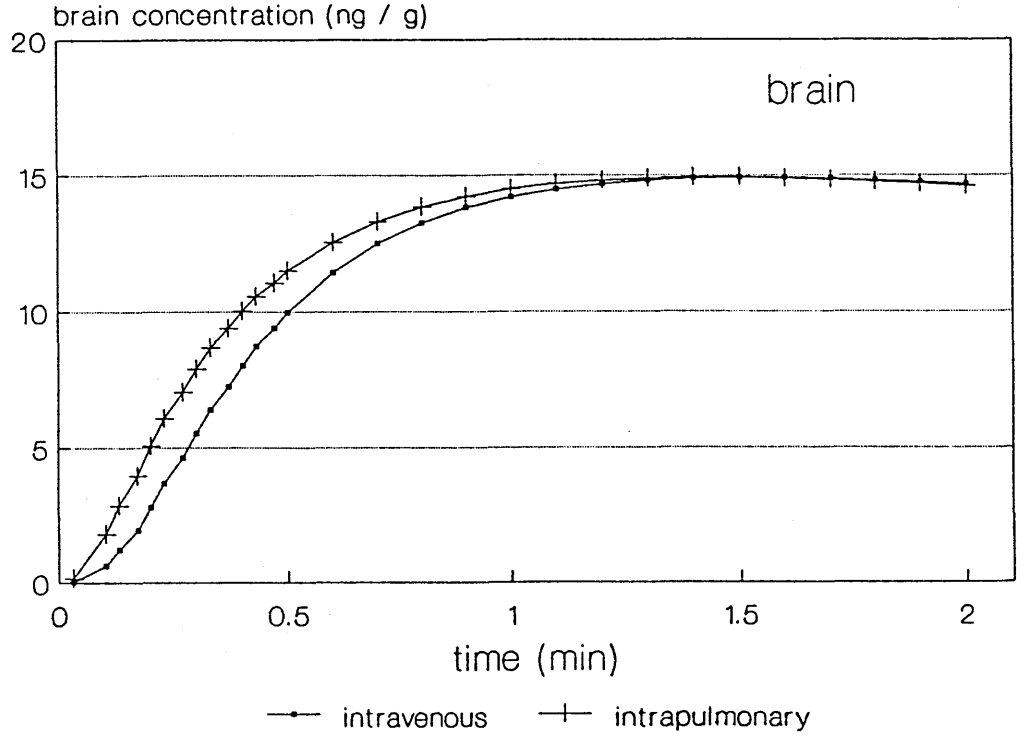


Figure 15.21

Concentrations of Fentanyl in Brain and Arterial Plasma Following Simulated Intravenous and Intrapulmonary Administration (100 µg over 1 s)





circulating blood approximates the volume of the injection pool, the difference between the routes is virtually eliminated.

Tissue and Plasma Binding

The characterisation of all tissue binding in terms of a distribution between water, protein and fat is conceptually elegant. However one cannot help but have reservations as to the practicality of fully quantifying such a scheme given the heterogeneity of body proteins (and to a lesser extent fats), and the difficulties with such a circumscribed and accessible system as binding to plasma proteins.

It may be that the present scheme could be simplified and that information on whole cell binding would be easier to collect experimentally. For fentanyl, and probably other potent lipid soluble drugs, protein binding of unionised drug at pharmacological concentrations can apparently be approximately characterised by a simple partition coefficient (see chapter 13). Since most of the protein-bound and all the lipid-dissolved drug is in the unionised form it should be possible to characterise distribution of unionised drug between cell and extra cellular fluid (ecf) in terms of a single combined partition coefficient. Ionised drug would be distributed between cell water and ecf water according to the respective pH.

The question of how to proceed is essentially empirical since it depends on how detailed a characterisation is necessary before the model begins to fulfil its functions satisfactorily. If the model is to be quantitatively useful one measure of this must be its ability to outperform the predictive ability of conventional compartmental models.

In the short term a measure of the solubility of pethidine and

fentanyl in human or animal lipids is clearly needed. Experiments would involve measuring aqueous / lipid partition coefficients over a range of pH for standard industrial solvents and extracted subcutaneous and intra-abdominal fat, ideally using tritiated drug. Since this would allow a series of estimates of the effective pKa (see chapter 13) the opportunity could also be taken to estimate pKa directly by electrometric titration [133] **using exactly the same pH-measuring system.** The results should either convincingly resolve the uncertainty in the present published data or else delineate real discrepancies for future investigation.

Elimination

Metabolism: In life, clearance will vary with factors such as liver blood flow, perhaps drug concentration and probably plasma pH through its effects on drug dissociation and tissue distribution. The overall model is specifically constructed to simulate the effect of these influences on drug distribution. By contrast the simulation of metabolism in both fentanyl and pethidine models is based on values of total body clearance derived from conventional group pharmacokinetic analysis. This seems a relative weakness in terms of the model's adaptability to individual physiological conditions.

As the hepatic extraction ratio in the models is fixed metabolism will in fact increase with increased hepatic blood flow. However this relationship is unlikely to be quantitatively correct. In life, for a first order elimination process, as hepatic blood flow increases, clearance would be expected to increase towards intrinsic clearance but the extraction ratio to decrease [167].

An experimentally validated sub model of metabolism based on hepatocyte drug concentration would be ideal but certainly for fentanyl there is no data at present with which to build one.
It would be possible however to incorporate existing clearance data into the model in a slightly more elaborate way than at present by relating it to intrinsic clearance and thence calculating an internally consistent relationship between rate of metabolism and hepatic drug concentration.
Thus for instance:

(Eq 15.2) $\text{Clearance(blood)}_{int} = \text{Clearance(blood)} \;/\; (1 - \text{ER})$

where $\text{Clearance(blood)}_{int}$ is intrinsic hepatic clearance expressed in terms of concentration in blood, Clearance(blood) is the measured blood clearance and ER is the extraction ratio [167].
But, by definition

(Eq 15.3) $\text{Clearance(blood)}_{int} = V \;/\; C_{blood}$

where V is the rate of elimination and C_{blood} is the concentration of drug in blood.

combining equations 15.2 and 15.3 and rearranging

(Eq 15.4) $$V = \frac{\text{Clearance(blood)} \times C_{blood}}{(1 - \text{ER})}$$

For a first order process <u>at relatively low substrate concentration</u> [134]

(Eq 15.5) $$\frac{V}{C_{hep}} = \frac{V_{max}}{Km}$$

where C_{hep} is the concentration of drug in the hepatocyte, V_{max} is the maximum attainable reaction velocity and Km is the Michaelis constant.

Thus substituting for V in equation 15.5 from equation 15.4

$$\text{(Eq 15.6)} \quad \frac{V_{max}}{Km} = \frac{\text{Clearance(blood)} \times C_{blood}}{(1 - ER) \times C_{hep}}$$

V_{max} / Km may be calculated using the steady state tissue / arterial blood ratio generated by the model for C_{blood} / C_{hep}. (By steady state value is meant the value to which this ratio tends once the distribution phase is over: see tissue distribution above). Finally equation 15.5 could be inserted into the model to calculate metabolism in the place of the present extraction ratio.

Essentially the process is that of calculating (by using the model to compute a numerical solution) the ratio V / C_{hep} associated with the measured experimental clearance given the prevalent model conditions and assumptions. The advantage is that in the case of deviations from these conditions (changes in liver blood flow or tissue / arterial concentration ratios) metabolism will now behave according to a theoretically correct model. It may be argued that the procedure is too contrived yet conversion of the experimentally measured metabolic clearance to an extraction ratio is similarly contrived in that liver perfusion was not measured at the time of the experiments but the model value assumed.

Excretion A theoretical as opposed to an empirical submodel was adopted for fentanyl urinary excretion from necessity and it

remains speculative and untested. It has the merits however of varying urinary excretion with urine flow , urine pH , kidney blood flow and plasma fentanyl concentration in a manner that by analogy with urea [168] is qualitatively plausible. For instance increased urine flow will be associated with increased fentanyl excretion but decreased urinary fentanyl concentration. Excretion will increase with higher plasma fentanyl concentration or higher renal blood flow.

With the exception of renal blood flow the physiological variables used in the sub-model are relatively easily measurable and experimental assessment should thus be possible. One difficulty is the concentrating factor which I introduced as a constant but might be expected to vary with renal blood flow or glomerular filtration rate. Experiments to study fentanyl renal excretion would be justified by the present almost complete lack of data.

Summary

The model fulfils admirably its qualitative function of providing a disciplined framework for considering drug disposition. By this I mean not only that it illustrates how basic principles lead to results that may not be immediately intuitively obvious, as for instance in the detailed time course of tissue / arterial plasma ratios under different conditions, but also that the natural examination of the model's limits and assumptions promotes a closer scrutiny of physical mechanisms. The overall performance of the fentanyl model in simulating plasma concentrations to match published studies appears at this brief initial assessment to be quite reasonable. However ideally further development would be undertaken before prospective

evaluation is performed. In particular a better estimate should be made of solubility in body lipids in view of the large part this plays in drug distribution. The present total volume of distribution is probably slightly too large.

There is also evidence that the affinity of the lung for fentanyl is too low at present. Certainly, on account of its massive perfusion, distribution to lung plays a disproportionately large part in determining plasma concentrations in the first few minutes after intravenous and intrapulmonary administration. In addition the lung and its circulation are routinely and uniquely accessible in vivo allowing a direct assessment of predictions made from laboratory data. Proper determination of binding to lung tissue should probably therefore have a high priority.

The model was less useful for detailed realistic simulation of the very transient concentration changes that occur immediately after drug administration. (The study of inhaled fentanyl and its associated theoretical background reported in part one had created the suggestion that the pattern of these transient changes might have a bearing on drug effect). It would be straightforward to build a more detailed circulatory model, perhaps using Mapleson's model M as a starting point, but to model the transient changes accurately it would also be necessary to design a more sophisticated model of blood / tissue exchange incorporating a representation of capillary volume and probably, concentration gradients within the tissues. It would thus involve a considerable increase in computational complexity.

CHAPTER 16

Summary and Conclusions

1. An existing physiological model of intravenously and intramuscularly administered pethidine has been extended to allow intranasal and intrapulmonary administration and adapted to describe fentanyl.

2. The original model exists as a computer program in several different versions. During the present modifications, with help from the original author, several errors were identified in these programs. Some of these are present in published versions. Simulation results which had been used for initial evaluation of the model are affected.

3. A preliminary evaluation of the fentanyl model against published data suggests that simulated arterial and venous plasma concentrations are similar to those obtained experimentally.

4. Elements of the model have been examined individually and its future development discussed.

5. The model proved only of limited use for simulating the transient concentration changes which occur in the few seconds following drug administration, the examination of which was suggested by work reported in part 1 of this thesis. Despite this the model is a useful conceptual tool in the examination of questions arising from the development of new routes of drug delivery.

5. A lack of agreement between published studies of aspects of fentanyl pharmacology has been noted. This was particularly surprising for the physiochemical variables lipid solubility and pKa.

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APPENDIX 1

Program Listing

This is a word-processed version of the listing used for the enclosed compiled program. Some long program lines have been broken up using the Turbo Basic line continuation sign "_" and comments have been underlined.

```
DEFDBL A-Z                  'default variable type double precision floating point

                            'Define basic data.

                            'The model man: values Davis 87 with
                            'additions Higgins 90
nc = 11                :      nc$ = "no. of compartments "
totw = 70.001          :      totw$ = "body mass Kg "
qdot = 6.48            :      qdot$ = "cardiac output l/min (default)"
bden = 1.06            :      bden$ = "density of blood Kg/l "
pden = 1.03            :      pden$ = "density of plasma Kg/l "
rden = 1.10            :      rden$ = "density of erythrocytes Kg/l "
fwtb = 0.07857         :      fwtb$ = "Kg blood / Kg body mass "
fr = 0.4500            :      fr$ = "Kg erythrocyte /Kg blood "
fppkg = 0.0680         :      fpp$ = "Kg protein / Kg plasma "
fwp = 0.9155           :      fwp$ = "Kg water /Kg plasma "
DIM fwt#(1:nc)         :      fwt$ = "Kg tissue / Kg total mass"
RESTORE numericaldata
FOR x = 1 TO nc
  READ fwt#(x)
NEXT x
numericaldata:
DATA 0.006628,0.00000,0.004057,0.0198700,0.02459
DATA 0.02686,0.52446,0.19557,0.00010,0.00014,0.0001186
DIM fc#(1:nc)          :      fc$ = "Kg cells / Kg tissue"
FOR x = 1 TO nc
  READ fc#(x)
NEXT x
DATA 0.8000,0.0000,0.8000,0.8000,0.8000
DATA 0.8000,0.8000,0.9250,0.7000,0.8000,0.8000
DIM fl#(1:nc)          :      fl$ = "Kg lipid / Kg tissue"
FOR x = 1 TO nc
  READ fl#(x)
NEXT x
DATA 0.0120,0.0000,0.0520,0.0570,0.0670
DATA 0.1050,0.0450,0.8000,0.1100,0.0200,0.0617
DIM fp#(1:nc)          :      fp$ = "Kg protein / Kg tissue"
FOR x = 1 TO nc
  READ fp#(x)
NEXT x
DATA 0.1800,0.0000,0.1700,0.1410,0.1800
DATA 0.0970,0.1910,0.0490,0.0800,0.1700,0.1333
DIM fw#(1:nc)          :      fw$ =  "Kg water / Kg tissue"
FOR x = 1 TO nc
  READ fw#(x)
NEXT x
DATA 0.7500,0.0000,0.7700,0.7840,0.7200
DATA 0.7740,0.7470,0.1490,0.7900,0.7900,0.7917
DIM fpekg#(1:nc)       :      fpe$ = " Kg protein / Kg ECF"
FOR x = 1 TO nc
  READ fpekg#(x)
NEXT x
DATA 0.0350,0.0350,0.0350,0.0350,0.0350
DATA 0.0350,0.0350,0.0350,0.0100,0.0350,0.0350
DIM fwe#(1:nc)         :    fwe$ = "Kg water / Kg ECF"
FOR x = 1 TO nc
```

```
  READ fwe#(x)
NEXT x
DATA 0.9650,0.9650,0.9650,0.9650,0.9650
DATA 0.9650,0.9650,0.9650,0.9900,0.9650,0.9650
fra = 0.1300            :  fra$ = "fraction of blood volume arterial pool "
frf = 0.1010            :  frf$ = "fraction of blood volume fat pool "
fri = 0.1300            :  fri$ = "fraction of blood volume injection pool "
frl = 0.3380            :  frl$ = "fraction of blood volume lean pool "
frp = 0.1710            :  frp$ = "fraction of blood volume portal pool "
frv = 0.1300            :  frv$ = "fraction of blood volume mixed venous pool "
DIM f#(1:nc)            :  f$ = "fraction of total cardiac output" 'to tissue block"
FOR x = 1 TO nc
  READ f#(x)
NEXT x
DATA 1.00000,0.15100,0.18800,0.17100,0.06900
DATA 0.19100,0.17690,0.05300,0.00105,0.00003,0.000640
                          'correction factor to make sum fractional stroke
                          'volumes = 1.0"
scalefactor = 1.0 / 1.00162
FOR x = 2 TO nc           'corrected fractions"
  f#(x) = f#(x) * scalefactor
NEXT x
mwp = 91000          :     mwp$ = "mean molar mass plasma proteins "
mwe = 91000          :     mwe$ = "mean molar mass ECF proteins "
mwc = 91000          :     mwc$ = "mean molar mass of tissue cell proteins"
php = 7.4000         :     php$ = "pH plasma (default) "
DIM aph#(1:nc)       :     aph$ = "offsets intracellular pH equation" 'tissues
FOR x = 1 TO 11
  READ aph#(x)
NEXT x
DATA -0.40,0.00,-0.30,-0.60,-0.30
DATA -0.60,-0.46,-0.20,-0.30,-0.46,-0.30
bph = -0.80          :    bph$ = "slope intracellular pH equation "
phu = 6.2            :    phu$ = "urine pH (default) "
urout = 100.0        :    urout$ = "urine water output g / h (default) "
fconc = 17.0         :    fconc$ = "urine concentrating factor "
wtmuc = 4.0 / 10000  :    wtmuc$ = "Kg mucous in nasal absorbtion site "
protmuc = 6.77 / 1000:    protmuc$ = "Kg protein / Kg mucous  "
fwm = 0.94           :    fwm$ = "Kg water / Kg mucous  "
mwtmuc = 91000       :    mwtmuc$ = "av mol wt mucous proteins "
phm = 6.0            :    phm$ = "pH nasal secretions "
dt = 1.0             :    dt$ = "cycle time seconds (default)"

                          'drug variables:
metf = 0.55          :    metf$ = "metabolic extraction fraction "
lamlw = 500.0        :    lamlw$ = "lipid/water partition coefficient for fentanyl "
pka = 7.85           :    pka$ = "pKa fentanyl "
acid = 1             :    acid$ = "acid-base drug indicator "
dfac = 1.0 / 336.60  :    dfac$ = "dose conversion factor ug fentanyl base to umol "
eprat = 0.908
eprat$ = "umol drug per Kg erythrocyte / umol drug per Kg plasma "
nki = 0.001246       :    nki$ = "protein binding constant ionisd form "
nks = 0.019121       :    nks$ = "protein binding constant unionised form "

                     'Dimension arrays for intermediate values
DIM fwc#(1:nc)       'Kg water / Kg tissue cells
DIM fpc#(1:nc)       'umol or Kg protein / Kg tissue cells
DIM flc#(1:nc)       'Kg lipid / Kg tissue cells
DIM wf#(1:nc)        'mass stroke vol fractions Kg
DIM wt#(1:nc)        'mass tissue blocks Kg
DIM phc#(1:nc)       'pH tissue cells
```

```
DIM x#(1:nc)          'convenience variables see sub phcalc
DIM b#(1:nc)
DIM e#(1:nc)
DIM c#(1:nc)
DIM csold#(1:nc)      'umol unionised drug / Kg water before equilibration
DIM cs#(1:nc)         'umol unionised drug / Kg water after equilibration
DIM af#(1:nc)         'umol drug in blood entering tissue blocks
DIM aab#(1:nc)        'umol drug in blood fraction after equilibration
DIM aatold#(1:nc)     'umol drug in tissue block before equilibration
DIM aat#(1:nc)        'umol drug in tissue block after equilibration
DIM aabt#(1:nc)       'umol drug in blood and tissue after equilibration

        'calculate some intermediate variables for use in first or
        'subsequent simulations
FOR i = 1 TO nc
  IF NOT i = 2 THEN                        'Kg water / Kg tissue cells
    fwc#(i) = (fw#(i) - (fwe#(i) * (1 - fc#(i)))) / fc#(i)
                                           'Kg protein / Kg tissue cells
    fpc#(i) = (fp#(i) - (fpekg#(i) * (1 - fc#(i)))) / fc#(i)
                                           'Kg lipid / Kg tissue cells
    flc#(i) = (fl#(i) - (fle * (1 - fc#(i)))) / fc#(i)
END IF
NEXT i
fpp = (fppkg / mwp) * EXP10(9.0)           'umol protein / Kg plasma
fpm = (protmuc / mwtmuc) * EXP10(9.0)      'umol protein / Kg mucus
DIM fpe#(1:nc)
FOR  i = 1 TO nc
  fpe#(i) = (fpekg#(i) / mwe) * EXP10(9.0) 'umol protein / Kg ECF
  fpc#(i) = (fpc#(i) / mwc) * EXP10(9.0)   'umol protein Kg tissue cell
NEXT i
wpa = totw * fwtb * fra                    'mass arterial pool Kg
wpv = totw * fwtb * frv                    'mass mixed venous pool Kg
wpi = totw * fwtb * fri                    'mass injection pool Kg
wpp = totw * fwtb * frp                    'mass portal pool Kg
wpf = totw * fwtb * frf                    'mass fat pool Kg
wpl = totw * fwtb * frl                    'mass lean pool Kg
FOR i = 1 TO nc
  wt#(i) = totw * fwt#(i)                  'mass tissue blocks Kg
NEXT i
again = 0                        'indicates first simulation
begining:                        'program restarts here for second and
SCREEN 0                         'subsequent simulations
CLS
nul$ = INKEY$
e$ = ""
IF again = 1 THEN
  DO UNTIL UCASE$(e$) = "Y" OR UCASE$(e$) = "N"
    LOCATE 2,1
    PRINT "Another simulation Y or N?"     'input routine to continue rerun
    e$ = INKEY$
  LOOP
  IF UCASE$(e$) = "N" THEN GOTO finish
  qdot = 6.48                              'resets variable default values
  php = 7.40                               'for repeat simulation
  phu = 6.2
  dt = 1.0
  urout = 100.0
END IF
                   'initialises or resets some intermediate variables
apa = 0            'umol drug arterial pool
apv = 0            'umol drug mixed venous pool
```

```
api = 0                 'umol drug injection pool
apl = 0                 'umol drug lean pool
apf = 0                 'umol drug fat pool
app = 0                 'umol drug portal pool
aam = 0                 'umol drug nasal mucus
aabtm = 0               'umol drug nasal blood,tissue and mucous
FOR i = 1 TO nc
  aat#(i) = 0           'umol drug in tissue block
  aatold#(i) = 0
  aab#(i) = 0           'umol drug in blood fraction
  aabt#(i) = 0          'uml drug in blood and tissue combined
  cs#(i) = 0            'umol unionised drug / Kg water
  csold#(i) = 0
NEXT i
csa = 0                 'unionised drug umol / l water arterial pool
cscv = 0                'unionised drug umol / l water central venous pool
cspv = 0                'unionised drug umol / l water peripheral venous blood
csi = 0                 'unionised drug umol / l water injection pool
csp = 0                 'unionised drug umol / l water portal pool
csl = 0                 'unionised drug umol / l water lean pool
csf = 0                 'unionised drug umol / l water fat pool
css = 0                 'unionised drug umol / l water shunt blood
baba = 0                'umol drug / l arterial pool blood
babcv = 0               'umol drug / l central venous blood
babpv = 0               'umol drug / l peripheral venous blood
babi = 0                'umol drug / l injection pool blood
babp = 0                'umol drug / l portal pool blood
babl = 0                'umol drug / l lean pool blood
babf = 0                'umol drug / l fat pool blood
babs = 0                'umol drug / l shunt blood
bapa = 0                'umol drug / l arterial pool plasma
bapcv = 0               'umol drug / l central venous plasma
bappv = 0               'umol drug / l peripheral venous plasma
bapi = 0                'umol drug / l injection pool plasma
bapp = 0                'umol drug / l portal pool plasma
bapl = 0                'umol drug / l lean pool plasma
bapf = 0                'umol drug / l fat pool plasma
baps = 0                'umol drug / l shunt plasma
totalamtexc = 0         'cumulative umol drug excreted
totalamtmet = 0         'cumulative umol drug metabolised
ttime = 0               'simulation length seconds
rtime = 0               'elapsed time seconds
ptime = 0               'elapsed time since dose or parameter change seconds
dstot = 0               'umol drug administered
vdleft = 0              'unadministered portion of iv dose umol
ldleft = 0              'unadministered portion of intrapulmonary dose umol
ndleft = 0              'unadministered portion of intrnasal dose umol
vdurn = 0               'duration of administration of iv dose
ldurn = 0               'duration of administration of pulmonary dose
ndurn = 0               'duration of administration of nasal dose
vdinc = 0               'current iv dose increment umol
ldinc = 0               'current intrapulmonary dose increment umol
ndinc = 0               'current intranasal dose increment umol
ivdose = 0              'current iv dose umol
imdose = 0              'current im dose umol
nosdose = 0             'current intranasal dose umol
lungdose = 0            'current intrapulmonary dose umol
ivnum = 0               'number of iv doses
imnum = 0               'number of im doses
nosnum = 0              'number of intranasal doses
lungnum = 0             'number of intrapulmonary doses
```

```
unum = 0                'number of urine pHs if default changed
pnum = 0                'number of plasma phs if default changed
qnum = 0                'number of cardiac outputs if default changed
tnum = 0                'number of cycle times if default changed
uroutnum = 0            'number of urine outputs if default changed
maxnum = 0              'number of possible concentration maximums + 1

                        'initialises or resets some indicators and counters
rcount = 0              'counter for results(aat#(1)) printing
imcount = 0             'position indicator for pending imdose array
ivcount = 0             'position indicator for pending ivdose array
noscount = 0            'position indicator for pending nasal dose array
lungcount = 0           'position indicator for pending pulmonary dose array
ucount = 0              'position indicator for pending urine ph array
pcount = 0              'position indicator for pending plasma ph array
qcount = 0              'position indicator for pending cardiac output array
tcount = 0              'position indicator for pending cycle time array
uroutcount = 0          'position indicator for pending urine output array
graph1 = 0              'indicators for results graph display selection
graph2 = 0
graph3 = 0
graph4 = 0
graph5 = 0
graph6 = 0
paper = 0               'indicator for printer display selection
disk1 = 0               'indicators for results file selection
disk2 = 0
leglean = 0             'indicators show whether results graph labels in place
legfat = 0
legcv = 0
legp =  0
legbrain = 0
legart = 0
x1% = 0                 'x coordinates for results graph labels
x2% = 0
x3% = 0
x4% = 0
x5% = 0
x6% = 0
y1% = 0                 'y coordinates for results graph labels
y2% = 0
y3% = 0
y4% = 0
y5% = 0
y6% = 0
p% = 0                  'indicator for use in sub dsentry
                        'Prints initial instructions on screen and organises
                        'methods of program output
CLS
PRINT "          FENTANYL PHARMACOKINETIC SIMULATION"
PRINT ""
PRINT "Simulation Output: The program will present results graphically"
PRINT "to the screen and/or enter detailed information in a results file"
PRINT ""
PRINT ""
PRINT "File:"
PRINT ""
PRINT "The program calculates, at intervals, drug concentration in each tissue"
PRINT "block and the drug concentrations in blood and plasma at various locations"
PRINT "in the circulation. Times of concentration maxima and blood/tissue"
PRINT "concentration ratios are also given"
```

```
PRINT ""
PRINT "Two sampling schemes are available a standard scheme and a more"
PRINT "frequent scheme. The frequent scheme allows concentration changes"
PRINT "consequent on drug distribution to be followed very closely but"
PRINT "generates a large amount of data"
LOCATE 24,45
PRINT "Press any key to continue...";
WHILE NOT INSTAT:WEND
nul$ = INKEY$
CLS
PRINT "Screen: The program will  plot drug  concentrations to an appropriate"
PRINT "monitor screen as the simulation progresses."
PRINT ""
PRINT "Printer: If you have a printer the program will print details of drug"
PRINT "doses, and variable changes during the simulation."
PRINT ""
PRINT ""
PRINT "A star * at the bottom left of the screen"
PRINT "indicates the simulation is complete"
PRINT ""
PRINT ""
PRINT "At the end of the simulation press any key to return to the program."
PRINT ""
PRINT "";
LOCATE 24,45
PRINT "Press any key to continue...";
WHILE NOT INSTAT:WEND
nul$ = INKEY$
e$ = ""
CLS
DO UNTIL UCASE$(e$) = "Y" OR UCASE$(e$) = "N"
  LOCATE 2,1
  PRINT "Do you have a printer?"
  PRINT "Press Y or N";
  e$ = INKEY$
LOOP
IF UCASE$(e$) = "N" THEN paper = 0          'instructs the program
IF UCASE$(e$) = "Y" THEN                    'whether printer in use or not
  paper = 1
  LOCATE 5,1
  PRINT "DO NOT FORGET TO SWITCH THE PRINTER ON"
  LOCATE 24,45
  PRINT "press any key to continue..."
  WHILE NOT INSTAT:WEND
END IF
nul$ = INKEY$
e$ = ""
CLS
DO UNTIL  UCASE$(e$) = "Y" OR UCASE$(e$) = "N"
  LOCATE 1,1
  PRINT "Do you want results stored on disk during the simulation?"
  PRINT "Press Y or N";
  e$ = INKEY$
LOOP
IF UCASE$(e$) = "N" THEN
   disk1 = 0                                'instructs program  results output
   disk2 = 0                                'to datafile not required
   LOCATE 24,60
   PRINT "please wait....";
ELSE
  nul$ = INKEY$
```

```
e$ = ""
DO UNTIL UCASE$(e$) = "S" OR UCASE$(e$) = "F"
  LOCATE 5,1
  PRINT "                      time from dose              results"
  PRINT "                    or variable change "
  PRINT ""
  PRINT "STANDARD scheme:       0 to 1 hour                 1 min"
  PRINT "                                                   5 min"
  PRINT "                                                 every 5 min"
  PRINT "                     1 hour onwards              every 30 min"
  PRINT ""
  PRINT "FREQUENT scheme:      0 to 2 min                 every few seconds"
  PRINT "                      2 min to 20 min            every 30 s"
  PRINT "                      20 min to 1 hour           every 2 min"
  PRINT "                      1 hour to 6 hours          every 10 min"
  PRINT "                      6 hours onwards            every 30 min";
  LOCATE 21,1
  PRINT "NB The frequent scheme generates a large amount of data";
  LOCATE 24,1
  PRINT "Press S for the Standard scheme or F for the Frequent scheme";
  e$ = INKEY$
LOOP
IF UCASE$(e$) = "S" THEN disk1 = 1
IF UCASE$(e$) = "F" THEN disk1 = 2
e$ = ""
DO UNTIL UCASE$(e$) = "Y"
  CLS
  LOCATE 2,1
  PRINT "Give the full name of a file to store the main results."
  PRINT "Use the standard format  drive:\directory\filename.filetype"
  PRINT "to specify the drive, subdirectorie(s) (optional) and"
  PRINT "filename extension (optional)"
  PRINT ""
  PRINT "e.g. b:\dir1\results.dat"
  PRINT "     a:results"
  PRINT ""
  INPUT, file1$
  PRINT "";
  nul$ = INKEY$
  e$ = ""
  disk2 = 0
  DO
    LOCATE 12,1
    PRINT "If you wish the program will also list to a separate file"
    PRINT "the basic data on which the model is based."
    PRINT ""
    PRINT "Do you want this Y or N?";
    e$ = INKEY$
  LOOP UNTIL UCASE$(e$) = "Y" OR UCASE$(e$) = "N"
  IF UCASE$(e$) = "Y" THEN
    disk2 = 1
    nul$ = INKEY$
    e$ = ""
    LOCATE 17,1
    PRINT "Give the full name of a file to store the data."
    PRINT "Use the standard format  drive:\directory\filename.filetype"
    PRINT ""
    INPUT, file2$
  END IF
  nul$ = INKEY$
  e$ = ""
```

```
    DO UNTIL UCASE$(e$) = "Y" OR UCASE$(e$) = "N"
      LOCATE 22,1
      PRINT "Happy with filename(s) Y or N?";
      e$ = INKEY$
    LOOP
  LOOP
  LOCATE 24,60
  PRINT "please wait....";
                                'enters key in main results file
  OPEN file1$ FOR OUTPUT AS #1
  PRINT #1,"FENTANYL SIMULATION : RESULTS"
  PRINT #1,""
  PRINT #1,"abreviations:"
  PRINT #1,"ca   central arterial (pool)      pa    peripheral arterial (shunt)"
  PRINT #1,"cv   central venous pool          lean  lean pool"
  PRINT #1,"por  portal pool                  pv    peripheral venous pool"
  PRINT #1,"fat  fat pool m                   inj   injection pool"
  PRINT #1,"tissue  1: lungs"
  PRINT #1,"tissue  2: peripheral shunt (no tissue)"
  PRINT #1,"tissue  3: kidneys"
  PRINT #1,"tissue  4: portal bed (gut and spleen)"
  PRINT #1,"tissue  5: liver"
  PRINT #1,"tissue  6: other viscera"
  PRINT #1,"tissue  7: muscle"
  PRINT #1,"tissue  8: fat"
  PRINT #1,"tissue  9: sample brain"
  PRINT #1,"tissue 10: i.m. injection site"
  PRINT #1,"tissue 11: nasal absorbtion site"
  PRINT #1,"unionised aq - aqueous concentration unionised drug ug / Kg water"
  PRINT #1,"tis:arterial blood -"
  PRINT #1,"ug drug/Kg arterial blood : ug drug/Kg tissue before equilibration"
  CLOSE #1
END IF
                                'enters values and definitions of basic data
                                'used by by program in program data file
IF disk2 = 1 THEN
  OPEN file2$ FOR OUTPUT AS #1
  PRINT #1,"FENTANYL PHARMACOKINETIC SIMULATION"
  PRINT #1,""
  PRINT #1,"Basic data:"
  PRINT #1,""
  PRINT #1,"tissue  1: lungs"
  PRINT #1,"tissue  2: peripheral shunt (no tissue)"
  PRINT #1,"tissue  3: kidneys"
  PRINT #1,"tissue  4: portal bed (gut and spleen)"
  PRINT #1,"tissue  5: liver"
  PRINT #1,"tissue  6: other viscera"
  PRINT #1,"tissue  7: muscle"
  PRINT #1,"tissue  8: fat"
  PRINT #1,"tissue  9: sample brain"
  PRINT #1,"tissue 10: i.m. injection site"
  PRINT #1,"tissue 11: nasal absorbtion site"
  PRINT #1,""
  PRINT #1, USING "&###";nc$;nc
  PRINT #1, USING "&###.####";totw$;totw
  PRINT #1, USING "&###.##";qdot$;qdot
  PRINT #1, USING "&#.##";bden$;bden
  PRINT #1, USING "&#.##";pden$;pden
  PRINT #1, USING "&#.##";rden$;rden
  PRINT #1, USING "&.####";fwtb$;fwtb
  PRINT #1, USING "&.##";fr$;fr
```

```
PRINT #1, USING "&.###";fpp$;fppkg
PRINT #1, USING "&.####";fwp$;fwp
PRINT #1, USING "tissue     &";fwt$
FOR i = 1 TO nc
  PRINT #1,USING "  ##               #.######";i;fwt#(i)
NEXT i
PRINT #1,USING "tissue     &";fc$
FOR i = 1 TO nc
  PRINT #1,USING "  ##            #.###";i;fc#(i)
NEXT i
PRINT #1, USING "tissue     &";fl$
FOR i = 1 TO nc
  PRINT #1,USING "  ##            #.###";i;fl#(i)
NEXT i
PRINT #1, USING "tissue     &";fp$
FOR i = 1 TO nc
  PRINT #1,USING "  ##             #.###";i;fp#(i)
NEXT i
PRINT #1, USING "tissue     &";fw$
FOR i = 1 TO nc
  PRINT #1,USING "  ##            #.###";i;fw#(i)
NEXT i
PRINT #1, USING "tissue     &";fpe$
FOR i = 1 TO nc
  PRINT #1, USING "  ##              #.###";i;fpekg#(i)
NEXT i
PRINT #1, USING "tissue     &";fwe$
FOR i = 1 TO nc
  PRINT #1, USING "  ##            #.###";i;fwe#(i)
NEXT i
PRINT #1, USING "&#.###";fra$;fra
PRINT #1, USING "&#.###";frf$;frf
PRINT #1, USING "&#.###";fri$;fri
PRINT #1, USING "&#.###";frl$;frl
PRINT #1, USING "&#.###";frp$;frp
PRINT #1, USING "&#.###";frv$;frv
PRINT #1,USING "tissue     &";f$
FOR i = 1 TO nc
  PRINT #1,USING "  ##                    #.#####";i;f#(i)
NEXT i
PRINT #1, USING "&#####";mwp$;mwp
PRINT #1, USING "&#####";mwe$;mwe
PRINT #1, USING "&#####";mwc$;mwc
PRINT #1, USING "&#.###";php$;php
PRINT #1,USING "tissue     &";aph$
FOR i = 1 TO nc
  PRINT #1,USING "  ##                  -#.##";i;aph#(i)
NEXT i
PRINT #1, USING "&-#.##";bph$;bph
PRINT #1, USING "&#.###";phu$;phu
PRINT #1, USING "&###";urout$;urout
PRINT #1, USING "&###";fconc$;fconc
PRINT #1, USING "&#.#####";wtmuc$;wtmuc
PRINT #1, USING "&#.#####";protmuc$;protmuc
PRINT #1, USING "&#.##";fwm$;fwm
PRINT #1, USING "&#####";mwtmuc$;mwtmuc
PRINT #1, USING "&#.##";phm$;phm
PRINT #1, USING "&##";dt$;dt
PRINT #1, USING "&#.###";metf$;metf
PRINT #1, USING "&###";lamlw$;lamlw
PRINT #1, USING "&#.###";pka$;pka
```

```
  PRINT #1, USING "&#";acid$;acid
  PRINT #1, USING "&#.######";nki$;nki
  PRINT #1, USING "&#.######";nks$;nks
  PRINT #1, USING "&#.###";eprat$;eprat
  PRINT #1, USING "&#.#####";dfac$;dfac
  CLOSE #1
END IF
              'Prints heading if printer in use
IF paper = 1 THEN
  LPRINT "              FENTANYL PHARMACOKINETIC SIMULATION"
  LPRINT ""
END IF
              'entry routines for runtime data:
              'simulation duration
CLS
DO UNTIL ttime > 0
  LOCATE 1,1
  INPUT "Give the total simulation time in minutes    ",ttime
  IF NOT ttime > 0 THEN
    LOCATE 1,1
    INPUT "please re-enter simulation time (minutes)       ",ttime
  END IF
LOOP
ttime = ttime * 60                    'simulation time seconds
KEY 1, "1"                            'labels function keys for input
KEY 2, "2"                            'and clears keyboard buffer
KEY 3, "3"
KEY 4, "4"
nul$ = INKEY$
e$ = ""
                                      'drug dose details
CLS
WHILE -1
  LOCATE 4,1
  PRINT "You may give as many intravenous doses, intramuscular doses,"
  PRINT "intranasal doses, or intrapulmonary doses as you wish, in any "
  PRINT "combination:"
  PRINT ""
  PRINT ""
  PRINT "F1  intravenous"
  IF ivnum > 0 THEN                   'prints * sign on screen display
    LOCATE 9,24                       'next to chosen dose routes
    PRINT "*"
  END IF
  PRINT "F2  intramuscular"
  IF imnum > 0 THEN
    LOCATE 10, 24
    PRINT "*"
  END IF
  PRINT "F3  intranasal"
  IF nosnum > 0  THEN
    LOCATE 11,24
    PRINT "*"
  END IF
  PRINT "F4  intrapulmonary"
  IF lungnum > 0  THEN
    LOCATE 12,24
    PRINT "*"
  END IF
  PRINT ""
  PRINT ""
```

```
PRINT "Press the corresponding function button to enter dose details for"
PRINT "any route"
PRINT ""
PRINT "NB You may come back to this screen as often as you wish."
PRINT "If you have already entered doses by a particular route "
PRINT "retyping the appropriate function key will erase them"
PRINT ""
PRINT "When all doses are entered press RETURN to continue the program"
e$ = INKEY$

  'dose details are stored in temporary arrays as they are entered.
  'two temporary arrays are used. acummulated  dose details are copied
  'from one to the other before each additional dose is entered, the
  'recipient array having being erased and redimensioned large enough to
  'also accomodate details of the new dose to be entered. When all
  'doses have been entered for  given route details are transfered
  'to a separate array for use later in the program
SELECT CASE e$
CASE "2"
  CLS
  PRINT "You may enter as many im doses as you want. Doses must be entered in "
  PRINT "order from the start of the simulation"
  CALL scr("im")            'sets dose entry display up on screen and
                            'initialises dose times and durations
  IF imnum > 0 THEN ERASE pimdose#        'erases any previuos array
                                          'before entering loop
  imcount = 0                             'initialises dose counter
  WHILE -1
    imcount = imcount + 1                 'increments dose counter
    IF imcount > 1 THEN ERASE pimdose1#   'erases previous temp. array 1
    DIM pimdose1#(1:imcount,1:3)          'dimensions new temp. array 1
                            'dimensions temp array 2 if first pass through loop
    IF imcount = 1 THEN DIM pimdose2#(1:imcount,1:3)
                                          'entry routine for dose details
    CALL dsentry(1,mdurn,imcount,"im",pimdose1#(),pimdose2#())
    IF p% > 0 THEN EXIT LOOP              'indicates all im doses entered
    imcount = imcount + 1                 'increments dose counter
    ERASE pimdose2#                       'erases previous temp. array 2
    DIM pimdose2#(1:imcount,1:3)          'dimensions new temp. array 2
                                          'entry routine for dose details
    CALL dsentry(2,mdurn,imcount,"im",pimdose2#(),pimdose1#())
    IF p% > 0 THEN EXIT LOOP              'indicates all im doses entered
  WEND
  imnum = imcount - 1                     'total number of doses
  imcount = 1               'resets dose counter as position indicator
  IF imnum > 0 THEN
    DIM pimdose#(1:imnum,1:3)
    FOR x% = 1 TO imnum                   'enters dose details
      SELECT CASE p%                      'into array for use
      CASE 1                              'in program
        pimdose#(x%,1) = pimdose2#(x%,1)
        pimdose#(x%,2) = pimdose2#(x%,2)
      CASE 2
        pimdose#(x%,1) = pimdose1#(x%,1)
        pimdose#(x%,2) = pimdose1#(x%,2)
      END SELECT
    NEXT x%
  END IF
  ERASE pimdose1#                         'erases temporary arrays
  ERASE pimdose2#
  CLS
```

```
CASE "4"
  CLS
  PRINT "The pulmonary dose is the dose of drug  deposited in the"
  PRINT "terminal airways. The Program does not model the processes"
  PRINT "which determine the proportion of a given dispensed dose"
  PRINT "reaching this region"
  PRINT ""
  PRINT ""
  PRINT "You may enter as many doses as you want. Doses must be entered in "
  PRINT "order from the start of the simulation."
  PRINT "Intrapulmonary doses must not overlap each other"
  CALL scr("ip")            'sets dose entry display up on screen and
                            'initialises dose times and durations
  IF lungnum > 0 THEN ERASE plungdose#    'erases any previous array
                                          'before entering loop
  lungcount = 0                           'initialises dose counter
  WHILE -1
    lungcount = lungcount + 1             'increments dose counter
    IF lungcount > 1 THEN ERASE plungdose1#       'erases previous
                                                  'temporary array 1
    DIM plungdose1#(1:lungcount,1:3)      'dimensions new temp. array 1
                            'dimensions temp array 2 if first pass through loop
    IF lungcount = 1 THEN DIM plungdose2#(1:lungcount,1:3)
                                          'entry routine for dose details
    CALL dsentry(1,ldurn,lungcount,"ip",plungdose1#(),plungdose2#())
    IF p% > 0 THEN EXIT LOOP              'indicates all ip doses entered
    lungcount = lungcount + 1             'increments dose counter
    ERASE plungdose2#                     'erases  previous temp array 2
    DIM plungdose2#(1:lungcount,1:3)      'dimensions new temp array 2
                                          'entry routine for dose details
    CALL dsentry(2,ldurn,lungcount,"ip",plungdose2#(),plungdose1#())
    IF p% > 0 THEN EXIT LOOP              'indicates all ip doses entered
  WEND
  lungnum = lungcount - 1                 'total number of doses
  lungcount = 1             'resets dose counter as position indicator
  IF lungnum > 0 THEN
    DIM plungdose#(1:lungnum,1:3)
    FOR x% = 1 TO lungnum
      SELECT CASE p%
      CASE 1
        plungdose#(x%,1) = plungdose2#(x%,1)      'enters dose details
        plungdose#(x%,2) = plungdose2#(x%,2)      'into array for use
        plungdose#(x%,3) = plungdose2#(x%,3)      'in program
      CASE 2
        plungdose#(x%,1) = plungdose1#(x%,1)
        plungdose#(x%,2) = plungdose1#(x%,2)
        plungdose#(x%,3) = plungdose1#(x%,3)
      END SELECT
    NEXT x%
  END IF
  ERASE plungdose1#                       'erases temporary arrays
  ERASE plungdose2#
CLS

CASE "3"
KEY OFF
CLS
PRINT "The program assumes that nasal doses are dispensed as a dry aerosol"
PRINT "of fentanyl citrate or are dissolved in a small volume of solution"
PRINT "and are dispersed widely over the nasal mucosa"
```

```
PRINT ""
PRINT ""
PRINT "You may enter as many doses as you want. Doses must be entered in "
PRINT "order from the start of the simulation"
PRINT "Intranasal doses must not overlap each other"
CALL scr("in")              'sets dose entry display up on screen and
                            'initialises dose times and durations
IF nosnum > 0 THEN ERASE pnosdose#      'erases any previous array
                                        'before entering loop
noscount = 0                            'initialises dose counter
WHILE -1
  noscount = noscount + 1               'increments dose counter
  IF noscount > 1 THEN ERASE pnosdose1# 'erases previous temp array 1
  DIM pnosdose1#(1:noscount,1:3)        'dimensions new temp array 1

                            'dimensions temparay 2 if first pass through loop
  IF noscount = 1 THEN DIM pnosdose2#(1:noscount,1:3)
                            'entry routine for dose details
  CALL dsentry(1,ndurn,noscount,"in",pnosdose1#(),pnosdose2#())
  IF p% > 0 THEN EXIT LOOP              'indicates all in doses entered
  noscount = noscount + 1               'increments dose counter
  ERASE pnosdose2#                      'erases previous temp array 2
  DIM pnosdose2#(1:noscount,1:3)        'dimensions new temp array 2
                            'entry routine for dose details
  CALL dsentry(2,ndurn,noscount,"in",pnosdose2#(),pnosdose1#())
  IF p% > 0 THEN EXIT LOOP              'indicates all in doses entered
WEND
nosnum = noscount - 1                   'total number of doses
noscount = 1              'resets dose counter as position indicator
IF nosnum > 0 THEN
  DIM pnosdose#(1:nosnum,1:3)
  FOR x% = 1 TO nosnum
    SELECT CASE p%
    CASE 1
      pnosdose#(x%,1) = pnosdose2#(x%,1) 'enters dose details
      pnosdose#(x%,2) = pnosdose2#(x%,2) 'into array for use
      pnosdose#(x%,3) = pnosdose2#(x%,3) 'in program
    CASE 2
      pnosdose#(x%,1) = pnosdose1#(x%,1)
      pnosdose#(x%,2) = pnosdose1#(x%,2)
      pnosdose#(x%,3) = pnosdose1#(x%,3)
    END SELECT
  NEXT x%
END IF
ERASE pnosdose1#                        'erases temporary arrays
ERASE pnosdose2#
CLS

CASE "1"
KEY OFF
CLS
PRINT "You may enter as many intravenous doses as you want. "
PRINT "Doses must be entered in order from the start of the simulation"
PRINT "Intravenous doses must not overlap each other"
CALL scr("iv")              'sets dose entry display up on screen and
                            'initialises dose times and durations
IF ivnum > 0 THEN ERASE pivdose#        'erases any previous array
                                        'before entering loop
ivcount = 0                             'initialises dose counter
WHILE -1
  ivcount = ivcount + 1                 'increments dose counter
```

```
    IF ivcount > 1 THEN ERASE pivdose1#     'erases previous temp array 1
    DIM pivdose1#(1:ivcount,1:3)            'dimensions new temp array 1
                              'dimensions temp array 2 if first pass through loop
    IF ivcount = 1 THEN DIM pivdose2#(1:ivcount,1:3)
                              'entry routine for dose details
    CALL dsentry(1,vdurn,ivcount,"iv",pivdose1#(),pivdose2#())
    IF p% > 0 THEN EXIT LOOP                'indicates all iv doses entered
    ivcount = ivcount + 1                   'increments dose counter
    ERASE pivdose2#                         'erases previous temp array 2
    DIM pivdose2#(1:ivcount,1:3)            'dimensions new temporary array 2
                              'entry routine for dose details
    CALL dsentry(2,vdurn,ivcount,"iv",pivdose2#(),pivdose1#())
    IF p% > 0 THEN EXIT LOOP                'indicates all iv doses entered
  WEND
  ivnum = ivcount - 1                       'total number of doses
  ivcount = 1                 'resets dose counter as position indicator
  IF ivnum > 0 THEN
    DIM pivdose#(1:ivnum,1:3)
    FOR x% = 1 TO ivnum
      SELECT CASE p%
      CASE 1
        pivdose#(x%,1) = pivdose2#(x%,1)    'enters dose details into
        pivdose#(x%,2) = pivdose2#(x%,2)    'array for use in
        pivdose#(x%,3) = pivdose2#(x%,3)    'program
      CASE 2
        pivdose#(x%,1) = pivdose1#(x%,1)
        pivdose#(x%,2) = pivdose1#(x%,2)
        pivdose#(x%,3) = pivdose1#(x%,3)
      END SELECT
    NEXT x%
  END IF
  ERASE pivdose1#                           'erases temporary arrays
  ERASE pivdose2#
  CLS

  CASE CHR$(13)
    IF imnum > 0 OR ivnum > 0 OR lungnum> 0 OR nosnum> 0 THEN
      EXIT LOOP
    ELSE
      CLS
      LOCATE 22,1
      PRINT "NO DOSES HAVE BEEN ENTERED YET";
      PRINT "ENTER DOSE(S) TO CONTINUE PROGRAM";
      PRINT ""
      EXIT SELECT
    END IF
  CASE ELSE
    EXIT SELECT
  END SELECT
WEND

  'allows certain basic variables to be changed at runtime:
  'phu, php, qdot, dt,  urout
  'details are stored in temporary arrays as they are entered.
  'two temporary arrays are used. acummulated  details are copied
  'from one to the other before each additional change is entered,  the
  'recipient array having being erased and redimensioned large enough to
  'also accomodate details of the new change to be entered. when all
  'changes have been entered for  given variable details are transfered
  'to a separate array for use later in the program
WHILE -1
```

```
KEY 1, "W"
KEY 2, "U"
KEY 3, "P"                          'Defines function keys and clears
KEY 4, "Q"                          'keyboard buffer
KEY 5, "T"
nul$ = INKEY$
e$ = ""
CLS
PRINT "You may change the following  variables."
PRINT "The current values are as shown:"
LOCATE 4,1
IF uroutnum > 0 THEN
  PRINT "* F1  Urine water output (approximates to urine volume): AS ENTERED  *"
ELSE
  PRINT USING_
  "F1  Urine water output (approximates to urine volume) = #### ml / hour";urout
END IF
IF unum > 0 THEN
  PRINT "* F2  Urine pH: AS ENTERED        *"
ELSE
  PRINT USING "F2  Urine pH = #.#";phu
END IF
IF pnum > 0 THEN                                        'screen display
  PRINT "* F3  Plasma pH: AS ENTERED       *"
ELSE
  PRINT USING "F3  Plasma pH = #.####";php
END IF
IF qnum > 0 THEN
  PRINT "* F4  Cardiac output: AS ENTERED  *"
ELSE
  PRINT USING "F4  Cardiac output = #.## l / min";qdot
END IF
IF tnum > 0 THEN
  PRINT "* F5  Cycle time: AS ENTERED      *"
ELSE
  PRINT USING "F5  Calculation cycle time = ## s";dt
END IF
LOCATE 11,1
PRINT "Press the corresponding function key to enter details of the"
PRINT "variable you want to change"
LOCATE 15,1
PRINT "NB You may come back to this screen as often as you wish."
PRINT "If you have already made changes to a particular variable, retyping"
PRINT "the appropriate function key will erase them and restore the default"
LOCATE 19,1
PRINT "Press RETURN when you have finished entering changes"
PRINT "and are ready to go with the program "
WHILE NOT INSTAT:WEND
e$ = INKEY$
SELECT CASE e$
 CASE "U"
  CALL vscr("urine pH",phu)          'sets entry display up on screen and
                                     'initialises variable change times
  IF unum > 0 THEN  ERASE pphu#      'erases any previous array before
                                     'entering loop
  ucount = 1                         'initialises variable change counter
  WHILE  -1
    ucount = ucount + 1              'increments counter
    IF ucount > 2 THEN ERASE pphu1#       'erases temporary array 1
    DIM pphu1#(1:ucount,1:2)              'dimensions temp array 1
                'dimensions temp array 2 if first pass through loop
```

```
    IF ucount = 2 THEN DIM pphu2#(1:ucount,1:2)
                  'variable-change entry routine
    CALL varentry(1,ucount,"urine pH","",phu,pphu1#(),pphu2#())
    IF p% = 1 THEN EXIT LOOP          'indicates all phu changes entered
    ucount = ucount + 1               'increments counter
    ERASE pphu2#                      'erases temp array 2
    DIM pphu2#(1:ucount,1:2)          'dimensions temp array 2
                   'variable-change entry routine
    CALL varentry(2,ucount,"urine pH","",phu,pphu2#(),pphu1#())
    IF p% = 2 THEN EXIT LOOP          'indicates all phu changes entered
  WEND
  unum = ucount -1                    'total number of changes
  ucount = 1                          'resets counter as position indicator
  IF unum > 0 THEN
    DIM pphu#(1:unum,1:2)
    FOR x% = 1 TO unum
      SELECT CASE p%
      CASE 1                                  'enters details into
        pphu#(x%,1) = pphu2#(x%,1)            'array for use in
        pphu#(x%,2) = pphu2#(x%,2)            'program
      CASE 2
        pphu#(x%,1) = pphu1#(x%,1)
        pphu#(x%,2) = pphu1#(x%,2)
      END SELECT
    NEXT x%
  END IF
  ERASE pphu2#                         'erases temporary arrays
  ERASE pphu1#
CASE "P"
  CALL vscr("plasma pH",php)          'sets entry display up on screen and
                                      'initialises variable change times
  IF pnum > 0 THEN  ERASE pphp#       'erases any previous array before
                                      'entering loop
  pcount = 1                          'initialises variable change counter
  WHILE  -1
    pcount = pcount + 1               'increments counter
    IF pcount > 2 THEN ERASE pphp1#       'erases temporary array 1
    DIM pphp1#(1:pcount,1:2)              'dimensions temp array 1
                  'dimensions temp array 2 if first pass through loop
    IF pcount = 2 THEN DIM pphp2#(1:pcount,1:2)
                  'variable-change entry routine
    CALL varentry(1,pcount,"plasma pH","",php,pphp1#(),pphp2#())
    IF p% = 1 THEN EXIT LOOP          'indicates all php changes entered
    pcount = pcount + 1               'increments counter
    ERASE pphp2#                      'erases temp array 2
    DIM pphp2#(1:pcount,1:2)          'dimensions temp array 2
                  'variable-change entry routine
    CALL varentry(2,pcount,"plasma pH","",php,pphp2#(),pphp1#())
    IF p% = 2 THEN EXIT LOOP          'indicates all php changes entered
  WEND
  pnum = pcount -1                    'total number of changes
  pcount = 1                          'resets counter as position indicator
  IF pnum > 0 THEN
    DIM pphp#(1:pnum,1:2)
    FOR x% = 1 TO pnum
      SELECT CASE p%
      CASE 1                                  'enters details into
        pphp#(x%,1) = pphp2#(x%,1)            'array for use in
        pphp#(x%,2) = pphp2#(x%,2)            'program
      CASE 2
        pphp#(x%,1) = pphp1#(x%,1)
```

```
        pphp#(x%,2) = pphp1#(x%,2)
      END SELECT
    NEXT x%
  END IF
  ERASE pphp2#                        'erases temporary arrays
  ERASE pphp1#

CASE "W"
  CALL vscr("urine water output",urout)   'sets entry display up on screen and
                                          'initialises variable change times
  IF uroutnum > 0 THEN  ERASE purout#     'erases any previous array before
                                          'entering loop
  uroutcount = 1                          'initialises variable change counter
  WHILE  -1
    uroutcount = uroutcount + 1           'increments counter
    IF uroutcount > 2 THEN ERASE purout1#       'erases temporary array 1
    DIM purout1#(1:uroutcount,1:2)              'dimensions temp array 1
                'dimensions temp array 2 if first pass through loop
    IF uroutcount = 2 THEN DIM purout2#(1:uroutcount,1:2)

                'variable-change entry routine
    CALL varentry(1,uroutcount,"urine water output",_
    " ML PER HOUR",urout,purout1#(),purout2#())
    IF p% = 1 THEN EXIT LOOP              'indicates all urout changes entered
    uroutcount = uroutcount + 1           'increments counter
    ERASE purout2#                        'erases temp array 2
    DIM purout2#(1:uroutcount,1:2)        'dimensions temp array 2
                'variable-change entry routine
     CALL varentry(2,uroutcount,"urine water output",_
     " ML PER HOUR",urout,purout2#(),purout1#())
    IF p% = 2 THEN EXIT LOOP              'indicates all urout changes entered
  WEND
  uroutnum = uroutcount -1                'total number of changes
  uroutcount = 1                          'resets counter as position indicator
  IF uroutnum > 0 THEN
    DIM purout#(1:uroutnum,1:2)
    FOR x% = 1 TO uroutnum
      SELECT CASE p%
       CASE 1                                     'enters details into
        purout#(x%,1) = purout2#(x%,1)            'array for use in
        purout#(x%,2) = purout2#(x%,2)            'program
       CASE 2
        purout#(x%,1) = purout1#(x%,1)
        purout#(x%,2) = purout1#(x%,2)
      END SELECT
    NEXT x%
  END IF
  ERASE purout2#                          'erases temporary arrays
  ERASE purout1#

CASE "T"
  CALL vscr("cycle time",dt)          'sets entry display up on screen and
                                      'initialises variable change times
  IF tnum > 0 THEN  ERASE pdt#        'erases any previous arrays before
                                      'entering loop
  tcount = 1                          'initialises variable change counter
  WHILE  -1
    tcount = tcount + 1               'increments counter
    IF tcount > 2 THEN ERASE pdt1#          'erases temporary array 1
    DIM pdt1#(1:tcount,1:2)                 'dimensions temp array 1
                'dimensions temp array 2 if first pass through loop
```

```
    IF tcount = 2 THEN DIM pdt2#(1:tcount,1:2)
                  'variable-change entry routine
    CALL varentry(1,tcount,"cycle time "," SECONDS",dt,pdt1#(),pdt2#())
    IF p% = 1 THEN EXIT LOOP          'indicates all dt changes entered
    tcount = tcount + 1               'increments counter
    ERASE pdt2#                       'erases temp array 2
    DIM pdt2#(1:tcount,1:2)           'dimensions temp array 2
                  'variable-change entry routine
     CALL varentry(2,tcount,"cycle time"," SECONDS",dt,pdt2#(),pdt1#())
    IF p% = 2 THEN EXIT LOOP          'indicates all dt changes entered
  WEND
  tnum = tcount -1                    'total number of changes
  tcount = 1                          'resets counter as position indicator
  IF tnum > 0 THEN
    DIM pdt#(1:tnum,1:2)

    FOR x% = 1 TO tnum
      SELECT CASE p%
      CASE 1                                  'enters details into
        pdt#(x%,1) = pdt2#(x%,1)              'array for use in
        pdt#(x%,2) = pdt2#(x%,2)              'program
      CASE 2
        pdt#(x%,1) = pdt1#(x%,1)
        pdt#(x%,2) = pdt1#(x%,2)
       END SELECT
    NEXT x%
  END IF
  ERASE pdt2#                         'erases temporary arrays
  ERASE pdt1#

CASE "Q"
  CALL vscr("cardiac output",qdot)    'sets entry display up on screen and
                                      'initialises variable change times
  IF qnum > 0 THEN  ERASE pqdot#      'erases any previous arrays before
                                      'entering loop
  qcount = 1                          'initialises variable change counter
  WHILE  -1
    qcount = qcount + 1               'increments counter
    IF qcount > 2 THEN ERASE pqdot1#        'erases temporary array 1
    DIM pqdot1#(1:qcount,1:2)               'dimensions temp array 1
                  'dimensions temp array 2 if first pass through loop
    IF qcount = 2 THEN DIM pqdot2#(1:qcount,1:2)
                  'variable-change entry routine
    CALL varentry(1,qcount,"cardiac output"," LITRES PER MINUTE",qdot,pqdot1#(),pqdot2#(
    IF p% = 1 THEN EXIT LOOP          'indicates all qdot changes entered
    qcount = qcount + 1               'increments counter
    ERASE pqdot2#                     'erases temp array 2
    DIM pqdot2#(1:qcount,1:2)         'dimensions temp array 2
                  'variable-change entry routine
    CALL varentry(2,qcount,"cardiac output"," LITRES PER MINUTE",qdot,pqdot2#(),pqdot1#(
    IF p% = 2 THEN EXIT LOOP          'indicates all qdot changes entered
  WEND
  qnum = qcount -1                    'total number of changes
  qcount = 1                          'resets counter as position indicator
  IF qnum > 0 THEN
    DIM pqdot#(1:qnum,1:2)
    FOR x% = 1 TO qnum
      SELECT CASE p%
      CASE 1                                  'enters details into
        pqdot#(x%,1) = pqdot2#(x%,1)          'array for use in
        pqdot#(x%,2) = pqdot2#(x%,2)          'program
```

```
        CASE 2
          pqdot#(x%,1) = pqdot1#(x%,1)
          pqdot#(x%,2) = pqdot1#(x%,2)
        END SELECT
      NEXT x%
    END IF
    ERASE pqdot2#                        'erases temporary arrays
    ERASE pqdot1#

  CASE CHR$(13)
    IF ucount = 1 OR tcount = 1 OR qcount = 1 OR pcount = 1 OR uroutcount = 1 THEN
      EXIT LOOP
    ELSE
      LOCATE 23,1
      PRINT "NO CHANGES MADE YET";
      e$ = ""
      nul$ = INKEY$
      DO UNTIL UCASE$(e$) = "Y" OR UCASE$(e$) = "N"
        LOCATE 24,1
        PRINT "Do you want to make changes Y or N?";
        e$ = INKEY$
      LOOP
      SELECT CASE UCASE$(e$)
      CASE "N"
        EXIT LOOP
      CASE "Y"
        EXIT SELECT
      END SELECT
    END IF
  CASE ELSE
    EXIT SELECT
   END SELECT
WEND

DIM results#(1:50,1:77)              'array to store main results
                                     'number of possible concentration maxima + 1
maxnum = imnum + ivnum + nosnum + lungnum + pnum + 1
DIM tissuemax(1:9,1:maxnum)
DIM tmax%(1:9)
DIM maxcount%(1:9)
CALL graphscale                      'sets up screen display for ploting results
                                     'calculates dependant variables from basic
IF pnum = 0 THEN CALL phcalc         'variables where default values still apply
IF qnum = 0 AND tnum = 0 AND wonum = 0 THEN CALL qdtcalc

                                     'main calculation cycle
WHILE rtime < (ttime + dt)
                                     'whilst unexecuted changes remain in arrays
                                     'calls pend to check whether change of variable
                                     'value due, make appropriate changes and
                                     'recalculate dependant variables if appropriate
  IF ucount < (unum + 1) AND unum > 0 THEN_
  CALL pend(pphu#(),phu,ucount,"urine pH","")
  IF pcount < (pnum + 1) AND pnum > 0 THEN_
  CALL pend(pphp#(),php,pcount,"plasma pH","")
  IF qcount < (qnum + 1) AND qnum > 0 THEN_
  CALL pend(pqdot#(),qdot,qcount,"cardiac output","l /s")
  IF tcount < (tnum + 1) AND tnum > 0 THEN_
  CALL pend(pdt#(),dt,tcount,"cycle time","s")
  IF uroutcount < (uroutnum + 1) AND uroutnum > 0 THEN_
  CALL pend(purout#(),urout,uroutcount,"urine water output","ml/hour")
```

```
  IF rtime = 0 THEN
    IF paper = 1 THEN
      LPRINT "initial conditions:"      'initial conditions to printer
      LPRINT ""
      LPRINT USING "Cardiac output = ##.## l/min";qdot
      LPRINT USING "Urine pH = #.#####";phu
      LPRINT USING "Urine water output #### ml / hour";urout
      LPRINT USING "Plasma pH = #.#####";php
      LPRINT USING "Cycle time = ###.# s ";dt
      LPRINT USING "Simulation duration = ######.# min";ttime / 60
      LPRINT ""
    END IF
    IF disk1 > 0 THEN
      OPEN file1$ FOR APPEND AS #1      'initial conditions to results file
      PRINT #1,""
      PRINT #1, "initial conditions:"
      PRINT #1, USING "Cardiac output = ##.## l/min";qdot
      PRINT #1, USING "Urine pH = #.#####";phu
      PRINT #1, USING "Urine water output #### ml / hour";urout
      PRINT #1, USING "Plasma pH = #.#####";php
      PRINT #1, USING "Cycle time = ###.## s";dt
      PRINT #1, USING "Simulation duration = ######.# min";ttime/60
      CLOSE #1
    END IF
  END IF
                          'calls dospend to check current drug dose status
                          'whilst unadministered doses remain in arrays
  IF ivcount < (ivnum + 1) AND ivnum > 0 THEN_
  CALL dospend(vdurn,ivdose,"intravenously",vdleft,ivcount,pivdose#())
  IF imcount < (imnum + 1) AND imnum > 0 THEN_
  CALL dospend(mdurn,imdose,"intramuscularly",mdleft,imcount,pimdose#())
  IF noscount < (nosnum + 1) AND nosnum > 0 THEN_
  CALL dospend(ndurn,nosdose,"intranasally",ndleft,noscount,pnosdose#())
  IF lungcount < (lungnum + 1) AND lungnum > 0 THEN_
  CALL dospend(ldurn,lungdose,"intrapulmonarily",ldleft,lungcount,plungdose#())
                          'calculates iv,ip and in dose increments
  IF vdleft > 0 THEN CALL dose(vdleft,ivdose,vdurn,vdinc)
  IF ldleft > 0 THEN CALL dose(ldleft,lungdose,ldurn,ldinc)
  IF ndleft > 0 THEN CALL dose(ndleft,nosdose,ndurn,ndinc)
  CALL model                'main calculation
  CALL results              'output routines
  ptime = ptime + dt        'increments times
  rtime = rtime + dt
  imdose = 0                'resets im dose for next cycle
  ndinc = 0                 'resets dose increments for next cycle
  ldinc = 0
  vdinc = 0
WEND
LOCATE 25,1
nul$ = INKEY$               'clear keyboard buffer
PRINT "*";
WHILE NOT INSTAT:WEND       'wait for keystroke
again = 1                   'indicates first simulation complete
ERASE results#              'erase results and maximum concentration
ERASE tissuemax#            'indicator arrays from completed simultion
ERASE tmax%
ERASE maxcount%
IF ivnum > 0 THEN ERASE pivdose#          'erase any arrays containing drug
IF imnum > 0 THEN ERASE pimdose#          'doses and variable changes
IF nosnum > 0 THEN ERASE pnosdose#        'from completed simulation
IF lungnum > 0 THEN ERASE plungdose#
```

```
IF tnum > 0 THEN ERASE pdt#
IF qnum > 0 THEN ERASE pqdot#
IF unum > 0 THEN ERASE pphu#
IF pnum > 0 THEN ERASE pphp#
IF uroutnum > 0 THEN ERASE purout#
GOTO begining                'restart program from label begining
finish:
END
$INCLUDE "fsubs1.bas"        'compiling instruction



        '*********************************************



        'Subroutines:  file fsubs1.bas

SUB model
        'main calculation cycle
  SHARED af#(),api,apv,apa,apf,app,apl,wpf,wpl,wpa,wpv,wpi,wpp,svw
  SHARED wf#(),wt#(),cs#(),csold#(),aat#(),aatold#(),aab#(),aabt#(),nc,eprat
  SHARED lamlw,acid,fr,fc#(),nks,nki,dstot,metf,totalamtexc,totalamtmet
  SHARED fwp,fwc#(),fwe#(),flc#(),fpc#(),fpe#(),fpp,xe,xp,x#(),xm,wu,pka,phu
  SHARED aabtm,wtmuc,fwm,fpm,aam,ndinc,imdose,ldinc,vdinc,fconc,b#(),c#(),e#()
  SHARED m,u
  LOCAL afh,af5old,afl,aff,afv,afp,p,q,r,s,t,amtexc,amtmet,i
          'amounts of drug in blood fractions leaving  upstream pool
  af#(1) = api * svw / wpi
  af#(2) = apa * wf#(2) / wpa
  af#(3) = apa * wf#(3) / wpa
  af#(4) = apa * wf#(4) / wpa
  afh = apa * wf#(5) / wpa          'fraction leaving art pool for liver
  af#(6) = apa * wf#(6) / wpa
  af#(7) = apa * wf#(7) / wpa
  af#(8) = apa * wf#(8) / wpa
  af#(9) = apa * wf#(9) / wpa
  af#(10) = apa * wf#(10) / wpa
  af#(11) = apa * wf#(11) / wpa
  afp = app * wf#(4) / wpp          'fraction leaving portal pool for liver
  afl = apl * wf#(7) / wpl
  aff = apf * wf#(8) / wpf
  afv = apv * svw / wpv
          'amounts of drug left behind in pools
  apa = apa - (af#(2) + af#(3) + af#(4) + afh + af#(6) + af#(7) + af#(8)_
  + af#(9) + af#(10) + af#(11))
  apv = apv - afv
  api = api - af#(1)
  apl = apl - afl
  apf = apf - aff
  app = app - afp
  af5old = afh + afp                'adds amounts in hepatic and portal fractions
  wf#(5) = wf#(5) + wf#(4)          'add  hepatic and portal blood
  amtmet = metf * af5old            'calculates amount metabolised
  af#(5) = af5old - amtmet          'subtracts from blood entering liver
  FOR i = 1 to nc
    csold#(i) = cs#(i)              'records cs and aat before equilibration
    aatold#(i) = aat#(i)            'for results calculations
  NEXT i
```

```
          'tissue equilibration
  FOR i = 1 TO nc
    SELECT CASE i
    CASE 1,4 TO 10
      aabt#(i) = af(i) + aat#(i)     'total amount of drug in blood and tissue
      cs#(i) = aabt#(i) / (wf#(i)*b#(i) + wt#(i)*(c#(i) + e#(i)))
                                     'amount in blood at concentration equilibrium
      aab#(i) = cs#(i) * wf#(i)*b#(i)
                                     'amount in tissue at concentration equilibrium
      aat#(i) = cs#(i) * wt#(i)*(c#(i) + e#(i))
    CASE 2
      aab#(i) = af#(i)               'peripheral shunt
      aat#(i) = 0
      aabt#(i) = af#(i)
      cs(i) = af#(i) / (wf#(i)*b#(i))
    CASE 11
      aabtm = af#(i) + aat#(i) + aam
      cs(i) = aabtm / (wf#(i)*b#(i) + wt#(i)*(c#(i) + e#(i)) + m)
      aab#(i) = cs#(i) * wf#(i) * b#(i)       'nasal absorbtion compartment
      aat#(i) = cs#(i) * wt#(i) * (e#(i) + c#(i))
      aam = cs#(i) * m
      aabt#(i) = aab#(i) + aat#(i)
    CASE 3
      aabt#(i) = af#(i) + aat#(i)
      b#(i) = b#(i)/fconc : c#(i) = c#(i)/fconc : e#(i) = e#(i)/fconc
      cs(i) = aabt#(i) / (wf#(i)*b#(i#) + wt#(i)*(c#(i) + e#(i)) + u)
      aab#(i) = cs#(i) * wf#(i)*b#(i)
      aat#(i) = cs#(i) * wt#(i)*(c#(i) + e#(i))
      amtexc = cs#(i) * u            'renal compartment
      cs#(i) = cs#(i)/fconc
      b#(i) = b#(i) * fconc : c#(i) = c#(i) * fconc : e#(i) = e#(i) * fconc
    END SELECT
  NEXT i
  wf#(5) = wf#(5) - wf#(4)           'reset mass hepatic fraction for next cycle
          'amounts of drug in downstream pools after admixture
  api = api + afv
  apv  = apv + aab#(2) + aab#(3) + aab#(5) + aab#(6) + aab#(9)_
         + aab#(10) + aab#(11) + afl + aff
  apa = apa + aab#(1)
  apl = apl + aab#(7)
  apf = apf + aab#(8)
  app = app + aab#(4)
          'calculate cumulative amount excreted metabolised and remaining in body:
  totalamtexc = totalamtexc + amtexc
  totalamtmet = totalamtmet + amtmet
  dstot = dstot + ldinc + vdinc + imdose + ndinc
  api = api + vdinc                          'add iv dose
  aat#(10) = aat#(10) + imdose               'add im dose
  aat#(1) = aat#(1) + ldinc                  'add intrapulmonary dose
  aam = aam + ndinc                          'add intranasal dose
END SUB


SUB phcalc
          'calculates variables dependant on plasma or mucus pH
  SHARED phe,php,phc#(),phm,aph#(),bph,xp,xe,x#(),xm,acid,pka,nc,b#(),c#(),e#()
  SHARED eprat,fr,fpp,nks,nki,fwp,fc#(),flc#(),lamlw,fpc#(),fwc#()
  SHARED fpe#(),fwe#(),wtmuc,fwm,fpm,m
  LOCAL i
  phe = php                                  'pH of ecf
  FOR i = 1 TO nc
```

```
   phc#(i) = phe + aph#(i) + bph * (phe - 7.4) 'pH of tissue cells
  NEXT i
  xp = EXP10(acid * (pka - php))                'conveniance variables
  xe = EXP10(acid * (pka - phe))                'for tissue distribution
  xm = EXP10(acid * (pka - phm))                'calculation
  m = wtmuc * (fwm * (1 + xm) + fpm * (nks + xm * nki))
  FOR i = 1 TO nc
    x#(i) = EXP10(acid * (pka - phc(i)))
    b#(i) = (eprat * fr + 1 - fr) * (fpp * (nks +  nki * xp) + fwp * (1 + xp))
    c#(i) = fc#(i) * (flc#(i) * lamlw + fpc#(i) * (nks + nki * x#(i))_
    + fwc#(i) * (x#(i) + 1))
    e#(i) = (1 - fc#(i)) * (fpe#(i) * (nks + nki * xe) + fwe#(i) * (1 + xe))
  NEXT i
END SUB


SUB qdtcalc
   'calculates urine water output Kg / cycle
   'calculates (fractional) stroke volume(s) and checks them against
   'pool sizes making adjustment if necessary
  SHARED wqdot,qdot,bden,svw,f#(),wpa,wpi,wpl,wpf,wpp,wpv,dt,wf#(),nc
  SHARED wu,urout,paper,disk1,file1$,rtime
  STATIC dtchange%
  LOCAL test,i
  wu = (dt * urout) / (1000.0 * 60.0 * 60.0)   'urine water output Kg / cycle
  wqdot = (qdot * bden) / 60.0     'cardiac output Kg / s
  svw = wqdot * dt                 'mass of stroke volume Kg
  FOR i = 1 TO nc
    wf#(i) = svw * f#(i)           'mass of fractional stroke volumes Kg
  NEXT i
  IF wpa > wpi THEN                'smallest pool chosen from the main pools
    test = wpi
  ELSE
    test = wpa
  END IF
  IF wpv < test THEN test = wpv
           'if any pool smaller than relevant (fractional) stroke volume
           'then decrease cycle time
  IF svw > test OR wf#(4) > wpp OR wf#(7) > wpl OR wf#(8) > wpf THEN
    dt = dt - 1.0
    dtchange% = 1                  'indicator that dt has been changed
    CALL qdtcalc                   'recall sub to calculate stroke volumes
  END IF                           'and recheck against pool volumes
  IF dtchange% = 1 THEN            'until satisfactory
    IF paper = 1 THEN
      LPRINT ""                    'notifies printer
      LPRINT USING "time ####.# min: stroke volume size error:";rtime/60
      LPRINT USING "Cycle time automaticaly reset to ##.## s";dt
    END IF
    IF disk1 > 0 THEN              'notifies results file
       OPEN file1$ FOR APPEND AS #1
       PRINT #1, ""
       PRINT #1, USING "time ####.# min: stroke volume size error:";rtime/60
       PRINT #1, USING "Cycle time automaticaly reset to ##.## s";dt
       CLOSE #1
     END IF
     dtchange% = 0                 'reset indicator
  END IF
  CALL ucalc                       'calculates wu dependant variable
END SUB
```

```
SUB ucalc
   'calculates urine pH dependant conveniance variable for tissue
   'distribution calculation
  SHARED u,wu,acid,pka,phu
  u = wu * (1 + EXP10(acid * (pka - phu)))
END SUB


SUB blconc(cs,ab,wb,bab,bap)
   'sub calculates total drug concentrations in blood plasma and
   'erythrocytes (umol/l or nmol/ml) and aqueous concentration
   'of unionised drug (umol/Kg water) from the total mass of drug
   'and blood present
  SHARED eprat,fr,fwp,fpp,nks,nki,xp,bden,pden
  bab = ab / wb          'total conc in blood umol / Kg
                         'aqueous conc unionised drug umol / Kg water
  cs = bab / ((eprat * fr + 1 - fr) * (fpp * (nks +  nki * xp) + fwp * (1 + xp)))
  bap = bab / (eprat * fr + 1 - fr)
  bab = bab * bden       'umol/l (nmol/ml) blood
  bap = bap * pden       'umol/l (nmol/ml) plasma
END SUB


$INCLUDE "fsubs2.bas"  'compilinng instruction

                       'file f2subs.bas
SUB results
        'sub calculates and organises results
  SHARED rtime,ptime,ttime,nc,dfac,dt,eprat,fr,bden,rden,pden
  SHARED aam,dstot,totalamtmet,totalamtexc,maxnum,file1$,disk1,paper
  SHARED csold#(),wf#(),aat#(),aab#(),cs#(),wt#(),tmax%(),maxcount%(),tissuemax()
  SHARED wpv,apv,apa,api,app,apl,apf,babs,babcv,baps,bapcv,css,cscv
  SHARED graph1,graph2,graph3,graph4,graph5,graph6
  SHARED legart,legp,legfat,leglean,legbrain,legcv,ysize,xsize
  SHARED x1%,x2%,x3%,x4%,x5%,x6%,y1%,y2%,y3%,y4%,y5%,y6%,g$
  LOCAL aat,aab,concblood,conctissue,concplasma,i,n,y%
        'call blconc to calculate drug concentrations: blood, plasma,
        'erythrocyte (umol/l), aqueous unionised (umol / Kg water)
        'in peripheral shunt (arterial),lean, fat, and central venous pool,
        'and notional peripheral venous blood
  CALL blconc(css,aab#(2),wf#(2),babs,baps)    'arterial and central
  CALL blconc(cscv,apv,wpv,babcv,bapcv)        'venous plasma concenrations
  IF disk1 > 0 THEN
    FOR i = 1 TO 9                        'detect time of maximum concentration
      IF csold#(i) > cs#(i) THEN          '(ie start of falling concentration)
        IF tmax%(i) = 0 THEN              'for each tissue and read into array
          maxcount%(i) = maxcount%(i) + 1
          tissuemax(i,maxcount%(i)) = (rtime - dt) / 60
          IF maxcount%(i) = maxnum THEN
            OPEN file1$ FOR APPEND AS #1
            PRINT #1,""
            PRINT #1,"tissue      concentration maxima(simulation time min)"
            PRINT #1,""
            FOR i = 1 TO 9
              FOR n = 1 TO maxcount%(i)
                PRINT #1,"    #                          ####.##                  ";_
                i;tissuemax(i,n)
              NEXT n
            NEXT i
            FOR i = 1 TO 9
              maxcount%(i) = 0
```

```
            FOR n = 1 TO maxnum
              tissuemax(i,n) = 0
            NEXT n
          NEXT i
        END IF
        tmax%(i) = 1
      END IF
    END IF
    IF cs#(i) > csold#(i) THEN tmax%(i) = 0   'reset max concentartion indicator
  NEXT i
END IF
                    'plot selected results graphs on screen
IF graph1 = 1 THEN
  IF rtime > = 1 AND baps > 0 THEN
    IF g$ = "1" THEN PSET (LOG10(rtime),LOG10(baps/dfac))  'time logarithmic
    IF g$ = "2" THEN PSET (rtime,LOG10(baps/dfac))         'time linear
    IF legart = 0 THEN                          'calculate coordinates
                                                'for graph label
      y% = CINT(PMAP(LOG10(baps/dfac),1))
      IF y% MOD ysize > ysize*2/3 OR y% MOD ysize < ysize/3 THEN
        y1% = 0
      ELSE
        y1% = (y% \ ysize) + 1
      END IF
      IF g$ = "1" THEN x1% = (PMAP(LOG10(rtime),0) \ xsize) + 1
      IF g$ = "2" THEN x1% = (PMAP(rtime,0) \ xsize) + 1
      IF x1% > 10 THEN CALL legend(x1%,y1%,legart,"a")  'legend prints label
    END IF                                              'if coordinates suitable
  END IF
END IF
IF graph2 = 1 THEN
  IF rtime > = 1 AND aat#(1) > 0 THEN
    IF g$ = "1" THEN PSET (LOG10(rtime),LOG10((aat#(1)/wt#(1))/dfac))
    IF g$ = "2" THEN PSET (rtime,LOG10((aat#(1)/wt#(1))/dfac))
    IF  legp = 0  THEN
      y% = CINT(PMAP(LOG10((aat#(1)/wt#(1))/dfac),1))
      IF y% MOD ysize > ysize*2/3 OR y% MOD ysize < ysize/3 THEN
        y2% = 0
      ELSE
        y2% = (y% \ ysize) + 1
      END IF
      IF g$ = "1" THEN x2% = (PMAP(LOG10(rtime),0) \  xsize) + 1
      IF g$ = "2" THEN x2% = (PMAP(rtime,0) \  xsize) + 1
      IF x2% > 10 AND y2% > 0 THEN CALL legend(x2%,y2%,legp,"p")
    END IF
  END IF
END IF
IF graph3 = 1 THEN
  IF rtime >= 1 AND aat#(8) > 0 THEN
    IF g$ = "1" THEN PSET (LOG10(rtime),LOG10((aat#(8)/wt#(8))/dfac))
    IF g$ = "2" THEN PSET (rtime,LOG10((aat#(8)/wt#(8))/dfac))
    IF legfat = 0 THEN
      y% = CINT(PMAP(LOG10((aat#(8)/wt#(8))/dfac),1))
      IF y% MOD ysize > ysize*2/3 OR y% MOD ysize < ysize/3 THEN
        y3% = 0
      ELSE
        y3% = (y% \ ysize) + 1
      END IF
      IF g$ = "1" THEN x3% = (PMAP(LOG10(rtime),0) \ xsize) + 1
      IF g$ = "2" THEN x3% = (PMAP(rtime,0) \ xsize) + 1
      IF x3% > 10 THEN CALL legend(x3%,y3%,legfat,"f")
```

```
    END IF
  END IF
END IF
IF graph4 = 1 THEN
  IF rtime >= 1 AND aat#(7) > 0 THEN
    IF g$ = "1" THEN PSET (LOG10(rtime),LOG10((aat#(7)/wt#(7))/dfac))
    IF g$ = "2" THEN PSET (rtime,LOG10((aat#(7)/wt#(7))/dfac))
    IF leglean = 0 THEN
      y% = CINT(PMAP(LOG10((aat#(7)/wt#(7))/dfac),1))
      IF y% MOD ysize > ysize*2/3 OR y% MOD ysize < ysize/3 THEN
        y4% = 0
      ELSE
        y4% = (y% \ ysize) + 1
      END IF
      IF g$ = "1" THEN x4% = (PMAP(LOG10(rtime),0) \  xsize) + 1
      IF g$ = "2" THEN x4% = (PMAP(rtime,0) \  xsize) + 1
      IF x4% > 10 THEN CALL legend(x4%,y4%,leglean,"1")
    END IF
  END IF
END IF
IF graph5 = 1 THEN
  IF rtime >= 1 AND bapcv > 0 THEN
    IF g$ = "1" THEN PSET (LOG10(rtime),LOG10(bapcv/dfac))
    IF g$ = "2" THEN PSET (rtime,LOG10(bapcv/dfac))
    IF legcv = 0 THEN
      y% = CINT(PMAP(LOG10(bapcv/dfac),1))
      IF y% MOD ysize > ysize*2/3 OR y% MOD ysize < ysize/3 THEN
        y5% = 0
      ELSE
        y5% = (y% \ ysize) + 1
      END IF
      IF g$ = "1" THEN x5% = (PMAP(LOG10(rtime),0) \ xsize) + 1
      IF g$ = "2" THEN x5% = (PMAP(rtime,0) \ xsize) + 1
      IF x5% > 10 THEN CALL legend(x5%,y5%,legcv,"c")
    END IF
  END IF
END IF
IF graph6 = 1 THEN
  IF rtime >= 1 AND aat#(9) > 0 THEN
    IF g$ = "1" THEN PSET (LOG10(rtime),LOG10((aat#(9)/wt#(9))/dfac))
    IF g$ = "2" THEN PSET (rtime,LOG10((aat#(9)/wt#(9))/dfac))
    IF legbrain = 0 THEN
      y% = CINT(PMAP(LOG10((aat#(9)/wt#(9))/dfac),1))
      IF y% MOD ysize > ysize*2/3 OR y% MOD ysize < ysize/3 THEN
        y6% = 0
      ELSE
        y6% = (y% \ ysize) + 1
      END IF
      IF g$ = "1" THEN x6% = (PMAP(LOG10(rtime),0) \ xsize) + 1
      IF g$ = "2" THEN x6% = (PMAP(rtime,0) \ xsize) + 1
     IF x6% > 10 THEN CALL legend(x6%,y6%,legbrain,"b")
    END IF
  END IF
END IF
IF disk1 = 1  AND rtime < ttime THEN              'store reads results into
  IF ABS(60 - ptime) <= (dt/2) THEN CALL store    'array for storage
  SELECT CASE ptime
  CASE <= 3600                                    'standard sampling scheme
    IF ABS((ptime/300 - CINT(ptime/300))*300) <= dt/2 THEN CALL store
  CASE > 3600
    IF ABS((ptime/1800 - CINT(ptime/1800))*1800) <= dt/2 THEN CALL store
```

```
  END SELECT
END IF
IF disk1 = 2 and rtime < ttime THEN
  IF ptime <= 120 THEN
    IF dt > 2.0 THEN
      CALL store                                    'frequent sampling scheme
    ELSEIF ABS((ptime/2 - CINT(ptime/2))*2) <= dt/2 THEN
      CALL store
    END IF
  END IF
  IF ptime > 120 AND ptime <= 1200 THEN
    IF ABS((ptime/30 - CINT(ptime/30))*30) <= dt/2 THEN CALL store
  END IF
  IF ptime > 1200 AND ptime <= 3600 THEN
    IF ABS((ptime/120 - CINT(ptime/120))*120) <= dt/2 THEN CALL store
  END IF
  IF ptime > 3600 AND ptime <= 21600 THEN
    IF ABS((ptime/600 - CINT(ptime/600))*600) <= dt/2 THEN CALL store
  END IF
  IF ptime > 21600 THEN
    IF ABS((ptime/1800 - CINT(ptime/1800))*1800) <= dt/2 THEN CALL store
  END IF
END IF
IF rtime > = ttime  THEN
  aat = 0                                   'calculate totals
  FOR i = 1 TO nc
    aat = aat#(i) + aat
  NEXT i
  aab = apa + apv + app + apf + apl + api 'total amount drug blood
  IF paper = 1 THEN                         'final summary to printer
    LPRINT ""
    LPRINT USING "ug drug remaining in tissues ######.#";aat/dfac
    LPRINT USING "ug drug remaining in blood ######.#";aab/dfac
    LPRINT USING "ug drug excreted ######.#";totalamtexc/dfac
    LPRINT USING "ug drug metabolised ######.#";totalamtmet/dfac
    LPRINT "
    LPRINT "error check: (ug in body + ug eliminated - ug administered)  ";_
    (aam + aab + aat + totalamtexc + totalamtmet - dstot)/dfac
    LPRINT ""
    LPRINT ""
    LPRINT "END"
  END IF
  IF disk1 > 0 THEN                         'final results and
    CALL store                              'concentration maxima
    CALL disk                               'to results file
    OPEN file1$ FOR APPEND AS #1
    PRINT #1,""
    PRINT #1,"tissue      concentration maxima(simulation time min)"
    PRINT #1,""
    FOR i = 1 TO 9
       FOR n = 1 TO maxcount%(i)
         PRINT #1, USING"   #                        ####.##                ";_
         i;tissuemax(i,n)
       NEXT n
    NEXT i                                  'equilibrium tissue/blood
    PRINT #1,""                             'conc. ratios to results file
    PRINT #1,"Ratios at concentration equilibrium (ug/Kg):"
    PRINT #1,"tissue     tissue/blood     tissue/plasma"
    FOR i = 1 TO nc
      SELECT CASE i
      CASE 2
```

```
        EXIT SELECT
      CASE 1,3 TO nc
        IF i = 5 THEN
          concblood = aab#(5) / (wf#(5)+wf#(4))
        ELSE
          concblood = aab#(i) / wf#(i)
        END IF
        conctissue = aat#(i) / wt#(i)
        concplasma = concblood / (eprat * fr + 1 - fr)
        PRINT #1, USING "  ##          ##.##            ##.##";_
        i;conctissue/concblood;conctissue/concplasma
      END SELECT
    NEXT i
    PRINT #1,""
    PRINT #1, USING "ug drug/l erythrocytes : ug drug/l plasma = #.###";_
    eprat*rden/pden
    PRINT #1, USING "ug drug/l blood : ug drug/l plasma = #.###";babs/baps
    PRINT #1,""
    PRINT #1,""
    PRINT #1, "ERROR CHECK (ug in body+ug eliminated)-ug administered ";_
    (aam + aab + aat + totalamtexc + totalamtmet - dstot)/dfac
    PRINT #1,"END"
    CLOSE #1
  END IF
 END IF
END SUB


SUB store
  'sub calculates results not available from sub results
  'reads results into storage array
 SHARED rcount,results#(),rtime,totalamtmet,totalamtexc,dstot
 SHARED dfac,cs#(),aat#(),aab#(),aatold#(),wt#(),wf#(),nc,eprat,fr
 SHARED csi,api,wpi,babi,bapi,csp,app,wpp,babp,bapp
 SHARED csa,apa,wpa,baba,bapa,cscv,apv,wpv,babcv,bapcv
 SHARED css,babs,baps,csf,wpf,apf,babf,bapf,csl,apl,wpl,babl
 SHARED bapl,cspv,amtpv,wtpv,babpv,bappv,bden,aam
 LOCAL i%,x%,aab,aat
 CALL blconc(csi,api,wpi,babi,bapi)        'calculate drug concentrations
 CALL blconc(csp,app,wpp,babp,bapp)        'in those pool bloods not
 CALL blconc(csa,apa,wpa,baba,bapa)        'available from SUB results
 CALL blconc(csf,apf,wpf,babf,bapf)
 CALL blconc(csl,apl,wpl,babl,bapl)
 amtpv = (babs + babf + babl) / (3 * bden)
 wtpv = 1
 CALL blconc(cspv,amtpv,wtpv,babpv,bappv)
 aat = 0                                   'calculate total drug
 FOR i% = 1 TO nc                          'in all blood and tissue
   aat = aat#(i%) + aat
 NEXT i%
 aab = apa + apv + app + apf + apl + api   'total amount drug blood
       'read results into results storage array
       'concentrations as ng/ml
 rcount = rcount + 1
 results#(rcount,1) = rtime/60
 results#(rcount,2) = dstot/dfac
 results#(rcount,3) = aab/dfac
 results#(rcount,4) = aat/dfac
 results#(rcount,5) = totalamtmet/dfac
 results#(rcount,6) = totalamtexc/dfac
 results#(rcount,7) = aat#(1)/dfac
```

```
  results#(rcount,8) = aat#(1)/(dfac*wt#(1))
  results#(rcount,9) = cs#(1)/dfac
  i% = 3
  FOR x% = 10 TO  34 STEP 3                    'concentrations and
    results#(rcount,x%) = aat#(i%)/dfac        'amounts in tissues
    results#(rcount,x%+1) = aat#(i%)/(dfac*wt#(i%))
    results#(rcount,x%+2) = cs#(i%)/dfac
    i% = i% + 1
  NEXT x%                        'tissue/blood and tissue plasma concentration ratios
  IF aab#(2) > 0 THEN results#(rcount,37) = (aatold#(1)/(wt#(1)))/(aab#(2)/(wf#(2)))
  IF aab#(2) > 0 THEN results#(rcount,38) = (aatold#(1)/(wt#(1)))/(aab#(2)/(wf#(2)_
  *(eprat * fr + 1 - fr)))
  i% = 3
  FOR x% = 39 TO 51 STEP 2
    IF aab#(2) > 0 THEN results#(rcount,x%) = (aatold#(i%)/(wt#(i%)))/_
    (aab#(2)/(wf#(2)))
    IF aab#(2) > 0 THEN results#(rcount,x%+1) = (aatold#(i%)/(wt#(i%)))/_
    (aab#(2)/(wf#(2)*(eprat * fr + 1 - fr)))
    i% = i% + 1
  NEXT x%                                  'concentrations in blood and plasma
  results#(rcount,53) = baba/dfac
  results#(rcount,54) = bapa/dfac
  results#(rcount,55) = csa/dfac
  results#(rcount,56) = babs/dfac
  results#(rcount,57) = baps/dfac
  results#(rcount,58) = css/dfac
  results#(rcount,59) = babcv/dfac
  results#(rcount,60) = bapcv/dfac
  results#(rcount,61) = cscv/dfac
  results#(rcount,62) = babpv/dfac
  results#(rcount,63) = bappv/dfac
  results#(rcount,64) = cspv/dfac
  results#(rcount,65) = babp/dfac
  results#(rcount,66) = bapp/dfac
  results#(rcount,67) = csp/dfac
  results#(rcount,68) = babl/dfac
  results#(rcount,69) = bapl/dfac
  results#(rcount,70) = csl/dfac
  results#(rcount,71) = babf/dfac
  results#(rcount,72) = bapf/dfac
  results#(rcount,73) = csf/dfac
  results#(rcount,74) = babi/dfac
  results#(rcount,75) = bapi/dfac
  results#(rcount,76) = csi/dfac
  results#(rcount,77) = aam/dfac
  IF rcount = 50 THEN CALL disk            'results to file when array full
END SUB


SUB disk
     'sub reads results storage array to results file
  SHARED results#(),rcount,file1$
  LOCAL x%,y%,i%
  OPEN file1$ FOR APPEND AS #1
  FOR x% = 1 TO rcount
    PRINT #1,""
    PRINT #1, USING "time ####.## min          ug administered#####.#";_
    results#(x%,1);results#(x%,2)
    PRINT #1, USING "ug in blood#####.#        ug in tissue#####.#";_
    results#(x%,3);results#(x%,4)
    PRINT #1, USING "ug metabolised#####.#     ug excreted#####.# ";_
```

```
    results#(x%,5);results#(x%,6)
    PRINT #1,"tissue   total drug    ug drug/Kg tissue   unionised aq"
    i% = 1
    PRINT #1,USING "  ##      #####.##       ######.####      ####.####";_
    i%;results#(x%,7);results#(x%,8);results#(x%,9)
    i% = 3
    FOR y% = 10 TO 34 STEP 3
       PRINT #1,USING "  ##      #####.##       ######.####      ####.####";_
       i%;results#(x%,y%);results#(x%,y%+1);results#(x%,y%+2)
       i% = i% + 1
    NEXT y%
    PRINT #1, USING "mucous     ####.##";results#(x%,77)
    PRINT #1,"tissue     ratios   tis:art blood    tis:art plasma"
    i% = 1
    PRINT #1, USING "  ##                    ##.####          ##.####";_
    i%;results#(x%,37);results#(x%,38)
    i% = 3
    FOR  y% = 39 TO 51 STEP 2
      PRINT #1, USING "  ##                     ##.####          ##.####";_
      i%;results#(x%,y%);results#(x%,y%+1)
      i% = i% + 1
    NEXT y%
    PRINT #1,"          ug/l blood         ug/l plasma         unionised aq"
    PRINT #1, USING "ca        ####.####           ####.####           ####.####";_
    results#(x%,53);results#(x%,54);results#(x%,55)
    PRINT #1, USING "pa        ####.####           ####.####           ####.####";_
    results#(x%,56);results#(x%,57);results#(x%,58)
    PRINT #1, USING "cv        ####.####           ####.####           ####.####";_
    results#(x%,59);results#(x%,60);results#(x%,61)
    PRINT #1, USING "pv        ####.####           ####.####           ####.####";_
    results#(x%,62);results#(x%,63);results#(x%,64)
    PRINT #1, USING "por       ####.####           ####.####           ####.####";_
    results#(x%,65);results#(x%,66);results#(x%,67)
    PRINT #1, USING "lean      ####.####           ####.####           ####.####";_
    results#(x%,68);results#(x%,69);results#(x%,70)
    PRINT #1, USING "fat       ####.####           ####.####           ####.####";_
    results#(x%,71);results#(x%,72);results#(x%,73)
    PRINT #1, USING "inj       ####.####           ####.####           ####.####";_
    results#(x%,74);results#(x%,75);results#(x%,76)
  NEXT x%
  rcount = 0                          'reset results array counter
  CLOSE #1
END SUB


SUB graphscale
    'sub sets up screen for graphical display of results
  SHARED graph1,graph2,graph3,graph4,graph5,graph6,leglean,legfat
  SHARED legcv,legp,legart,legbrain,xsize,ysize,ttime,g$
  LOCAL y,y1,y2,x,x1,x2,e$,p,nul$
  CLS
  PRINT "Screen output:"              'input routine for graph selection
  PRINT ""
  PRINT "the program will plot drug concentration in any combination of"
  PRINT "the following to a suitable screen during the simulation:"
  PRINT ""
  PRINT "F1  arterial plasma "
  PRINT "F2  central venous plasma"
  PRINT "F3  fat tissue"
  PRINT "F4  lean tissue (muscle)"
  PRINT "F5  lung tissue"
```

```
PRINT "F6  brain tissue"
PRINT ""
KEY 1, "1"                          'labels function keys
KEY 2, "2"
KEY 3, "3"
KEY 4, "4"
KEY 5, "5"
KEY 6, "6"
KEY 9, "9"
nul$ = INKEY$                       'clears keyboard buffer
e$ = ""
PRINT "Press the function key(s) corresponding to the graph(s) you want to plot"
PRINT "Press F9 to erase and redo"
PRINT "Press RETURN when you have finished"
WHILE -1
  e$ = INKEY$                       'reads input
  SELECT CASE e$
    CASE "1"
    graph1 = 1                      'sets indicator for appropriate graph
    legart = 0                      'initialises indicator for graph legend
    LOCATE 6,32
    PRINT "*"                       'indicates selection on screen
  CASE "5"
    graph2 = 1
    legp = 0
    LOCATE 10,32
    PRINT "*"
  CASE "3"
    graph3 = 1
    legfat = 0
    LOCATE 8,32
    PRINT "*"
  CASE "4"
    graph4 = 1
    leglean = 0
    LOCATE 9,32
    PRINT "*"
  CASE "2"
    graph5 = 1
    legcv = 0
    LOCATE 7,32
    PRINT "*"
  CASE "6"
    graph6 = 1
    legbrain = 0
    LOCATE 11,32
    PRINT "*"
  CASE "9"
    graph1 = 0                      'resets graph indicators
    graph2 = 0
    graph3 = 0
    graph4 = 0
    graph5 = 0
    graph6 = 0
    LOCATE 11,32
    PRINT " "
    LOCATE 10,32                    'removes * from screen display
    PRINT " "
    LOCATE 9,32
    PRINT " "
    LOCATE 8,32
```

```
    PRINT " "
    LOCATE 7,32
    PRINT " "
    LOCATE 6,32
    PRINT " "
  CASE CHR$(13)
    IF graph1 = 0 AND graph2 = 0 AND graph3 = 0 AND graph4 = 0 AND graph5 = 0_
    AND graph6 = 0 THEN
      nul$ = INKEY$
      e$ = ""
      DO UNTIL UCASE$(e$) = "Y" OR UCASE$(e$) = "N"
        LOCATE 20,1
        PRINT "No graphs have been chosen"
        PRINT "Do you want graphs Y or N?"
        e$ = INKEY$
      LOOP
      IF UCASE$(e$) = "N" THEN
         CLS
         LOCATE 12,30
         PRINT "Simulation underway"
         EXIT LOOP
      ELSE
        LOCATE 20,1
        PRINT "                                        "
        PRINT "                                        "
        e$ = ""
      END IF
    ELSE
      EXIT LOOP
    END IF
  END SELECT
WEND
IF graph1 = 1 OR graph2 = 1 OR graph3 = 1 OR graph4 = 1 OR graph5 = 1_
OR graph6 = 1 THEN
  CLS
  g$ = ""
  nul$ = INKEY$
  DO UNTIL g$ = "1" OR g$ = "2"
    LOCATE 1,1
    PRINT "Drug concentration is plotted on a logarithmic scale"
    PRINT ""
    PRINT "Time may be logarithmic or linear"
    PRINT ""
    PRINT "Press  F1 for logarithmic time scale"
    PRINT "OR     F2 for linear time scale"
    g$ = INKEY$
  LOOP
  CLS
  e$ = ""
  nul$ = INKEY$
  DO UNTIL e$ = "1" OR e$ = "2" OR e$ = "3" OR e$ = "4" OR e$ = "5"
    LOCATE 1,1
    PRINT ""
    PRINT "Monitor type:"
    PRINT ""
    PRINT "F1  Monochrome or Colour                  640 * 200 pixels"
    PRINT "F2  Colour EGA high resolution            640 * 200 pixels"
    PRINT "F3  Monochrome high resolution            720 * 348 pixels"
    PRINT "F4  Colour EGA enhanced high resolution   640 * 350 pixels"
    PRINT ""
    PRINT "Press the function button corresponding to your monitor"
```

```
  PRINT "If you inadvertantly make an incompatible choice the program"
  PRINT "may crash and you will have to start again"
  PRINT ""
  PRINT "F5  disables the screen display and enables the program"
  PRINT "    to proceed without screen output"
  e$ = INKEY$
LOOP
SELECT CASE e$
CASE "5"
  graph1 = 0
  graph2 = 0
  graph3 = 0
  graph4 = 0
  graph5 = 0
  graph6 = 0
  CLS
  LOCATE 12,30
  PRINT "Simulation underway"
  EXIT IF
CASE "1"
  SCREEN 2
  KEY OFF                          'define area of screen to be used for
  VIEW (48,12) - (639,179)         'graph: assumes 640 * 200 pixels
  ysize = 8                        'scaling factors to convert pixel to
  xsize = 8                        'character coordinates for graph labels
CASE "2"
  SCREEN 8
  KEY OFF
  VIEW (48,12) - (639,179)         'assumes 640 * 200 pixels
  ysize = 8
  xsize = 8
CASE "3"
   SCREEN 2
   KEY OFF
   VIEW (54,21) - (719,314)        'assumes 720 * 348 pixels
   ysize = 14
   xsize = 9
CASE "4"
   SCREEN 9
   KEY OFF
   VIEW (48,21) - (639,314)        'assumes 640 * 350 pixels
   ysize = 14
   xsize = 8
END SELECT
y1 = LOG10(0.1)                    'define coordinate system
y2 = LOG10(1000)                   'for axes
SELECT CASE g$
CASE "1"
  x1 = LOG10(1)
  x2 = LOG10(3600 * 24.0)
CASE "2"
  x1 = 0
  IF ttime <= 120 THEN x2 = 120
  IF ttime > 120 AND ttime <= 300 THEN x2 = 300
  IF ttime > 300 AND ttime <= 3600 THEN x2 = 3600
  IF ttime > 3600 AND ttime <= 21600 THEN x2 = 21600
  IF ttime > 21600 AND ttime <= 86400 THEN  x2 = 86400
  IF ttime > 86400 THEN x2 = ttime
END SELECT
WINDOW (x1,y1) - (x2,y2)
                                   'draw dotted  horizontal lines
```

```
FOR y = -1 TO 3
  FOR x = PMAP(x1,0) TO PMAP(x2,0) STEP 3
    PSET(PMAP(x,2),y)
  NEXT x
NEXT y
                                'label axes and draw vertical lines
LOCATE 1,7
IF graph6 = 0 AND graph3 = 0 AND graph4 = 0 THEN
  PRINT "Plasma concentration (nanogram / ml)";
ELSEIF (graph6 = 1 OR graph3 = 1 OR graph4 = 1) AND  graph5 = 0 AND_
graph1 = 0 AND graph2 = 0 THEN
  PRINT "Tissue concentration (nanogram / g)";
ELSE
  PRINT "Plasma conc (nanogram / ml)   Tissue conc (nanogram / g)";
END IF
LOCATE 23,4
PRINT "0.1";
LOCATE 18,4
PRINT "1.0";
LOCATE 12,3
PRINT "10.0";
LOCATE 7,2
PRINT "100.0";
LOCATE 2,1
PRINT "1000.0";
LOCATE 25,76
PRINT "Time";
SELECT CASE g$
CASE "1"
  LINE (LOG10(1),-4) - (LOG10(1),3)
  LINE (1,-4) - (1,3)
  LINE (LOG10(60.0),-4) - (LOG10(60.0),3)
  LINE (LOG10(600.0),-4) - (LOG10(600.0),3)
  LINE (LOG10(3600.0),-4) - (LOG10(3600.0),3)
  LINE (LOG10(3600.0 * 6.0),-4) - (LOG10(3600.0 * 6.0),3)
  LINE (LOG10(3600.0 * 24.0),-4) - (LOG10(3600.0 * 24.0),3)
  LOCATE 24,7
  PRINT "1 s";
  LOCATE 24,20
  PRINT "10 s";
  LOCATE 24,31
  PRINT "1 min";
  LOCATE 24,45
  PRINT "10 min";
  LOCATE 24,59
  PRINT "1 h";
  LOCATE 24,70
  PRINT "6 h";
  LOCATE 24,77
  PRINT "24 h";
CASE "2"
  LINE (0,-4) - (0,3)
  LOCATE 24,7
  PRINT "0";
  SELECT CASE x2
  CASE 120
    LINE (20,-4) - (20,3)
    LINE (40,-4) - (40,3)
    LINE (60,-4) - (60,3)
    LINE (80,-4) - (80,3)
    LINE (100,-4) - (100,3)
```

```
    LINE (120,-4) - (120,3)
    LOCATE 24,17
    PRINT "20 s";
    LOCATE 24,29
    PRINT "40 s";
    LOCATE 24,41
    PRINT "60 s";
    LOCATE 24,54
    PRINT "80 s";
    LOCATE 24,66
    PRINT "100 s";
    LOCATE 24,76
    PRINT "120 s";
  CASE 300
    LINE (60,-4) - (60,3)
    LINE (120,-4) - (120,3)
    LINE (180,-4) - (180,3)
    LINE (240,-4) - (240,3)
    LINE (300,-4) - (300,3)
    LOCATE 24,20
    PRINT "1 m";
    LOCATE 24,35
    PRINT "2 m";
    LOCATE 24,49
    PRINT "3 m";
    LOCATE 24,64
    PRINT "4 m";
    LOCATE 24,78
    PRINT "5 m";
  CASE 3600
    LINE (600,-4) - (600,3)
    LINE (1200,-4) - (1200,3)
    LINE (1800,-4) - (1800,3)
    LINE (2400,-4) - (2400,3)
    LINE (3000,-4) - (3000,3)
    LINE (3600,-4) - (3600,3)
    LOCATE 24,17
    PRINT "10 m";
    LOCATE 24,29
    PRINT "20 m";
    LOCATE 24,41
    PRINT "30 m";
    LOCATE 24,54
    PRINT "40 m";
    LOCATE 24,66
    PRINT "50 m";
    LOCATE 24,77
    PRINT "60 m";
  CASE 21600
    LINE (3600,-4) - (3600,3)
    LINE (7200,-4) - (7200,3)
    LINE (10800,-4) - (10800,3)
    LINE (14400,-4) - (14400,3)
    LINE (18000,-4) - (18000,3)
    LINE (21600,-4) - (21600,3)
    LOCATE 24,17
    PRINT "1 h";
    LOCATE 24,29
    PRINT "2 h";
    LOCATE 24,41
    PRINT "3 h";
```

```
    LOCATE 24,54
    PRINT "4 h";
    LOCATE 24,66
    PRINT "5 h";
    LOCATE 24,78
    PRINT "6 h";
  CASE 86400
    LINE (14400,-4) - (14400,3)
    LINE (28800,-4) - (28800,3)
    LINE (43200,-4) - (43200,3)
    LINE (57600,-4) - (57600,3)
    LINE (72000,-4) - (72000,3)
    LINE (86400,-4) - (86400,3)
    LOCATE 24,17
    PRINT "4 h";
    LOCATE 24,29
    PRINT "8 h";
    LOCATE 24,41
    PRINT "12 h";
    LOCATE 24,54
    PRINT "16 h";
    LOCATE 24,66
    PRINT "20 h";
    LOCATE 24,77
    PRINT "24 h";
  CASE >86400
    LINE (ttime,-4) - (ttime,3)
    LOCATE 24,73
    PRINT USING "###.# h";ttime/3600;
  END SELECT
END SELECT
p = 0
IF graph2 = 1 THEN                    'print legend for individual
  LOCATE 2,66                         'graph labels
  PRINT "p pulmonary";
  p = 1
END IF
IF graph5 = 1 THEN
  LOCATE (2 + p),66
  PRINT "c cen venous";
  p = p + 1
END IF
IF graph1 = 1 THEN
  LOCATE (2 + p),70
  PRINT "a arterial";
  p = p + 1
END IF
IF graph3 = 1 THEN
  LOCATE (2 + p),73
  PRINT "f fat";
  p = p + 1
END IF
IF graph4 = 1 THEN
  LOCATE (2 + p),73
  PRINT "l lean";
  p = p + 1
END IF
IF graph6 = 1 THEN
  LOCATE (p + 2),73
  PRINT "b brain";
END IF
```

```
  END IF
END SUB


SUB legend(x%,y%,leg,leg$)
     'labels individual graphs
     'places label near the start of each graph wherever it occurs on screen
     'ensures labels do not overprint each other
  SHARED legart,legp,legfat,leglean,legbrain,legcv
  SHARED x1%,x2%,x3%,x4%,x5%,x6%,y1%,y2%,y3%,y4%,y5%,y6%
  IF y% > 0 AND y% < 23 THEN
    IF y% <= y1%+1 AND y% >= y1%-1 AND legart = 1 AND x% < x1% + 4 THEN EXIT SUB
    IF y% <= y2%+1 AND y% >= y2%-1 AND legp = 1 AND x% < x2% + 4 THEN EXIT SUB
    IF y% <= y3%+1 AND y% >= y3%-1 AND legfat = 1 AND x% < x3% + 4 THEN EXIT SUB
    IF y% <= y4%+1 AND y% >= y4%-1 AND leglean = 1 AND x% < x4% + 4 THEN EXIT SUB
    IF y% <= y5%+1 AND y% >= y5%-1 AND legcv = 1 AND x% < x5% + 4 THEN EXIT SUB
    IF y% <= y6%+1 AND y% >= y6%-1 AND legbrain = 1 AND x% < x6% + 4 THEN EXIT SUB
    LOCATE y%,x%
    PRINT leg$;
    leg = 1
  END IF
END SUB

$SEGMENT                        'compiling instruction

SUB scr(route$)
    'sub sets dose entry display up on screen
    'initialises variables for time and duration
    'of dose administration
  SHARED time,lasttime,vdurn,ldurn,ndurn,mdurn
  LOCAL nul$
  LOCATE 15,40
  PRINT "Press any key to continue..."
  WHILE NOT INSTAT:WEND
  nul$ = INKEY$
  CLS
  LOCATE 1,58
  PRINT "time";
  LOCATE 1,65
  PRINT "dose";
  IF route$ = "iv" OR  route$ = "ip"  OR route$ = "in" THEN
    LOCATE 1,72
    PRINT "duration";
  END IF
  time = 0                      'time of start of dose administration
  lasttime = -1                 'sets time of previous dose < time
  IF route$ = "iv" THEN vdurn = 0
  IF route$ = "ip" THEN ldurn = 0
  IF route$ = "in" THEN ndurn = 0
  IF route$ = "im" THEN mdurn = 0
END SUB


SUB dsentry(n%,durn,count,route$,temp1#(2),temp2#(2))
    'sub is entry routine for dose  details
    'forces dose entry in temporal order
    'prevents  overlapping doses
  SHARED p%,lasttime,ttime,time,dfac
  LOCAL dentry$,nul$,x%
  STATIC v%
  p% = 0                        'initialise indicator
```

```
LOCATE 12,1
PRINT "Enter dose in MICROGRAM fentanyl base";
LOCATE 13,1
INPUT; "Or press plain RETURN to finish   ",dentry$
IF dentry$ = "" THEN
  p% = n%                        'indicates no dose entered and
                                 'location of leaving input routine
  EXIT SUB
END IF
DO
  LOCATE 15,1
  INPUT; "Enter time dose commences; MINUTES from start ",time
  IF NOT route$ = "im" THEN
    DO
      LOCATE 17,1
      INPUT; "Enter time over which dose given; SECONDS ",durn
      IF NOT durn > 0 THEN
        LOCATE 17,1
        PRINT "                                                    ";
        LOCATE 23,1
        PRINT "Duration must be greater than 0. Please re-enter";
      END IF
    LOOP UNTIL durn > 0
  END IF
  IF (time*60 + durn) > (ttime + 0.1) OR (time*60) <= lasttime THEN
    LOCATE 23,1
    PRINT "time(s) incorrect please re-enter                ";
    LOCATE 15,1
    PRINT "                                                 ";
    LOCATE 17,1
    PRINT "                                                 ";
  END IF
LOOP UNTIL (time*60 + durn) < (ttime + 0.1) AND time*60 > lasttime
lasttime = time*60 + durn     'set time of dose ending
LOCATE 12,1
PRINT "                                                 ";  'clear
LOCATE 13,1                                                 'screen
PRINT "                                                 ";
LOCATE 15,1
PRINT "                                                 ";
LOCATE 17,1
PRINT "                                                 ";
LOCATE 23,1
PRINT "                                                 ";
IF count > 1 THEN
  FOR x% = 1 TO (count - 1)
    temp1#(x%,1) = temp2#(x%,1)      'transfers details between
    temp1#(x%,2) = temp2#(x%,2)      'temporary arrays
    temp1#(x%,3) = temp2#(x%,3)
  NEXT x%
END IF
temp1#(count,1) = VAL(dentry$)*dfac
temp1#(count,2) = time*60            'adds new dose details
temp1#(count,3) = durn
v% = 2
FOR x% = 1 TO count
  LOCATE v%,58                       'prints dose details on
  PRINT temp1#(x%,2)/60;             'screen display
  LOCATE v%,65
  PRINT temp1#(x%,1)/dfac;
  IF NOT route$ = "im" THEN
```

```
      LOCATE v%,72
      PRINT temp1#(x%,3);
    END IF
    v% = v% + 1
    IF v% > 22 then v% = (v% MOD 22) + 1
  NEXT x%
  IF lasttime > = ttime THEN
    LOCATE 13,1
    PRINT "Dose accepted; but no more simulation time left"
    PRINT "for further drug administration by this route"
    PRINT "Press RETURN to continue"   'prevents program sticking
    IF n% = 1 THEN p% = 2              'when previous
    IF n% = 2 THEN p% = 1              'dose does not finish before
    count = count + 1                  'ttime and user has attempted
    WHILE NOT INSTAT:WEND              'to enter a further dose
    nu1$ = INKEY$
  END IF
END SUB


SUB pend(hold#(2),var,count,var$,unit$)
    'sub changes current variable value to that stored in array when run time
    'equals administration time or, if administration time not divisible exactly
    'by cycle time, at the first run time which exceeds administration time
    'calls subs to recalculate dependant intermediate variables
  SHARED rtime,disk1,file1$,dt,paper,ptime
  IF (rtime + dt) > hold#(count,2) OR ABS(rtime - hold#(count,2)) < EXP10(-9.0) THEN
    var = hold#(count,1)
    ptime = 0                          'reset counter for results frequency
    count = count + 1                  'advance pending array position indicator
    IF var$ = "plasma pH" THEN CALL phcalc
    IF var$ = "urine pH" THEN CALL ucalc
    IF var$ = "cardiac output" OR var$ = "cycle time" OR_
    var$ = "urine water output" THEN CALL qdtcalc
    IF rtime > 0 THEN
      IF paper = 1 THEN                'sends details to priner
        LPRINT ""
        LPRINT USING "& changed to ####.##### & at #####.# min ";_
        var$;var;unit$;rtime/60
      END IF
      IF disk1 > 0 THEN                'sends details to results file
        CALL disk                      'sends main results so far to results file
        OPEN file1$ FOR APPEND AS #1
        PRINT#1,""
        PRINT #1, USING "& changed to ####.##### & at #####.# min";_
        var$;var;unit$;rtime/60
        CLOSE #1
      END IF
    END IF
  END IF
END SUB


SUB dospend(durn,dose,dose$,dleft,count,dosehold#(2))
    'sub changes current dose to that stored in array when run time equals
    'administration time or, if administration time not divisible exactly
    'by cycle time, at the first run time which exceeds administration time
  SHARED rtime,ptime,dfac,disk1,paper,file1$,dt
  IF (rtime + dt) > dosehold#(count,2) OR_
  ABS(rtime - dosehold#(count,2)) < EXP10(-9.0) THEN
    dose = dosehold#(count,1)
```

```
    durn = dosehold#(count,3)
    dleft = dose                    'set dose remainder = dose
    count = count + 1               'advance array position indicator
    ptime = 0                       'reset results() frequency counter
    IF paper = 1 THEN
      LPRINT ""
      IF dose$ = "intramuscularly" THEN
        LPRINT USING "####.# ug given & at ####.# min";dose/dfac;dose$;rtime/60
      ELSE
        LPRINT USING " ####.# ug given & over ###### s starting #####.# min";dose/dfac;_
        dose$;durn;rtime/60
      END IF
      LPRINT ""                     'record dose details on printout
    END IF
    IF disk1 > 0 THEN
      Call disk                     'sends main results so far to results file
      OPEN file1$ FOR APPEND AS #1
      PRINT #1,""
      IF dose$ = "intramuscularly" THEN
        PRINT #1, USING "####.# ug given & at ######.# min";dose/dfac;dose$;rtime/60
      ELSE
        PRINT #1, USING " ####.# ug given & over ######.# s starting #####.# min";_
        dose/dfac;dose$;durn;rtime/60
      END IF
      CLOSE #1

    END IF
  END IF
END SUB


SUB dose(dleft,dose,durn,dinc)
  'sub calculates dose increment to be administered each cycle
  SHARED dt
  LOCAL aliq
  aliq = dose * (dt / durn)
  IF aliq > dose THEN aliq = dose
  IF aliq > dleft THEN aliq = dleft
  dleft = dleft - aliq              'decrement remaining portion of dose
  dinc = aliq
END SUB


SUB vscr(var$,var)
    'sub sets variable-change entry display up on screen
    'initialises variables for times of changes
  SHARED time,lasttime
  LOCAL nul$
  KEY OFF
  CLS
  PRINT "You may enter as many different ";var$;"s as you like"
  PRINT "The new values must be entered in the order they occur"
  PRINT "from the start of the simulation"
  PRINT "If you don't give a new value for time 0 the default"
  PRINT "will be used up to the time of the first change"
  LOCATE 15,40
  PRINT "Press any key to continue..."
  WHILE NOT INSTAT:WEND
  nul$ = INKEY$
  CLS
  LOCATE 1,65
```

```
  PRINT "time";
  LOCATE 1,72
  PRINT "variable"
  LOCATE 2,65
  PRINT "0";
  LOCATE 2,72
  PRINT var;
  time = 0
  lasttime = -1
END SUB


SUB varentry(n%,count,var$,unit$,var,hold1#(2),hold2#(2))
    'sub is entry routine for changes of variable values
    'forces  entry of changes in  temporal order
  SHARED ttime,time,lasttime,p%
  LOCAL entry$,x%
  STATIC v%
  IF  n% = 1 and count = 2 THEN           'if first pass through loop
    hold1#(1,1) = var                     'enters default values into
    hold1#(1,2) = 0                       'temporary array
  END IF
  p% = 0                                  'initialises indicator
  DO
    LOCATE 11,1
    PRINT "Enter new ";var$;unit$;
    LOCATE 12,1
    INPUT; "Or press plain RETURN to finish changes  ",entry$
    IF entry$ = "" THEN
      IF n% = 1 AND count = 2 THEN count = 1    'adjust counter to prevent
                                                'counting default as change
      p% = n%      'indicates entry complete and position of leaving routine
      EXIT SUB
    ELSEIF (NOT VAL(entry$) > 0 ) AND (NOT var$ = "urine water output") THEN
      LOCATE 12,1
      PRINT "                                                              ";
      LOCATE 22,1
      PRINT var$;" must be greater than 0 Please re-enter";
    END IF
  LOOP UNTIL entry$ = "" OR VAL(entry$) > 0 OR var$ = "urine water output"
  LOCATE 22,1
  PRINT "                                                                  ";
  DO
    LOCATE 14,1
    PRINT "Enter time of new value taking effect:";
    LOCATE 15,1
    INPUT; "MINUTES from start  ",time
    IF time*60  >= ttime OR time*60 <= lasttime THEN
      LOCATE 15,1
      PRINT "                                                              ";
      LOCATE 22,1
      PRINT "time incorrect please re-enter";
    ELSE
      LOCATE 11,1
      PRINT "                                                              ";
      LOCATE 12,1
      PRINT "                                                              "; 'clear
      LOCATE 14,1                                                            'text
      PRINT "                                                              ";
      LOCATE 15,1
      PRINT "                                                              ";
```

```
      LOCATE 22,1
      PRINT "                                                              ";
    END IF
  LOOP UNTIL time*60 < ttime AND time*60 > lasttime
  lasttime = time*60                          'sets lasttime for next entry
  IF time = 0 THEN count = 1
  IF count > 2 OR (count = 2 AND n% = 2) THEN
    FOR x% = 1 TO (count - 1)                 'unless first time through sub
      hold1#(x%,1) = hold2#(x%,1)             'transfers details between
      hold1#(x%,2) = hold2#(x%,2)             'temporary arrays
    NEXT x%
  END IF
  hold1#(count,1) = VAL(entry$)               'adds new details
  hold1#(count,2) = time*60
  v% = 2
  LOCATE 2,64                                 'clears default values
  PRINT "                 ";                  'from display
  FOR x% = 1 TO count
    LOCATE v%,65
    PRINT hold1#(x%,2)/60;
    LOCATE v%,72                              'displays details on screen
    PRINT hold1#(x%,1);
    v% = v% + 1
    IF v% > 23 then v% = (v% MOD 23) + 1
  NEXT x%
END SUB
```

APPENDIX 2

Running the Program

Instructions For Using The Programs

The programs should run on any IBM compatible micro-computer under MS-DOS or PC-DOS 2.0 or above. A Printer is optional.

1. To run the fentanyl simulation insert the program disk into the computer and type FENTSIM followed by the RETURN key.
2. To run the pethidine simulation insert the program disk into the computer and type PETHSIM followed by the RETURN key.

A star * in the bottom left hand corner of the display screen indicates the simulation has finished. The display will remain in the screen until pressing any key restarts the program for a new simulation. Pressing SHIFT-PrtScr at this stage will not remove the display, thereby allowing a "Screen Dump" to a suitable printer.
There is no provision for interrupting the program once a simulation is underway.

Errors: The following errors may crash the program without warning:

- printer not turned on after "printer available" option chosen.
- inaccessible results file e.g. filename of wrong form, non-existent diskdrive specified, "write protect" on floppy disk, disk full (beware when using frequent results scheme) etc.
- wrong screen specification selected for graphical display. (may also cause a distorted display)

Summary

This thesis concerns two investigations into the opioid analgesic fentanyl. The first is a clinical investigation into the safety and efficacy of fentanyl administered by inhalation. The second is a theoretical assessment of the feasibility and merits of applying an alternative form of pharmacokinetic model to the drug. The thesis is in two related but essentially self-contained parts preceded by a review of fentanyl pharmacology.

The first part describes a study of nebulised inhaled fentanyl for post-operative pain relief. One of three concentrations of fentanyl citrate solution (318 $\mu g\ ml^{-1}$, 159 $\mu g\ ml^{-1}$, or 64 $\mu g\ ml^{-1}$) was administered to each of 30 patients from Lifecare Micro-Neb disposable jet nebulisers. There were no major side effects. Within the limits of the trial design the study suggests possible efficacy of nebulised inhaled fentanyl for postoperative pain relief. The disadvantages and limitations of the drug delivery regimen employed in the trial are described and improvements in trial design discussed. Aspects of nebulisation and aerosol delivery and the measurement of pain by linear visual analogue are reviewed.

The second part describes the extension of an existing physiological model of intravenously and intramuscularly administered pethidine to allow intranasal and intrapulmonary administration and adaptation of the model to describe fentanyl.

The original pethidine model exists as a computer program in several different versions. During the present modifications,

with help from the original author, several errors were identified in these programs. Some of these are present in published versions.

A preliminary evaluation of the fentanyl model against published data suggests that simulated arterial and venous plasma concentrations are similar to those obtained experimentally.

Elements of the model are examined individually and its future development discussed.

The model proved only of limited use for simulating the transient concentration changes which occur in the few seconds following drug administration, the examination of which was suggested by work reported the first part of this thesis, nevertheless, the model is a useful conceptual tool in the examination of questions arising from the development of new routes of drug delivery.

A lack of agreement between published studies of aspects of fentanyl pharmacology is noted.