HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND PHARMACOKINETICS OF SOME ANTIBIOTICS

A thesis submitted by David Keith Scott for the degree of Doctor of Philosophy.

The University of Aston in Birmingham

May 1981

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SUMMARY

The principles of High Performance Liquid Chromatography (HPLC) and pharmacokinetics were applied to the use of several clinically-important drugs at the East Birmingham Hospital. Amongst these was gentamicin, which was investigated over a two-year period by a multi-disciplinary team. It was found that there was considerable intra- and inter-patient variation that had not previously been reported and the causes and consequences of such variation were considered. A detailed evaluation of available pharmacokinetic techniques was undertaken and 1- and 2-compartment models were optimised with regard to sampling procedures, analytical error and model-error. The implications for control of therapy are discussed and an improved sampling regime is proposed for routine usage.

Similar techniques were applied to trimethoprim, assayed by HPLC, in patients with normal renal function and investigations were also commenced into the penetration of drug into peritoneal dialysate. Novel assay techniques were also developed for a range of drugs including 4-aminopyridine, chloramphenicol, metronidazole and a series of penicillins and cephalosporins.

Stability studies on cysteamine, reaction-rate studies on creatinine-picrate and structure-activity relationships in HPLC of aminopyridines are also reported.

Keywords

HPLC, pharmacokinetics, gentamicin, 4-aminopyridine, peritoneal dialysis.

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The contributions of the other members of the gentamicin research group are noted elsewhere but I am particularly grateful to Dr. Igor Gonda for much advice and debate in the mathematical areas of this thesis.

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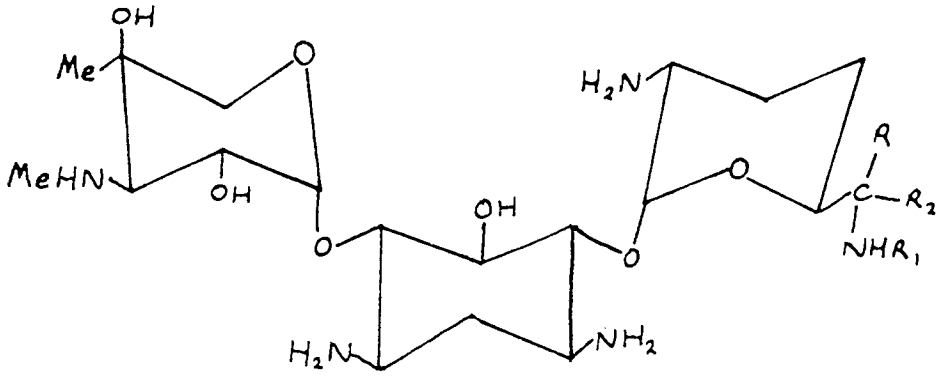
INTRODUCTION

Pharmacokinetics has become increasingly well documented with regard to theory and mathematical background, but only recently has there been a significant increase in the practical application of such work in clinical practice. This discrepancy is primarily due to the late development of specific, rapid and sensitive assays suitable for use in body fluids. High Performance Liquid Chromatography (HPLC) has advanced to such a degree in recent years that it may be regarded as a highly satisfactory method of drug analysis and it is the aim of this thesis to investigate the applicability of HPLC and pharmacokinetics to clinically-important antibiotics.

Different drugs were investigated as clinical interest permitted, but whilst some were amenable to HPLC analysis, others were not and pharmacokinetics alone was applied to data obtained from other analytical laboratories. Such a drug was gentamicin, which was the subject of a two-year multi-disciplinary investigation and which forms the basis for the first portion of the thesis.

Other drugs investigated in patients include trimethoprim and 4-aminopyridine whilst a number of assays were developed to a pre-clinical stage or applied to pharmaceutical formulations.



Gentamicin	R	R_1	R_2
C_1	Me	Me	H
C_2	Me	H	H
C_{2a}	H	H	Me
C_{2b}	H	Me	H
C_{1a}	H	H	H

Fig 1.1 Structure of gentamicin C components

(after Byrne et al)

The earlier aminoglycosides (streptomycin, neomycin and kanamycin) have a more restricted antibacterial spectrum, or are more toxic, than gentamicin and the more recent drugs (tobramycin, amikacin, sisomicin and netilmicin) are more expensive whilst lacking proven superiority to gentamicin. The only exceptions are for cases of gentamicin-resistance and possibly for long (or repeated) courses where some recent work (6) has shown that tobramycin has a reduced incidence of toxicity. Toxicity has manifested itself principally as ototoxicity and nephrotoxicity although neuromuscular blockade and hypersensitivity are occasionally reported(7).

1.2. Ototoxicity

In human studies the incidence of aminoglycoside ototoxicity has been found to be 2-50% (8,9,10). Attempts to correlate various factors with ototoxicity have been made (Table 1.1). Although the peak levels of some patients who developed ototoxicity were excessive, high peak levels alone cannot be ototoxic since an iv bolus regimen, which produces transiently high peaks, is no more dangerous than an intramuscular regimen (11,78). Similarly, although high trough levels were associated with ototoxicity in some cases, the incidence of ototoxicity in 44 patients on continuous intravenous infusion regimens assessed prospectively was only 6-8% despite having sustained serum concentrations of 4-5 µg/ml for gentamicin, 3-5 µg/ml for sisomicin and 14-16 µg/ml for amikacin (11).

	Correlation between total dose and toxicity		Correlation between duration of treatment and toxicity		Correlation between trough levels and toxicity		Correlation between peak levels and toxicity	
	Present	Absent	Present	Absnt	Present	Absent	Present	Absent
OTOTOXICITY	Fee 1978(16)	Winkel 1978(10)	Black 1976(147)	Winel 197(10)	Black 1976(140)	Winkel 1978(10)	Black 1976(147)	Nordstrom 1978(9)
	Black 1976(147)	Nordstrom 1973(9)	Nordstrom 1973(9)		Mawer 1974(108)			Winkel 1978(10)
		Jackson 1971(8)	Line 1970(80)		Nordstrom 1973(9)		Jackson 1971(8)	Lynn 1974(70)
		Meyers 1970(149)			Line 1970(80)			
NEPHROTOXICITY	Fee 1978(16)	Smith 1978(17)	Smith 1978(17)	Keeling 197(12)	Smith 1978(17)		Smith 1978(17)	Dahlgren 1975(18)
		Hoffman 1977(148)		Hoffman 197(148)	Lerner 1977(20)		Lerner 1976(20)	Goodman 1975(19)
		Goodman 1975(19)		Goodman 195(19)	Dahlgren 1975(18)			
					Goodman 1975(19)			

Table 1.1 The relationships of total dose, duration of treatment and serum levels to aminoglycoside toxicity

Animal work using guinea pigs has provided some interesting information on ototoxicity in relation to the perilymph levels of different aminoglycosides after chronic treatment. Neomycin accumulated in the perilymph of guinea pigs secondary to accumulation in serum, whereas kanamycin accumulated in perilymph without accumulating in serum (13). Similarly, gentamicin accumulates in blood and perilymph, producing ototoxicity, whereas sisomicin, amikacin and tobramycin accumulate in perilymph without accumulating in blood; sisomicin, whose perilymph accumulation is greater than that for amikacin and tobramycin, produces greater toxicity (14). Netilmicin has a longer half life than gentamicin in perilymph, yet it does not accumulate and produces minimal toxicity (15). These findings suggest that aminoglycoside ototoxicity follows accumulation of the drug in perilymph and that this accumulation may be produced either by accumulation in the blood, presumably following nephrotoxicity, or by some local factor in the inner ear itself. It has been postulated that the aminoglycosides cause cell damage which impairs their release from perilymph and this enhances their accumulation (13).

In summary, aminoglycoside accumulation in blood evidenced by rising peak or trough concentrations should alert the clinician to the possibility of ototoxicity. However, accumulation in the perilymph is only partially dependent on blood accumulation. Individual idiosyncracy in susceptibility to perilymph accumulation is an important possibility.

1.3 Nephrotoxicity

The nephrotoxicity of gentamicin has an incidence of 10-24% (12, 16, 17). The correlation between total dose, duration of treatment and nephrotoxicity is summarised in Table 1.1.

A number of studies have reported increased trough levels occurring before serum creatinine rises (17, 18, 19, 20), but this could obviously be an early manifestation of toxicity rather than the cause of it. The amount of gentamicin calculated to be in the peripheral compartment of a two-compartment model (detailed in subsequent chapters) in six patients developing nephrotoxicity was higher in all cases than in forty-seven patients with no toxicity, although eight of these patients had equally high trough levels (21).

The correlation between the cortical concentrations of aminoglycosides and the degree of toxicity produced by each drug is conflicting (22, 23, 24, 25, 26). Sodium depletion, however, increases both gentamicin cortical concentration and renal damage (27) whereas the concurrent administration of cephalosporins with gentamicin has the opposite effect (22).

Thus nephrotoxicity is also related to the concentration of drug in the target organ, which may be subject to individual idiosyncracy. An example of disease related factors appparently influencing nephrotoxicity is the finding of a higher incidence of nephrotoxicity in febrile leukaemic and cancer patients with positive bacteriology than in those with fever of unknown origin (12). Recent work shows that sensitive indicators of renal tubular damage, such as the urinary excretion of β2 microglobulin and lysosomal enzymes, may be valuable in identifying patients at risk from toxicity several days before changes in creatinine clearance occur (28, 29).

1.4 Efficacy

Using a standard eight hour dose interval, peak blood levels of gentamicin of less than 4 µg/ml were associated with treatment failure for Pseudomonas bacteraemia (30) and soft tissue and urinary tract infections due to a variety of organisms (31). Peak levels of 8 µg/ml have been shown to be desirable in gram-negative penumonia and septicaemia (31). Pseudomonas-infected cystic fibrotics may also require high levels (74). Attempts to correlate the sensitivity of the organisms treated to serum levels or the back titration of blood or other body fluids to find the maximum dilution which inhibits the organism *in vitro* have been only moderately successful (32, 33, 34). Similarly, sputum levels of tobramycin correlated partly with the eradication of pseudomonas from the lungs of patients with cystic fibrosis (35). Several host factors have been shown to influence outcome independent of the sensitivity of the organism or the adequacy of blood levels; these include the site of infection (12, 36), the number of neutrophils in the patient's blood at the start of treatment (12, 34, 35, 37, 38), whether neutrophils increase or decrease during treatment (12, 38) and the presence of shock, abscesses, fistulae and foreign bodies (36, 37).

Recently the use of continuous intravenous infusions for treatment of neutropenic patients has been reported (12, 38, 39). The results have been favourable and in one study (12) continuous infusion was shown to be as successful as six-hourly injections for sisomicin. The serum concentrations aimed for with these regimens were 4-5 µg/ml for gentamicin, 3-4 µg/ml for sisomicin and 14-16 µg/ml for amikacin. These indicate that sustained concentrations of aminoglycosides are a viable alternative to the usual intermittent injections.

1.5 Pharmacokinetics and Bioavailability

Gentamicin is poorly absorbed orally and is generally used parenterally, by intravenous (iv) or intramuscular (im) injection. Intraventricular administration has been advocated (40) because gentamicin does not penetrate the blood-brain barrier unless the meninges are inflamed, but has proved too hazardous (41). Subconjunctival, intrathecal or peritoneal injections (42) and topicals (creams, ointments, eye-drops, bone-cement and polymethylmethacrylate beads (43)) may be used in appropriate cases. Other forms of administration have been reviewed (44). These preparations normally produce only low serum levels although the serum level following 10-20 mg administered sub-conjunctivally has been reported to be as high as 1 µg/ml, one hour after dosing.

Intramuscular injection leads to complete absorption (as judged by comparison of the integral of the concentration-time curve, area under the curve (AUC), with that of iv injections) and after one hour serum levels are similar (but slightly higher, section 5) than those from iv injections. Elimination is by the kidneys, 70-90% of a single dose is recovered unchanged in a twenty-four hour urine collection (45, 46) whilst Schentag et al (47, 48, 49) have shown that up to 99% may be recovered by extending the collection for several days. There is no evidence of metabolism or significant biliary excretion. Secretion into sputum (50) and cervical and menstrual fluid (51) has been shown.

The evidence for aminoglycoside binding to plasma proteins is conflicting. The quoted range of binding for gentamicin is 0-30% (52, 53, 54, 55). It has been shown that 30% plasma protein binding will not significantly affect the accuracy of estimation of either total free drug in the body, or of

volume of distribution, based on levels of unbound drug in blood (56). However, protein binding to any extent will have an effect on renal elimination so the question is of more than theoretical interest. Part of the variation between studies can be accounted for by the concentrations of calcium and magnesium in the test system (53, 57). Even controlling these factors 0-20% binding has been found (53, 55, 57). Ramirez-Ronda (53) demonstrated 6-20% binding using different methods. The problem is therefore one of methodology and requires further study.

The precise mode of renal elimination of gentamicin is not clear, although glomerular filtration plays a major role. A study of renal clearance of gentamicin during treatment (47) showed that at the beginning of treatment the clearance was less than creatinine clearance but that with repeated dosing gentamicin clearance rose to the same level. This was interpreted as evidence that gentamicin undergoes tubular reabsorption in the kidney and that this mechanism becomes saturated with repeated dosing. There is also evidence from animal studies to support this hypothesis. Very high concentrations of gentamicin have been found in homogenates of renal cortex from rats (23, 58, 59) and dogs (60). Since high concentrations of drug are found in the urine these results could have been produced by urinary contamination. However, Whelton showed that urinary contamination could not explain his results (60).

Animal studies have shown that gentamicin uptake into the renal cortex is blocked by infusing amino acids, which presumably compete for proximal tubular reabsorption (26). Further evidence that tubular reabsorption is at least partially responsible for cortical uptake has come from

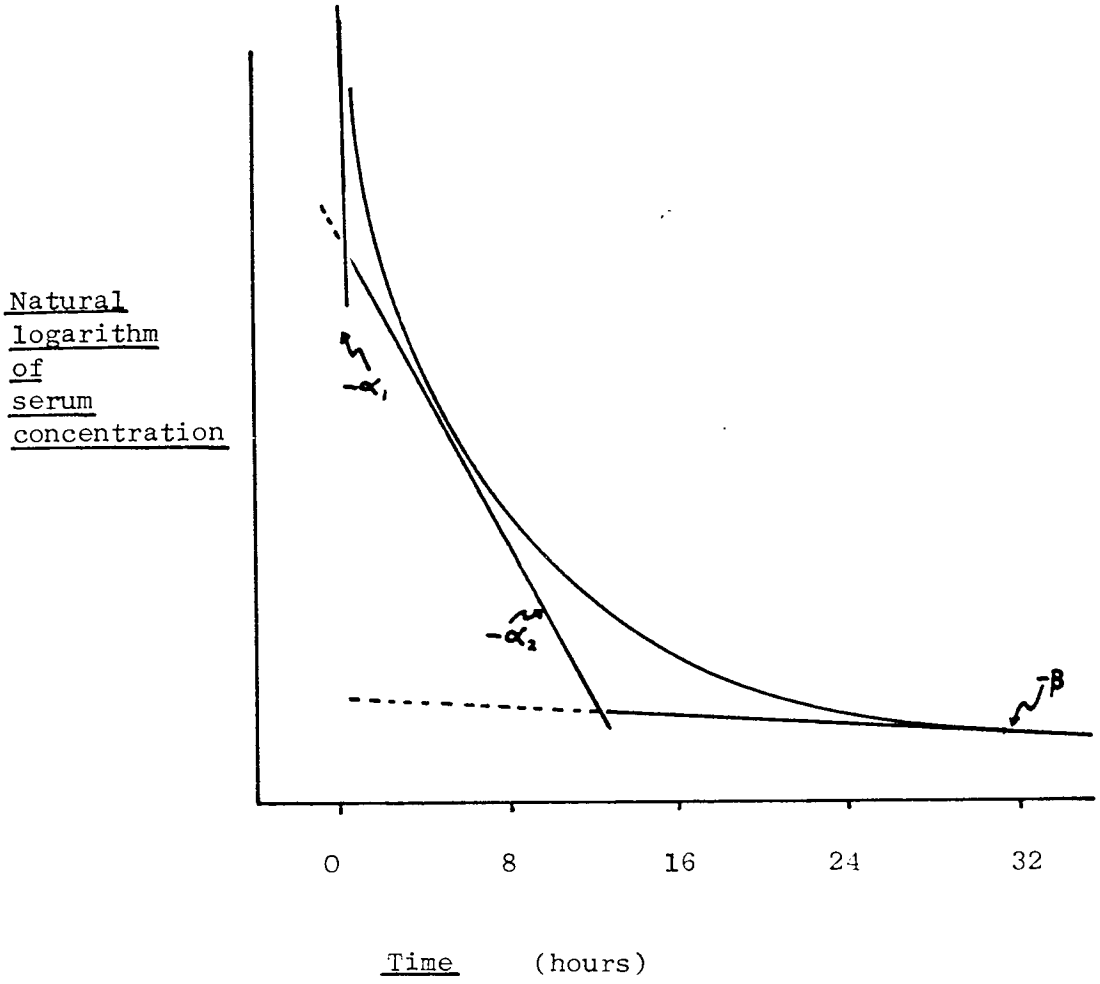


Fig 1.2 iv dose showing parameters obtained by curve stripping

autoradiographic studies in rats (61).

Tissues other than blood have been poorly studied with regard to gentamicin kinetics, most studies using only a few sampling points and comparing them with serum levels, without regard to relative half-lives. Animal data on aminoglycosides is, however, listed in table 1.2. Human tissue fluid cage experiments have shown kinetics similar to those of serum, as has work on synovial fluid. Lung, cerebro-spinal and menstrual studies have already been quoted.

A commonly used measure of the distribution of a drug is the apparent volume of distribution (V litres). If a drug is widely distributed, or highly concentrated in some tissue other than blood, then the volume will appear to be large, since the blood concentration will be low.

The volume, normalised as a percentage of body weight, or lean body weight or ideal body weight calculated from actuarial tables, varies greatly from patient to patient, as seen from Table 7.1 . It has been reported to increase (62), decrease (63) or stay the same (64) as renal function decreases. It is increased in inappropriate ADH secretion, obesity and ascites (65), surgery, shock, trauma and burns (66).

Values from literature are given in Table 1.3.

The serum concentration-time curve following an iv bolus is typically that of Figure 1.2 and may be described by the equation

$$C = A_1 e^{-\alpha_1 t} + A_2 e^{-\alpha_2 t} + B e^{-\beta t} \qquad (100)$$

where C is concentration of drug (µg/ml) at time t (hours), α_1, α_2 and β are rate constants specific to each individual and A_1, A_2 and B are also constants specific to an individual. (The relationships between these values and the rate-constants of compartmental models are discussed in section 4).

Renal Cortex	98-166 hours	Fabre et al 1978 (131)
	60-109 hours	Luft et al 1974 (59)
Renal Medulla	55 hours	Fabre et al 1978 (131)
Perilymph	10-12 hours	Brummett et al 1978 (14)
	5 hours	Federspil et al 1976 (150)
Tissue fluid cage	2 hours	Kozak et al 1977 (248)
Aqueous humour of the eye	2 hours	Federspil et al 1976 (150)

Table 1.2 Half life of gentamicin in animal tissues and body fluids

Range of k_{el}	Range of $t_{\frac{1}{2}}$ (Numbers of subjects given in parentheses)	Range of V as % body weight	Reference
0.718-0.139	0.97-4.97(21)	13.8-70(42)	Barza et al 1975 (118)
0.406-0.3	1.7-2.3(-)	-	Cutler et al 1972 (106)
0.406-0.3	1.7-2.3(-)	14-42(18)	Gyselynck et al 1971 (45)
0.43-0.29	1.6-2.4(7)	18-35(7)	Jahre et al 1978 (46)
0.43-0.19	1.6-3.6(5)	-	McHenry et al 1971 (126)
0.69-0.25	1.0-2.7(13)	-	Riff et al 1977 (140)
0.38-0.28	1.8-2.44(4)	24-35.4(4)	Regamey et al 1973 (141)
0.859-0.408 ($Hours^{-1}$)	1.2-3.5(10) Hours	10-23(10)	Walker et al 1979 (142)

Table 1.3 Range of K_{el} and $t_{\frac{1}{2}}$ in subjects with creatinine clearances of 70ml/min and range of V from studies of gentamicin

Since, by definition, $\alpha_1 > \alpha_2 > \beta$, after a certain length of time the first two terms of (100) become negligible and the curve is described by the equation

$$C = Be^{-\beta t} \qquad (101)$$

which may be expressed as

$$\ln (C) = \ln(B) + (-\beta t) \qquad (102)$$

A rectilinear plot of this portion of the curve will yield a straight line from which β and B may be determined (Figure 1.2). Subtraction of this term from the rest of the data will yield a plot of

$$C = A_1 e^{-\alpha_1 t} + A_2 e^{-\alpha_2 t} \qquad (103)$$

from which A_2 and α_2 may be similarly determined and then too A_1 and α_1. This process, known as 'feathering' or 'curve-stripping', becomes more accurate as the ratios α_1/α_2 and α_2/β become large.

All three phases have been observed in rabbits by Huang and Chiou (67) using very large doses. In humans, however, the terminal phase is only normally observable after the last dose (or a single test dose) because for effective therapy the drug is usually administered at dose intervals of 12 hours or less. Schentag (47, 48, 49) and Follath (68) have described the α_2 and β phases (with different nomenclature) whilst Mawer and Lynn have described the first two phases (78, 70). Typical rate constants for humans (with good renal function) are:

$\alpha_1 = 4h^{-1}$, $\alpha_2 = 0.3h^{-1}$, $\beta = 0.015h^{-1}$

α_2 is closely related to renal function (48, 70) but although β is also well correlated with renal function (48) it is thought to represent the rate of transfer of drug from the tissues to serum. A significant problem in measuring the terminal (β) phase is that the microbiological assays

traditionally used are insensitive at the low levels attained and the more sensitive radio-immune assay techniques (RIA) are relatively imprecise and need measurements taken over a long period to obtain reliable results (see discussion in section 5).

Intramuscular dosing gives similar results but the α_1 phase is unreported, instead an absorption phase is seen, lasting for the whole of the equivalent iv α_1 phase and part of the α_2 phase. The absorption rate constant $Ka(h^{-1})$ may be included in a modified equation (100) as follows:

$$C = Le^{-Kat} + Me^{-\alpha t} + Ne^{-\beta t} \qquad (104)$$

(L is usually negative; L,M,N,α,β,Ka are determined as previously)

Jeliffe has reported a value for Ka of $2.16h^{-1}$, whilst Mawer (72) used $2.5h^{-1}$. Many authors have used a one-compartment model in which for an iv bolus

$$C = \frac{D}{V}e^{-kt} \qquad (105)$$

where D is the dose (mg), V the apparent volume of distribution (l) and K the elimination rate constant (h^{-1}). K corresponds approximately to α_2 or α in the above models. Various determinations of V and K, together with the half-life ($t_{\frac{1}{2}} = \ln(2)/K$) are given in Table 1.3. These show great variation between individuals, demonstrating a need for the individualisation of drug therapy in order to ensure efficacy whilst preventing toxicity. To individualise treatment it is necessary to construct a model of the drug-patient system.

2. MODELLING

2.1 General Principles

Models are generally formulated for one of two reasons, either (i) to identify and characterise a system as completely as possible or (ii) to control a system.

These are not mutually exclusive aims and both imply a predictive ability, but the data-gathering techniques may have to be varied to suit the purpose. Reliable identification and characterisation of a system will require a considerable amount of data collected over a wide-range of conditions. Control of a system, however, implies a time-limited procedure in which access to data may be limited. An essential feature of control models is that they must be used prospectively to predict, and then perhaps to modify, future states of the system. The better characterised is the system, the better will be the control, but full characterisation may require a period of observation which extends beyond the period which is to be controlled.

Two other common, practical but not essential, features of control models are that they should be verifiable in use and that they should require minimal effort in design and implementation. In pharmacokinetic or physiological systems where the well-being and the comfort of human patients are involved, these features assume a greater significance.

Examples of modelling in physiological systems include:

(i) The investigation of physiological and biochemical structure, and the plausibility of various proposed systems, concerning glucose and insulin metabolism in the liver (83).

(ii) The use of parameter estimation in a standard model of bilirubin metabolism to identify a transition

from normal to abnormal liver function (75).

(iii) The design and correlation of groups of biochemical tests to assist in clinical diagnosis of thyroid disease (76).

(iv) Numerous descriptive studies have been made of the behaviour of drug-body systems, mostly by means of serum drug concentration measurements but also by measurements in other body fluids and tissues. A relatively small number have been designed as control models, those relevant to aminoglycosides are listed in Chapter 1, but general examples will be found in the journals, Clinical Pharmacokinetics, Journal of Pharmaceutical Sciences and Journal of Pharmacokinetics and Biopharmaceutics.

2.2 Types of model applicable to physiological systems

Numerous mathematical and non-mathematical techniques have been applied to biological and medical systems, but many of these have only limited or specialised applicability. Logistic and linguistic models have been used (77) but these are most applicable to pharmacodynamic and whole-body studies respectively, and of little use in pharmacokinetics, *per se*. Linear and non-linear mathematical models, with or without oscillatory characteristics, have also been described for widely varying systems (e.g.77). Two general approaches have been used for such models, with a range of relative emphasis placed on the two approaches in any one model. The first approach is that of the unit-process, where a few components of the whole system (be these body organs, chemicals or physiological fluids) are held to function in isolation from the rest of the system. This is a useful approach since it enables comparatively simple mathematical models to be formulated and tested. It is, however, liable to considerable over-simplification and the other approach, the global approach, often applied to behaviour, may be useful in bringing other processes to bear upon the chosen model. Unfortunately, however, for reasons similar to those given to delineate characterisation and control models, the amount of information available will limit the universality of the global approach, and the need for aggregation of systems and functions may lead again to over-simplification.

Models may be empirical, where only system inputs and outputs are considered, with no reference to physiological knowledge, or may involve *a priori* and theoretical considerations. *A priori* considerations will include knowledge of the physiology, metabolism and biochemical structure of the system and these will lead to the formulation of a model which may

be validated by experimental methods.

The a priori considerations may be modified empirically to give a better fit to the experimental data, and this may lead to a re-evaluation of the underlying structure of the system.

In pharmacokinetics the most commonly used models have been linear compartmental types, described below, where the approach may range from the empirical (purely descriptive) to the more complex a priori models involving numerous compartments representing individual physiological states. Often there is considerable aggregation of biological entities, justifying the 'global' description, as one or two compartments are used to describe a whole body. Sometimes, however, such models are used to describe smaller parts of the body in greater detail, Di Stefano has used 27 compartments to describe thyroid hormone regulation (79).

The solution of such models may be expressed in several ways. Tracer studies on metabolic systems frequently use concepts of 'mean transit time' or 'mean residence time' to estimate the turn-over of electrolytes, drugs or other body components (77) whilst the influence of control engineering on some of the theoretical aspects of compartmental modelling has lead to the use of transfer functions to relate mathematically the input and output of any part of a system without reference to the mechanism of such changes (77). More commonly, however, linear differential equations are used to describe the system and curve-fitting techniques are employed to estimate the model parameters.

The concept of linearity (i.e. that the response may be related to the stimulus by a purely linear combination of factors dependent upon the stimulus) is essential to the

Lithium

Phenytoin, ethosuximide, carbamazepine

Digoxin, digitoxin, propranolol

Lignocaine, procainamide, quinidine

Theophylline

Nortryptiline, imipramine, clomipramine

Gentamicin

Table 2.1 Drug Assays of Accepted Therapeutic Value (Marks, 1979)

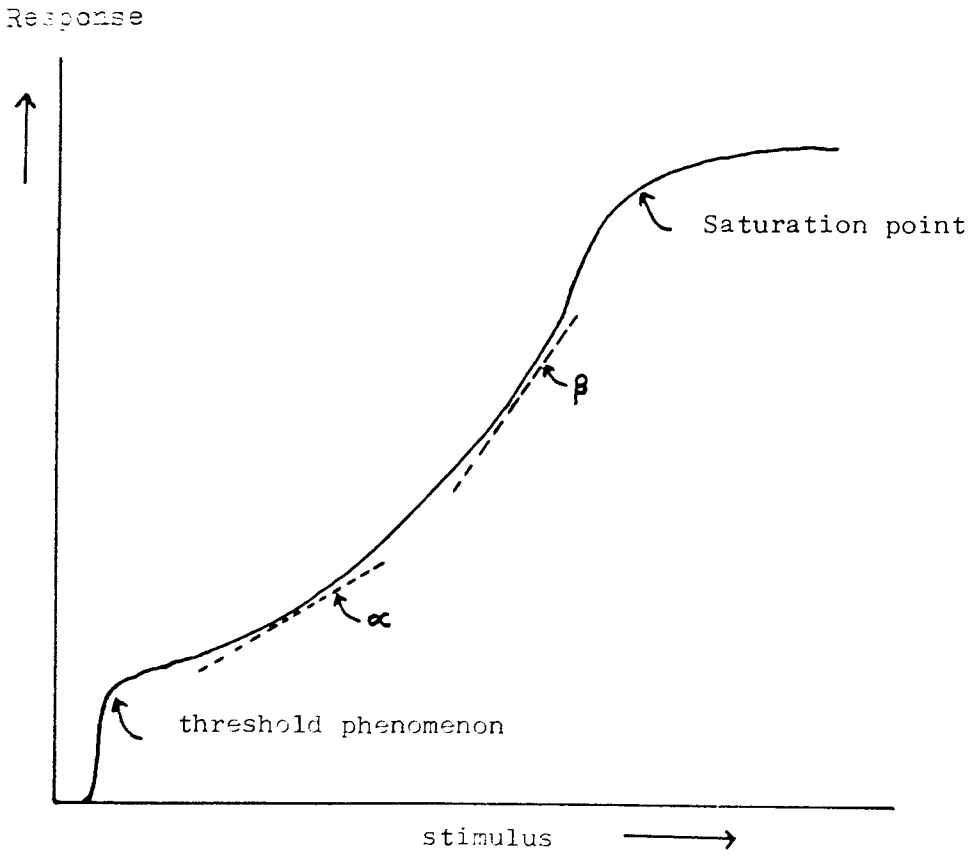


Fig 2.1 Response curve illustrating some non-linear phenomena

design of these models but may be invalidated by a number of biological features. Discontinuities (e.g. switching or threshold phenomena) are known to exist (e.g. glomerular glucose excretion) whilst lag-times apply to many processes because the stimulus must be transmitted across cells or between organs before a response can occur.

Saturation (e.g. of enzymes metabolising alcohol or paracetamol) can occur and these processes may sometimes be described by Michaelis-Menton kinetics (82) whilst, more generally, the response curve may simply exhibit curvature of sometimes unknown origin.

Such processes, very common in metabolic systems but less common in drug studies at normal dose levels, may however be linearised by approximation over short ranges of stimulus.

Thus in Figure 2.1, the slopes α and β might be used to linearise the general response curve in the appropriate regions.

Other common assumptions in compartmental analyses are that the parameters are time-invariant (i.e. that the system response to any given stimulus will always be the same) and that the perturbation due to sampling or stimulus (e.g. drug administration) does not affect the response of any other part of the system. Thus in tracer studies the injection of radio-labelled hormone is assumed not to alter the body's response to that hormone.

It is further assumed that the transfer of drug (or metabolite) from one compartment (i) to another (j) is dependent upon the concentration of drug in (i) and is independent of the concentration in (j) or in any other compartment. This donor-controlled flow eliminates the possibility of

conventional engineering control theory where a separate 'controller' governs the processes in any given compartment (77)

In 1937 Teorell presented the first major application of compartmental modelling in pharmacokinetics, using a three-compartment model to describe drug disposition (83, 84). He did not, however, use the term 'compartment', that description seems to be attributable to Shepherd (85).

Since then many applications have been made, using one to three compartments for most drug studies but up to 27 for some metabolic studies, as described previously. For all further references a compartment will refer to a real or hypothetical location for a drug, or its metabolites. Distinct forms of the drug (protein-bound, free, conjugated, ionised, unionised etc.) will be treated separately from the compartmental model, following normal pharmacokinetic practice rather than typical metabolic descriptions.

2.3 Therapeutic Drug Monitoring.

Most modern drugs are both efficacious and toxic, depending upon the dose administered, and iatrogenic disease is not uncommon. As the potency of drugs has tended to increase over recent years, so too has the desire to prevent toxicity and attain efficacy by exerting closer control over an individual drug therapy. Some drugs have a rapid and clinically observable effect (e.g. intravenous short-acting barbiturate) and can be so monitored, but others (e.g. antibiotics) have no rapidly-observable therapeutic effect and perhaps no rapid warning of impending toxicity (e.g. aminoglycosides).

In order to supplement clinical observations, and biochemical or physiological tests, quantification of drug in body tissues has been attempted for a wide range of drugs and diseases (e.g. 86, 87). In most cases the model has been simple, one threshold level is effective, a second, higher level is toxic. A knowledge of the distribution of the drug is important, however, since (for example) tricyclic anti-depressants may exert a toxic effect at very low serum levels due to the preferential disposition in neural and muscular tissues (88).

Cardiovascular, anxiolytic and anti-epileptic drugs have been monitored and controlled by various pharmacokinetic means for some time (e.g. 89, 90) and Marks (91) has defined a list of therapeutic drugs where assays are of accepted value (Table 2.1).

Apart from the above examples Breimer (92) and others have suggested indications for drug monitoring including:

(1) hepatic or gut disease; to monitor the effects of changing metabolism or absorption.

(2) renal disease; to monitor changing elimination.

(3) multiple drug treatment; to monitor the effects of interactions, especially on protein-binding.

(4) verification of compliance; especially in elderly or disturbed patients.

(5) suspected drug intoxication; e.g. alcohol, anti-depressants, analgesics.

Paracetamol overdosage is often treated according to nomograms based on serum levels (93). Gentamicin and other aminoglycosides have received much attention from those anxious to institute a more rigorous type of therapeutic control because of the drugs' small therapeutic indices and great inter-individual variability. Such control has frequently involved the use of computers.

2.4. Computer assisted drug monitoring

Computer programs used to control or evaluate drug therapy can do no more than any operator, given enough time and freedom from error, could do by hand.

The models they use, therefore, fall into the same categories as the manual models and may be summarised as follows:

(i) Empirical models Doses are calculated without reference to serum drug levels or previous therapy, but may use other patient data. Such models must be used

prior to treatment and fall into two classes;

(a) Group mean models Data from previously-studied populations are used to calculate doses, based on, say, weight (e.g. 2 mg/Kg/8 hours) or on weight and non-parametric characteristics (e.g. age-group, sex, disease-state).

(b) Individualised models The group mean approach is modified by considering a measured parameter (e.g. serum creatinine level) and relating that parameter to dose by regression analysis of such data in previous studies. Several nomograms exist (and are discussed in Section 3), for the use of serum creatinine in calculating gentamicin dosage, mostly assuming a 1-compartment model and relating serum creatinine (with or without other parameters) to the gentamicin elimination rate constant.

(ii) Compartmental models Estimates are made of the various parameters of a chosen compartmental model and these are used to predict the serum levels that any given regimen would be expected to yield and regimens are calculated to generate any desired levels. The parameter estimates may be partly group means (e.g. V(1) = 0.3 Weight (Kg)) or taken wholly from serum data.

Either type of model may be fixed, in that there is no facility for accommodating data subsequent to the initial set, or adaptive, where predictions and dosages are modified according to such data.

Programs of various types have been implemented for the control of cardiac glycosides (e.g. 90, 94), anti-convulsants (e.g. 95) anticoagulants (81) and amino-glycosides (96, 97, 98) and for paediatric intravenous feeding (99). Mawer has reviewed programs in these

areas (100, 1976) whilst Blois (101, 1980) has reviewed the wider use of such programs, including diagnostic procedures and clinical assessment routines, as well as commenting on the proper role of such work in relation to clinical judgment.

Cer (S-N) male	$\frac{W}{C}(1.251 \times 10^{-7} x^4 - 3.049 \times 10^{-5} x^3 + 0.002553 x^2 - 0.09888 x + 2.9476)$
Cer (S-N) female	$\frac{W}{C}(-2.93 \times 10^{-7} x^4 + 7.25 \times 10^{-5} x^3 - 0.00634 x^2 + 0.217 x - 1.091)$
Cer (J) male	$95.94/(C^{1.387})$
Cer (J) female	$70.31/(C^{1.307})$
Cer (CG) male	$\frac{W}{C}(\frac{140 - x}{72})$
Cer (CG) female	$\frac{W}{C}(\frac{140 - x}{72})\ 0.85$

Cer Creatinine clearance (ml/min),

C Serum creatinine concentration (mg/100 ml),

W weight (Kg),

x age (years),

S-N Method of Siersback-Nielsen et al (112),

J method of Jadrný (111),

CG method of Cockcroft and Gault (110).

Table 5.1 Numerical conversion of serum creatinine to creatinine clearance

3. APPLICATION OF MODELLING TO GENTAMICIN THERAPY

3.1. Empirical Models

.1 Creatinine

Commonly advocated dosage regimens for abnormal renal function are the full dose (based on body weight) every three half-lives or the full dose once only followed by half the dose every half-life (102). These rules may be modified (103, 104) for more rigorous application. The easiest method for converting serum creatinine (mg/100 ml) to gentamicin half life (hours) is to multiply the creatinine value by 3 or 4 (105, 106).

The production of creatinine, by muscle metabolism, varies with the age and sex of the patient (107) and nomograms, or equations, which take these factors into account are preferable (108).

Nomograms by Chan et al (109), Mawer et al (108), Cockcroft and Gault (110), Jadrný (111), Siersbaek-Nielsen et al (112) and Jeliffe (113) have been applied to adult patients. Numerous others have been applied to paediatrics, for a brief review see Ng (114).

Those of Chan, Mawer and Jeliffe are specifically for gentamicin, but the other three require conversion of creatinine clearance (ml/min) to gentamicin elimination rate constant (K) and thence to $t_{\frac{1}{2}}$. The formula of Jeliffe,

$$K = 0.012 + 0.034\ Cr_{Cl} \qquad (301)$$

where Cr_{Cl} is creatinine clearance, is convenient.

Whilst each of these methods provides a good general guide to the value of $t_{\frac{1}{2}}$ over a wide range of creatinine concentrations, they become very poor over narrow ranges of creatinines, especially at normal levels of renal function. At creatinine clearances greater than 60 or 80 ml/minute the correlation with

$t_{\frac{1}{2}}$ becomes poor (44, 109).

Creatinine clearances are difficult to measure directly due to problems in ensuring a complete urine collection and serum creatinines are slow to reach a new steady state level following a sudden change in glomerular filtration (115) and so a determination, by either method, of creatinine clearance may well reflect the glomerular function of some hours, or as much as a day, previously and not the current state.

.2 Miscellaenous estimates of $t_{\frac{1}{2}}$

Changes in gentamicin serum levels unrelated to changes in renal function have been reported due to changes in body temperature (116,117), haematocrit (52, 117, 118) and hypoxaemia (119). Changes of $t_{\frac{1}{2}}$ in patients stabilised on haemodialysis, or with constant renal function may be as much as 44% (117, 120) during treatment.

.3 Estimates of V

The studies listed in Table 1.3 show a wide range of V, expressed as a percentage of body weight, within, and between, studies. The 95% confidence limits for peak levels produced by a dose of 1.7 mg/Kg were 2.1 and 9.7 µg/ml in one study (121). Although a dose of 60 mg/m^2 of body surface area has been reported to produce peak levels of 4-8 µg/ml in 80% of patients (122), the variation in peak levels produced by doses derived from body weight or from surface area was identical in another study on children (123). Changes in V may occur in a variety of circumstances and are reviewed by Klotz (124).

3.2 Compartmental Models

Jeliffe *et al* (97, 113) used both empirical (creatinine and compartmental models for the prediction and control of blood levels. Most of this work has been with the one compartment model, although they have recently used a three-compartment model, considering the injection site to be a distinct compartment. The parameters for the models are selected from group-mean data (Schentag *et al*) taken from patients with a wide range of renal function. No allowance is made for renal function in the initial estimates of these parameters, contrary to the practice of Schentag. The aim of the model is to control peak and trough (immediately pre-dose) levels. Any blood data available is incorporated in a Niels-Elder simplex which modifies the initial parameter estimates until an acceptable fit has been found. The model is, however, heavily dependent upon serum creatinine measurements for estimates of the changes in elimination rate, a dependence which is probably unwarranted in view of the above data on creatinine.

Mawer *et al* (98) have employed less ambitious compartmental models to good effect, using principles described above.

Other published studies using compartmental models have been concerned with retrospective analysis rather than prospective control. Schentag *et al* (28, 47, 48, 49) have used a two-compartment model to control therapy, but have published their work prinicipally from a retrospective point of view. They have used the model to describe tissue deposition and to identify features which may predispose to nephrotoxicity. Much of the development and characterisation of the two-compartment model for aminoglycosides is attributable to Schentag and has been reported only after the author

commenced this work.

3.3. Other Models

Orr *et al* (125) advocated the Occupancy Principle as a means of measuring and controlling levels of antibiotics. The principle is derived from control engineering and is essentially identical to the Mean Residence Time principle discussed previously. The Occupancy is calculated from the area under the curve by a technique similar to that of the linear trapezoidal rule discussed in Chapter 5 . This, together with the desired mean level, leads to the dose to be administered. Orr *et al* gave only theoretical examples and recommended drug levels that would be considered unwise by most workers, but nonetheless the principle is a useful one. It may be demonstrated that the occupancy is the reciprocal of the drug clearance (the product of V and K) in the one compartment model. A related principle, albeit one of more general applicability, was formulated by the author and others, who were at that time unaware of Orr's proposed method, and is described in this thesis.

1-hour level ($\mu g/ml$)	Elimination rate constant (K) (h^{-1})	half-life ($t_{\frac{1}{2}}$) (h)	trough level ($\mu g/ml$)	Time (tm) of MEC ($4\mu g/ml$) (hours)	Mean level, t = 0-8h ($\mu g/ml$)	Mean steady-state level ($\bar{C}_{ss}$) ($\mu g/ml$)
8	0.173	4.0	2.38	5.0	5.15	6.87
8	0.346	2.0	0.71	3.0	3.83	4.09
8	0.460	1.5	0.32	2.5	3.37	3.44
10	0.173	4.0	3.00	6.3	6.42	8.59
10	0.346	2.0	0.89	3.6	4.79	5.10
10	0.460	1.5	0.40	3.0	4.19	4.30

Table 3.2 Calculated trough and mean levels associated with given peaks at normal values of K

3.4. Adjustment of dosage regimens

Much of the literature quoted uses the peak (one-hour) and trough (pre-dose) levels as indicators of efficacy and toxicity. These are, however, difficult to relate for a number of theoretical and practical reasons, despite their ease of measurement. Some of the practical problems (timing of the preceding doses and analytical error) are discussed in Section 4, with analyses of their effect on the values observed. It may not be possible, using the conventional 8-hour dosing interval, to attain the commonly recommended levels for peak and trough. Assuming, for example, the aim of a peak of 8 µg/ml and a trough less than 2 µg/ml, with a monoexponential decay curve between one and eight hours, (this would describe an iv injection quite adequately, even in a 2 or 3 compartment model, between these times) to fall from 8 µg/ml to 2 µg/ml (a factor of $\frac{1}{4}$) in seven hours, the half-life must be 3.5 hours at most. This is within the normal range of a number of studies (Table 1.3) including this one. Thus some patients, even without renal impairment, could not meet these criteria. Table 3.2 lists the troughs obtained from peaks of 8 and 10 µg/ml at values of K taken from the range of patients in this study with normal renal function, and it will be observed that there is a wide range in these patients.

From equation (105) it may be shown that

$$D\ (mg/Kg) = \frac{(\text{desired trough level}) \times V(l/Kg)}{e^{-K\tau}} \qquad (302)$$

where τ is the dosing interval (hours).

In a patient with moderate renal impairment ($t_{\frac{1}{2}}$ = 10h, K = 0.0693), a volume of distribution (l) of 25% body weight; and dosing interval τ = 8h;

$$D\ (mg/Kg) = \frac{\text{desired trough level}}{2.298} \qquad (303)$$

For a trough of 2 μg/ml, the dose would be 0.87 mg/Kg yielding a one-hour level of 3.2 μg/ml. To attain a one-hour level of 8 μg/ml the dose must be 2.17 mg/Kg, yielding an eight hour level of 4.98 μg/ml and 2 μg/ml at 20 hours. In such cases the criteria must be changed, or the dosing interval adjusted. If the latter course is adopted, however, the levels will be below 4 μg/ml (a level associated with treatment failure) for 10 hours at a time. This illustrates a dilemma that will occur at some level of renal function, whatever the chosen peak and trough levels.

If the minimum effective concentration (MEC) occurs at time tm, and the one hour level is C_1, then

$$MEC = C_1 \, e^{-K(tm-1)}$$

and

$$tm = \ln \frac{\left(\frac{C_1}{MEC}\right)}{K} + 1 \qquad (304)$$

For any given values of C_1 and MEC, tm will depend inversely on k. Values of tm are also listed in table 3.2 assuming MEC = 4 μg/ml. (It should be noted that although an iv bolus has been considered, the use of im injection, iv infusion, or the assumption of a β phase would render higher troughs for any given peak level and thus the therapeutic dilemma occurs at a higher degree of renal function than implied here).

Some nomograms advocate adjusting only the dose (109), some the interval (126) whilst some adjust both (108, 127). For a bactericidal drug, such as gentamicin, low levels for short periods may be less critical than for a bacteriostatic drug (104) but the only prospective trial of variable-dose and variable-interval regimes was poorly matched and too small to be conclusive (19).

It may be shown (128) that the mean steady-state level

($\bar{c}ss$) is related to τ, V, K and D (units as above) by

$$\bar{c}_{ss} = \frac{D}{KV\tau} \quad (\mu g/ml) \qquad (305)$$

(This is the mean level at steady-state, not the first dose as considered here, but may be caulated from the first dose).

Values of this mean level are also tabulated, demonstrating a wide range.

The value of the product VK is the clearance from a one-compartment system and is the inverse of Orr's occupancy (Section 3.3). The value D/KV is equal to the AUC for any linear model, not only for a 1-compartment system (128).

Thus,

$$\bar{c}_{ss} = \frac{AUC}{\tau} = \frac{\int_{t=0}^{\infty} c}{\tau} \qquad (306)$$

As a means of reliable control, this is probably the easiest concept to use. Use of tm to indicate efficacy is difficult to implement, tm having a non-linear dependence upon K, and peaks and troughs are likely to give inadequate therapy in renal impairment.

The determination of $\bar{c}_{ss}$ may also be less model-dependent, provided that a sufficient number of samples are drawn, but this may be a significant drawback.

Apart from Orr's theory, the only work on the concept of AUC seems to be that of Mawer et al (108) who related the area under the troughs (throughout dosing) to toxicity and noted a positive correlation and Line et al (80) who found a similar relationship for streptomycin.

4. AIMS AND METHODOLOGY

4.1. Aims

With such a diversity of views and data on the merits and demerits of various aminoglycosides and their properties it was decided to set up a prospective clinical trial to gather as much information as possible about a small group of patients, attempting to counteract the usual problems of small trials by a complete data collection on both patients and concurrently selected controls.

Since the problems were wide-ranging, the interests of those principally concerned with the project were disparate:

Dr. E.S.Harpur - overall control of the project with a particular interest in toxicity.

Dr. P.G.Davey - research medical registrar, responsible for clinical management and investigations

Dr. I.D.Farrell - Principal microbiologist, E.B.H., responsible for antibiotic assays by bacteriological methods.

Miss F. Jabeen - Ph.D student specialising in audiology testing.

Dr. I. Gonda and D.K.Scott - pharmacokinetics. Also (DKS) provision of statistical and computing services to all aspects of the project.

The aims of the study were:

(1) To study the incidence, nature and identification of toxicity in patients treated with aminoglycosides, and in particular gentamicin.

(2) To evaluate predictive and testing procedures for toxicity.

(3) To investigate the variability of pharmacokinetic

Steady state is assumed to be attained.

Method 1 shows a typical sampling profile for a patient in this study (Doses at 8 hour intervals)

Method 2 shows larger doses given at 24 hour intervals.

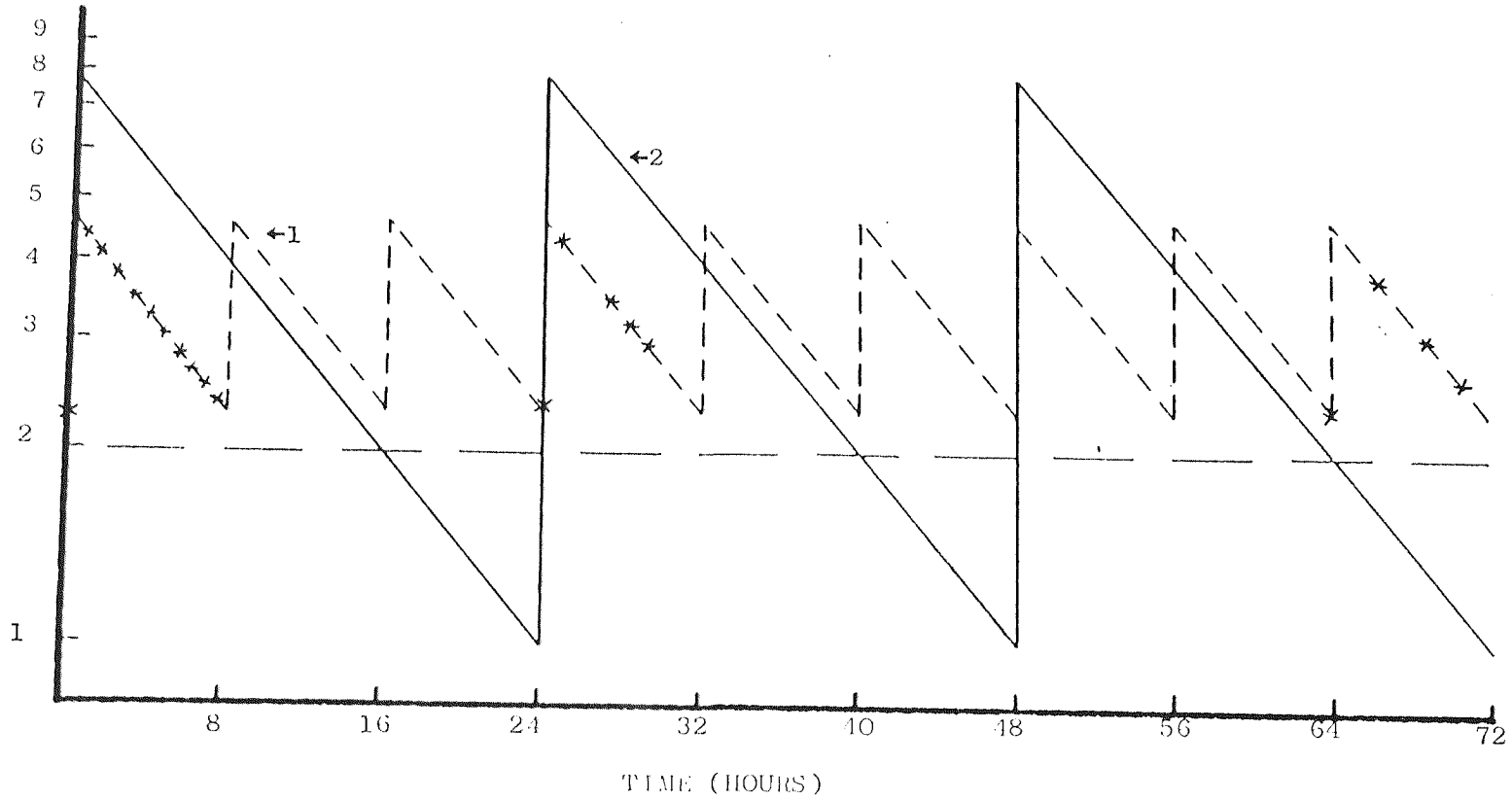


Fig 4.1 Alternative iv dosing regimens achieving the same mean steady state level in a one-compartment system

response to aminoglycosides in a group of hospital patients.

(4) To investigate and evaluate methods of control of aminoglycoside dosing in clinical practice with regard to utility and closeness of control and to relate these to toxicity and efficacy.

Full reports of the toxicity testing will be found in the theses of P.G.Davey (129) and F.Jabeen (130) and are not given here.

Computer programs written by DKS used in the analysis of these data are listed in Appendix 2.

As a secondary therapeutic goal (assuming the primary one to be curing the patient {without doing them harm}) a mean steady-state level was chosen and all patients were to be stabilised to this level. The possibility of creating two groups, one with a dose interval of six hours and the other 12 hours, was considered in order to differentiate between the effects of high peaks and low troughs (12 hour interval) and low peaks with high troughs (6 hour interval). (See Figure 4.1 for a representation of the differences between such regimens). This was not implemented due to the small numbers of patients that we would receive into the trial, there being no real possibility of achieving statistical significance for differences in toxicity or efficacy. It was also found that the assay service could not supply results quickly enough to enable a sufficiently rapid adjustment of dose in patients with a six hour dosing interval. At best, three doses would have elapsed before a correction could be made, and more often six doses.

The selection of a mean level was not easy, since there were no published examples. Continuous infusion in neutropenics (36) was effective at 4 µg/ml, but this was felt to be

too high for intermittent dosing. It was decided to use the manufacturers recommended dose, together with published mean values of V and K to determine $\bar{c}_{ss}$ by the formula already given:

$$\bar{c}_{ss} = \frac{D\ (mg/Kg)}{\tau KV\ (l/Kg)} \qquad (305)$$

Thus, at D = 1.7, τ = 8h, V = 0.21 (Michelson et al (54)), K = .346 h^{-1}, $\bar{c}_{ss}$ = 2.9 µg/ml.

It became apparent, however, that in the first few patients this level was not being met. It was found that the average volume was higher (0.3 l/Kg) than published values. This may well be due to the greater obesity amongst American patients, since fat is less well penetrated by gentamicin than other tissues. Whatever the cause, however, the average remained at approximately 30% throughout the study. The desired level was dropped accordingly, whilst raising the starting dose to 2 mg/Kg, to

$$\bar{c}_{ss} = 2.5\ \mu g/ml$$

Whilst for most patients the adoption of this policy would effectively mean disregarding the peak and trough values, this could not be done for patients with very poor renal function. The reason for that being that an effective level may not be achieved for a satisfactory length of time. Based on continuous infusion studies already cited (36), it was decided that the levels should exceed 4 µg/ml during the dose interval, and be less than 4 µg/ml for no longer than 12 hours continuously. Thus in a few patients a mean level of 4 µg/ml was employed with doses given every 12 or 24 hours.

An ethical problem occurred in several patients, in that their therapy was in the charge of the appropriate consultant (usually not from Communicable Diseases) and most of these were extremely reluctant to increase the dose to attain a desired level when the patient was not deteriorating clinically. (This was not an unreasonable attitude, but did restrict the trial)

4.2 Sampling

Once a patient became available for the trial, a ten or eleven point kinetic profile was taken as soon as possible in treatment around an iv infusion of five minutes duration. Samples were taken before the dose and at 15, 30, 45 and 60 minutes after the dose commenced and then a further six were taken between one and eight hours after the dose. This was the maximum number that the microbiological assay service could handle at one time. The assay procedure was that of Broughall (132) using a fresh overnight culture of *Klebsiella edwardsii* (NCTC 10896) in 37-zone agar diffusion plates. Standards and samples were run five times each giving an overall coefficient of variation of 2.2%. This did not vary significantly with concentration over the range 1.5 to 10 µg/ml.

In most cases intravenous infusions were used to avoid the need to estimate Ka (see chapter 5) and to prevent the inadvertent loss of a major part of the dose into the peri-vascular space. (It is possible to 'lose' up to 0.5 ml of injection fluid without realising it and this would form a major part of an iv bolus (80 mg/2ml) but not of a short infusion (dose given in 20 ml normal saline over 5 minutes). Such loss apparently occurred from an iv bolus on at least one occasion).

Administration was via a 'Venflon' cannula and sampling performed via a two-part 'Butterfly' cannula, usually on the fore-arm or hand. Heparin was used to keep the cannulae patent between sampling times, appropriate volumes of blood were discarded prior to the acceptance of a sample to avoid interference with the assay procedure.

When intramuscular doses were given subsequently a site in the gluteal musculature was used as first choice,

although some patients who had received numerous injections were in such poor condition that other sites (e.g. scapular muscles) were used. The effect of the quality of the site is discussed in section 7.

Further full (11-point) or reduced (usually 5-point, 0, 0.5, 2, 3, 4 hours after dosing) serum profiles were taken in as many patients as possible on subsequent days, and a number of individual or pairs of samples taken in some cases.

A typical sampling profile is illustrated in Figure 4.1. The β phase from the last dose was not sampled for two reasons:

(i) Most patients were discharged before sufficient suitable measurements could be made.

(ii) The assays used were not sufficiently sensitive and RIA techniques investigated were not sufficiently precise. Discussion of this latter point, with theoretical calculations, is made in chapter 5.

Reasons for failure to obtain measurements subsequent to the first full curve included

cessation of therapy,

death,

transfer to another hospital,

patient's protestations against further venepuncture.

To prevent inter-batch variation in the administered gentamicin having an effect, the whole hospital was issued with a single batch of gentamicin for six month periods (Roussell, 'Cidomycin' Inv. 3333).

Calculations upon the data were performed using computer programs written in BASIC (Beginners All-Symbolic Instruction Code) installed on either a Hewlett-Packard 2000 Access

System (University of Aston Computing Service) or, after the initial period, a Data General NOVA 3 (Cardiology Department East Birmingham Hospital). These calculations and programs are described in subsequent chapters. The change to the NOVA 3 was primarily for logistic reasons, keeping the analysis of data on the same site as the collection of data.

Bilateral, pure-tone audiometry (250-8000 Hz) was performed throughout therapy by Miss F. Jabeen and Dr. P.G. Davey. Total urine collections were made and sent for β2-microglobulin and enzyme assays to assess renal damage. Full serum electrolyte and haematological profiles were performed and the results recorded together with the patient's sex, age, weight, height, race, previous medical history, diagnosis, course of illness, previous and current drug therapy (so far as is known) and any details of occupation or family history that might be pertinent to the possible toxic properties of aminoglycosides.

Serum and urinary creatinine levels were used to assess creatinine clearance but these results proved to be most erratic and clearly indicated incomplete urine collections, despite strenuous efforts to ensure a satisfactory procedure.

For toxicity testing a set of control patients were matched, so far as was possible, with the gentamicin-treated set for age, sex, weight and disease or surgical procedure. These patients were monitored for changes in indices of aural and renal function including serum creatinine levels.

A number of healthy volunteers were also monitored for auditory and vestibular function.

For reasons elaborated upon in section 7, a volunteer study was started to enable more detailed information to be obtained on the pharmacokinetics and toxicity of gentamicin in healthy male volunteers.

Subjects were to be screened for auditory and renal function and then given nine doses of 80 mg gentamicin at eight hour intervals. One dose on each day would be sampled eleven times as described above and all other doses would be sampled three times between two and four hours. The second of the full profiles would be performed on an im dose whereas the others would be done on iv infusions. Auditory threshold values would be determined at six frequencies (250 to 8000 Hz) every two hours during full profile doses and total urine collections made every two hours during daytime and every four hours at night.

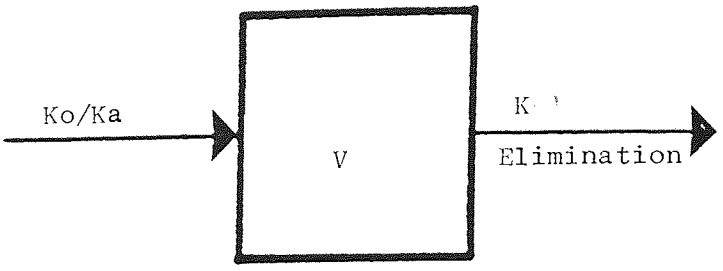
Subjects were to be treated in pairs until statistically significant information was obtained, or until it became apparent that the sample population required would be too large.

The first pair however, (ESH and PGD, caucasian males 30-35 years) developed a marked high frequency hearing loss within two hours of the first dose. This became progressively worse up to six hours and they were withdrawn from the trial, although the monitoring procedures were continued.

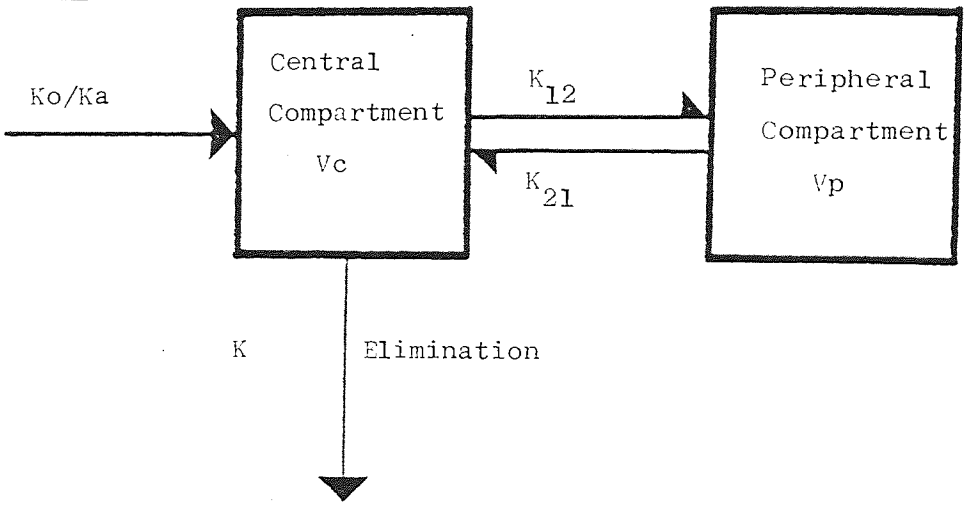
Despite the complete reversal of the hearing loss within a few hours, ethical permission to continue the trial was withdrawn because of this previously unreported acute onset and reversal of ototoxicity.

Investigations into the acute effects in patients who necessarily had to receive the drug were hampered by the conditions under which the tests were performed and were not conclusive. The trial was therefore abandoned.

1 - compartment model



2 - compartment model



K is the rate of elimination (first order, hr^{-1})

Ka is the rate of absorption (first order, hr^{-1})

Ko is the rate of infusion (zero order, mg/hour)

K_{12}, K_{21} are first order rate constants

V, Vc, Vp are the apparent volumes of the respective compartments

Arrows denote movement of drug.

Fig 4.2 Compartmental Models

4.3. Model

Compartmental models used in pharmacokinetics depend on estimating the rates at which the drug enters , leaves and distributes around the system.

Some of these rate processes are described by zero-order kinetics, that is the rate of the process is independent of the concentration of drug, e.g. intravenous infusion. (The rapid intravenous bolus is a special case where the duration of the infusion is held to be negligible).

Many biological processes exhibit first-order kinetics, that is, the rate of the process depends upon the concentration of drug or other material, viz;

$$\frac{dx}{dt} = -Kx \qquad (401)$$

where x is the amount of drug in the system, k is the first-order rate constant and t is time.

The rate of drug elimination by renal glomerular filtration is such a process, although renal excretion may not be first-order.

Integration of (401) gives,

$$x_t = x_o e^{-Kt} \qquad (402)$$

where the subscript denotes the time of measurement x.

Eq (402) may be transformed to give:

$$\ln x_t = \ln x_o - Kt \qquad (403)$$

In drug-body systems it is more convenient to consider concentrations, where the system volume = V and the resultant concentration (C) is denoted by

$$C = \frac{x}{V} \qquad (404)$$

Thus eq (402) becomes

$$C_t = C_o e^{-Kt} \qquad (405)$$

with volumes cancelling.

It is known that the body does not act as a uniform system in relation to a drug, but that drug concentration varies from one type of tissue to another (e.g. 128). The drug must move, therefore, from one tissue to another by (definable) rate-processes, and these processes are normally considered to be exponential (first-order) in form.

Serum may thus lose drug by first-order renal excretion and also by first-order deposition into muscle and possibly also by similar processes into numerous other tissues and tissue-types. Serum may also gain drug by similar processes from these tissues or from the site of an intramuscular injection.

Almost all drugs will require a multicompartment model to describe fully their kinetics, but many may be adequately described for purposes of administration by a one-compartment model. Gentamicin was long-held to be such a drug.

Metabolism of a drug may also appear to be a first-order process but will not be considered here since there is no evidence that aminoglycosides are metabolised.

The compartments may or may not correspond to any particular tissue or group of tissues but at least one (usually the central compartment in these mamilliary models) should correspond to the tissue that is sampled, normally blood, although that compartment may represent more than just the blood. If another tissue or fluid is sampled then at least one other compartment should correspond to that site. The effect of un-sampled compartments on the sampled compartment may well not be negligible, and may be large both in terms of quantity and physiological effect (e.g. intravenous barbiturate anaesthetics).

A two-compartment model, as in Figure 4.2, may be

described by two exponential terms, as given previously,

$$C = Ae^{-\alpha t} + Be^{-\beta t} \qquad (406)$$

4.4. Estimates of rate-constants

The parameters A, B, α and β are related to the parameters of the 2-compartment model by the equations:

$$\alpha\beta = K_{21}K_{10} \qquad (407)$$

$$\alpha+\beta = K_{12} + K_{21} + K_{10} \qquad (408)$$

$$A = \frac{D}{V}\frac{(\alpha-K_{21})}{(\alpha-\beta)} \qquad (409)$$

$$B = \frac{D}{V}\frac{(K_{21}-\beta)}{(\alpha-\beta)} \qquad (410)$$

In order to determine these parameters, one of two techniques may be used:

(1) Curve-stripping (described in Chapter 1)

(2) Nonlinear regression

The latter requires a digital computer of considerable size, or a long time on a smaller machine, and this was not thought possible with the computers and languages available. NONLIN (133) was available for batch processing on a CDC 7600 at UMRCC, Manchester, but the four-hour turn-round time was not acceptable. It was also found that it did not

fit bi-exponential curves well in the presence of experimental error. A good fit would be declared whilst still some way from the true parameters, and the parameters found to fit the data would depend on the initial estimates made by the operator. Similar observations, about NONLIN and other programs, have been published by others (134, 135).

It was also found that the NOVA 3 had such a limited core (approx. 12K bytes RAM) that the program transferred from HP2000 would not run and it had to be edited severely in order to make it small enough to run as a single program.

For reasons given in Chapter 2 it was not thought that two-compartmental anlysis was going to be important and so data were analysed using a 1-compartment model by means of linear least mean square regression on data two hours or more after dosing. At a later stage, however, it was necessary (Chapter 6) to divide the program into two, linked semi-automatically from within the programs, and this enabled the inclusion of a curve-stripping subroutine (Chapter 1).

For purposes of comparison, and for patients with no available blood-level data, calculations were also performed upon serum creatinine measurements. Of those nomograms listed in Chapter 3, those of Jadrny , Cockcroft and Gault, and Siersbaek-Nielsen et al (110, 111, 112) were employed. The conversion formula of Jeliffe (131) was used to convert creatinine clearance to gentamicin half-life but the Jeliffe nomogram itself was not used. This was because it was normalised to the body surface area, and required the patient's height as input. Although known for our trial patients, it was not thought that this would be generally available and there was not enough evidence of the method's superiority over others to justify its use.

4.5 Determination of volume of distribution

Initial estimates were made based on body weight or ideal body weight (Documenta Geigy) using the methods previously described. These were used throughout for creatinine calculations but also initially with regression-calculated values of K. This practice was superceded by the use of directly-calculated values of V, but the former methods were continued for purposes of comparison. In the final versions, 30 per cent of body weight was used only if a calculated V was not available.

V may be calculated from blood data in several ways.

4.5.1 Intercept of regression lines

For a 1-compartment model, the regression intercept from a single dose (I) is related to D and V by

$$V = \frac{D}{I} \quad \text{(iv bolus)} \tag{411}$$

$$V = \frac{D}{I}\left(\frac{Ka}{Ka-K}\right) \quad \text{(im)} \tag{412}$$

$$V = \frac{D}{I}\left(1-e^{-KT}\right)\frac{e^{KT}}{KT} \quad \text{(iv infusion of T hours duration)} \tag{413}$$

For multiple dosing the value I must be replaced by (I-Tr) where Tr is the trough level from previous doses. This may be evaluated using the multiple dosing functions

$$\left(\frac{1-e^{-nK\tau}}{1-e^{-K\tau}}\right) \quad \text{and} \quad \left(\frac{1-e^{-nKa\tau}}{1-e^{-Ka\tau}}\right)$$

where n is the number of doses and all doses were identical and given at constant intervals, or by the superimposition technique (128), or by measuring the trough value.

This method is used by Zaske, Sawchuk et al (136, 137) for 60 minute infusion in burns patients. We found, however, that for short infusions (ca 5 minutes) the mixing phase was significantly longer than the duration of infusion and so

levels sampled from the alternate limb to the infusion site were lower immediately after infusion than they were 15-30 minutes later. Thus, this method was felt to be invalid, was not used routinely and was deleted from the programs.

A variation of this method, possibly employed by Zaske et al but not so stated, is to estimate t^1 by regression from later data, assuming no mixing phase (Eq 414). This is essentially identical to (413) but accounts for preceding doses.

$$V = \frac{Do}{t^1 K} \quad \frac{(1-e^{-Kt^1})}{\hat{c}e^{K(E-t^1)}} - t^1 e^{-Kt^1} \qquad (414)$$

where $\hat{c}$ is the interpolated value of C at the mean (E) of the times used for regression analysis.

Use of the intercept without accounting for the pre-dose level may give significant errors, as may the use of the trough calculated (or measured) from the current dose (a labour-saving procedure that makes use of the same data required for calculated of K).

The latter procedure will only be satisfactory if the patient is at steady-state and the preceding dose was given at the correct time and in the correct quantity.

The timing of the preceding dose may well vary according to the strictness of the nursing procedures and examples are quoted in section 7 where there were discrepancies of several hours between the prescribed and administered time of dosing. The significance of such errors are discussed in Section 5.

4.5.2.Calculation of V from area under the serum concentration-time curve (AUC)

It may be shown (128) that for any linear model, V is related to the integral of the serum concentration-time curve, the dose and elimination rate:

$$V = \frac{D}{k \int_{t=0}^{\infty} C.dt} \qquad (415)$$

If an accurate estimate of the integral can be made, this is clearly the method of choice, although it may mean more blood samples (and more discomfort for the patient).

The integral $\int_{t=o}^{\infty} C.dt$ is capable of solution for several models;

1-compartment iv bolus

$$C = \frac{D}{V} e^{-Kt} \qquad \int_{t=o}^{\infty} C.dt = \frac{D}{VK} = \frac{Co}{K} \qquad (416)$$

1-compartment im

$$C = \frac{D\ Ka}{V\ Ka-K} (e^{-Kt} - e^{-Kat}) \qquad \int_{t=o}^{\infty} C.dt = \frac{D\ Ka}{V(Ka-K)} \left(\frac{1}{K} - \frac{1}{Ka}\right)$$

$$= \frac{D}{VK} = \frac{Co(Ka-K)}{KKa} \qquad (417)$$

1-compartment infusion

$$C = \frac{Ko}{VK}(1-e^{-KT})e^{-Kt^{1}} \qquad \int_{o}^{\infty} C.dt = \frac{KoT}{VK} = \frac{D}{VK}$$

$$= Co\ \left(\frac{T}{e^{KT}-1}\right) \qquad (418)$$

It should be noted that whilst all integrals may be reduced to D/VK, the value of Co is different in each case.

Whilst the analytical solution of the integral is theoretically calculable, in practice the information required may not be available or may be difficult to obtain (e.g. Ka) or not justified by the constraints of the models employed (e.g. t^{1}). Thus an iterative approach using observed data is indicated. The method of linear trapezoids, commonly employed (e.g. 128), assumes that the curve follows a straight line between observations and calculates the area under that portion of the curve as the product of the mean level and the time elapsed. The various areas are summated to approximate to the $\int_{t=o}^{t=\tau} C.dt$. No account is taken either of the effect of the preceding dose or of the area due to the current dose beyond the last observation.

(Modifications that may be made to this procedure to account for these factors are described below).

The linear technique will give errors if used on monoexponential data (described in detail in Section 5) and the method of splines overcomes this problem by use of n-1 simultaneous equations for n observations. It is, however, subject to wide fluctuations and great errors if the data is in error. The method of Lagrange uses quadratic, cubic and quartic equations solved simultaneously but is also liable to fluctuations between observations. Yeh and Kwang (138) conclude that whilst both are superior to linear trapezoidal techniques they require good quality data.

A further disadvantage is that they need considerable computing power and yet produce answers which are only a little better than simpler techniques.

One such simple technique is that of logarithmic trapezoids (e.g. Chiou (139)). Here a mono-exponential curve is assumed and the area for a given trapezoid is defined as

$$AUC_{1-2} = \frac{(C_1-C_2)(telapsed)}{\ln C_1 - \ln C_2} \qquad (419)$$

This is accurate provided the data is accurate and collected in the post-absorption, post-distribution phase.

A possible modification to reduce the effect of analytical error is to note that

$$\frac{telapsed}{\ln C_1 - \ln C_2} = \frac{1}{K} \qquad (420)$$

$$\text{Hence} \quad AUC_{1-2} = \frac{(C_1-C_2)}{K} \qquad (421)$$

K is the result of a number of observations and so any error due to one erroneous observation will be minimised.

The potential errors involved in using these various methods when data points are in error are described in Section 5 where it may be seen that the linear technique is least prone to error.

It was further noted that the logarithmic methods are not valid for the early part of the curve, where their assumptions are not true. Here, use of the linear technique for im data will yield some under-estimates of the area to part-compensate for later over-estimates (although that is not to suggest that the errors are equal in magnitude).

The errors may, however, be quite considerable if the time interval between observations is long. For an iv bolus or short infusion the error in assuming that the first observation is zero may also be considerable. These errors are analysed in section 5.

For the portion of the curve between zero-time and the first other observations, semi-analytical procedures were used.

For iv doses an extrapolation was performed using the calculated regression intercept and slope to calculate the level at the end of the infusion and the linear technique employed between that and the first observation. The area under the infusion was calculated analytically using the measured K and 30% of body weight for volume.

The area from the last observation to infinity was calculated by integral calculus using the measured K and assuming a mono-exponential curve (Eq 416). Initially the last serum level was used as Co but since this renders the result entirely dependent on the accuracy of this value, and the error thus incurred could be considerable, cf 5.55, the procedure was modified to use the regression coefficients and

calculate the integral between the last observation and infinity.

The relative importance of each part of the curve varies according to the rate constants and calculations are given in Section 5. The effect of the previous dose (if any) however, is not thus eliminated. To account for this, the pre-dose level is decayed according to the elimination rate constant determined from the current dose and the resultant levels are subtracted from each observation and from the regression-calculated intercept.

The possibility of measuring the total area between two doses, rather than the above procedure, was considered. This, however, makes two major assumptions.

(i) The patient is at steady state (ss) (or else the mean level calculated does not reflect the mean steady state level.

(ii) The doses are identical in magnitude and route and are evenly spaced.

The possible errors resulting from these assumptions are discussed in connection with the intercept calculations in section 5. The percentage errors for the trapezoidal segments, for the iv extrapolated segments and the im tail segment would all be the same as for the intercept approach. Only the semi-analytical first portion of the im curve will be unaffected. Thus a non-ss procedure must be used, preferably with a measured pre-dose level.

Intercept-based calculations were used as additional output in later programs.

Programs for the prediction of subsequent post-distribution-phase serum concentrations.

Whilst the estimation of distribution volume and appropriate rate constants had been accomplished by several methods,

the values varied considerably for any one patient (e.g., table 7.5) and a method of validation by prediction was sought.

Since serum samples could be taken subsequent to the first-studied dose, a 1-compartment model was used to predict post-distribution data by the super-imposition principle.

This method had the advantage that it employed the slope and zero-time intercept of the regression line and did not require any other estimate of the volume. It applied only to post-distribution data, however, and required that the dosing interval was constant, that the initial data was from the first dose and that a constant mode of administration was employed in addition to the previously-mentioned constraints of the 1-compartment model since it was a multiple dosing function to add the troughs from all previous doses to the current dose curve treated as a monoexponential.

Subsequently values of K estimated by the three creatinine methods were also used but the value of the zero time intercept was necessarily constant, since the creatinine did not involve regression analyses.

As anticipated from this theoretical flaw, the predictions using K from serum creatinine were rarely close to the serum level measured at the time chosen.

In an effort to find whether the patient characteristics had changed between the measured serum concentration profile and the predicted level, an iterative routine was established (Flow Chart 6.7)using K estimated from serum data.

From the initial prediction an estimate of the error between that and the measured value was made. If this exceeded 2% of the measured value, the elimination rate constant (K) was

modified in the appropriate direction by 2% and the calculation repeated. This was done 10 times or until the error was less than 2%, at which stage the correct value of K and the predicted serum level was output. If the error was still greater than 2% the value of A was altered by 10% and the procedure repeated, modifying K by up to a further ten intervals.

In practice, this was not a very satisfactory routine, for several reasons.

(1) Analytical error (not known at that stage) meant that 2% was far too narrow a tolerance.

(2) It was assumed that K could change whilst the intercept remained constant.

(3) It assumed that the change was instantaneous and effective throughout the course of treatment (an assumption known to be in error but probably reasonable in larger courses of treatment where a new value for K was maintained for several doses).

(4) Only post-distribution data could be used.

(5) The fit depended on predicting a single value. This could have been overcome by a least-squares fit to several points but in view of the other objections this was not attempted.

General Predictive routines

The super-imposition method was discarded in favour of analytical equations which described the whole of the curve but which required the appropriate rate constants and V.

Initially the iterative procedures were arranged such that predictions could only be made for one dose and one set of regression parameters. They were then expanded to perform

predictions for that one dose using the regression rate constant (with calculated V) and the three creatinine-calculated rate constants (with 'fixed-percentage' V) in turn.

Facilities were added to vary the dose interval for each of these values. It was often desirable, however, to predict levels in several different future doses and this was achieved by nesting the predictive loop in another loop giving the number of doses. Thus all predictions for any one set of parameters were performed before a new elimination rate was selected. This was rather restrictive, however, since for each prediction the dose and interval were requested by the program. The effectof previous doses was accounted for by using the trough after the last prediction.

This became very time-consuming to operate, became unwieldy in the number of arrays and variables required and used large quantities of output paper. It became most unmanageable when a routine for making calculations based on a continuously changing renal function were inserted. These did not work satisfactorily in all circumstances and a change in structure was felt necessary.

A new segment was written in which predictions were made using whatever parameters were required for any one dose before making predictions for further doses. This included all features of the earlier model except that each prediction (i.e. for several elimination rate constants) used the same data for dose, route and interval. In addition a value of K could be input from the keyboard, using the calculated volume of distribution. This method had very simple input requests, better formatted output and whilst it used more arrays it reduced the number of miscellaneous variables and was easier to

test and maintain.

Selection of data for regression and AUC calculations

On input each data pair was assigned a code-number from 1 onwards, in order of input. To select the values for use for regression the desired code-numbers were input and working arrays created from the back-up arrays using the codes held in their own array. This was thought to be better than setting arbitrary time limits for the selection of points. It was not felt worthwhile to shuffle the data into chronological order prior to assigning codes because the data would mostly be chronological anyway and it would entail either outputting a copy of the new order to the console VDU or forcing the operator to do mental re-ordering.

Traps were written into the input and AUC routines to warn the operator of non-chronological data or a non-zero initial point. Order is unimportant for linear regression.

Initially all points were used for AUC calculations.

To warn the operator of poor data, correlation coefficients and standard errors were calculated and output to the console. A graph of data and regression line was plotted on either the VDU screen or teletype roll using a discrete digital field system.

These devices were unfortunately not very sensitive to poor data, making it difficult to assess whether a poor correlation might be due to one point a long way from the best-fit line, all points having a small amount of scatter or data exhibiting two straight but dis-jointed lines (due to differences in the microbiological assay plates).

As the core became the limiting factor in further development, the graph plotting routine was removed, to be

revised and replaced at a later date when the program was subdivided. This time it used the matrix-printer as the output medium, providing a much more sensitive index due to the increased size of the plotting field. As it became apparent that not all data was equally reliable it was felt necessary to extend the selection facilities to enable the selection of codes for AUC calculations and this was achieved in a similar fashion to that for regression analysis. When two exponential terms were being fitted similar selection procedures were used for each term.

5. ERROR ANALYSES AND SIMULATION STUDIES

5.1 Use of 1-hour post-dose level as im peak level

Calculations were performed to find the peak time and concentration following im dosing and to compare these values with those found one hour after dosing in order to evaluate the common assumption that they are identical.

Single Dose Studies

For a single dose, the time of the concentration (tmax) may be calculated (128) as

$$tmax = \frac{1}{(Ka-K)}\left(\ln \frac{Ka}{K}\right) \qquad (501)$$

and the peak concentration (Cmax) as

$$Cmax = \frac{D}{V} e^{-Ktmax} \qquad (502)$$

(A special case occurs when Ka=K and a different approach is required).

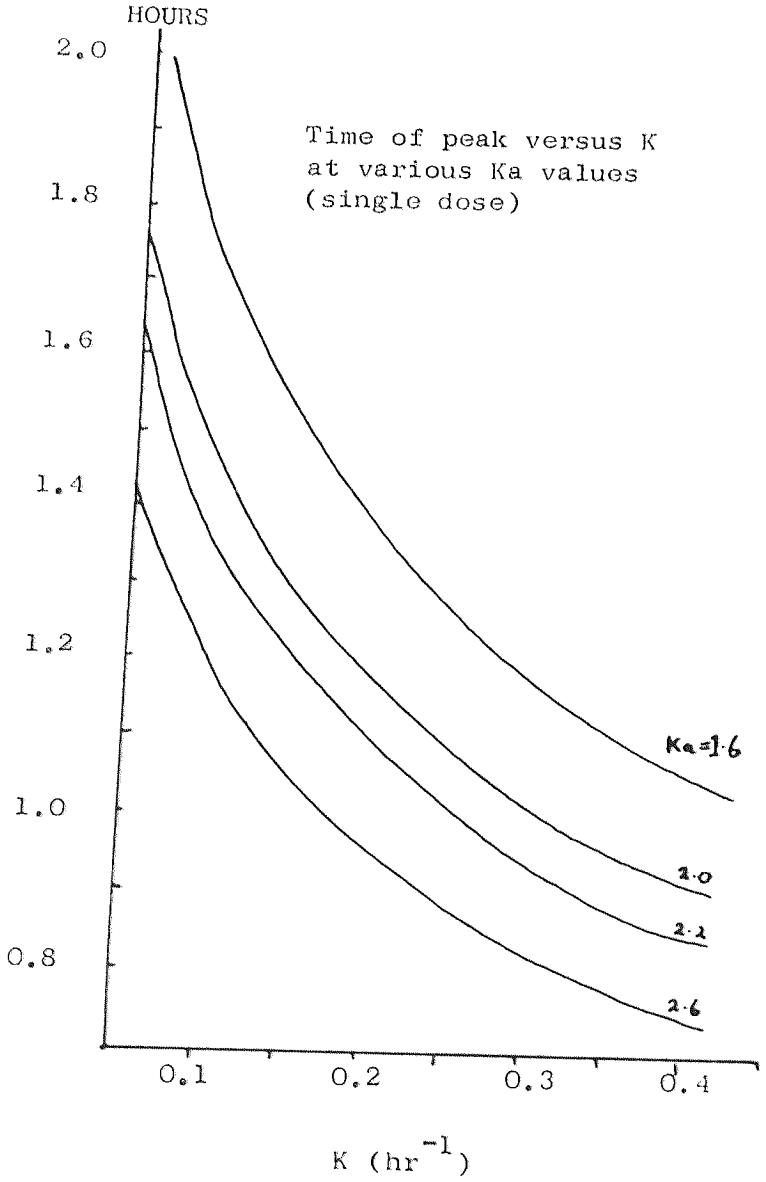
Using values of Ka and K shown by literature to be appropriate to gentamicin, tp and Cmax were calculated, as well as the level at one hour (C_1). Graphs of tp and the percentage error in using C_1 as Cmax,

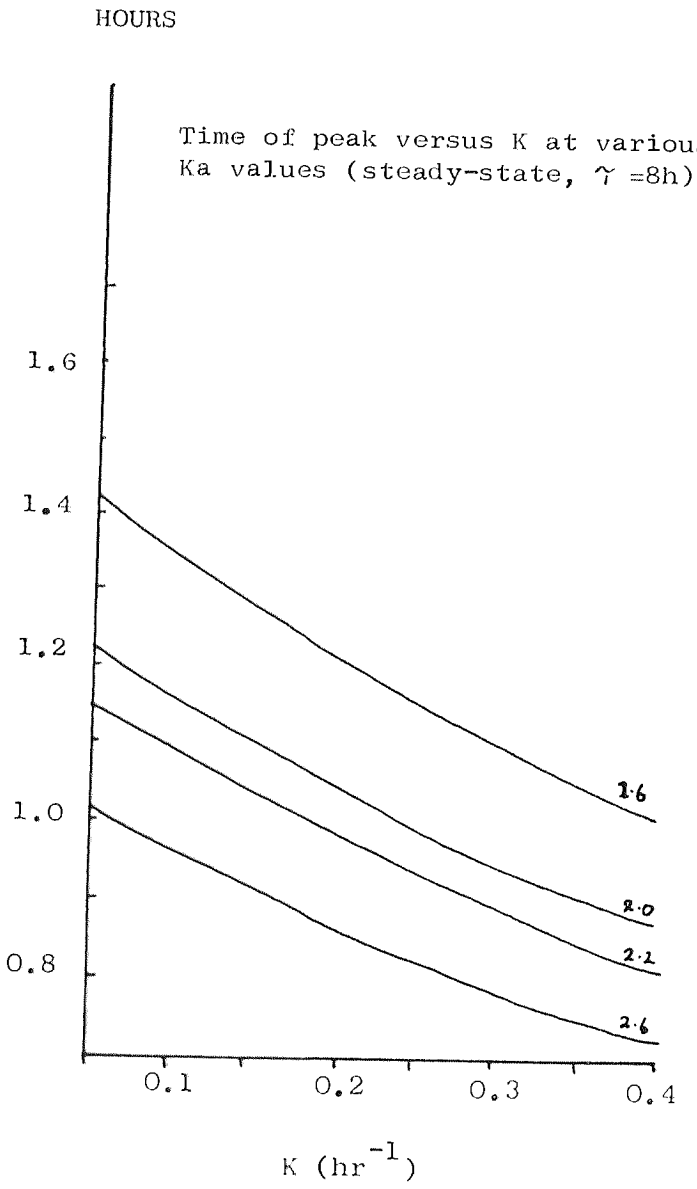
$$\% \text{ error} = \frac{Cmax-C_1}{Cmax} \times 100 \qquad (503)$$

are given in Figure 5.1.

It will be noted from Figure 5.1 that whilst tmax might well range from 0.85 to 1.9 hours in typical patients with tmax increasing rapidly as the ratio Ka/K increases, a plot of Cmax versus K appears paraboloid for any given value of Ka and the errors are quite small.

Almost all errors in humans would be less than 5% ($K > 0.1\ hr^{-1}$) and only 8% if Ka = 2.0 and K = 0.05. This compares favourably with intra-and inter-individual variations





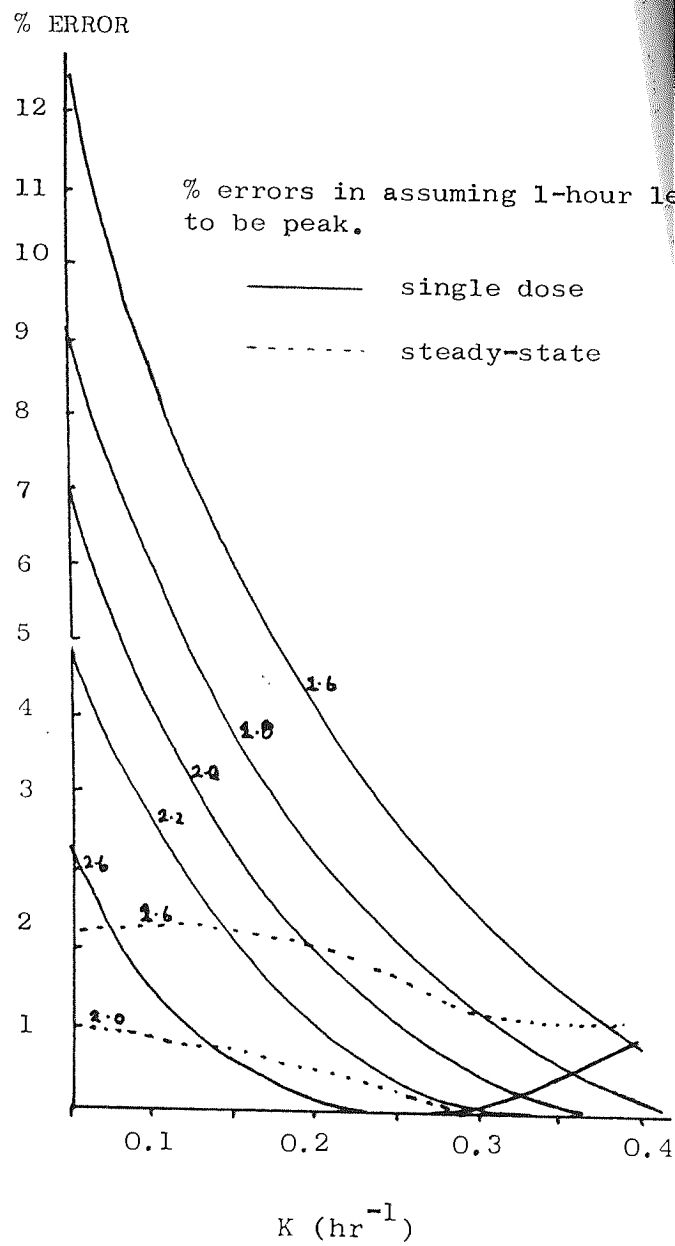


Fig 5.1 Simulations of im 1-compartment data

discussed in Chapters 1 and 7.

Steady-state Studies

The peak time and concentration at steady-state (or any multiple-dosing regimen) depends both on the current and previous doses.

Thus, at steady-state,

$$tmax = \frac{\ln(Ka(1-e^{-K\tau})/K(1-e^{-Ka\tau}))}{Ka-K} \qquad (504)$$

$$Cmax = \frac{D}{V}\left(\frac{1}{1-e^{-K\tau}}\right)e^{-Ktmax} \qquad (505)$$

tmax has a narrower range at common Ka, K values at steady state than after a single dose (0.83-1.22 hours for the range quoted above) and the error in using C_1 for Cmax is also greatly reduced, although the plots of error versus K are no longer simple (Figure 5.1). Errors would be much less than 2% for typical patients.

Thus, despite the great variation in tmax over a range of Ka, K values, the error in using a one-hour level would be minimal. This is probably due to the flattening of the plateau in the concentration-time curve as the peak occurs further from one hour (high Ka and low K or low Ka and high K)

5.2 The effect of route of administration on serum levels

In a 1-compartment system with 1st order absorption (e.g. im), drug levels in the first dose interval are represented by

$$C_{tim} = \frac{D}{V}\frac{Ka}{Ka-K}\left(e^{-Kt}-e^{-Kat}\right) \qquad (506)$$

As t increases, e^{-Kat} becomes negligible (absorption is complete and

$$C_{tim} = \frac{D}{V}\frac{Ka}{Ka-K}\left(e^{-Kt}\right) \qquad (507)$$

With intravenous bolus administration (regarded as an

instantaneous input)

$$C_{tiv} = \frac{D}{V} e^{-Kt} \tag{508}$$

It is clear therefore that the ratio between the levels resulting from an im and an iv dose to the same patients will be:

$$\frac{C_{tiv}}{C_{tim}} = \frac{\frac{D}{V}(e^{-Kt})}{\frac{D}{V}(\frac{Ka}{Ka-K})(e^{-Kt})} \quad \text{when } e^{-Kat} \text{ is negligible} \tag{509}$$

thus

$$\frac{C_{tiv}}{C_{tim}} = \frac{Ka-K}{Ka} \tag{510}$$

Similarly, if e^{-Kat} is not negligible,

$$\frac{C_{tiv}}{C_{tim}} = \frac{Ka-K}{Ka(1-e^{-(Ka-K)t})} \tag{511}$$

The ratios for uniform multiple dosing may be derived:

$$C_{tim} = \frac{D}{V}\frac{Ka}{Ka-K}\left(\frac{1-e^{-nK\tau}}{1-e^{-K\tau}} e^{-Kt} - \frac{1-e^{-nKa\tau}}{1-e^{-Ka\tau}} e^{-Kat}\right) \tag{512}$$

$$C_{tiv} = \frac{D}{V}\left(\frac{1-e^{-nK\tau}}{1-e^{-K\tau}} e^{-Kt}\right) \tag{513}$$

Hence,

$$\frac{C_{tiv}}{C_{tim}} = \frac{Ka-K}{Ka}\left(\frac{1}{1-\frac{(1-e^{-nKa\tau})(1-e^{-K\tau})e^{-Kat}}{(1-e^{-nK\tau})(1-e^{-Ka\tau})e^{-Kt}}}\right) \tag{514}$$

If n is large, (i.e., dosing at steady-state such that $n\tau > 5t_{\frac{1}{2}}$)

$$\frac{C_{tiv}}{C_{tim}} = \frac{Ka-K}{Ka}\left(\frac{1}{1-\frac{(1-e^{-K\tau})e^{-Kat}}{(1-e^{-Ka\tau})e^{-Kt}}}\right) \tag{515}$$

If t is large enough for e^{-Kat} to be negligible, then $e^{-Ka\tau}$ will also be negligible and

$$\frac{C_{tiv}}{C_{tim}} = \frac{Ka-K}{Ka} \tag{516}$$

Thus the same ratio would obtain for the latter part of any dose interval, whether the first or at steady-state.

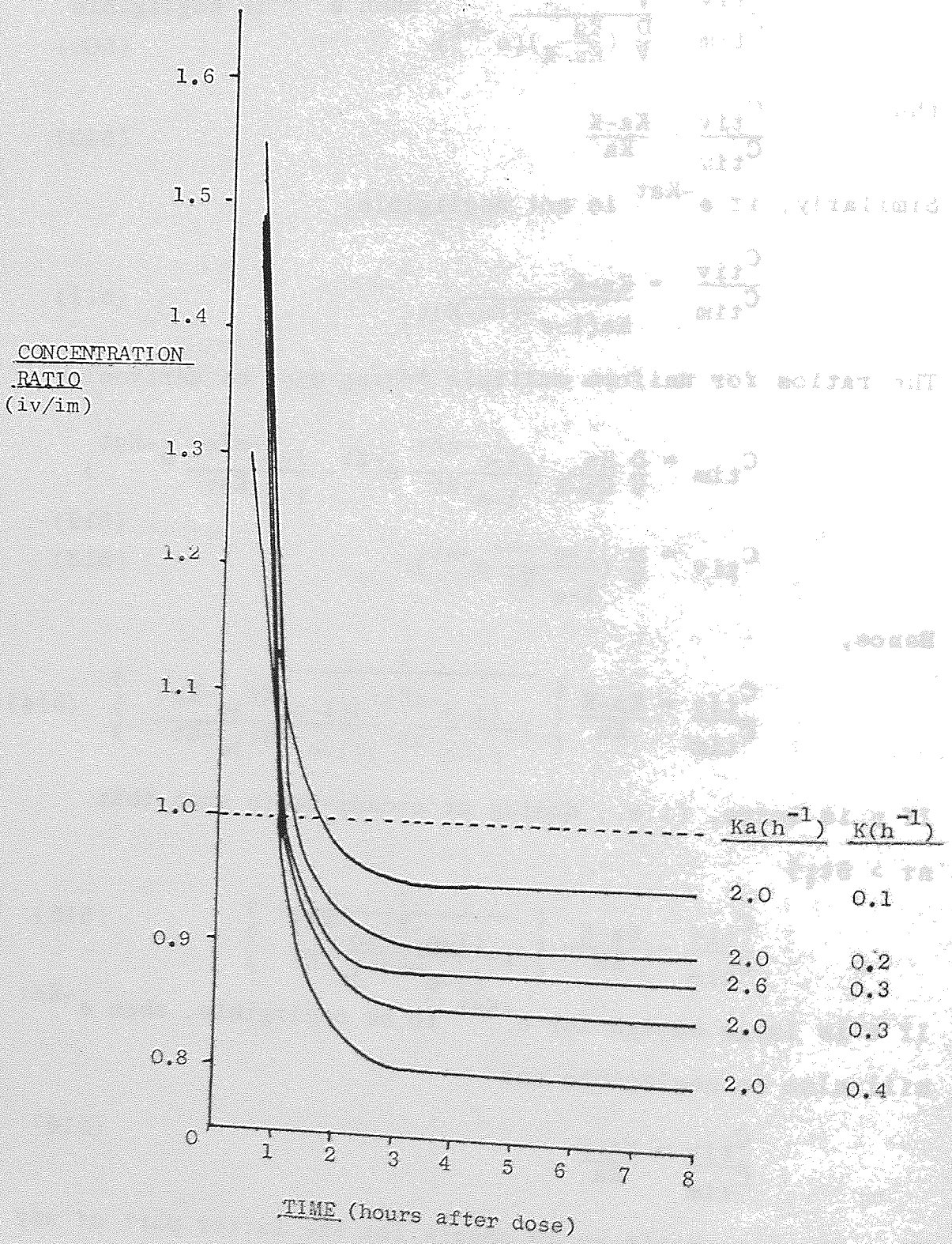


Fig 5.2 Ratio of iv concentration to im concentration from a single dose at various Ka and K values

Data were generated using 510, 511, 514 and 516 to determine the importance of correct analysis of the blood-level data, particularly with regard to AUC calculations (and hence the mean steady-state level).

Results for typical values of Ka and K are plotted in graphs 5.2 and 5.3 and listed in Table 5.1.

The discrepancies between steady-state im and iv levels in one subject becomes more significant as Ka is reduced and as K increases. At the average values of each parameter, from our study, there is a 17% difference between steady-state, post-absorption levels due to im and iv dosing and discrepancies of more than 20% would not be unexpected at other common parameter values. It is clear, therefore, that separate calculations are required for the two routes of administration, both for predictive purposes and also for interpolation in AUC calculation. The latter procedure is crucial in the period immediately after a dose, before the first serum level (cf. section 5.5). The errors in this region of the profile may be even larger, as much as 50% in typical cases.

Use of one hour and eight hour levels to determine K following im administration will produce significant errors, quite apart from the contribution of analytical error, due to the incomplete absorption at one hour. Data are given in Table 5.1, and discussed further in Section 5.3.2.

Ka (h^{-1})	K (h^{-1})	% differences in levels ((Civ-Cim)/Cim) 0.5h (1st dose)	8h (steady-state)	1 hour iv and 3 hour im levels to calculate K
2.0	0.1	54.9	- 5.0	-23.0
2.0	0.2	51.7	-10.0	-13.0
2.0	0.3	48.4	-15.0	- 9.3
2.0	0.4	45.3	-20.0	- 8.5
2.2	0.1	46.8	- 4.5	-18.8
2.2	0.2	43.8	- 9.1	-10.6
2.2	0.3	40.8	-13.6	- 7.5
2.2	0.4	37.9	-18.2	- 6.6
2.6	0.1	34.8	- 3.8	-12.1
2.6	0.2	32.1	- 7.7	- 6.6
2.6	0.3	29.4	-11.5	- 5.3
2.6	0.4	26.8	-15.4	- 4.4

Table 5.1 Discrepancies between serum levels from two routes of administration

5.2.2. im and iv infusions

A similar approach may be adopted for comparing iv infusions with im doses.

If

$$C_{tinf} = \frac{D}{V}\left(\frac{1}{KT}\right)(1-e^{-KT})e^{-Kt'} \qquad (517)$$

where T is the duration of the infusion and t' the time elapsed from the end of the infusion to sampling (128), then the multiple-dosing equation for the nth uniform dose is:

$$C_{tinf} = \frac{D}{VKT}(1-e^{-KT})\left(\frac{1-e^{-nK\tau}}{1-e^{-K\tau}}\right) e^{-Kt'} \qquad (518)$$

The equivalent of equation (514) may be derived as

$$\frac{C_{tinf}}{C_{tim}} = \frac{Ka-K}{KaKT}\left(\frac{(1-e^{-KT})\frac{e^{-Kt'}}{e^{-Kt}}}{\left(1 - \frac{(1-e^{-nKa\tau})(1-e^{-K\tau})e^{-Kat}}{(1-e^{-Ka\tau})(1-e^{nK\tau})e^{-Kt}}\right)}\right) \qquad (519)$$

If n is large then

$$\frac{C_{tinf}}{C_{tim}} = \frac{Ka-K}{KaKT}\left(1 - \frac{(1-e^{-KT})\frac{e^{-Kt'}}{e^{-Kt}}}{\frac{(1-e^{-K\tau})e^{-Kat}}{(1-e^{-Ka\tau})e^{-Kt}}}\right) \qquad (520)$$

If t is large enough for e^{-Kat} to be negligible,

$$\frac{C_{tinf}}{C_{tim}} = \frac{Ka-K}{Ka}\left(\frac{1-e^{-KT}}{KT}\right) e^{KT} \qquad (521)$$

This also leads to a value for the ratio,

$$\frac{C_{tinf}}{C_{tiv}} = \frac{(1-e^{-KT})e^{KT}}{KT} \qquad (522)$$

Values of this ratio are given in Table 5.2. from which it is clear that for short infusions (e.g. 5 minutes) the difference between iv bolus and iv infusion serum levels will be small but near to im values. This is to be expected since both im and iv infusion routes represent

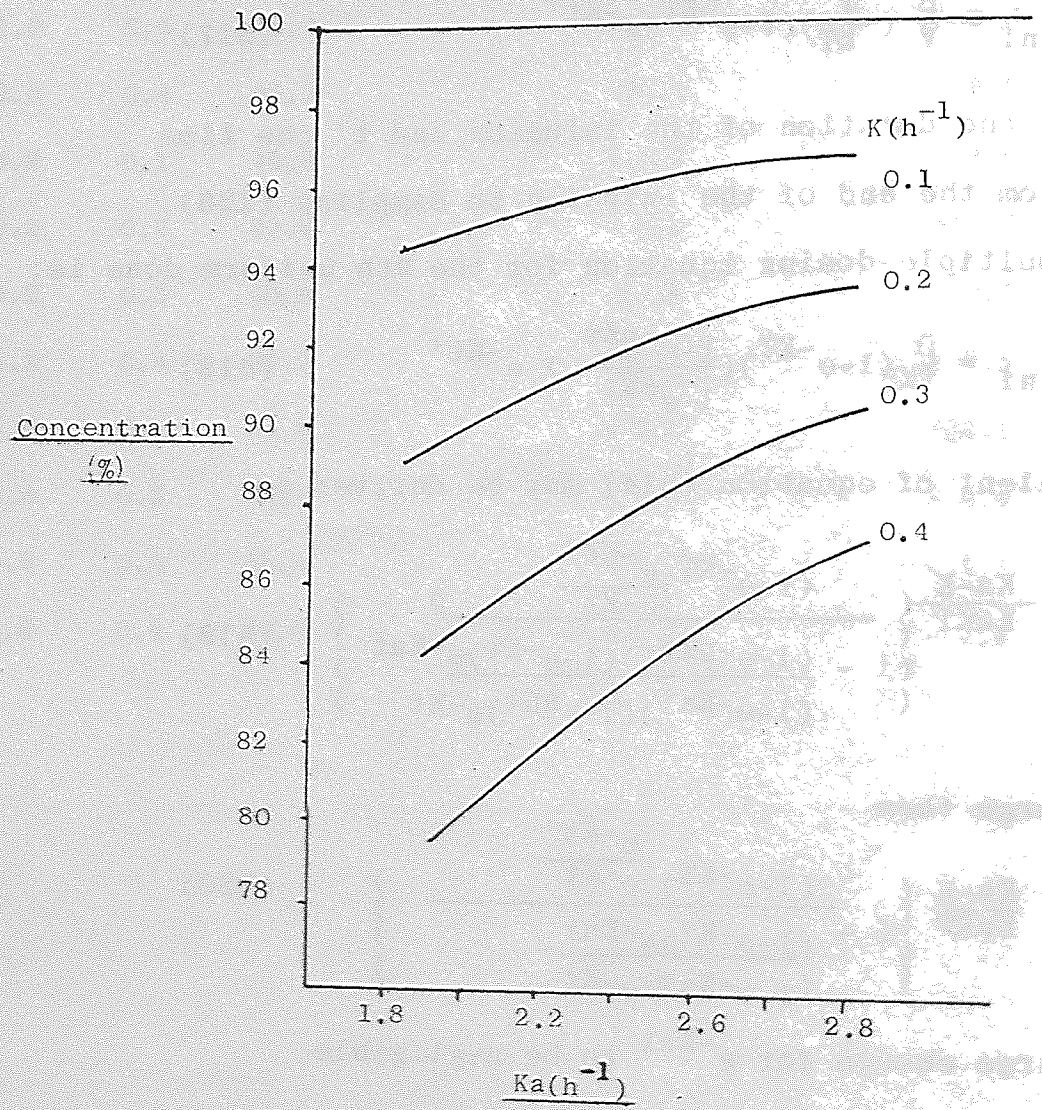


Fig 5.3 Concentration 8 hours after 5th iv dose as percentage of equivalent im level at various values of K and Ka.

K (hr^{-1})	T (mins)	$\frac{Ctinf}{Ctiv}$ (%)
0.1	5	100.41
	10	100.84
0.2	5	100.84
	10	101.71
0.3	5	101.26
	10	102.54
0.4	5	101.71
	10	103.41
	20	106.97

Table 5.2 Ratio of levels following iv infusion and bolus doses at steady state and large values of t

a deviation from the instantaneous input of an iv bolus.

Simulations were performed to verify the relationship and examine the likely effects on parameter estimations.

5.3. Determination of parameters from doses administered by different routes

5.3.1. Determination of K, AUC and V from iv bolus data by iv infusion techniques.

Although a dose may be intended as an iv infusion over a given length of time (5 minutes in this trial) it is difficult to gauge the time exactly and so the time may vary by a few minutes. It may also happen that a bolus or very rapid infusion be administered, by accident or design, and it may be necessary to account for this in the pharmacokinetic procedures employed.

To evaluate this possibility, simulations were performed, analysing iv bolus data by an infusion technique with 1-10 minute infusions, values of K from 0.05 to 0.4 h^{-1} and times for the first sampling point ranging from 15 to 60 minutes.

Since the determination of K will be unaffected by the mode of input, provided determination commences after input is complete, only errors in the calculation of AUC will adversely affect clearance or volume values.

Table 5.3 gives values selected to show that using a

Duration of infusion (T) (mins)	K (hr^{-1})	First point (t) (mins after dose)	AUC inf. / AUC bolus (%)	AUC inf (T mins) / AUC inf (5 mins) (%)
1	0.3	30	99.77	100.93
3	0.3	30	99.31	100.46
3	0.4	15	99.05	100.64
3	0.4	30	99.10	100.60
5	0.05	30	99.79	-
5	0.1	30	99.59	-
5	0.2	30	99.21	-
5	0.3	30	98.85	-
5	0.4	15	98.42	-
5	0.4	30	98.51	-
5	0.4	45	98.59	-
5	0.4	60	98.67	-
7	0.3	30	98.39	99.53
7	0.4	15	97.80	99.37
7	0.4	30	97.91	99.39
9	0.1	15	99.26	99.77
9	0.4	15	97.17	98.73

Table 5.3 AUC calculated by infusion techniques from iv bolus data

five-minute infusion equation the maximum error in determining AUC on iv bolus data is less than 1.6%, with an unusually high K and an early first sample. The difference between that calculation and one assuming a three-minute infusion is 0.64%, and 0.63% compared with a seven-minute infusion. Since these are maximum likely errors, and most patients would exhibit parameters which would reduce the error considerably, it seemed acceptable to use a single five-minute infusion equation for all iv doses, to reduce the core requirement and complexity of the program and to reduce the operator input. Also, in practice, the operator may well be unaware of the precise duration of the infusion, or even whether or not it was an infusion rather than a bolus.

5.3.2. Calculation of K from im and iv data

.1 regression analysis

The best-fit slope of a linear least mean squares regression line may be denoted as;

$$K = \frac{\Sigma(x_i-\bar{x})y_i}{\Sigma(x_i-\bar{x})^2} \qquad (523)$$

where x_i and y_i are the ith values for time and concentration respectively and $\bar{x}$ is the mean time for the data set (143). (The summation sign is assumed throughout this chapter to apply to all values of the subscripted variable).

This expression may be reduced by setting

$$A_i = x_i-\bar{x} ,$$

$$\text{thus } K = \frac{\Sigma\ A_i y_i}{\Sigma\ A_i^2} \qquad (524)$$

$$\text{If } R_i = \frac{e^{-Kat_i}}{e^{-Kt_i}}$$

and the formulae 506 and 502 describe the serum levels after single im and iv injections respectively, then,

$$K_{im}-K_{iv} = \frac{\Sigma\ \text{In}(\frac{D}{V}\frac{Ka}{(Ka-K)}(e^{-Kt_i}-e^{-Kat_i}))Ai-\Sigma\ \text{In}(\frac{D}{V}e^{-Kt_i})Ai}{\Sigma A_i^2} \qquad (525)$$

$$= \frac{\Sigma\ Ai\{\text{In}\ \frac{D}{V} + \text{In}\ (\frac{Ka}{Ka-K}) + \text{In}(1-Ri)-Kt_i-\text{In}\ \frac{D}{V} + Kt_i\}}{\Sigma\ A_i^2} \qquad (526)$$

where Kim and Kiv are the values of K calculated from im and iv data respectively using identical sampling times. This simplifies to,

$$K_{im} - K_{iv} = \frac{\Sigma Ai\{\text{In}(\frac{Ka}{Ka-K}(1-Ri))\}}{\Sigma A_i^2} \qquad (527)$$

If, instead of comparing im and iv data, one compares im data during absorption with post-absorption im data, then equation (527) becomes,

K	Initial time	Interval	Ka=1.8 n=3	Ka=1.8 6	Ka=2.2 3	Ka=2.2 6	Ka=2.6 3	Ka=2.6 6
0.1	1.5	1	66.9	65.3	38.5	37.2	21.9	20.9
0.2	1.5	1	38.3	37.5	22.1	21.5	12.6	12.1
0.35	1.5	1	26.7	26.2	15.6	15.2	8.9	8.6
0.2	2.5	1	7.4	7.2	2.9	2.8	1.1	1.1
0.35	2.5	1	5.9	5.8	2.4	2.3	0.9	0.9
0.1	2.0	2	16.4	15.2	7.4	6.7	3.4	2.9
0.2	2.0	2	10.0	9.3	4.5	4.1	2.0	1.8
0.35	2.0	2	7.6	7.2	3.5	3.2	1.6	1.4
0.1	1.0	2	97.8	89.8	64.4	57.7	42.5	37.1
0.35	1.0	2	36.3	33.8	23.9	21.7	15.7	14.0
0.2	1.5	3	15.7	13.8	8.5	7.2	4.6	3.8
0.35	1.5	3	11.3	10.1	6.1	5.2	3.3	2.7
0.1	1.0	4	50.4	41.4	32.6	25.9	21.4	16.5
0.2	1.0	4	28.1	23.3	18.2	14.5	11.9	9.2
0.35	1.0	4	19.0	16.0	12.9	9.9	7.9	6.2

Table 5.4 Percentage errors in estimate of K from n im data points, assuming absorption to be complete at time of sampling

$$K_{imabs} - K_{impost-abs} = \frac{\Sigma Ai\{\ln(1-R_i)\}}{\Sigma A_i^2} \qquad (528)$$

and hence the percentage error (E) becomes;

$$E = \frac{\Sigma Ai\{\ln(1-R_i)\}}{K\ \Sigma A_i^2} x100 \qquad (529)$$

Values of E, for various values of K, Ka, n (number of sampling points) and ranges of t are given in Table 5.4

It will be noted that, whilst the formulae are too complex to reduce the dependence of E on any one of these parameters to a simpler form, the following criteria are met by these formulae:

1. The error decreases as:
   either K increases,
   or Ka increases,
   or the sampling period moves away from dosing (the mean time and the first point are important here),
   or the number of sampling points in a given period increases.
2. The estimate of K is always less negative than the true K.

Figures 5.4 and Table 5.4 illustrate these features.

.2 2-point analysis

An alternative to the regression analysis approach is to consider using only two points for calculation of the slope.

Thus if equation (506) is reduced to

$$C = A_o(e^{-Kt} - e^{-Kat}) \qquad (530)$$

where $A_o = \frac{D}{V} \frac{Ka}{(Ka-K)}$

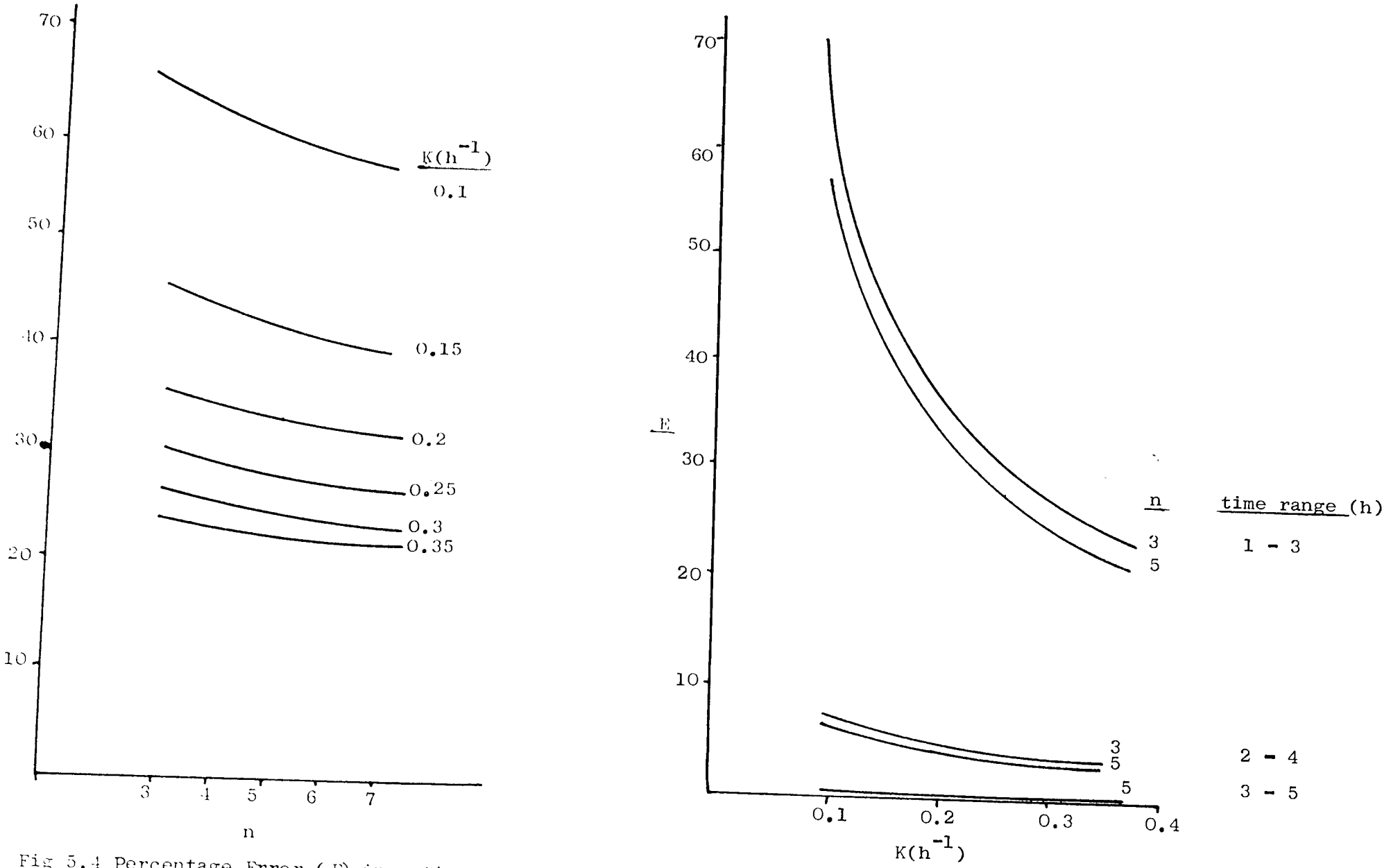


Fig 5.4 Percentage Error (E) in estimate of K from im doses (Ka = $2.2h^{-1}$) using n serum levels, assuming absorption to be complete

then the calculated slope (K^1) may be expressed;

$$K^1 = \frac{\ln(Ao(e^{-Kt_1}-e^{-Kat_1}))-\ln(Ao(e^{-Kt_2}-e^{-Kat_2}))}{t_2-t_1} \qquad (531)$$

where subscripts note order of sampling points.

If $R_i = \frac{e^{-Kati}}{e^{-Kt_i}}$

rearrangement of (531) gives:

$$K^1 = \frac{\ln((1-R_1)e^{-Kt_1})-\ln((1-R_2)e^{-Kt_2})}{t_2-t_1} \qquad (532)$$

$$= \frac{\ln(\frac{1-R_1}{1-R_2}) + K(t_2-t_1)}{t_2-t_1} \qquad (533)$$

$$= \frac{\ln(\frac{1-R_1}{1-R_2})}{t_2-t_1} + K \qquad (534)$$

Hence

$$\frac{K^1-K}{K} = \frac{\ln(\frac{1-R_1}{1-R_2})}{K\ (t_2-t_1)} \qquad (535)$$

and thus the percentage error (E) is

$$E = \frac{\ln(\frac{1-R_1}{1-R_2})}{K\ (t_2-t_1)} \times 100\ \%$$

This relationship also satisfies the necessary criteria:

1. E decreases as:
   either Ka increases
   or K increases
   or t_2 increases
   or (t_1-t_2) increases
2. As Ka → K; E → 0
3. K^1 is always less negative than K.

The results from these calculations are very similar to those of the regression technique, and may be derived directly from (529), assuming n = 2, although this is not a practicable

procedure because regression analysis fails if n = 2.

For both techniques sampling before two hours will lead to errors greater than 10% unless Ka, K and (t_1-t_2) are all large, but these cases are rare in practice.

Commencing sampling at two hours will usually give less than 10% error, but only if the second sample is taken at four hours or later and Ka > 2. (Again, exceptions occur but only at very low values of K where this rapid procedure would not be necessary).

Commencing at 2.5 hours and sampling for at least one hour gives E < 10% and 2.5 to 5 hours gives errors less than 5% for all common combinations of Ka and K.

Similarly samples taken at three hours or later, even over only one hour, will be satisfactory, with only a few (rare) combinations of Ka and K giving E > 5%. No patients in our study would have been thus affected. Sampling from three to five hours (E = 2.96 at K = 0.1, Ka = 1.8, n = 3) appears to be satisfactory under all conceivable circumstances, although sampling in anuric or severely impaired patients would normally require a longer sampling interval (see Section 5.6).

5.4 The effect of preceding doses on the measured dose

Since gentamicin is not totally excreted from the body in one dosing interval, the monitored levels will represent the summation of the residual levels from all previous doses. Incorrect assumptions about preceding doses with regard to their timing or route would produce errors in predictions of measured levels. There will be no error in estimates of K providing they are estimated by regression analysis, since slopes of curves, rather than absolute values, are important for these estimates. If V is estimated from the regression intercept, however, then the absolute values are important and errors may be made. The effect of the route and timing of preceding doses are outlined in the following sections, particularly in the light of known nursing errors in the timing of doses.

5.4.1 Assumption that preceding dose is of a given type

For predictive purposes, using multiple dose formulae, it is convenient to assume that all non-monitored doses are of a given type, i.e. all iv or all im. One might assume that they were of the same type as the monitored dose (e.g. all iv) or one might assume that they were all of one type, regardless of the type of monitored dose (e.g. all im, but monitored dose is iv). This may, however cause some error if the wrong type is selected.

From equation (516) we find that the ratio of trough levels from im and iv doses (R) is,

$$R = \frac{Ka}{Ka-K} \tag{537}$$

The ratio of an iv bolus zero-time intercept to the trough level (single dose study) (Rp) is given as

$$Rp = \frac{\frac{D}{V} e^{-K.0}}{\frac{D}{V} e^{-K\tau}} \tag{538}$$

Hence,

$$Rp = \frac{1}{e^{-K\tau}} \tag{539}$$

Thus the intercept from the second iv bolus (I_2) will be

$$I_2 = (1 + \frac{1}{e^{-K\tau}})(\text{trough level}) \tag{540}$$

If the preceding dose was not iv, but im, then from (537) and (540)

$$I_2' = (\frac{Ka}{Ka-K} + \frac{1}{e^{-K\tau}})(\text{trough level}) \tag{541}$$

where I'_2 is the zero-time intercept of the second dose.

Combining (540) and (541) and eliminating common terms, we find that

$$\frac{I_2' - I_2}{I_2} = \frac{\frac{Ka}{(Ka-K)} + \frac{1}{e^{-K\tau}} - 1 - \frac{1}{e^{-K\tau}}}{(1 + \frac{1}{e^{-K\tau}})} \tag{542}$$

Ka	K	$\frac{Ka}{Ka-K}$	$\frac{1}{e^{-K\tau}}$	% error
1.8	0.1	1.0588	2.22554	1.82
	0.35	1.2414	16.4446	1.38
2.0	0.1	1.0526	2.2255	1.63
	0.35	1.212	16.445	1.215
2.2	0.1	1.0476	2.2255	1.476
	0.35	1.1892	16.445	1.084
2.4	0.1	1.0435	2.2255	1.349
	0.35	1.1707	16.445	0.978
2.6	0.1	1.040	2.2255	1.240
	0.35	1.1555	16.445	0.8914

Table 5.5 Error in prediction of levels following on iv bolus due to use of im formulae instead of iv formulae for preceeding dose

Hence,

$$\text{Percentage error} = \frac{\frac{Ka}{Ka-K} - 1}{1 + \frac{1}{e^{-K\tau}}} \times 100\% \qquad (543)$$

(Section 5.2.1 shows that this error, as a percentage, will be constant throughout the dose interval).

Table 5.5 shows that these errors would be 1-1.5% in most cases. At steady state equation (543) will still apply because the multiple dosing function-

$$\left(\frac{1-e^{-nK\tau}}{1-e^{-K\tau}} \right)$$

will apply to the numerator and denominator and so cancel out. Thus using the wrong dose type would produce a small, but finite, error that is best avoided.

5.4.2. Errors in regression intercept due to errors in measurement of pre-dose level or to nursing error

As discussed in Section 4, it would be desirable to use the regression intercept to calculate V, rather than take extra samples to dermine AUC. The intercept, however, will include the trough due to the previous doses (unless measuring the first dose) and whilst the effect of this can be calculated and allowed for, any error in assay, or in timing of the previous dose, where the trough is not measured, will produce errors in the intercept and hence errors in V.

Calculations were performed to assess these errors as follows.

Assuming that a given dose should cause an intercept of D, which together with the preceding trough (Tr) gives a gross intercept of D_N, and that an error E, such that $\varepsilon = E/Tr$,

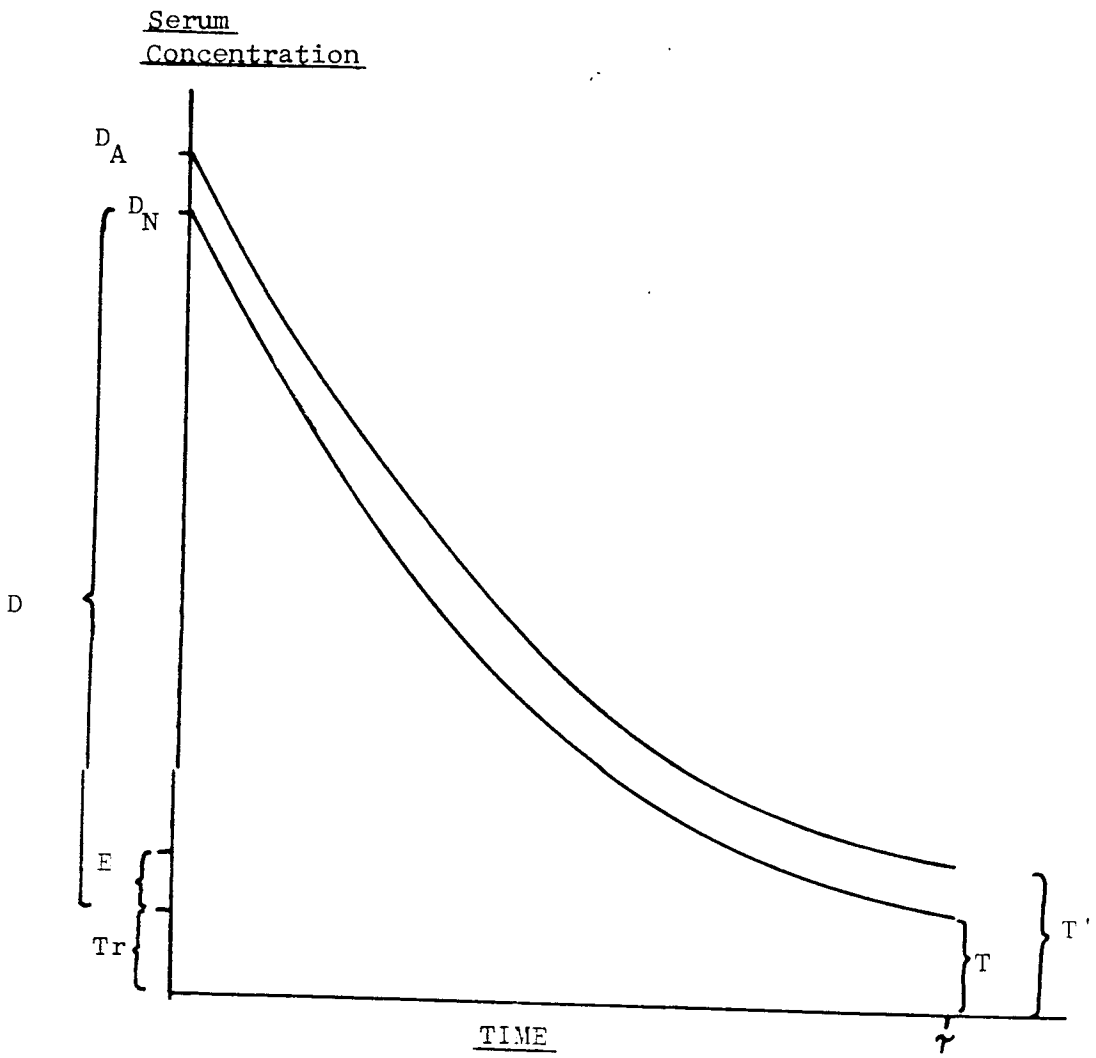


Fig 5.5 Profile of iv dose as analysed in section 5.4

increases the intercept to D_A and the post-dose trough from T to T' (illustrated in Figure 505) then the following equations are defined:

$$D_N = D + Tr \tag{544}$$

$$D_A = D + Tr + E = D + Tr\ (1+\varepsilon) \tag{545}$$

$$T' = D_A e^{-K\tau} \tag{546}$$

$$T = D_N e^{-K\tau} \tag{547}$$

Hence,

$$T' = \frac{T.D_A}{D_N} = \frac{T(D + Tr(1 + \varepsilon))}{D + Tr} \tag{548}$$

and

$$\frac{D_A}{D_N} = \frac{D + Tr(1 + \varepsilon)}{D + Tr} = 1 + \frac{E}{D + Tr} \tag{549}$$

Now, using D_A to calculate T', instead of D_N to yield T, and subtracting T' from D_A (intended to yield D, but actually yielding $(D_A - T')$ then from (545) and (548)

$$D_A - T' = D + Tr(1 + \varepsilon) - \frac{T\ (D + Tr\ (1 + \varepsilon))}{D + Tr} \tag{550}$$

Since the true intercept (having discounted previous doses) is D, the error (Er%) in using $D_A - T'$ is,

$$Er = \frac{D + Tr(1 + \varepsilon) - \frac{T\ (D + Tr(1+ \varepsilon))}{D + Tr} - D}{D} \times 100 \tag{551}$$

Cancelling and rearrangement gives

$$Er = \frac{(D + Tr)(1 + \varepsilon)\ Tr - TD - TTr(1 + \varepsilon)}{D\ (D + Tr)} \times 100 \tag{552}$$

$$= \frac{Tr\ (1 + \varepsilon)(D + Tr-T) - TD}{D\ (D + Tr)} \times 100 \tag{553}$$

At any given values of K and τ,

$$\text{let } R = e^{K\tau} \tag{554}$$

Thus from (547) and (554),

$$T = \frac{D_N}{R} \tag{555}$$

and $D = RT - Tr$ (556)

If the subject is not at steady-state but has had a constant dosage regimen, then

$$T = FTr \tag{557}$$

where

$$F = \frac{X_o \left(\frac{1-e^{-nK\tau}}{1-e^{-K\tau}}\right) e^{-K\tau}}{X_o \left(\frac{1-e^{-(n-1)K\tau}}{1-e^{-K\tau}}\right) e^{-K\tau}} \tag{558}$$

$X_o = \frac{D}{V}$ for iv boluses or $\frac{D\ Ka}{V(Ka-K)}$ for im administration,

or $\frac{D(1-e^{-KTinf})}{VKTinf}$ for iv infusions.

assuming $e^{-Ka\tau}$ is negligible for im administrations.

This expression may be reduced to

$$F = \frac{1-e^{-nK\tau}}{1-e^{(1-n)K\tau}} \tag{559}$$

Thus $D = RFTr - Tr$ (560)

By substitution from (557) and (560) for T and D, equation (553) becomes,

$$Er = \frac{Tr(1+\varepsilon)(Tr + RFTr - Tr - FTr) - FTr(RFTr - Tr)}{(RFTr - Tr)(RFTr - Tr + Tr)} \times 100 \tag{561}$$

Cancelling our T_r^2F and rearranging gives

$$Er = \frac{(1+\varepsilon)(R-1) - (RF-1)}{(RF-1)R} \times 100 \tag{562}$$

$$= \frac{R + \varepsilon R - 1 - \varepsilon - RF + 1}{(RF-1)R} \times 100 \tag{563}$$

$$= \frac{\varepsilon(1 - \frac{1}{R}) + 1 - F}{RF - 1} \times 100 \tag{564}$$

This equation represents the error in the intercept when using a trough level (T'), whether measured or calculated, in place of the true pre-dose level (εTr) when not at steady-state.

To verify this equation, let $\varepsilon = 0$ and $F = 1$, whence Er becomes zero, as is indeed the case at steady-state with correct timing. If at steady-state ($F = 1$) but with nursing error then

$$Er = \frac{\varepsilon(1 - \frac{1}{R})}{R - 1} \times 100 = \frac{\varepsilon}{R} \times 100 \qquad (565)$$

This may be verified by setting $T = Tr$ in equation (551) which then cancels down to $\frac{\varepsilon}{R} \times 100$.

If there is no nursing error ($\varepsilon = 0$) but the system is not at steady-state,

$$Er = \frac{1-F}{RF-1} \times 100 \qquad (566)$$

Expansion leads to

$$Er = \frac{Tr-FTr}{RFTr-Tr} - 100 \qquad (567)$$

$$= \frac{(D + Tr) - FTr - D}{D} \times 100 \qquad (568)$$

$$= \frac{D_N - T - D}{D} \times 100 \qquad (569)$$

This is clearly the correct result, namely the measured intercept (D_N) minus the measured post-dose trough (T) minus the single-dose intercept (D), all divided by the single-dose intercept.

The value of ε may be related to nursing error in the following manner:

From (547) and (557) we note that the pre-nth dose level Tr(where n is large) may be expressed for uniform dosing as:

$$Tr = D_N e^{-K\tau} \qquad (570)$$

and if the nursing error is x hours (positive if late, negative if early) then

$$Tr + E = D^* e^{-K(\tau-x)} \qquad (571)$$

where $D^* = D + Tre^{-Kx}$ replaces D_N.

Since,

$$\varepsilon = \frac{E}{Tr}$$

Doses (n)	2	3	4	5	6
K					
0.1	1.45 (0.550)	1.140 (0.797)	1.055 (0.909)	1.023 (0.960)	1.010 (0.982)
0.2	1.20 (0.800)	1.034 (0.959)	1.007 (0.991)	-	-
0.3	1.09 (0.909)	1.007 (0.992)	1.000 (1.000)	-	-
0.4	1.04 (1.000)	1.002 (1.000)	-	-	-

values for $\gamma = 8$, (R-1)/(RF-1) given in brackets

Table 5.6 Values of F and (R-1)/(RF-1) for various values of K and n

we find that

$$\varepsilon = \frac{D^* e^{-K(\tau - x)} - D^* e^{-K\tau}}{D^* e^{-K\tau}}$$

$$= e^{Kx} - 1 \qquad (572)$$

Thus (564) becomes

$$Er = \left(\frac{e^{Kx}(R-1)}{R(RF-1)} - \frac{1}{R} \right) \times 100 \qquad (573)$$

It should be noted that although this applies only to steady-state condition, it would in any case be irrelevant to the first dose (since there is no preceding dose) and for the second dose (n = 2) D* should be replaced by D in equation (571) which then cancels to give (573) in exactly the same manner as above. A non-steady state condition in gentamicin therapy, other than in the first two doses, is rare at dose intervals of eight or more hours (at K = 0.1 and τ = 8, 95% of steady-state levels have been achieved by the start of the third dose). Dosing intramuscularly yields the same error values since although the factor Ka/(Ka-K) must be applied to D, their values always cancel out.

Equation (573) may be verified in the following special cases:

$F = 0 \quad : Er = \frac{e^{Kx} - 1}{R} = \frac{\varepsilon}{R}$

$F = 0, \; x = 0 \; : Er = 0$

Relevant values of F, (R-1)/(RF - 1) and the consequent errors are listed in Tables 5.6 and 5.7.

K	Hours error in preceding dose time (+ dose given later)	% change in tr	% error in intercept due to ignoring trough (ss)	% error in using calc. trough (ss)	% error using calculated trough whilst not at ss (n doses)			
					n = 2	n = 3	n = 4	n = 5
0.1	0	0	81.6	0	-45.0	-14.0	-5.5	-2.3
	+1	10.5	90.2	4.7	-42.4	-10.2	-1.2	+2.2
	+2	22.1	99.7	9.9	-39.5	- 6.1	+3.5	+7.2
	+3	35.0	110.2	15.7	-36.4	- 1.5	8.8	12.8
0.2	0	0	25.3	0	-20.0	- 3.4	-0.7	-
	+1	22.1	30.9	4.5	-16.4	0.9	3.8	-
	+2	49.2	37.8	9.9	-12.1	6.1	9.1	-
	+3	82.2	46.1	16.6	- 6.7	12.5	15.7	-
	-1	-18.1	20.7	-3.6	-22.9	- 3.8	-3.6	-
0.3	0	0	10.0	0	- 9.9	- 0.7	-	-
	+1	35.0	13.5	3.2	- 6.2	2.5	-	-
	+2	82.2	18.2	7.5	- 2.3	6.7	-	-
	+3	146.0	24.5	13.2	+ 2.9	12.4	-	-
0.4	0	0	4.3	0	- 4.1	-	-	-
	+1	49.2	6.3	2.0	- 2.1	-	-	-
	+2	122.6	9.5	5.0	0.9	-	-	-
	+3	232.0	14.1	9.5	5.4	-	-	-

τ = 8:- indicates that % errors for non-ss = % errors for ss $\pm$ 1.0

Table 5.7 Errors in calculated value of intercept, before and during steady-state (ss), due to incorrect timing of preceding dose (iv dosing)

5.5 Procedures for calculating AUC:

5.51 Errors inherent in the trapezoidal rule

As described in Section 4, a trapezoidal approach was used for determining the AUC over the time period of sampling.

Three trapezoidal techniques were considered:

(1) Linear rule

Here,

$$AUC_{t_1-t_2} = \tfrac{1}{2}(C_1 + C_2)(t_2 - t_1) \qquad (574)$$

This has the advantage that it is not specific to any particular model, but the disadvantage that it fits none of them well. It is, however, simple to use.

(2) Logarithmic rule - I

This technique assumes a mono-exponential delay between two points and calculates the integral of the curve accordingly. As given by Chiou (139),

$$AUC_{t_1-t_2} = \frac{(C_1 - C_2)(t_2 - t_1)}{\ln C_1 - \ln C_2} \qquad (575)$$

If the curve may genuinely be described by a mono-exponential expression then this expression is superior to that of the linear rule, but if this is not the case (e.g. during im absorption phase) then the error may be greater than that of a linear rule.

(3) Logarithmic rule - II

For a monoexponential, where

$$C_t = C_o e^{-Kt} \qquad \text{(from 405)}$$

$$AUC_{t_1-t_2} = \int_{t_1}^{t_2} C = \frac{Co}{-K}\,(e^{-Kt_2} - e^{-Kt_1}) \qquad (576)$$

Hence

$$AUC_{t_1-t_2} = \frac{1}{K}\,(C_1 - C_2) \qquad (577)$$

$\left(\frac{\text{Sampling interval}}{\text{half life}}\right)$ (n)	% over-estimate of AUC
0.25	0.25
0.5	0.99
0.75	2.24
1.0	3.95
1.5	8.84
2.0	15.5

K (hr^{-1})	Sampling interval (hr)	% over-estimate
0.1	0.5	0.021
	1.0	0.084
0.2	0.5	0.084
	1.0	0.33
0.3	0.5	0.19
	1.0	0.75
	2.0	2.98
0.4	0.5	0.33
	1.0	1.33
	2.0	5.28

Table 5.8 Percentage over-estimates of AUC by using linear rule on mono-exponential data

This modified equation, which agrees with (575) if $\frac{(t_2-t_1)}{\ln C_1-\ln C_2}$ is expressed as $\frac{1}{K}$ it will reduce the dependence of the result upon any two data points, one or both of which may be in error. It is only usable, however, if an estimate of K has been made and has the general advantages and disadvantages of the logarithmic rule in common with equation (575).

Errors arising from use of trapezoidal models

The situations in which errors may occur for these three models have been described above. In considering the application to gentamicin, it is reasonable to assume that large sections of the curve will correspond to a mono-exponential decay curve and over these portions of the curve the logarithmic rules will work well, but the linear rule will overestimate the AUC. Chiou (139) has described the overestimate as,

$$\% \text{ error} = \frac{0.693n\ (1+e^{-0.693n}) - 2(1-e^{-0.693n})}{2(1-e^{-0.693n})} \times 100 \qquad (576)$$

where n = (sampling interval)/(half-life).

Some values of this model-error relevant to gentamicin are given in Table 5.8

5.5.2. Analytical error on a single point

These calculations. however, ignore the possibility of analytical error. Thus whilst one may determine the error due to the model, this error will vary with the quality of the data.

Let us suppose that we are concerned to determine the AUC between points t_1 and t_3, with measured concentrations

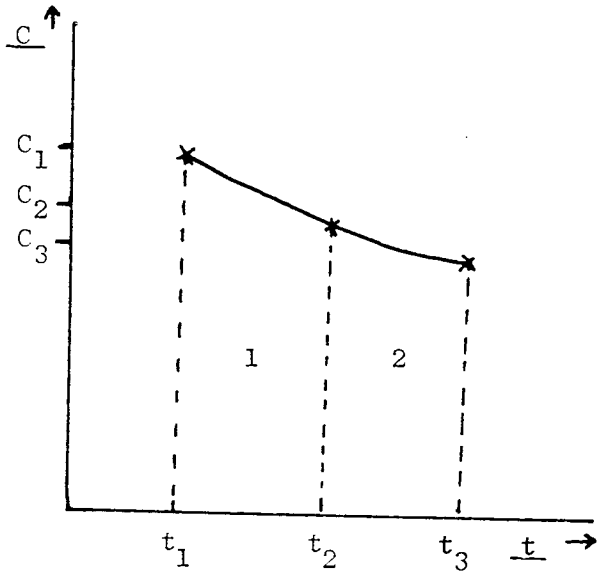


Fig 5.6 Data for AUC calculations

at points t_1, t_2, t_3. (Figure 5.6).

For each model we shall assume no model error, thus the linear rule will assume a straight line between t_1 and t_3 and the logarithmic rules will assume a monoexponential. We shall assume that C_1, C_3, t_1, t_2, t_3 are all accurate, but that C_2 is in error by a quantity ΔC and that t_1 to t_3 is part of a curve. This is equivalent to assessing the effect of one 'rogue' point, a common analytical problem.

(1) Linear rule

From equation (574) we find the total AUC (denoted AUC_T) to be

$$AUC_T = \tfrac{1}{2}(C_1 + C_3)(t_3 - t_1)$$

$$AUC_1 = \tfrac{1}{2}(C_1 + C_2 + \Delta C)(t_2 - t_1)$$

$$AUC_2 = \tfrac{1}{2}(C_2 + \Delta C + C_3)(t_3 - t_2)$$

The percentage error (E) is described by

$$E = \frac{AUC_1 + AUC_2 - AUC_T}{AUC_T} \qquad (577)$$

Thus,

$$E = \frac{\tfrac{1}{2}\{(C_1+C_2+\Delta C)(t_2-t_1) + (C_2+\Delta C+C_3)(t_3-t_2) - (C_1+C_3)(t_3-t_1)\}_{x100}}{\tfrac{1}{2}\{(C_1+C_3)(t_3-t_1)\}} \qquad (578)$$

By noting that

$$(C_1+C_3)(t_3-t_1) = (C_1+C_2)(t_2-t_1) + (C_2+C_3)(t_3-t_2)$$

(578) may be reduced to

$$E = \frac{\Delta C(t_3-t_1)}{(C_1+C_3)(t_3-t_1)} \times 100$$

and thus

$$E = \frac{\Delta C}{(C_1+C_3)} \times 100 \qquad (579)$$

Error in C_2 (%)	K (h^{-1})	t_1 (h)	t_2 (h)	t_3 (h)	Error in AUC (%) by methods (1)	(2)
-20	0.1	0.5	0.75	1.0	-10.00	-10.37
		0.5	0.63	1.0	-10.00	-10.41
		0.5	0.88	1.0	-10.00	-10.33
		1.0	1.5	2.0	-10.00	-10.37
	0.2	0.5	0.75	1.0	-10.00	-10.37
		0.5	0.63	1.0	-10.00	-10.45
		1.0	1.5	2.0	-10.00	-10.36
		1.5	2.25	3.0	-10.00	-10.35
-10	0.1	1.5	2.25	3.0	- 5.0	- 5.09
	0.2	1.5	2.25	3.0	- 5.0	- 5.08
	0.3	1.5	2.25	3.0	- 5.0	- 5.07
	0.3	1.5	2.25	4.5	- 5.0	- 5.31
+10	0.1	1.0	1.5	2.0	5.0	4.92
		1.5	2.25	3.0	5.0	4.92
	0.2	1.0	1.5	2.0	5.0	4.92
		1.5	1.88	3.0	5.0	4.91
	0.3	1.0	1.5	2.0	5.0	4.91
		1.5	2.25	3.0	5.0	4.90
		1.5	2.25	4.5	5.0	5.23

Table 5.9 Errors in AUC calculation due to error in one of three serum levels

(2) Logarithmic rule

A similar approach for the logarithmic rule yields, for model 3, an equation equivalent to (578) whereby,

$$E = \frac{\frac{1}{K}(C_1-C_2-\Delta C)+\frac{1}{K}(C_2-C_3+\Delta C)-\frac{1}{K}(C_1-C_3)}{\frac{1}{K}(C_1-C_3)} \times 100 \qquad (580)$$

which simplifies to

$$E = \frac{(\Delta C - \Delta C)}{(C_1 - C_3)} = 0$$

This, however, assumes K to be unaffected by the error in C_2. That this is not so is demonstrated by the equivalent equation for model 2,

$$E = \frac{\frac{(C_1-C_2-\Delta C)(t_2-t_1)}{\ln C_1-\ln(C_2+\Delta C)}+\frac{(C_2-C_3+\Delta C)(t_3-t_2)}{\ln(C_2+\Delta C)-\ln C_3}-\frac{(C_1-C_3)(t_3-t_1)}{\ln C_1-\ln C_3}}{\frac{(C_1-C_3)(t_3-t_1)}{\ln C_1-\ln C_3}} \times 100 \qquad (581)$$

The complex nature of the denominators prevents further useful reduction but Table 5.9 gives values of E for models 1 and 2 at various values of t_1, t_2 and t_3.

5.5.3. General Analytical error

Rather than consider just one 'rogue' point, however, it would be useful to consider a more general case where there is error on all measurements of C. It is only necessary here to consider one segment of the curve, that is, t_1 to t_2.

If n is the ratio of C_1 to C_2, without analytical error, and x and y are the ratios of the measured value of C to the true value of C at t_1 and t_2 respectively (i.e. the measured values are xC_1 and yC_2) then percentage errors (E) may be derived as follows.

Linear Rule

$$E = \left(\frac{\frac{1}{2}(xC_1+yC_2)(t_2-t_1)-\frac{1}{2}(C_1+C_2)(t_2-t_1)}{\frac{1}{2}(C_1+C_2)(t_2-t_1)} \right) \times 100$$

$$= \left(\frac{(nxC_2+yC_2)-(nC_2+C_2)}{nC_2+C_2} \right) \times 100$$

$$= \left(\frac{nx+y}{n+1} - 1 \right) \times 100 \qquad (582)$$

This conforms to intuitive criteria in that

if $x = y = 1$, $E = 0$

if $x = y \neq 1$, $E = (x-1)\times 100$

if $x = 2 - y$, $E = \left(\frac{2n-ny-y}{n+1} - 1\right) \times 100$

(i.e. errors are of same magnitutde but opposite sign)

Logarithmic rule

Model 2:

$$E = \left(\frac{\frac{(xC_1-yC_2)}{\ln(xC_1)-\ln(yC_2)} - \frac{(C_1-C_2)}{(\ln C_1-\ln C_2)}}{\frac{(C_1-C_2)}{(\ln C_1-\ln C_2)}} \right) \times 100$$

$$= \left(\frac{(xC_1-yC_2)(\ln C_1-\ln C_2)}{\{\ln(xC_1)-\ln(yC_2)\}(C_1-C2)} - 1 \right) \times 100$$

$$= \left(\frac{(nxC_2-yC_2)(\ln(nC_2)-\ln(C2))}{(\ln(nxC_2)-\ln(yC_2))(nC_2-C_2)} - 1 \right) \times 100$$

$$= \left(\frac{(nx-y)\ln(n)}{\ln(\frac{nx}{y})(n-1)} - 1 \right) \times 100 \qquad (583)$$

This complies with the special cases:

$x = y = 1$: $E = 0$

$x = y \neq 1$: $E = (x-1)\times 100$

$x = 2 - y$: $E = \left(\frac{n(2-2y)(\ln(n)}{\ln(\frac{2n}{y}-1)(n-1)} - 1\right) \times 100$

Ratio of levels (n)	Combinations of K and sampling intervals (Δt) that give n		Errors in levels (%)		Resultant errors in AUC (%) by methods		
	Δt(h)	K(h^{-1})	C_1	C_2	(1)	(2)	(3)
1.1	.25	.381	-10	-10	-10.0	- 10.0	- 10.0
	.5	.191	-10	0	- 5.2	- 5.2	-110.0
	.75	.127	-10	+10	- 0.5	- 0.5	-210.0
			+10	+10	10.0	10.0	10.0
			0	+10	4.8	-100.0	-100.0
1.2	.50	.365	-10	+10	- 0.9	- 0.6	-110.0
	.75	.243	0	-10	- 4.5	- 4.9	50.0
	1.0	.182	0	+10	4.5	4.8	- 50.0
	1.5	.122	+10	-10	0.9	0.0	110.0
1.3	.75	.350	-10	+10	- 1.3	- 0.8	- 76.7
	1.0	.262	-10	0	- 5.7	- 5.3	- 43.3
	1.25	.210	+10	+10	+10.0	10.0	10.0
	1.5	.175	0	0	0.0	0.0	0.0
1.4	1.0	.336	-10	0	- 5.8	- 5.4	- 35.0
	1.5	.224	-10	+10	- 1.7	- 0.9	- 60.0
	2.0	.168	+10	+10	10.0	10.0	10.0

Table 5.10 Errors in calculated area of a trapezoid due to errors in measurements

Model 3:

The assumption that K is unaffected by the errors in C_1 and C_2 leads to the equation:

$$E = \left(\frac{(nxC_2 - yC_2) - (nC_2 - C_2)}{(nC_2 - C_2)} \right) \times 100$$

$$E = \left(\frac{nx-y}{n-1} - 1 \right) \times 100 \qquad (584)$$

The same special cases are similarly verifiable.

Table 5.10 gives typical errors likely to occur in gentamicin data. It is clear that the logarithmic methods yield great fluctuations, with relative errors in AUC much greater than the relative analytical error in certain circumstances. These fluctuations easily outweigh the model-error for method 1 (Table 5.8) and so the linear trapezoidal method was selected for use.

5.5.4. Application of a linear trapezoidal rule to im doses.

The application of a linear model to iv dosing has already been described (139) but the application to im dosing is more complex.

From work described above (Section 5.2) it follows that the latter portion of the curve will be very similar to an iv curve, and this has been confirmed by simulations (described below). From Figure 5.7 it is clear that the trapezoidal rule will underestimate the AUC for the early part of the curve. The extent of the underestimate will get worse as the interval between sampling points increases, in particular the first trapezoid (effectively a triangle) will grossly underestimate the true area (See Table 5.11).

Simulations of the trapezoidal estimation of AUC at

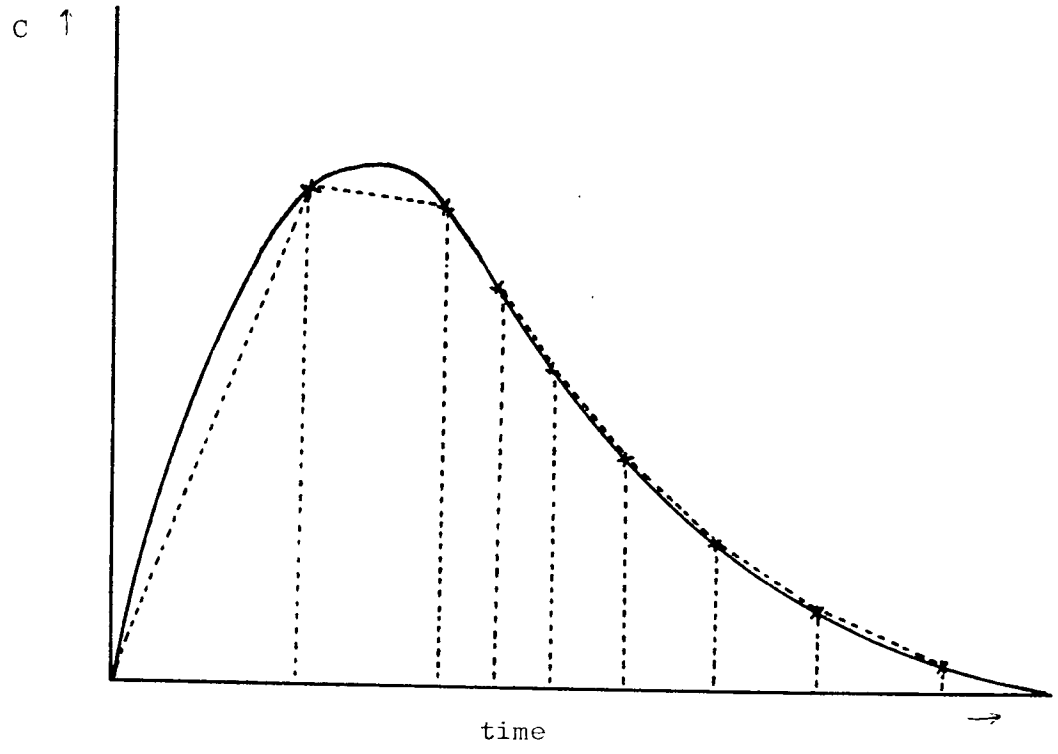


Fig 5.7 Application of trapezoidal rule to an im dose

sampling intervals of 0.5 hours and 1 hour, using computer-generated error-free im data and comparing with the calculated integral (true AUC), were performed at values of K from 0.1 to 0.4 hr^{-1} and values of Ka from 2.0 to 2.8 hr^{-1}. Selected calculations are listed in Table 5.11. These demonstrate that the errors in the first part of the curve depend upon both K and Ka, whilst those from later portions are independent of Ka and equal to the equivalent iv results (Table 5.8). Whilst it is useful that the slight overestimates of the latter part of the curve are partially compensated for by the underestimates in the middle of the curve, it is undesirable to have such large underestimates in the early part of the curve. It was therefore decided to use the trapezoidal method for im data only from the first post-dose level, using an analytical integral for the portion from t = 0 to t = t_1. This, from integration of (417) is,

$$AUC_{0-t_1} = \frac{D}{V}\frac{Ka}{(Ka-K)}\left(\frac{(1-e^{-Kt_1})}{K} - \frac{(1-e^{-Kat_1})}{Ka}\right) \qquad (585)$$

The calculation requires estimates of K, Ka and V. The latter was determined by using the body weight, adjusted to volume by population mean calculations, and K was found from the data as described elsewhere.

Ka was estimated as 2.16 hr^{-1}, for reasons given in Section 4. That this was a reasonable choice is demonstrated by table 5.12 which shows that the relative errors are very much smaller than those for the trapezoidal method (Table 5.11). Errors in the volume, however, would make an appreciable difference at high values of K (Section 5.5.5.) and so a two-stage iterative procedure was adopted using first 30% body weight as the volume and then the estimate of V that this produced. Further iterations were found to be of little

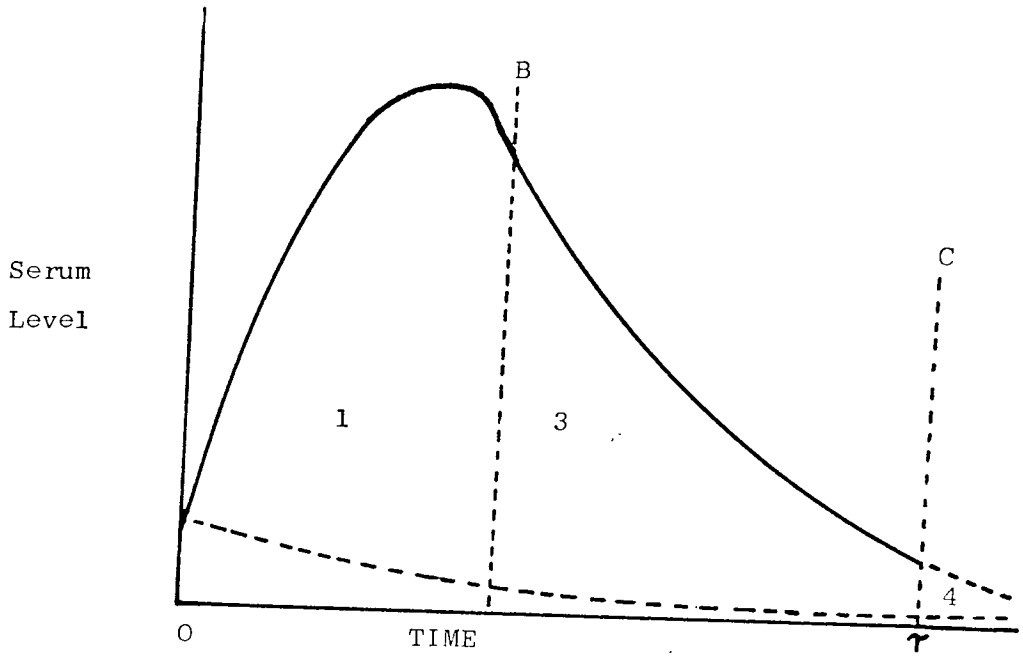
Ka (h^{-1})	K (h^{-1})	Trapezoidal Area as percentage of true area					
		0-1h	0-0.5h	0.5h-1h	1.5-2h	2.5-3h	5.5-6h
2.0	0.1	74.09	84.97	97.38	99.70	99.97	100.02
2.2	0.1	72.52	83.81	97.28	99.75	99.99	100.02
2.2	0.2	70.49	82.86	97.08	99.76	100.04	100.08
2.2	0.3	68.52	81.93	96.91	99.81	100.13	100.19
2.2	0.4	66.61	81.01	96.76	99.88	100.26	100.33
2.6	0.1	69.73	81.61	97.33	99.83	100.00	100.02
2.6	0.4	63.66	78.76	96.88	100.02	100.30	100.33
2.8	0.4	62.35	77.69	99.96	100.08	100.31	100.33

Table 5.11 AUC calculated by trapezoids from im data compared with true AUC

Ka (hr^{-1})	Relative AUC, 0-1hr, K=0.35
1.8	91.05
2.0	96.23
2.16	100.00
2.2	100.89
2.4	105.04
2.6	108.76
3.0	115.15

Table 5.12 Variation of AUC, calculated by integral, with Ka

(a)



(b)

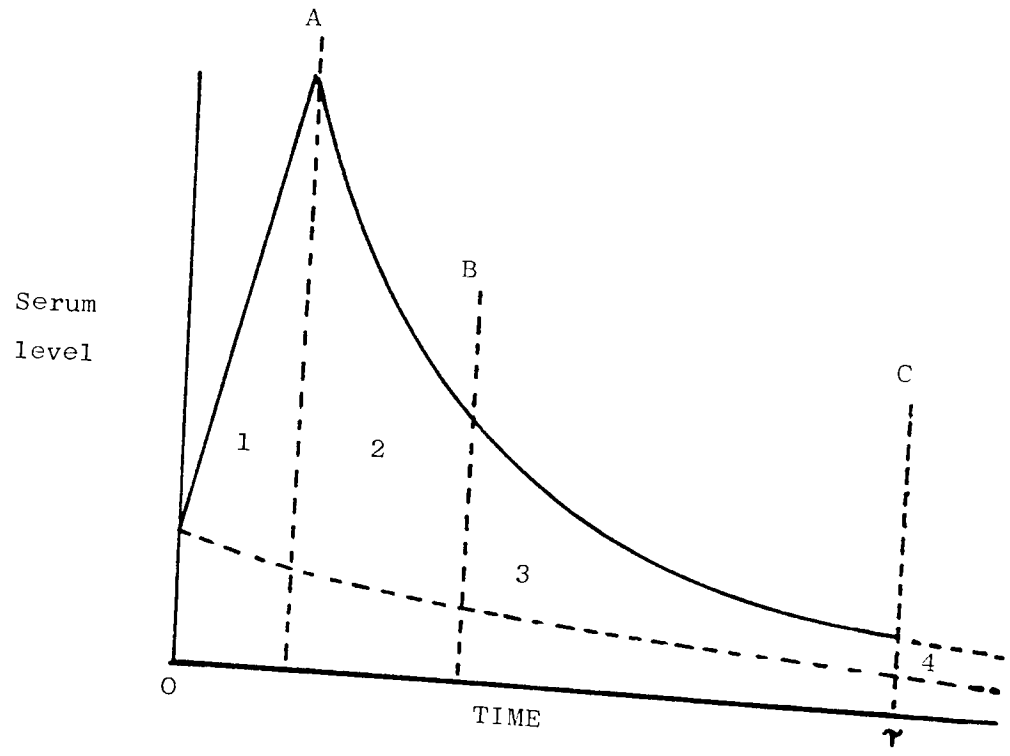


Fig 5.8 AUC calculations, segments of concentration-time curve

benefit, as for example in one of the worst possible cases (using data from this study):

Suppose that AUC_1 = 24 units, V = 50% LBW, AUC total = 100 units (Table 5.13).

Calculated values (using 30% LBW) are:

1st iteration: $AUC_1 = 24 \times \frac{50}{30} = 40$

AUC total = 116

V = 43.1%

2nd iteration: $AUC_1 = 40 \times \frac{50}{43.1} = 27.84$

AUC total = 102.84

V = 48.64%

3rd iteration: $AUC_1 = 27.84 \times \frac{43.1}{48.64} = 24.67$

AUC total = 100.67

V = 49.67%

5.5.5 Relative areas under the curve by sections

Figure 5.8 illustrates the methods used for determining AUC using three (im) or four (iv) methods of calculating AUC for different sections of the curve. Each method uses a linear trapezoidal method between the second and last data points (section 3 of the curve) and a mono-exponential extrapolation from the last point to infinity (section 4). For iv dosing section 1 represents an analytical solution to the integral of the infusion curve and section 2 the back-extrapolation from the second point to the end of the infusion. For im dosing section 2 is redundant and section 1 is an analytical solution to the integral from zero time to the first post-dose level.

It was important to ascertain the relative importance of each of these segments in order to ensure that the approximations being made would not unduly prejudice the result

Ka(hr^{-1}) or Ko (mg/min/L)	K(hr^{-1})	% of whole in each section 1	2	3	4
iv (sampling at 1 hour)					
16.667	0.1	0.08	9.56	56.2	34.1
"	0.2	0.13	15.74	69.3	14.9
"	0.4	0.22	26.25	70.08	3.45
2.083	0.1	0.55	8.88	56.33	34.23
"	0.2	0.96	14.60	69.51	14.93
"	0.4	1.81	24.24	70.48	3.47
0.980	0.1	1.17	8.01	56.49	34.39
"	0.2	2.04	12.98	69.85	15.00
"	0.4	3.82	21.42	71.04	3.50
im (sampling at 1.0 hour)					
1.5	0.1	4.97	-	41.86	53.16
"	0.2	10.24	-	61.58	28.17
"	0.4	21.55	-	70.61	7.84
2.25	0.1	6.06	-	42.03	51.91
"	0.2	12.15	-	61.03	26.82
"	0.4	24.13	-	68.79	7.07
2.5	0.1	6.33	-	42.00	51.66
"	0.2	12.63	-	60.80	26.57
"	0.4	24.82	-	68.24	6.94
im (sampling at 0.5 hours)					
2.25	0.1	1.65	-	39.01	59.34
"	0.2	3.25	-	54.88	41.87
"	0.4	7.24	-	68.82	23.94

Table 5.13 Relative AUC for iv and im doses by section

Simulations, sampling at 0.5 or 1 hour intervals up to seven hours with combinations of K and Ka or Ko, (ranges 0.1 - 0.4 hr^{-1}, 2.0 - 2.8 hr^{-1} and 0.9 to 17 mg/min/l respectively) were performed. Figures 5.9 show typical results with several sets given in table 5.13. In all cases, section 4 decreased and section 3 increased as K increased. Section 4 was also independent of Ka and Ko and changed only slightly as the total AUC changed due to differing relative errors in the trapezoidal section (This result is in accord with Chapter 5.2). The importance of section 3 increased as K increased, due mostly to the decrease of section 4 and, as a consequence, the total are As K approached 0.4, however, section 3 maintained a steady level and then decreased slightly in some cases, for similar reasons to the fall in section 4. This effect was greater at high values of Ka or Ko due to the increased importance of section 1 (and section 2 in iv doses). Section 1 increase markedly with K by both routes, but never exceeded 2% of the whole for iv dosing with a 4.8 minute infusion. Typical values for im dosing, however, were 5 - 20% AUC up to 1 hour, a little less than the equivalent iv period, where section 2 was dominant.

It was therefore necessary to pay careful attention to the trapezoidal and extrapolated (tail) areas which accounted for over 75% in almost all cases, whilst the infusion portion of the curve was of little importance, and approximations or assumptions here (e.g. of a fixed percentage of weight as volume) would have only minimal effects.

The earlier sampling commences, the more importance the trapezoidal area assumes.

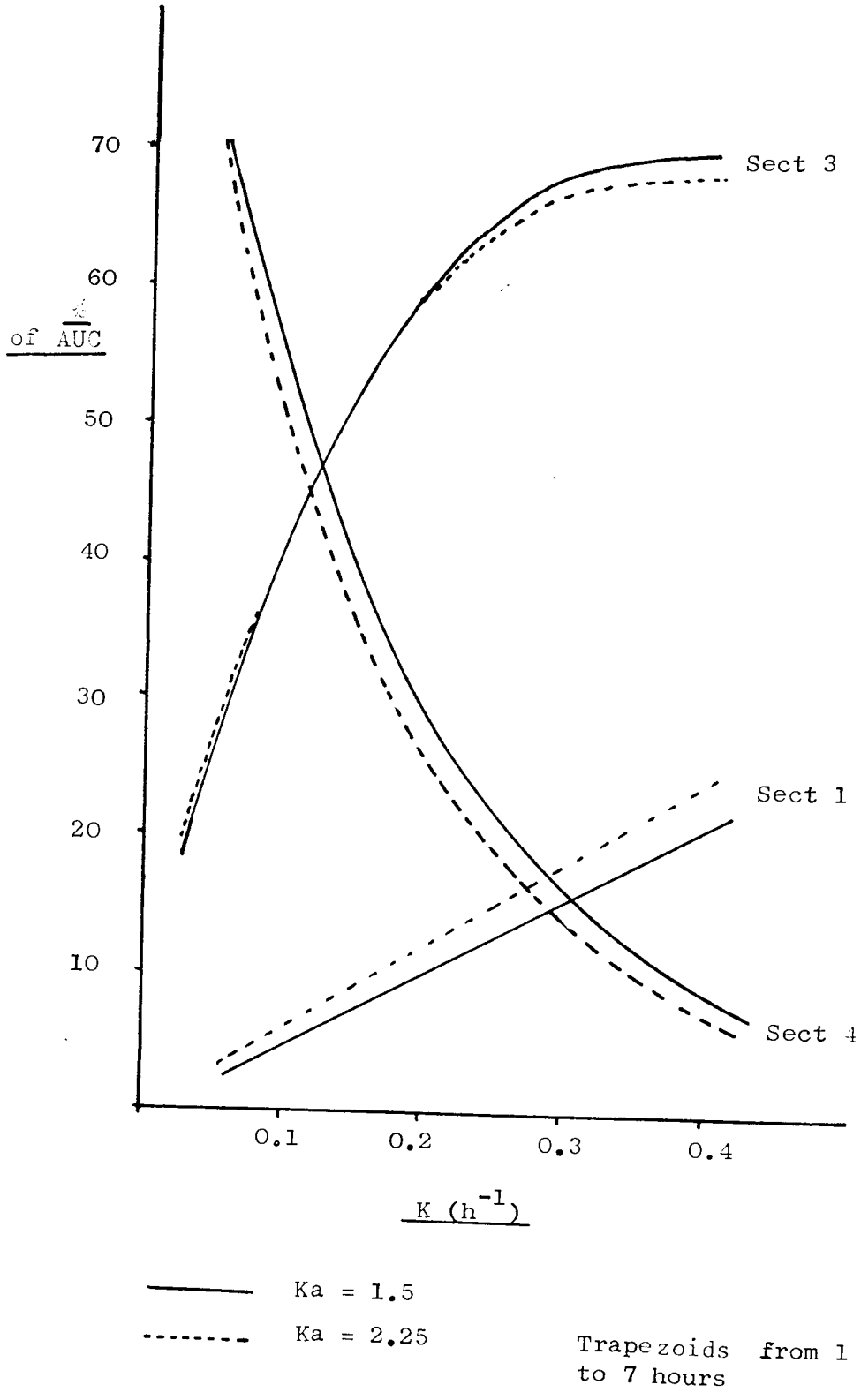


Fig 5.9 AUC from an im dose divided into sections (fig 5.8)

5.6 One compartment analyses of two compartment data

Several authors (e.g. 144, 145) have commented upon discrepancies between results from 1 compartmental analysis of data which truly fits a 2-compartment model. The accumulation in the central compartment during multiple dosing of a 2-compartment system is covered by standard texts (e.g. 128) and, since this is a time-dependent phenomenon, it is apparent that the effects of the accumulation on the measured characteristics using a 1-compartment analysis will also vary with time.

Since there are a large number of variables to consider in this situation, it was not possible to devise analytical mathematical formulae to examine these discrepancies and apparent changes in characteristics. Instead, it was decided to generate data by a 2-compartment model for each of 36 successive doses and analyse each of these doses by a 1-compartment model to obtain the apparent elimination rate constant. Each dose was analysed three times, using three points on each occasion at 1, 2, 3 hours, 2, 3, 4 hours and 3, 4, 5 hours respectively. (These times were considered reasonable for clinical use). Laboratory error was not included in the simulation because it was felt that this might well obscure the true trends in the data.

Early results showed great differences in the changes in measured K values for small changes in model parameters. These differences were complex in nature and did not lend themselves to simple mathematical description. To give a more realistic aspect to the calculations, parameters were selected from papers describing 2-compartment kinetics of aminoglycosides. Unfortunately, not only are there few such papers but most do not give individual patient data,

	α	β	K_{21}	K_{12}	K_{10}
GROUPS					
I	.053	.004	.009	-	-
II	.119	.008	.016	-	-
III	.175	.013	.016	-	-
IV	.195	.013	.015	-	-
INDIVIDUALS					
Case 1	.172	.008	.009	.018	.153
Case 2	.186	.004	.004	.000	.186
Case 4	.1833	.00468	.013	.109	.066
Case 7	.0867	.0063	.007	.0114	.0746
JA01	.2263	.0031	.0035	.0235	.2024*
MK25	.2168	.0038	.0046	.0369	.1791*
WR35	.1191	.0122	.0280	.0514	.0519*
HR66	.0777	.0083	.0105	.0141	.0614*
AD89	.1845	.0048	.0058	.0308	.1527*

* private communication

Table 5.14 Rate constants for two-compartment model using Schentag data

Group	$\alpha\beta$	$K_{10}K_{21}$	$\alpha+\beta$	$K_{10}+K_{12}+K_{21}$
I	2.12×10^{-4}	3.6×10^{-4}	0.057	0.030
II	9.52×10^{-4}	9.9×10^{-4}	0.127	0.059
III	2.275×10^{-3}	2.56×10^{-3}	0.188	0.223
IV	2.535×10^{-3}	2.7×10^{-3}	0.208	0.238

Table 5.15 Sums and products of parameters in four groups of patients (Schentag et al, 48)

preferring to give either group means (with large standard deviations) or incomplete sets of parameters. Table 5.14 lists the acceptable data found in the literature, including group means.

As anticipated, it was found that the group means of Schentag et al (48) did not conform to the criteria for an individual 2-compartment system where the following relationship should obtain (Eq. 407-408)

$$\alpha\beta = K_{10}K_{21}$$

$$\alpha+\beta = K_{10} + K_{12} + K_{21}$$

As shown in Table 5.15, there were considerable discrepancies in the sums and products obtained. Caution must therefore be exercised in the interpretation of these group data since the observed features may represent genuine features of the model or merely the disparity between individuals and their collective means.

The apparent changes in K (always negative, i.e. a decrease in the absolute value of K) increased in magnitude as the elapsed time between dosing and sampling increased. Thus if the magnitude of the change in K between dose 1 and dose n, sampling at 1, 2, 3 hours is $\Delta Kn;_{1,2,3}$.

$$\Delta Kn;_{1,2,3} < \Delta Kn;_{2,3,4} < \Delta Kn;_{3,4,5}$$

ΔK also increased asymptomatically with successive doses until a steady-state was achieved (4-5 β half-lives from the first dose, where dosing was in the post distributive phase), indpendent of the dosing interval and in accord with theory (128);

$$\Delta Kn;\ x,\ y,\ z \quad < \quad \Delta Kn + 1;\ x,\ y,\ z$$

The direction of the inequality is not, however, merely a function of elapsed time from the first dose since in all cases,

$$\Delta Kn;\ 3,4,5 \quad > \quad \Delta Kn + 1;\ 1,2,3.$$

Origin GROUP DATA	$\Delta_{1,2,3}$ n=7	$\Delta_{2,3,4}$ n=12	$\Delta_{2,3,4}$ n=24	$\Delta_{2,3,4}$ n=SS	$\Delta_{3,4,5}$ n=SS	% of total change to occur in 1 day (1, 2, 3)
I	11.7	20.7	33.0	39.9	49.6	12.2
II	17.2	27.1	36.5	42.6	44.4	23.3
III	6.5	10.8	14.0	15.1	17.1	27.1
IV	4.4	7.6	9.9	10.8	12.6	27.2
INDIVIDUALS						
Case 1	2.6	5.0	7.4	9.1	10.5	17.9
Case 2	0.0	0.0	0.0	0.0	0.0	0.0
Case 7	1.9	3.5	5.9	7.8	8.4	12.3
Case 4	19.5	32.3	44.9	58.0	60.7	19.9
JA01	1.1	2.4	4.3	9.5	11.6	7.1
MK25	2.4	5.0	8.5	15.6	18.5	9.2
WR35	25.2	35.0	42.1	44.7	45.6	34.8
HR66	5.2	9.2	13.8	17.2	18.1	15.9
AD89	4.0	7.6	12.4	20.1	23.1	11.4

Table 5.16 Percentage changes in K (Δ) as defined in text for Schentag data

The form of the plot of $\Delta Kn;\ 1,2,3$ for n = 1 to 36, versus either time (t hours) or doses was found to be

$$\ln\ (\ \Delta K_{ss} - \Delta K_t) = W - \gamma t \qquad (586)$$

where ss is steady state, not attained at time t, W is constant

Thus

$$\gamma = \frac{\ln(\ \Delta K_{ss} - \Delta K_{t1}) - \ln\ (\ \Delta K_{ss} - \Delta K_{t2})}{t_1 - t_2} \qquad (587)$$

No satisfactory relationship was found between γ or ΔK_{ss} and the rate constants of the 2-compartment model (Table 5.16) γ did not vary significantly with sampling times for a given set of parameters, despite the sometimes large changes in ΔK_{ss}.

From equation (587) it is clear that if γ is constant the change in K between any two doses will depend not only on the interval between those doses (denominator) but also on their relationship to the steady-state (numerator). Thus the greatest changes will be over the first few doses;

$$(\Delta Kn;\ -\ \Delta Km;) > (\Delta Kn+1;\ -\ \Delta Km + 1;)$$

It might, therefore, be better for control purposes to monitor not the first dose, but, say, the fourth (one day later at τ = 8h) thus eliminating some (usually 20-30%, Table 5.16) of the changes observed before steady-state. (Doing so, would, inicidentally, be more practicable for the laboratory and medical staffs).

It would also be best to measure levels as early as possible in the dose interval, taking care, however, to avoid the effects of a rapid (α_1) distribution phase or absorption phase if exhibited in any given case (cp. Section 5.3).

5.6.1 Associations amongst two-compartment parameters

Data have been published for four individuals (21, 48), all by Schentag, who also provided data on a further five patients by personal communication. These nine sets are listed in table 5.14. Each one was analysed by the author for the percentage change in the estimate of K during treatment to steady state. The data are shown with the group mean data in table 5.16. 'Case 2' had identical reported values for K_{21} and β, making Kn = 0, and showed no change in K at all. The percentage changes in K at steady-state (2, 3, 4 hours) were compared with other parameters for all except 'Case 2' and the rank orders are shown in table 5.17 (n = 9).

There was a significant positive correlation between K_{21} and β ($r = .76$, $p < .05$) and a negative one between K_{21} and α ($r = -.69$, $p < .05$). There was no significant correlation, however, between the changes in K and α, β, K_{10} or K_{21}. There was a significant correlation, however, between the changes and K_{12} ($r = .798$, $p < .05$). There was also a significant relationship between the changes and the difference between K_{21} and β ($r = .649$, $p < .05$) but a highly significant correlation between the changes and the fractional difference between K_{21} and β (ie $(K_{21}-\beta)/\beta$) ($r = .89$, $p < .01$) and the standardised difference between α and K_{10} (ie $(\alpha-K_{10})/\alpha$) ($r = .86$, $p < .01$). Although 'Case 2' was omitted, by virtue of the zero difference between K_{21} and β it would have fitted into this pattern and improved these correlation coefficients.

Seven of the nine cases had less than 10% change in K after 12 doses (2, 3, 4 hours) but the other two changes were in excess of 40%. There was no apparent method of predicting these cases without knowing the micro-constants from serum data but urinary data may yield useful results. From the volunteer study (section 7) it was found that K_{10} could be measured with four consecutive two-hour urine collections. Since α in the Schentag model is almost equal to K in our study, an estimate of $(\alpha-K_{10})/\alpha$ should be readily available if desired.

Patient Code	1	7	4	01	25	35	66	89
Parameter								
Total % change	7	8	1	6	5	2	4	3
α	5	7	4	1	2	6	8	3
β	3	4	6	8	7	1	2	5
K_{21}	4	5	2	8	7	1	3	6
K_{12}	6	8	1	5	3	2	7	4
K_{10}	3	5	6	1	2	8	7	4
$K_{21}-\beta$	4.5	2	7	1	3	8	6	4.5
$(K_{21}-\beta)/\beta$	7	8	1	6	4	2	3	5
$(\alpha-K_{10})/\alpha$	7	6	1	8	4.5	2	3	4.5

Table 5.17 Rank orders of 2-compartment parameters in Schentag data

5.6.2 Two-compartment parameters applied to patient data

Despite the objections to the use of group mean parameters, listed above, it was thought desirable to assess the effect that these parameters might be expected to exert upon the data obtained from the patients in this study. The mean values of β and K_{21} from Schentag's group IV (Table 5.14) were used, together with K for each patient to determine α and hence complete the characterisation of a two compartment system.

For each patient the percentage change in K was calculated, using only those doses where K had been measured from blood data, in a manner similar to that of section 5.6 above. The group of patients for whom mean changes in K had been calculated (Section 7.4) were also assessed by this method to compare the changes. It was found that whereas the measured K in this study changed by a mean of 23.0% (range 2-54%) the expected mean change in K due to use of an incorrect model was only -5.93% (sd = 2.52, n = 23, $<$ 50% change in serum creatinine between measurements). It is unlikely, therefore, that accumulation due to a two-compartment system could be the sole cause of the changes observed.

5.7 Monte Carlo Simulations

Estimates of the accuracy and precision of any technique subject to experimental error, or subject variation, are difficult to make without large numbers of subjects and measurements. One way to circumvent this problem, is to create a mathematical model that mimics, so far as is possible, the conditions of the experiment and use the data thus generated to evaluate the precision and performance of the analytical method (so-called, Monte Carlo Simulations).

A program simulating a 1-compartment model was written in FORTRAN, first on ICL 1904S, where its performance was evaluated in detail, and then on UMRCC CDC 7600. Serum levels, following on iv infusion of specified length, were generated at set intervals (usually 1/32nd of the dose interval) for the required number of doses (usually 20) in each of 20 or 40 patients. Each level, from an analytical formula (ref 128), was multiplied by a random factor to simulate experimental error. This factor wasgeneratedby a pseudo-random generator of the Numerical Algorithms Group (NAG) referenced by functions G05CBF (seed 0) and G05DDF, and had a mean of 1.00 and a standard deviation determined by the level of experimental error required. Dependent on the requirements of each investigation, data were generated using a range of values of K and analysed in a variety of ways, using the subroutines and methods employed by the interactive programs. The values of K, V and clearance calculated by each method from each dose were stored and evaluated statistically by the subroutine SDEV. (The programs are described more fully in section 6 and listed in appendix 2)

5.7.1 Variation of estimate of K

To evaluate the significance of the changes in K observed in this study, and to determine the effect of the numbers of sampling points, the levels of experimental error (expressed as coefficient of variation (cv, standard deviation as a percentage of mean)) and the 'true' K value,

M	K (h^{-1})	n	Range (h)	CV (%)	CVR (%)	CVK1 (%)	CVV1 (%)	CVC1 (%)	Notes
400	.346	6	5	5	3.52	4.40	5.33	2.24	V from AUC (11 points)
		6		7.5	5.3	6.68	8.08	3.36	
		6		10	7.12	9.04	10.91	4.50	
		6		20	14.89	19.75	23.81	9.18	
400	.346	3	2	5	10.62	10.91	9.52	3.86	V from AUC (5 points)
		3		7.5	15.97	16.43	14.67	5.99	
		3		10	21.37	22.03	20.49	8.44	
		3		20	42.21	44.43	42.70	21.97	
380	.1	6	5	2	4.85	6.08	4.38	3.25	V from AUC (11 points)
	.2				2.43	3.02	2.80	1.23	
	.3				1.62	2.01	2.26	0.91	
	.35				1.39	1.72	2.10	0.80	
760	.3	3	6	2	2.38	-	-	2.02	
		4			2.01	-	-	1.66	
		5			1.76	-	-	1.31	
		6			1.63	-	-	1.14	
380	.3	3	6	2	2.44	-	-	1.99	V from AUC (11 points)
		4			2.06	-	-	1.58	
		5			1.85	-	-	1.35	
		6			1.62	-	-	1.18	
380	.3	3	30	2	0.49	-	-	3.51	
		4			0.40	-	-	2.73	
		5			0.37	-	-	2.38	
		6			0.32	-	-	2.07	

M = number of doses sampled,
n = the number of blood samples per estimate of K
cvr = cv of ratio K'/K,
CVK1 = cv of K'/K^1
CVV1 = cv of V'/V^1
CVC1 = cv of cl'/Cl^1

Table 5.18 Typical values of statistics on estimates (K') of K to illustrate text

the first determination of K in each 'patient' was compared with all subsequent determinations (doses 2 to 20 or 2 to 40) and each determination compared with the 'true' K (i.e. the value used as the base for the calculations).

The mean of the ratio of the estimates of K, (K') to K,

$$\text{ratio} = \frac{K'}{K}$$

and the cv of this ratio (cvr) were examined. The mean was never significantly different from 1.0 (3 to 6 data points used for estimate of K), K = 0.05 to 0.40 in steps of 0.05, τ = 6, 8, 12 or 48, cv = 2, 5, 7.5, 10 or 20%.

The cvr, however, was observed to vary inversely with K, to decrease as the number of data points increased, to be independant of the number of simulations, and to increase as the sampling range decreased. (Table 5.18 lists values to illustrate these trends).

Calculations were performed to define these relationships and to reduce the number of simulations required.

cvr may be expressed by definition as:

$$\text{cvr} = \sqrt{\frac{\sum_{i=1}^{m} \left\{\frac{K'_i}{K} - \frac{\bar{K}'}{K}\right\}^2}{m-1}} = \frac{1}{K}\sqrt{\frac{\sum_{i=1}^{m} (K' - \bar{K}')^2}{m-1}} \qquad (588)$$

where $\bar{K}'$ is the mean of K' from m simulations.

To determine the effect of K upon cvr, the expression $(K' - \bar{K}')^2$ must be reduced to a factor of K, or else shown to be independant of K.

If C_j^i is the true jth serum level in the ith simulation and is measured as $F_j^i . C_j^i$ due to experimental error (F being independant of K and t) then

$$\ln C_j^i = f_j^i + \ln C_j^i$$

where f_j^i is the natural logarithm of F_j^i,

and $\ln C_j^i = f_j^i + I - Ktj$

where $I = \ln Co$

By linear regression,

$$-K_i' = \frac{n \sum_{j=1}^{n} tj\,(f_j^i - Ktj + I) - \sum tj \sum (f_j^i - Ktj + I)}{n \sum t_j^2 - (\sum tj)^2}$$

$$= \frac{n \sum tjf_j^i - \sum tj \sum f_j^i}{n \sum t_j^2 - (\sum tj)^2} - \frac{K(n \sum t_j^2 - (\sum tj)^2)}{n \sum tj^2 - (\sum tj)^2}$$

Hence $$K_i' = - \frac{n \sum tj\, f_j^i - \sum tj \sum f_j^i}{n \sum tj^2 - (\sum tj)^2} + K$$

Hence $(K_i' - K)$ is independant of K.

Since $\bar{K}'$ approaches K as m increases (verified by simulation)

$(Ki' - \bar{K}')^2$ is independant of K.

Hence $$cvr = \frac{1}{K} \sqrt{\frac{\sum_{i=1}^{i=m} \varepsilon_i^2}{m-1}}$$

where $$\varepsilon_i = \frac{\sum_{j=1}^{n} (tj - \bar{t})\, fj}{\sum_{j=1}^{n} (tj - \bar{t})^2}$$

and is the regression of f against t.

The term $\sum_{i=1}^{i=m} \varepsilon_i^2$ may be expressed as

$$m\bar{\varepsilon}_i^2$$

where $\bar{\varepsilon}_i^2$ is the mean of $(Ki' - \bar{K}')^2$, is always positive and independant of m.

Thus,

$$cvr = \frac{1}{K} \sqrt{\frac{m\bar{\varepsilon}_i^2}{m-1}}$$

cvr is therefore dependant upon $\sqrt{\frac{m}{m-1}}$, and for large values of m will be practically independant of m (the number of simulations).

The effect of the sampling range may be evaluated if the samples are taken in a standardised fashion such that the jth sample is always taken at a certain fraction of the sampling interval. (The first sample is always taken at time zero (let relative time $t_j = 0$), and the last sample at the end of the sampling interval (let tj=1)). A scale factor (Si) may be applied to convert relative times to absolute times, and the same Si will apply to all values of t in one simulation.

Thus
$$\bar{\varepsilon}_i = \frac{\sum_{j=1}^{n} Si\ (tj - \bar{t})\ fj}{\sum_{j=1}^{n} Si^2\ (tj - \bar{t})^2}$$

$$= \frac{1}{Si}\left(\frac{\sum_{j=1}^{n} (tj - \bar{t})\ fj}{\sum_{j=1}^{n} (tj - \bar{t})^2}\right) = \frac{\bar{\varepsilon}}{Si} \qquad (589)$$

Hence
$$cvr = \frac{1}{K} \cdot \frac{1}{Si} \sqrt{\frac{m\,\bar{\varepsilon}^2}{m-1}}$$

The effect of the number of serum levels (n) used in the estimate of K is more difficult to quantify. Both the numerator and denominator in (589) are summated over n observations, but whereas the denominator is always positive (and therefore increases as n increases) the terms in the numerator may be positive or negative (averaging zero) and so the combined result ($\bar{\varepsilon}$ i) is unpredictable but will tend to depend inversely upon n. cvr will, therefore, depend inversely upon n. (The same scale factor (Si) will operate upon all observations, regardless of the number of them).

K' determined by n points over a range of 2 hours with 'true' values (K) as shown. Predictions were made P doses ahead

Analytical cv = 2%

		K = .2		K = .3		K = .4	
P	n	mean	cv	mean	cv	mean	cv
3	3	11.3	89.5	9.0	99.2	8.1	94.3
	4	9.2	95.6	7.3	89.2	6.7	99.8
	5	8.7	94.0	6.9	87.6	6.3	98.5
	6	6.8	87.2	5.5	94.0	5.0	92.3
6	3	11.8	92.6	8.7	100.7	8.4	97.1
	4	9.7	106.7	7.2	88.9	7.0	108.2
	5	9.2	103.3	6.8	91.6	6.6	105.1
	6	7.1	86.8	5.3	88.7	5.2	92.1
9	3	11.8	90.8	9.0	105.2	8.4	95.7
	4	8.9	110.8	7.2	95.0	6.4	111.2
	5	9.1	102.8	6.7	94.8	6.6	104.1
	6	7.3	89.9	5.4	90.8	5.4	94.6
12	3	13.4	102.3	10.2	96.0	8.4	95.0
	4	10.7	100.7	8.0	99.6	6.4	97.5
	5	10.7	102.2	7.6	100.0	6.3	96.0
	6	9.8	111.1	6.2	96.5	5.3	103.5

Analytical cv = 5%

3	3	23.6	21.6
	4	17.4	17.8
	5	16.3	16.6
	6	13.9	12.9
6	3	22.6	22.7
	4	18.2	19.0
	5	16.1	18.0
	6	13.7	13.7
9	3	23.9	22.5
	4	16.3	17.3
	5	14.7	17.8
	6	15.3	14.1
12	3	23.6	21.9
	4	16.3	16.2
	5	15.4	16.2*
	6	15.5*	13.3

Table 5.19 Mean errors in prediction in Monte Carlo Analyses of 1-compartment data

The dependance of (589) upon the experimental error (fj) is similarly complex. The mean of a number of simulations of the numerator will depend not on the mean of $(tj - \bar{t})\, fj$ (which will be zero) but on the spread. The spread will depend on the spread of fj, but this is a logarithmic function (fj = ln Fj). The standard deviation of fj will be similar to the standard deviation of Fj, provided Fj is close to one. (e.g., ln 1.02 = 0.0198, ln 1.05 = 0.0488, ln 0.95 = -.051). Thus, $\bar{\bar{\varepsilon}}$ will be approximately proportional to the standard deviation due to experimental error.

Thus, approximately,

$$cvr \propto \frac{1}{K} \cdot \frac{1}{Si} \cdot \frac{1}{n} \cdot \frac{1}{cv}$$

5.7.2 Estimates of Volume of distribution and clearance

Calculations of the volume of distribution (by intercept or by AUC) are more complex. The trapezoidal rule has been dealt with above (5.5) and the terminal extrapolated portion of the AUC might be expected to be related to K, but summating these portions is too complicated for this type of analysis. Simulations, then, are the only practical means of evaluating the significance of the various parameters.

Table 5.18 shows that whilst the calculated volume has a similar variation to the elimination rate (both expressed as a ratio to the first determined value), the clearance has a much smaller spread, but both follow similar trends with regard to cv, n, m and K.

5.7.3 Predictions of serum levels

Predictions of subsequent serum levels were also made by simulations. All levels from doses 13 to 20 in each patient were predicted using the parameters calculated from 3, 6, 9 and 12 doses previously. Thus 5120 predictions (20 patients) were made at any one value of K, n and cv. The errors in the predictions were assessed as

$$\text{Error} = \frac{\text{Predicted} - \text{'Actual'}}{\text{'Actual'}}$$

K' determined by n points over a range of 6 hours with 'true' values of K as shown. Predictions made P doses ahead

Analytical cv = 2%

		K = 0.1		K = 0.2		K = 0.3	
P	n	mean	cv	mean	cv	mean	cv
3	3	6.04	81.0	3.7	88.1	3.2	85.5
	4	5.50	74.8	3.3	80.0	3.0	81.3
	5	4.67	79.7	3.0	81.1	2.6	84.1
	6	4.88	74.2	2.8	84.7	2.7	80.8
6	3	6.39	77.6	3.8	87.5	3.3	83.7
	4	5.39	72.6	3.2	79.8	2.9	80.0
	5	4.74	78.3	3.2*	86.6	2.7	82.2
	6	4.25	77.5	2.7	83.9	2.4	80.5
9	3	6.76	81.1	3.7	90.1	3.3	86.1
	4	5.58	73.8	3.3	83.9	2.9	81.1
	5	4.61	82.6	3.1	87.3	2.6	81.8
	6	4.50	77.5	2.8	82.4	2.4	80.6

Analytical cv = 5%

3	3	15.9	-	9.2	90.55	8.0	88.0
	4	14.1	-	8.2	80.1	7.4	81.6
	5	11.9	-	7.7	82.7	6.5	85.2
	6	12.7	-	7.1	87.4	6.8	82.7
6	3	17.0	-	9.6	90.5	8.3	86.7
	4	13.7	-	8.0	81.1	7.3	80.5
	5	12.0	-	8.1	90.6	6.6	82.7
	6	10.9	-	6.9	86.6	6.1	81.8
9	3	17.9	-	9.3	94.7	8.5	88.8
	4	13.7	-	8.3	86.5	7.2	81.6
	5	11.5	-	8.0	91.2	6.5	83.0
	6	10.9	-	6.9	84.2	6.1	81.1

All means are significantly different from horizontal and vertical neighbours except where marked *

Table 5.20 Mean errors in prediction in Monte Carlo Analyses of 1-compartment data

using both signed and absolute values for the calculation of means and standard deviations.

The signed data showed that there was no significant bias in the errors and that the mean error was not significantly different from zero.

The absolute errors showed a slight tendancy to increase as the interval between measured and predicted levels increased, but this was non-significant (tables 5.19, 5.20). Increasing the number of points for the estimation of K significantly improved the predictions at each increment. As K increased, or analytical cv decreased, the predictions improved. As the sampling period for the estimation of K increased ('Range') so the predictions improved.

6. COMPUTING TECHNIQUES REQUIRED TO IMPLEMENT PROGRAMS

6.1. Facilities available for computing

Programs were written to accommodate the various aims of the project on several machines. Initially the programs were written in BASIC on the University of Aston Hewlett Packard HP2000 Access System with 20Kbytes of available workspace. This machine was, however, both remote from the hospital site and in constant use by other users, factors which made effective use difficult.

Access to the Data General NOVA 3 system (approx. 12 Kbytes available core) owned by the East Birmingham Hospital Cardiology Department was obtained and this proved to be more satisfactory in both respects. BASIC was also available on this machine but there were a number of syntactic dissimilarities which necessitated considerable alterations not only in the program implementation but also in the organisation and flow of the procedures (discussed more fully in Section 6.2 below).

For reference and retrospective analysis a FØRTRAN (FØRmula TRANslation) language program was written and installed on the University of Aston ICL 1904S computer. FØRTRAN was also used for some of the simulation procedures discussed in Chapter 5 but these programs were written for the University of Manchester Regional Computer Centre (UMRCC) CDC 7600 computer to speed up job turnround and to economise on time and core usage charges. There are syntactical differences between the two versions of FØRTRAN but the major differences were in the data input/output procedures (more cumbersome at UMRCC because of the remote job entry system when used from Aston) and in the general philosophy of data handling. Such differences were also seen between

BASIC and FØRTRAN and between interactive and batch modes.

In batch processes it is common to collect data at the commencement of a program and assign it to assays which are accessed by the program as required. (It is not essential to do it this way, but input/output operations are relatively inefficient and are therefore usually minimised). In interactive programs the data is acquired only as needed, a little at a time,in response to program prompts issued to the operator. Interactive programs, therefore, might use a variable name and temporary location for several items in turn in order to economise on core space (normally at a premium in light interactive machines) whereas larger machines operating in batch mode would be more efficiently run by storing all the items in separate locations and so reducing access time as the program runs.

In addition, BASIC Programs are <u>interpreted</u> into machine (binary) code line by line as the program dictates. Thus, if a line is in a procedure to be repeated several times it will be interpreted on each occasion. FØRTRAN programs are <u>compiled</u> into binary code prior to the commencement of the program run, thus every line is compiled once only. CDC FORTRAN, however, runs the compiled version differently from ICL in that arrays are set up to contain the results of expressions that may be required more than once and if the same expression is to be evaluated again the result will be taken from the array and not recalculated. Similarly loop counters and small variables may not be assigned actual values but the values stored in working registers inaccessible to the programmer. It further optimises runtime by performing operations (e.g. arithmetic assignments) only if the result is required by another statement. Thus for a program segment

Line number	Statement
1	A = 11.0
2	X = 32.6
3	Y = 16*42*A
4	W = X*Y + A
5	Z = 16*42*A + W

The probable order of execution is,

(1), (2), (3), 1, 3, (4), 2, 4, 5. (line numbers in parenthesis are inspected but not executed).

For statement 5 only the sum of "16*42*A" and W would be evaluated since the expression 16*42*A would already have been recognised as being the same as the result of 3.

For the purposes of this project, inefficiencies on the CDC 7600 could be tolerated because of the machine's great capacity, but optimisation of the ICL Programs and the NOVA BASIC programs was vital (the latter eventually exceeded the capacity of the computer and had to be subdivided). A further difference between the two languages vital to the programming scheme is the handling of subroutines and variable names.

FØRTRAN (of any type) assigns a separate core location to each variable or array element in each segment of the program, unless otherwise instructed by the program segment description (e.g. by use of dummy variables or CØMMØN Statements). Thus a subroutine for multiple regression (say) could use arrays X and Y as dummy arrays for regressions upon (say) arrays A and B, B and C, X and A, X and Y (in turn) without any crossover of values or any programmed exchange of values or back-up arrays. In BASIC this facility is not available and any given variable (e.g. A or X(1)) will correspond to only one memory location throughout the program run. Thus to perform regression analysis upon two separate sets of arrays, whilst

preserving the arrays, a program must have either

(1) two regression routines

or (2) separate working arrays with interchanging of values

or (3) back-up temporary storage arrays, also with interchanging of values.

Such a procedure would be required in the analysis of data by a two-compartment model or in statistical assessment of data from simulation experiments.

In part-compensation, however, BASIC requires fewer statements for data control and subroutines may be entered at any number of points, whereas FØRTRAN requires more complicated ENTRY procedures.

An appreciation of the operational differences in machines and languages is essential to the proper programming of the various computers.

Operation	Permitted forms on HP2000	Permitted forms on NOVA 3
Multiple assignment	A = B = C = D = 6.2	not allowed
X to the power Y	X * * Y	X ↑ Y
Formatted output	1000 PRINT USING 1010; A, B, C 1010 IMAGE 2D.3D, 5X, 6D.D, 3X, 3D	1000 PRINT USING " ##.### ##### ### ", A,B,(or 999 S $= " ##.### ###### ### "
Prompted input	not allowed Use 1000 PRINT "This string is output to VDU"; 1010 INPUT X, Y, Z	Input "This string is output to VDU", X, Y, Z
	On excess input, ignore data not required	On excess input, stop program.
String literal assignment	see text, section 6.2	
dimensioning matrices	see text, section 6.2	
IF statement	see text, section 6.2	
Convert string characters to upper case	X $ = UPS $ (X$)	not allowed
Convert string characters to numeric equivalent	Converts all characters to ASCI I equivalent	Converts first-found string of characters 0 to 9 only. Stops program if other characters are found before a digit.
loop variable control FOR I = 0.2 TO 0.8 STEP 0.2 etc.	four cycles with I at 0.2, 0.4, 0.6, 0.8	three cycles, I = 0.2, 0.4, 0.6
Initialisation	All variables assigned zero or blank	variables assigned values they had when program was last 'SAVEd'.

Table 6.1 Differences in syntax between BASIC on NOVA 3 and BASIC on HP2000

6.2 Conversion of HP2000 BASIC to NOVA 3 BASIC

Many of the differences were minor and the changes required were tedious but simple (see Table 6.1).

Others were more significant, notably the 'IF' statement which resulted in duplication of some lines and considerable changes in direction of program flow.

Acceptable forms of the IF statement on the HP2000 include;

IF relational-expression GØTØ line number

IF relational expression ØR relational expression GØTØ line number

IF relational expression AND relational expression GØTØ line number

'ØR' and 'AND' are not acceptable on NOVA 3 machines (although AND may be imitated as illustrated below) but terminal statements other than 'GØTØ line number' are acceptable.

Thus the following are acceptable;

IF relational expression THEN GØTØ line number

IF relational expression THEN assignment statement

IF relational expression THEN IF relational expression THEN GØSUB line number

The unavailability of an 'ØR' operator is very restrictive

Character arrays on HP2000 are specified by giving the first and last element of the part of the array to be operated upon, or else the single element to be used. Thus, for a character strong A$ = "ABCDEFG", A$ (4) = "D" whereas in NOVA 3 BASIC A$ (4) = "DEFG", the string commencing at the specified point and including all characters to the end. On HP2000 strings may be examined by conversion to ASCII characters, but this is not possible on NOVA. Numerical matrices in HP2000 BASIC are dimensioned at the start of the program (by specific statement or by default) and any

subsequent input or manipulative statments will not affect the declared dimensions.

Thus, an array, x (6,5) will have thirty elements and a subsequent matrix statement such as MAT INPUT x (3,4) will assign twelve of those to the input values without affecting the remaining eighteen. In NOVA 3 BASIC such a sequence of statements would re-dimension x to x (3,4) and any values stored in the deleted parts would be lost. Any later attempt to assess one of the deleted elements, or to increase the dimensions, would be ruled illegal at the time of execution. (FØRTRAN handles such procedures quite differently in any case).

6.3 Installation of programs on NOVA 3

In addition to the syntax problems described above it became apparent that the HP2000 program was too large for the NOVA 3. This was temporarily solved by deleting the graph subroutine and two redundant subroutines from earlier versions of the program. As the program was expanded to enable output to be directed to the printer it again exceeded the core capacity and sub-division was considered necessary.

The core has to contain in Random Access Memory (RAM) both the program and the variables and assays thus created. If, therefore, a portion of the program containing, or referring to, arrays could be removed or isolated a disproportionately large portion of RAM might be cleared. The program used five major arrays at the time of program overflow (eight later on) but two of these five were back-up arrays and a third was used to create working arrays from the back-up arrays. This latter array, together with two input segments, was only used prior to the calculations and it was found that removal of the segments reduced the remaining program to an acceptable size. (Initially the back-up arrays were also removed but this later proved to be unnecessary and their restoration facilitated the restoration of the graph routine.

The sections thus removed were written into a separate program which determined the option required and the patient's dosing and assay information, assigned the working arrays and output the patient data to the printer. The data was then written to a disk file and the main program called into core. The main program read the data from the disk file and proceeded as previously until a new option was needed, when the reverse process would be performed. Unlike the HP2000, the

NOVA 3 does not retain the current status of the variables whilst changing programs, hence the need to use a disk file.

Details of the various subroutines and segments appear in the following sections, but the overall program flow is given in Figure 6.12.

6.4 Program Organisation

6.4.1. Interactive BASIC programs

It was realised from the inception of the project that the programs would require considerable modification and development as the investigations proceeded to cope with the changing demands made by the investigators and the various methods of data analysis. It was therefore decided to make each procedure as independent as possible, so that the program flow could be easily altered by means of programming changes or run-time operator instructions. To this end, the operator was required at various points in the program to select the options required. This was achieved either by using code-numbers to denote certain combinations of procedures or by entering a codon of Ys and Ns to initiate other procedures.

The code-numbers were used at the start of the program to select the mode of input (from the terminal or from the last set of data (i.e. further calculations on one patient's data)) and to opt for regression calculations, creatinine

calculations or both.

Thus the final version of the program has the following options:

1. Data from terminal, regression and creatinine calculations
2. Data from terminal, creatinine calculations only
3. " " " , new creatinine level for same patient (same age, sex, weight)
4. Data from last data set, regression calculations only.
5. Data from terminal, regression calculations only.
6. " " " , input values of K, V (or weight and desired percentage for V), Cr. Bypasses regression calculation.
7. Lists information on running program
8. Terminates program.

The codons were used after the initial regression and creatinine calculations. Thus in response to the prompt

Graph/AUC/Predict/Creatinine/Keyboard ?

the operator should input a codon of 3 to 5 letters, Y to opt for a procedure, N to reject it.

Thus

YYNNN

would initiate a graph-plotting routine and or AUC calculation but not the predictive procedures.

NYYYN

would not plot a graph but would do AUC calculations and predictions from the blood data and the creatinine data but not from a separately input value of K ("Keyboard" option).

$$b = \frac{n\Sigma x_i y_i - \Sigma x_i \Sigma y_i}{n\Sigma x_i^2 - (\Sigma x_i)^2}$$

$$a = \frac{\Sigma y_i - b\Sigma x_i}{n}$$

$$r = \frac{b}{|b|}\sqrt{\frac{(\bar{y} - \hat{y}_i)^2}{\Sigma(y_i - \bar{y})^2}}$$

$$Se = \sqrt{\frac{\Sigma(y_i - \hat{y}_i)^2}{n - 2}}$$

$$S_b = Se\sqrt{\frac{1}{\Sigma(x_i - \bar{x})^2}}$$

$$Sa = S_b\sqrt{\frac{x_i^2}{n}}$$

$$F = \frac{\Sigma x_i y_i - n\bar{x}\bar{y}}{n - 2}$$

n = number of data points, $\bar{x}$, $\bar{y}$ = mean values of x and y respectively, y_i = the interpolated value of y calculated from the ith x value, x_i = the sum of all x values between i - 1 and i = n, other symbols as described in text.

Table 6.2 Regression calculations

6.4.2. FORTRAN programs

Since there was no facility to accept user-control in a FØRTRAN background-mode program, it was necessary to design these programs to cover all eventualities. Thus, whilst the routines were essentially the same as their BASIC equivalents, they differed in certain operational respects viz:

(1) All interactive checks were removed (section 6.4.10)

(2) Variable FOR loops were replaced by fixed-cycle loops with control variables held in the data and read prior to the loop concerned.

(3) Flow to and from a segment was generally via the MASTER segment, not from another SUBROUTINE.

(4) Dummy variables were used within SUBROUTINES.

(5) Predictions were made for all levels in a dosing period at one call to the PREDICTOR SUBROUTINE, rather than define each dose to be predicted as the end of a dosing period.

(6) Back-up arrays were used to store results of previous calculations, rather than a single variable which changed its value as the program proceeded.

Flow charts for the overall program are given in Figure 6.7.

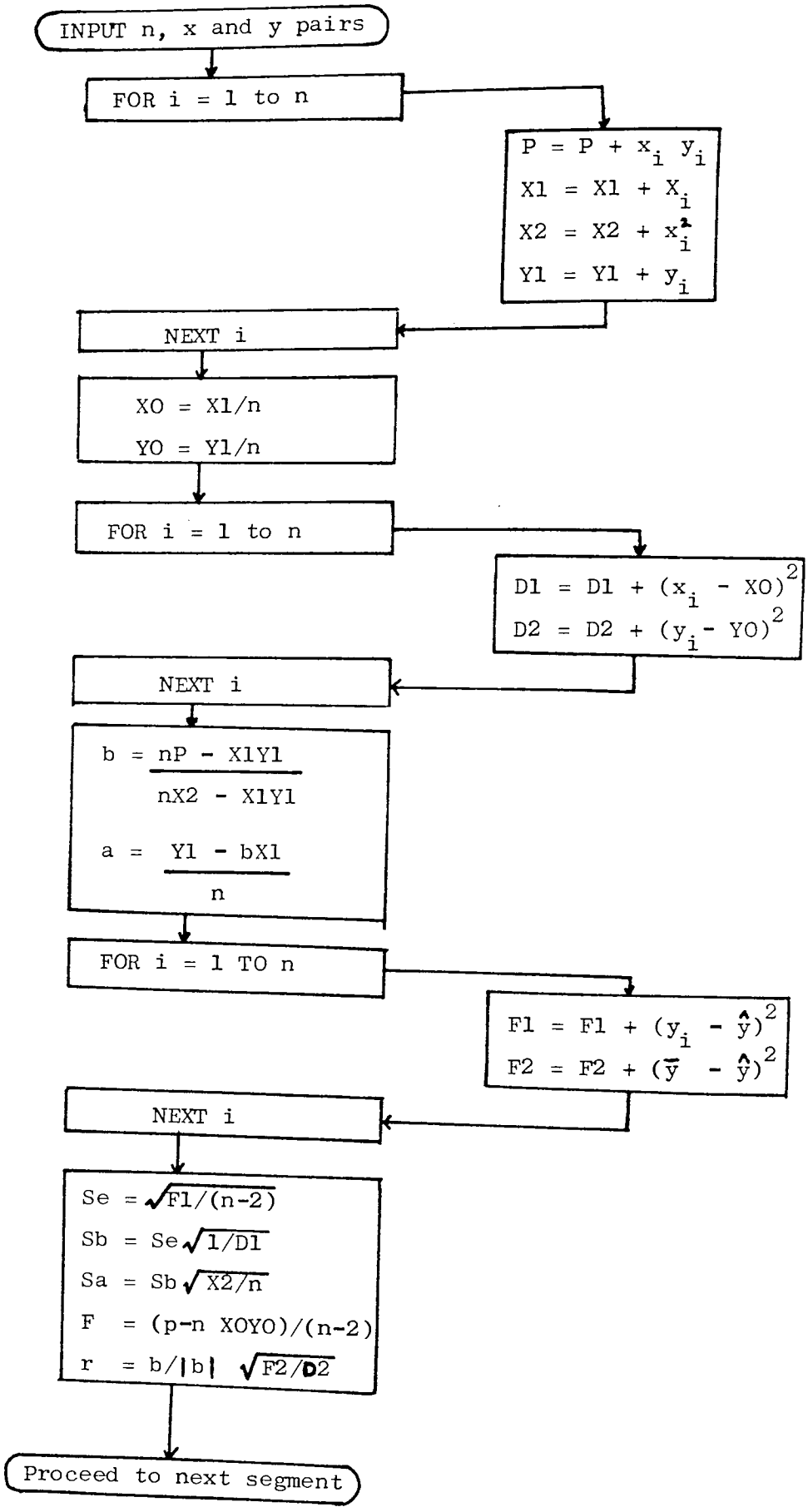


Fig 6.3 Regression subroutine

(Symbols as in table 6.2, other than computing variables)

6.4.3. Regression subroutine

The formulae of Davis et al (146) were used to construct a Least Mean Squares Linear Regression subroutine which remained virtually unchanged throughout the project. The statistics calculated included;

the slope and ordinate axis intercept of the calculated best-fit line (b and a, respectively),

the correlation coefficient (r),

the standard error of estimate (Se),

the standard deviations of slope and intercept (S_b, S_a respectively)

and the variance ratio (F).

The statistical formulae and BASIC statements are listed in table 6.2, the flow chart is given in Figure 6.3.

6.4.4 Creatinine subroutine

Four methods were used for the numerical conversion of serum creatinine to gentamicin half-life. The method of Jelliffe et al (54) required the use of the patient's height (in order to calculate body surface area) and was not used because heights are not routinely measured (although they were available for this project). Jelliffe, however, described a method for converting creatinine clearance values to gentamicin elimination rates, based on regression analysis of a group of patients, and this method was used in conjunction with the other three listed in Table 3.1.

All methods were based on linear regression techniques with groups of patients having a wide range of renal function. Jadrný (111) used solely the serum creatinine level whilst Siersbaek-Nielsen et al (112) and Cockcroft and Gault (110) included age and weight factors. The polynomials of Siersbaek-Nielsen represent a fairly complicated approach only really practicable with computer or calculators.

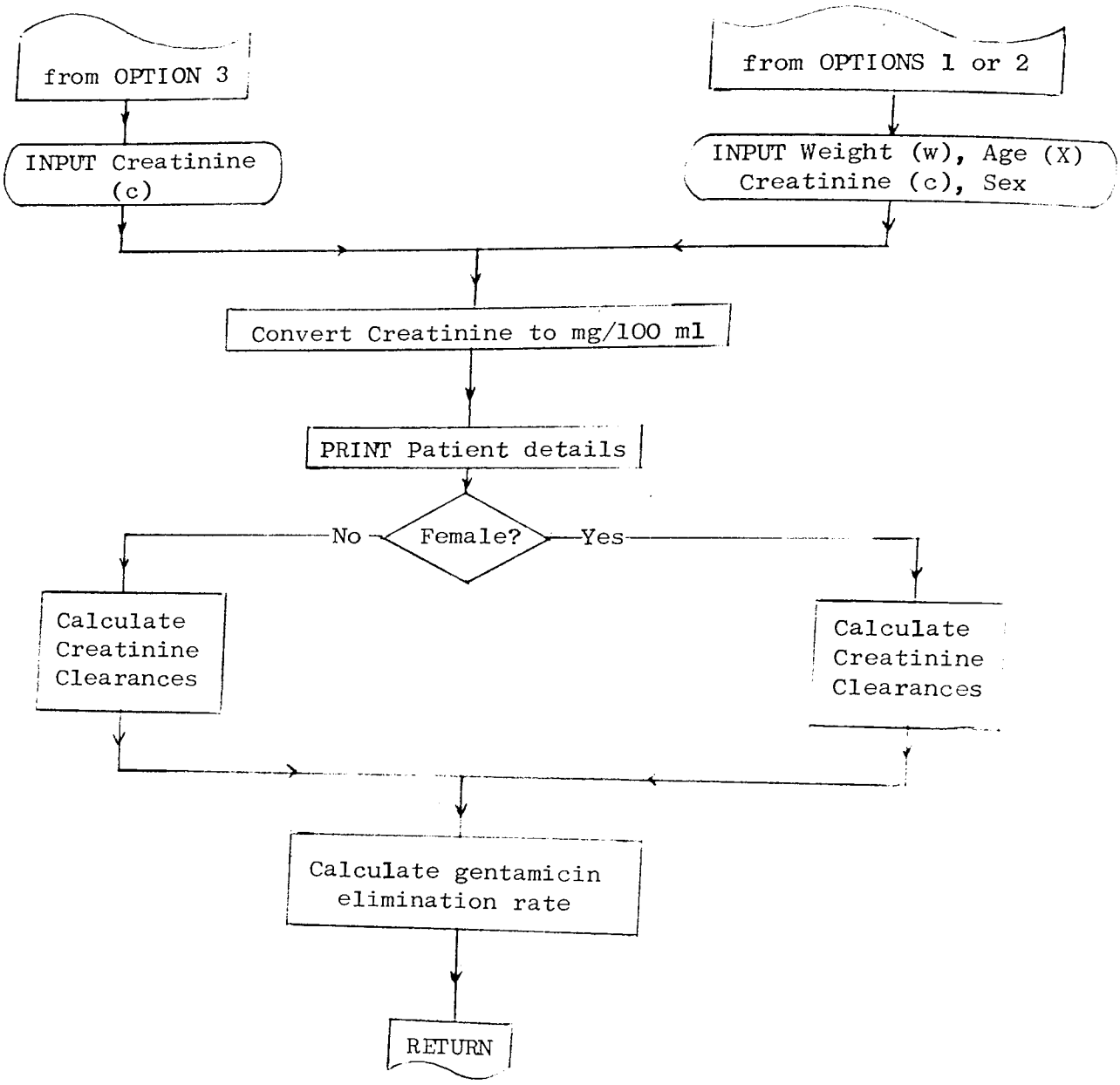


Fig 6.4 Creatinine calculations : flow chart

6.4.5. Graph Subroutine

The two early versions used a two-dimensional matrix large enough to fill the VDU screen and a lineprinter page respectively.

The serum levels, and interpolated levels according to the regression line, and the corresponding time value were converted to integer scale units and the appropriate matrix element assigned a print character accordingly. Axes and labels were similarly assigned and the whole matrix was output.

This matrix was too large, however, for the NOVA 3 core, causing program overflow. The segment was therefore omitted but since it was found to be desirable for checking the quality of regression a method of re-instating the segment was sought.

It was decided to reduce the matrix to a single-dimensiona array, assigning and outputting one row of the matrix before clearing the array and assigning the next row.

This was very costly in time and in nesting FOR-NEXT loops but reduced the core requirement greatly. A flow chart is give in Figure 6.5.

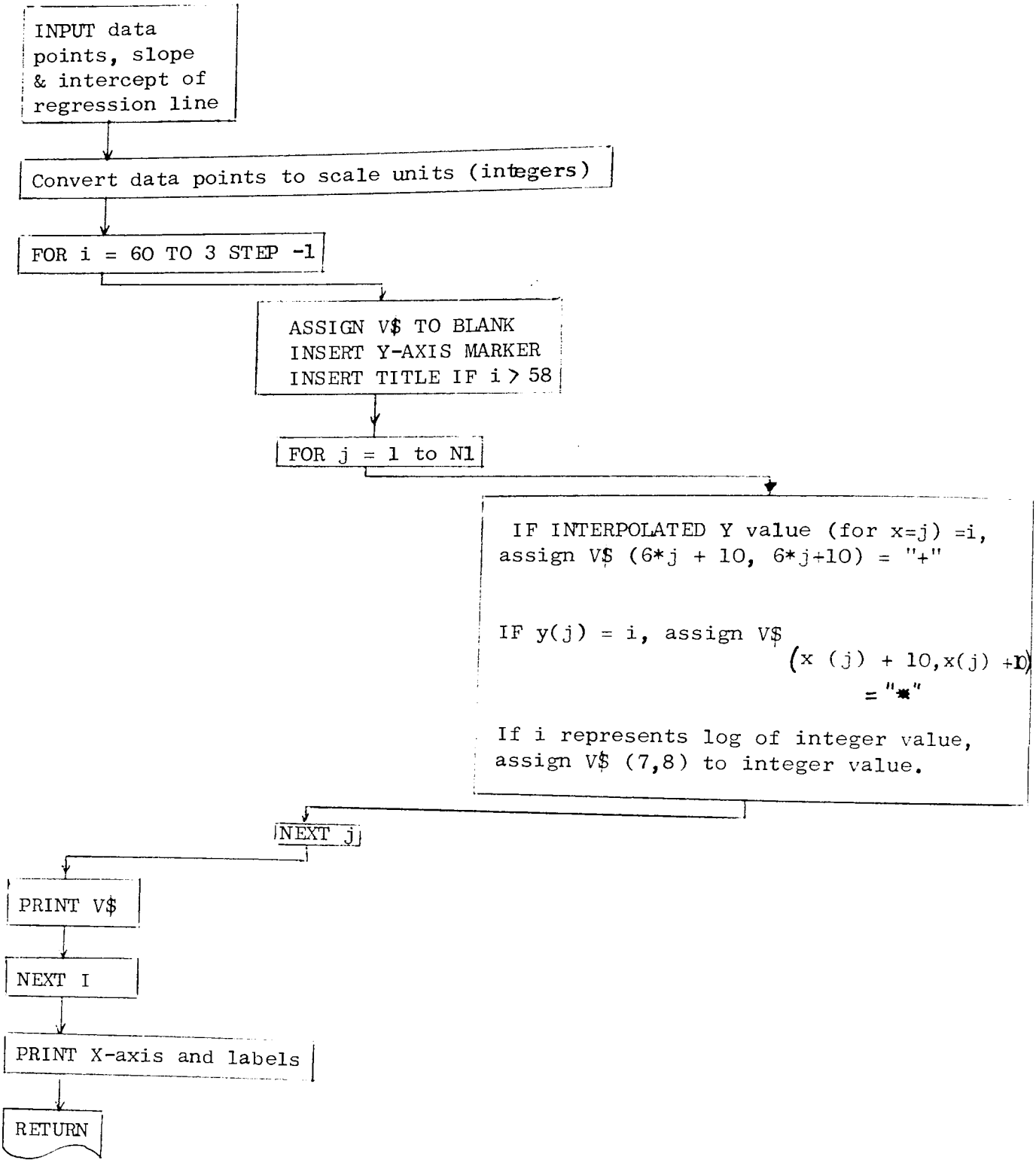


Fig 6.5 Graph subroutine : flow chart for BASIC

6.4.6. AUC segments

For calculation of AUC by the trapezoidal rule, the concentration time data were assumed to lie within the ranges denoted by the letters B and C in Figure5.8 , with the exception of the first point which was assumed to be a pre-dose measurement. All post-dose measurements were adjusted to remove the effect of previous doses by subtracting the pre-dose level which was extrapolated to the sampling time assuming a mono-exponential decay based on the calculated rate-constant. The same FOR-NEXT loop was used to calculate and summate the areas of the trapezoids between B and C.

The two extrapolated portions of the curve, before the second data point and after the last one, were then calculated and added to the total area. The calculation of V and the doses required to attain desired levels were performed and the results output to the printer.

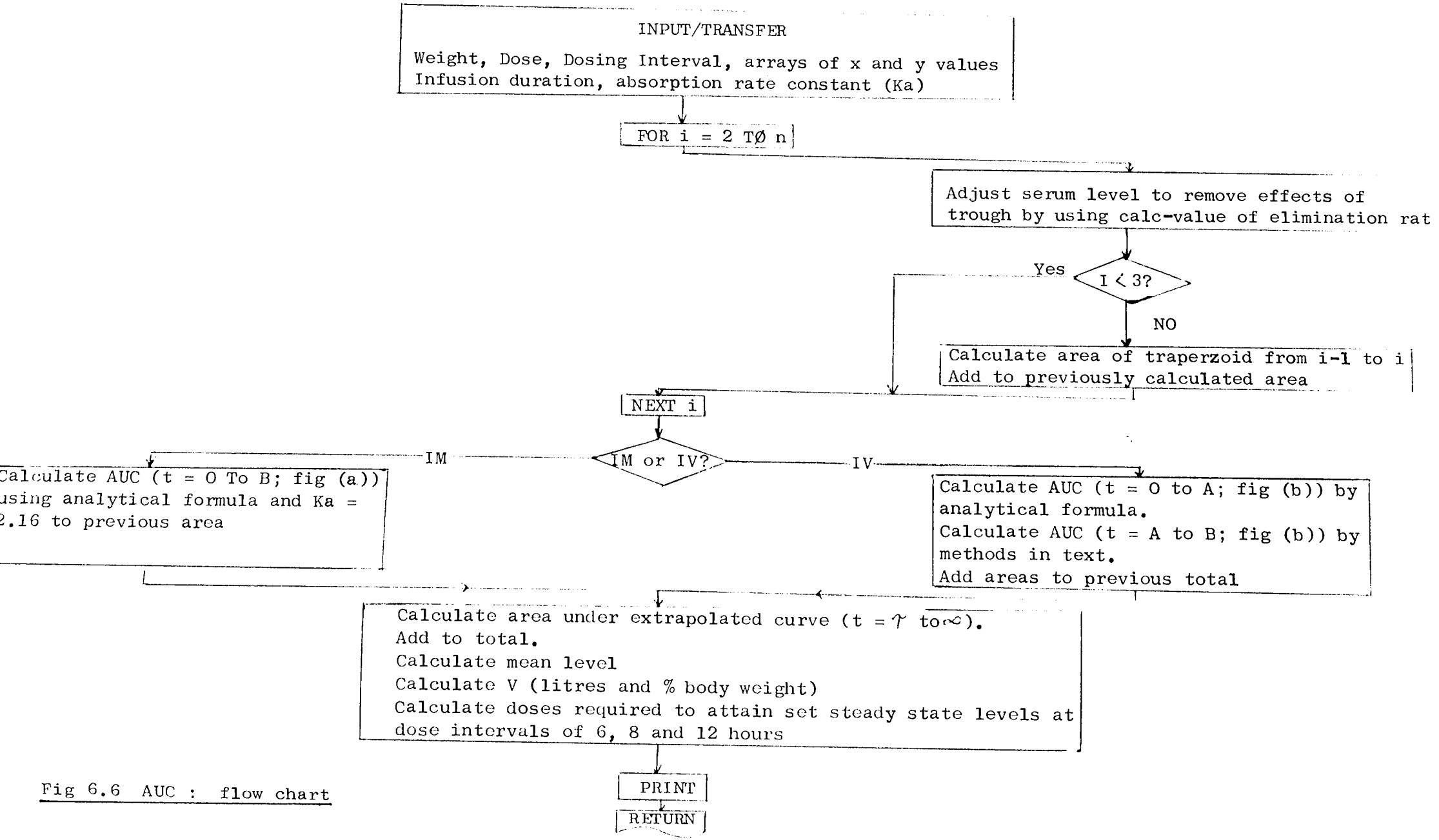


Fig 6.6 AUC : flow chart

6.4.7. Predictive routines

1. Superimposition principle, comparison with actual level

As described in Section 4 and flow-chart 6.7 this routine was developed on the HP2000 from a simple single-K routine.

The method was too sensitive for practical use, having very narrow limits for the anticipated changes in K or A (intercept of linear regression, changes presumed due to change in V) in comparison to analytical errors determined subsequently. This routine was never used on the NOVA 3.

2. Single-K, analytical formulae

A routine using analytical multiple-dose formulae was developed to avoid the use of the regression intercept and make use instead of the AUC calculations. Predictions could be made for any five times after a specified dose using the calculated V and K. If V was not known from AUC calculations a fixed percentage of body-weight was calculated. Controls were inserted to prevent zero volumes.

The ability to predict levels after several dosing periods was added by specifying the number of such periods in advance. A dosing period is defined as a period of uniform dosing, and ends when the dose or dose interval changes. A patient may have one or several such periods.

Flow charts are given in Figures 6.8 and 6.9.

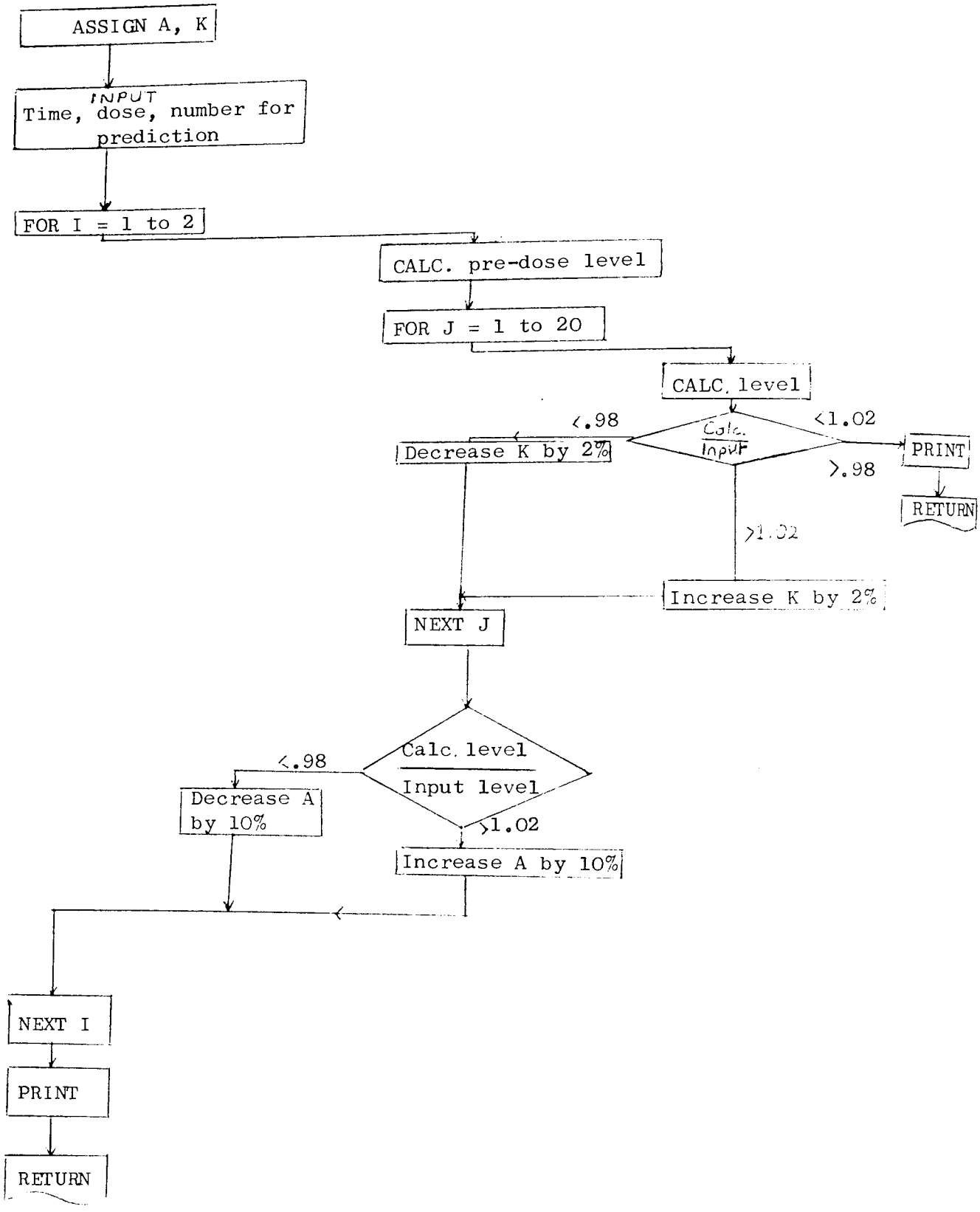


Fig 6.7 Superimposition prediction routines

6.4.7.3. Multiple-K routines

These prediction routines were arranged in two significantly different ways, distinguished by the order of the nesting of the FOR loops. The earlier version (GENTAM 2) was convenient for short or uncomplicated treatment regimens, dealt well with different K values, and followed on well from the single-K routines but became unwieldly as the complexity of predictions increased and was replaced by GENTAM 4.

GENTAM 2

It was assumed that several dosing periods might occur before any prediction was to be made, but that predictions would only be made on the last dose of the final period.

Calculations of the levels at given times were therefore nested inside the dosing periods which were nested inside the various values of K (from blood data, three creatinine methods and an input value).

Facilities existed to avoid predictions by any given means (controlled by an input codon) and there was great flexibility in being able to select different dosing periods and conditions for each K.

This meant, however, a considerable amount of operator input, on occasions requiring identical input four times. It also entailed using pre-period levels from only one value of K for calculations using any value of K.

GENTAM 4

To overcome the latter deficiency, to give greater flexibility for defining periods and to reduce input, at the expense of reduced flexibility between Ks the segment was re-arranged. The predictions for any time were made using all the previously requested values of K without operator intervention. Thus the dosing periods had to be the same for

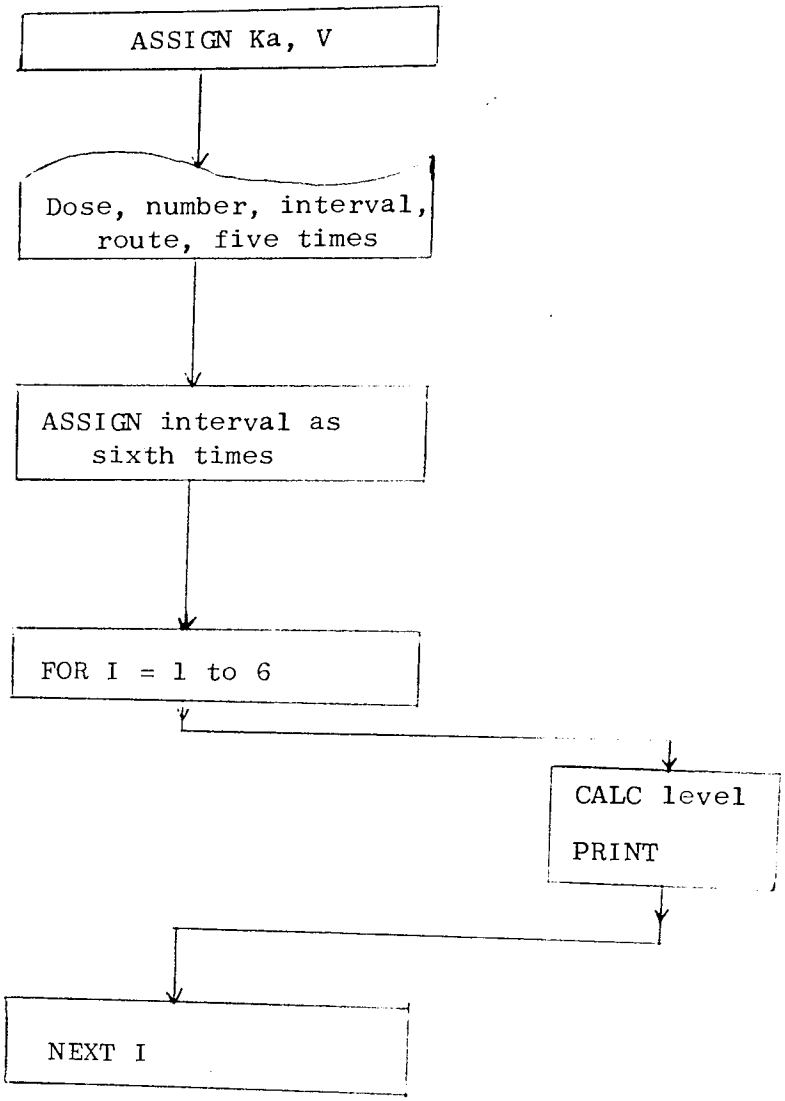


Fig 6.8 Single K analytical prediction routine (1)

each K, but predictions could be made at the end of any period if so desired.

A cumulative record of the number of doses administered, and of the different pre-period trough levels for each K, was kept.

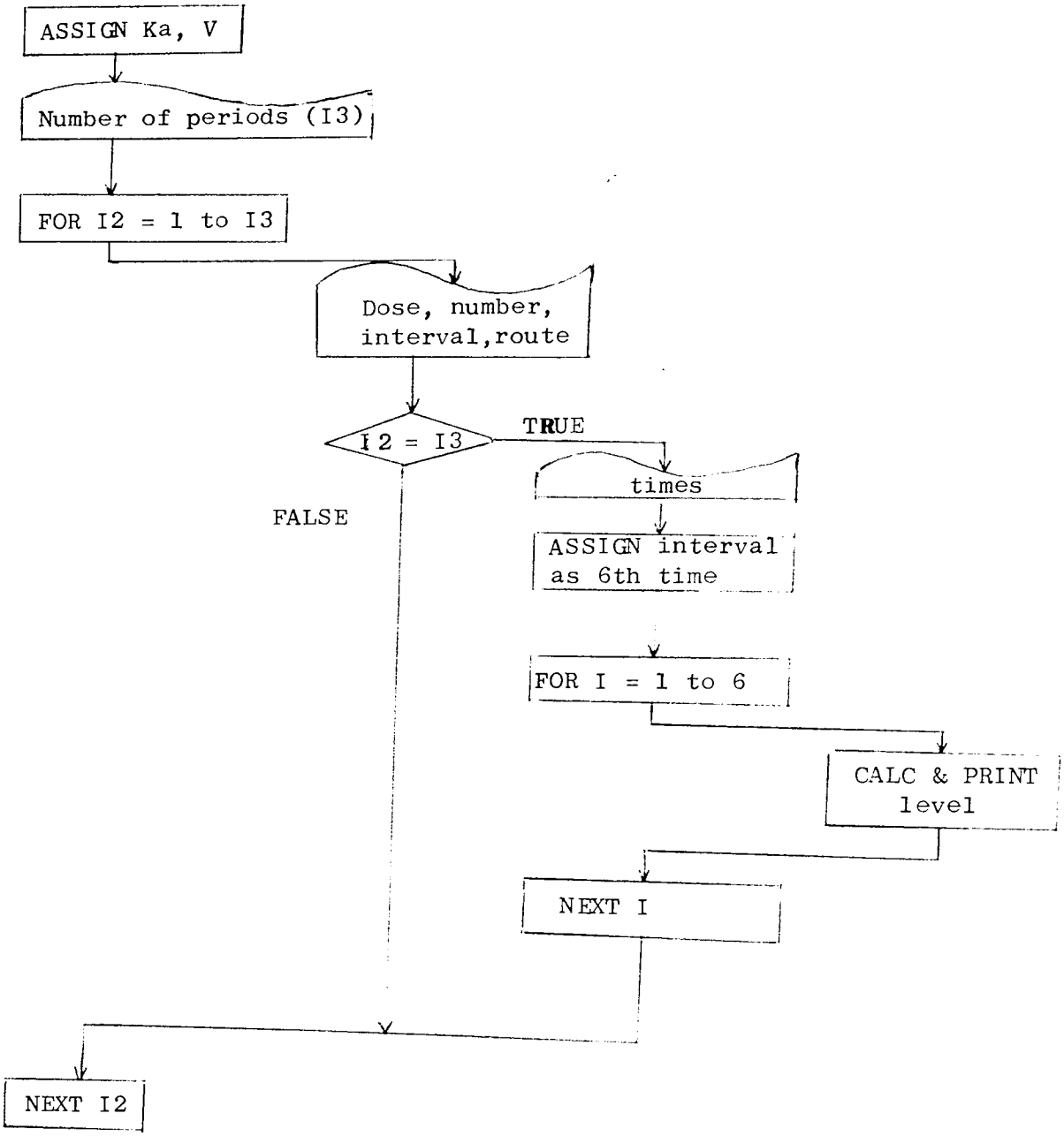


Fig 6.9

Single-K analytical prediction routine (II)

6.4.8 Input segments

.1 FORTRAN

Data were read in, one patient at a time, by an independent sub-routine called from the MASTER segment. A listing is given in appendix 2

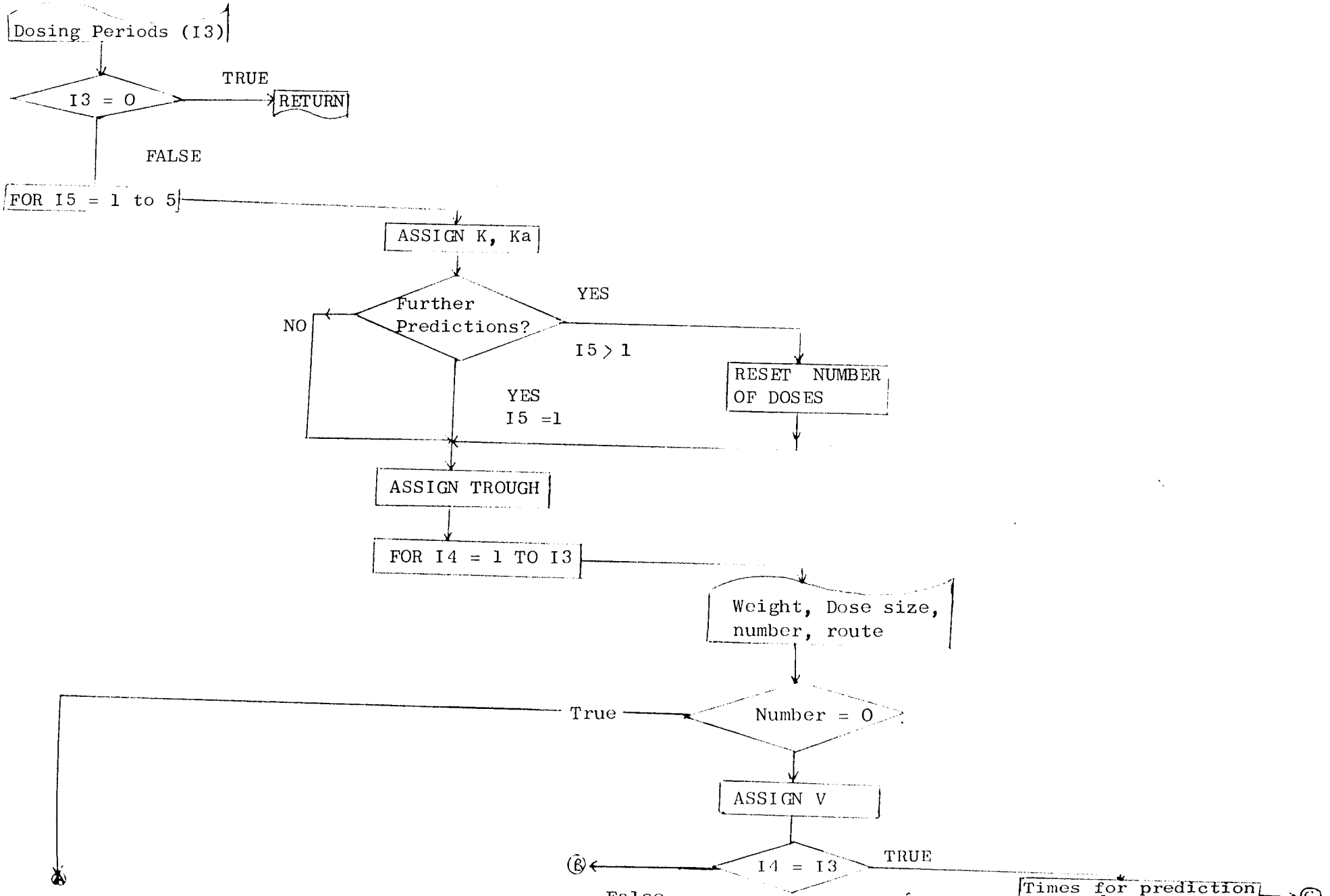
.2 BASIC-HP2000, DIRECT INPUT

A FOR-NEXT loop was used to input the paired time and concentration values for regression analysis. This had a control facility to abandon the run if the operator inserted a time value of 9999 and logged the serum levels before use. The loop terminated at the present value of n.

To permit selection of points for regression, from a previously input data set, thus saving operator time, a point selection segment was included. The data points were stored in arrays W and Z and assigned a code (1 to n) according to the order of input, and the operator asked for the number of points required. If that number was less than the total data set the operator was required to specify the code-numbers of the points required (N1). These were read into an array (E) and the required points transformed and assigned to new arrays X and Y which were used for regression. If the operator required to use all points for regression the program assigned the arrays X and Y automatically upon input of N1.

Traps were inserted to prevent the attempted use of less than three points, or more than n points or serum values of zero (which would produce errors upon logging). Time values were converted from hours and minutes (hh.mm) to hours (hh.hh) before assignation to array X.

When AUC segments were added the whole of arrays W and Z were used, together with additional information input via the AUC segment itself.


Dosing Periods (I3)
I3 = 0
TRUE
RETURN
FALSE
FOR I5 = 1 to 5
ASSIGN K, Ka
Further Predictions?
YES
NO
I5 > 1
RESET NUMBER OF DOSES
YES
I5 =1
ASSIGN TROUGH
FOR I4 = 1 TO I3
Weight, Dose size, number, route
True
Number = 0
ASSIGN V
I4 = I3
TRUE
Times for prediction

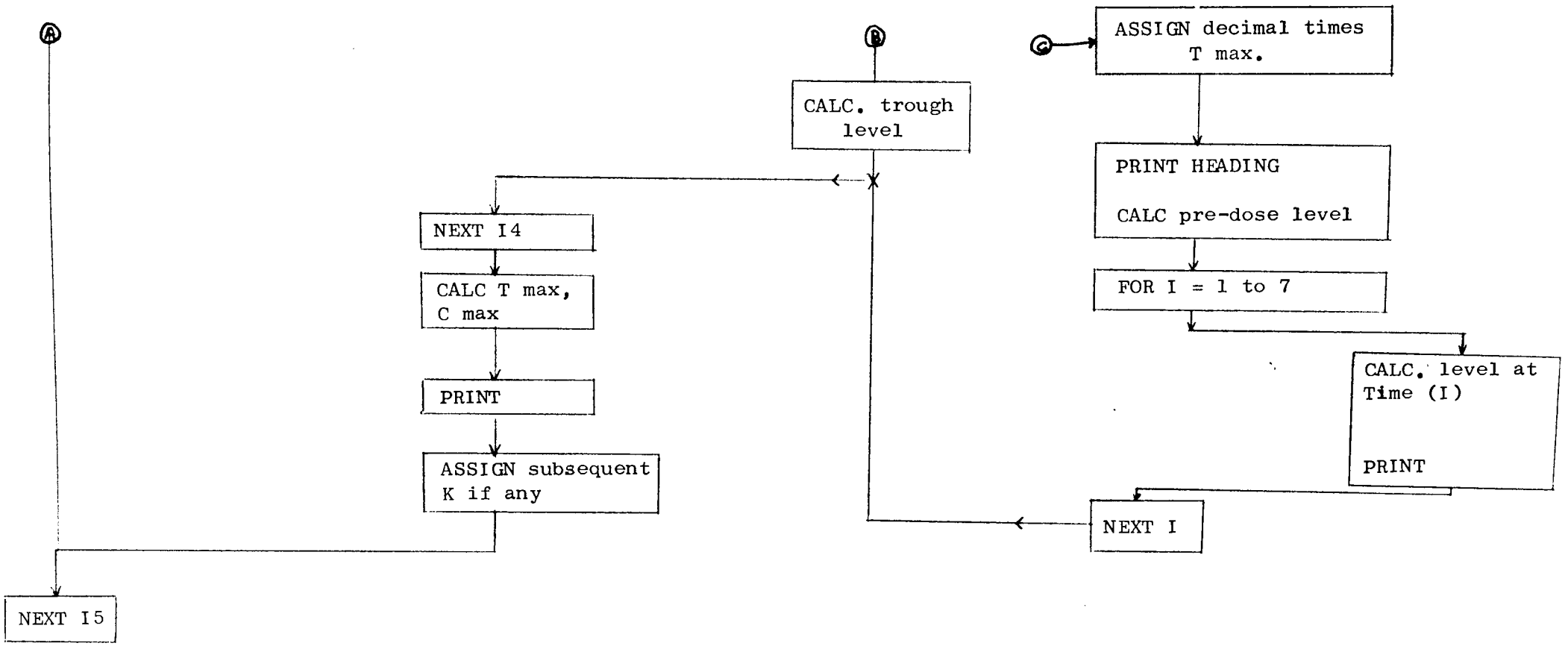
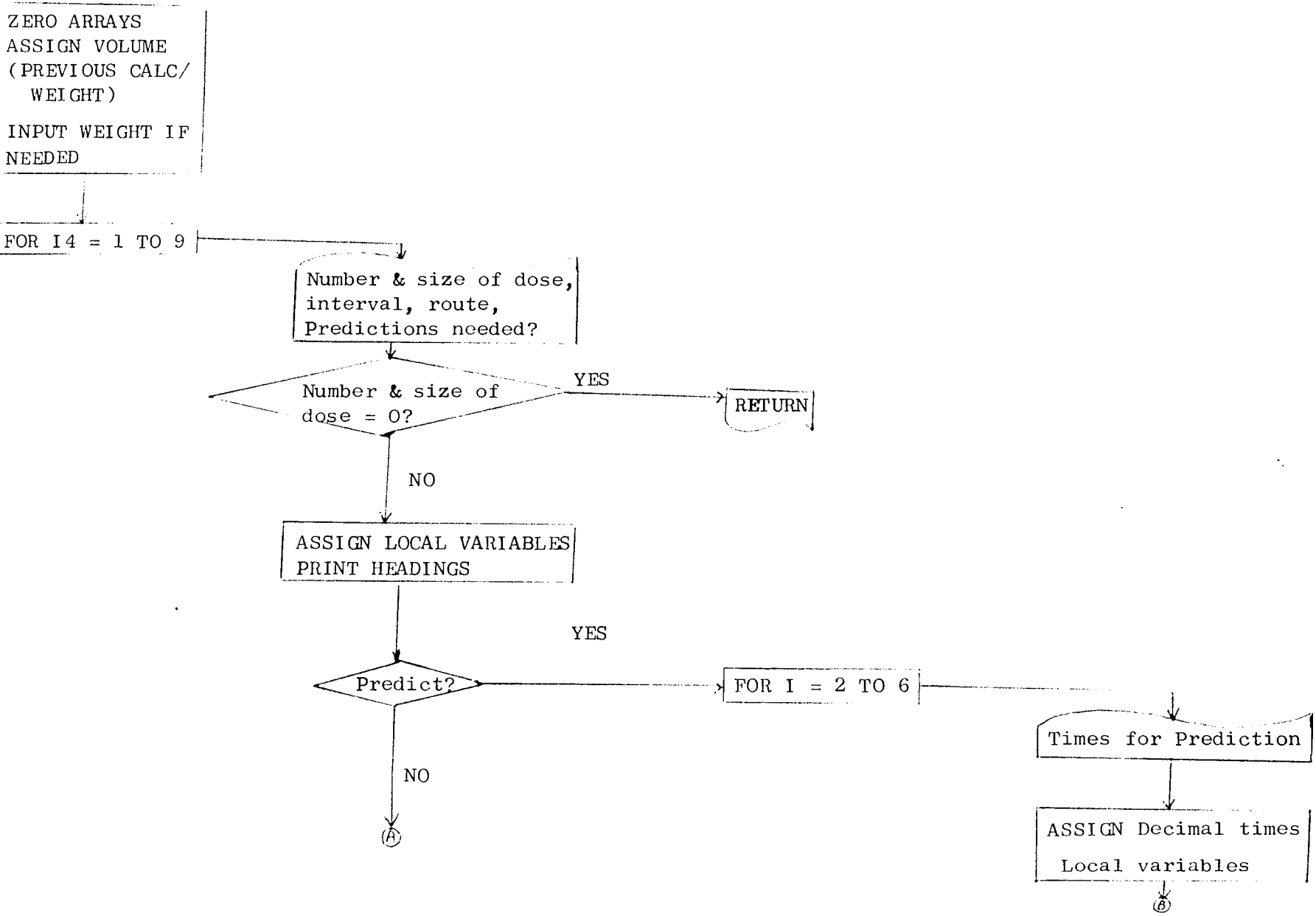


Fig 6.10 Predictive routine for 'GENTAM 2'


ZERO ARRAYS
ASSIGN VOLUME
(PREVIOUS CALC/
WEIGHT)
INPUT WEIGHT IF
NEEDED
FOR I4 = 1 TO 9
Number & size of dose,
interval, route,
Predictions needed?
Number & size of
dose = 0?
YES
RETURN
NO
ASSIGN LOCAL VARIABLES
PRINT HEADINGS
YES
Predict?
FOR I = 2 TO 6
NO
A
Times for Prediction
ASSIGN Decimal times
Local variables
B

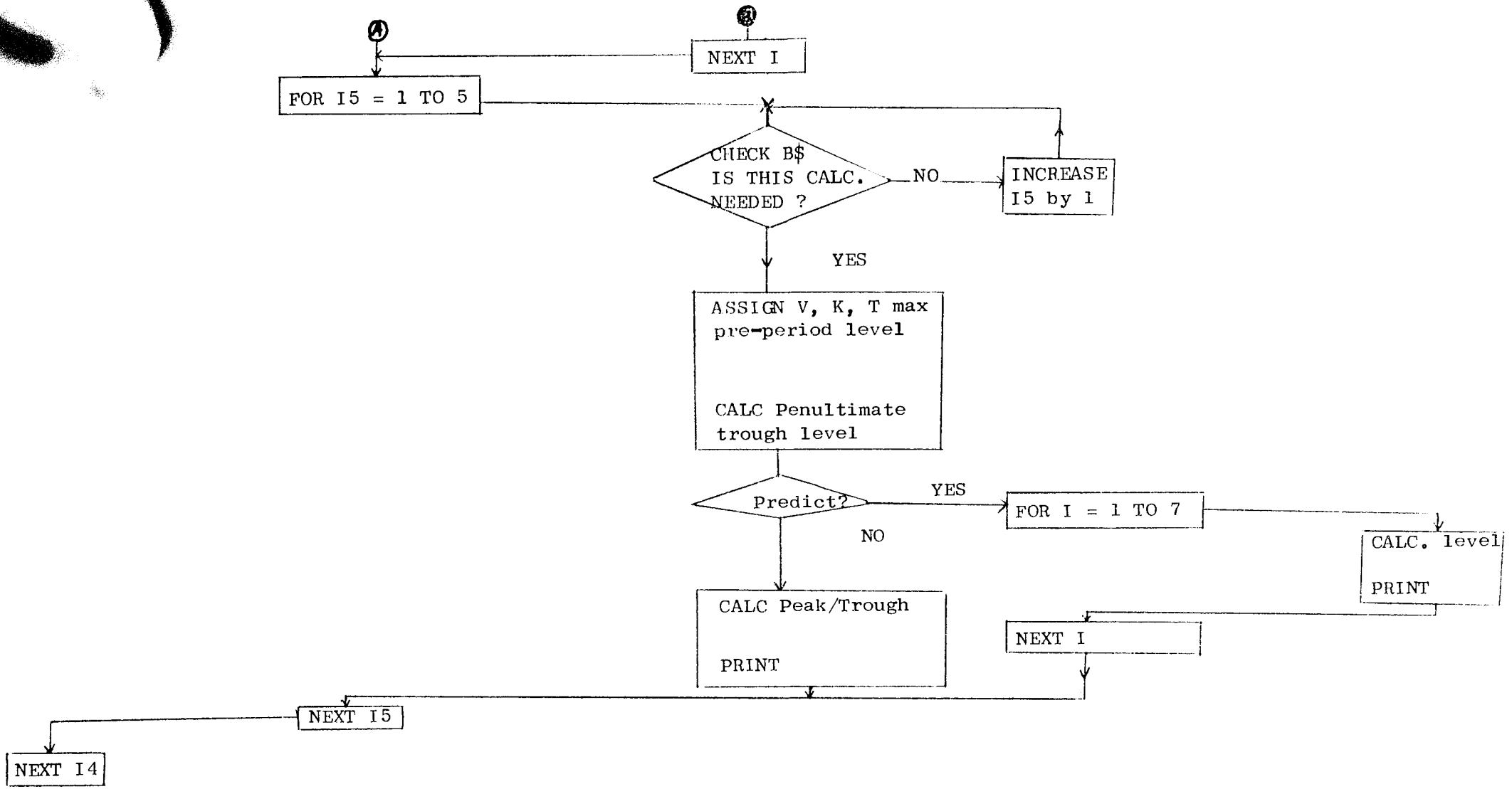


Fig 6.11 Predictive routine for GENTAM 4

(I levels may be predicted at the end of the I4th period using the I5th value of K)

6.4.8.3 BASIC - NOVA 3, DIRECT INPUT

The HP2000 routine was copied on to the NOVA 3 and used in similar fashion. It became desirable, however, to be able to select points from the data set for the AUC calculations, as well as for the regression analysis, to evaluate reduced data sets. Since the AUC calculations require a pre-dose level, as well as any points in the mixing phase, it was not possible to generate usefully the points for the AUC from those selected for regression and the versatility of the program would be severely curtailed by attempting to do so.

The array E had been input by a MAT INPUT statement on a single line, separating points by commas. Due to the shortage of variable names, it was necessary to convert E to a two dimensional array with row 1 for regression codes and row 2 for AUC codes. It was no longer possible, however, to use MAT INPUT statements since the code arrays for AUC and regression would always be of different lengths. Any attempt, therefore, to input the whole matrix at once would result in regression errors and any attempt to read in a single array would result in the loss of the other array and overflow problems, as described previously in section 6.2. It was therefore necessary to input from within a FOR-NEXT loop but to echo the input on a single line required the use of the carriage-return key (instead of a comma) and programmed suppression of carriage return and line feed. A single line was desirable to retain earlier data on the VDU screen for the operator's perusal, the NOVA 3 VDU having only a 19-line capacity.

The data for use in the AUC segment were held in a two-array matrix, M.

6.4.8.4 BASIC - NOVA 3, FILE INPUT

To facilitate later analysis or reference, file write/read segments were written to store the back-up arrays (W and Z) and certain other patient data in a serially-accessed file on magnetic disc. Two records of the file were assigned to each patient and these were located by means of a code-number assigned to each patient and stored in the file. When a new set of data was to be stored the current highest code would be read, incremented by one and re-written, together with the new patient data.

To access this data the operator ran the program as usual, but input the code for the required patient instead of a new name when requested to input a 'patient identifier'. The program would detect a numerical input, search the file for the required data, assign it to appropriate arrays and variables and list selected portions to the VDU and to the printer.

Selection of points for the working arrays proceeded as normal.

6.4.9 Output from Basic Programs

The output from the interactive programs was designed both to act as a check for the user (for verifying the input data as correct, for example) and to inform the user of the results of the calculations. Depending on the result, further calculations might be required and the output quickly exceeded the capacity of the VDU screens (HP 2000 Lynwood, 26 lines; NOVA 3, 19 lines). This necessitated the creation of a written record of the output with the attendant risks of inaccuracy and ommission that could cause difficulties at a later time. A program-generated hard-copy output was, therefore, required.

The HP 2000 had a hard-copy facility that was remote from the user and involved an over-night procedure controlled by computer centre staff. It was, therefore, somewhat limited. After application to the R.H.A. Research Committee, the NOVA 3 was equipped with a Lear Siegler 200A series ballistic printer within the user's control and the programs were expanded to utilise this device with a disc spool file and appropriate PRINT FILE commands.

Since the VDU screen width was only 80 characters it had previously been necessary to provide only essential output with a restricted format and to halt the output at intervals whilst awaiting a user-response to continue. The program-controllable, 120-character print field available on the printer was far more versatile but was restricted by the maximum permitted length of a BASIC statement (132 character). Since separate FØRMAT or IMAGE statements are not permitted in NOVA 3 BASIC (see table 6.1) the programmer had either to use a combination of string arrays (which used up valuable core) or PRINT FILE statements with suppression of line feed and carriage return.

Certain essential items were still output to the VDU, for verification, together with any warning messages issued by the program.

Output was, in general, performed as soon as possible after the

relevant operation had been performed, usually within the same program segment. A single output segment was used, however, for the creatinine and regression calculations.

6.4.10 Safeguards in Interactive Programs

To ensure the minimum of unintentional errors a range of checks and error-traps were installed in the program to obviate three main types of error, as given in the following sections.

A summary of the program flow, emphasising these checks, is given in figure 6.12. This diagram represents the viewpoint of an operator unfamiliar with the detailed working of the programs.

.1 Unfamiliarity with Computing Procedure

Early experience showed that operators unfamiliar with the operation of the computer did not always appreciate the significance of the data they were being asked to input and did not realise the consequences of any particular item. Thus, they might easily input a serum level of 0.0 μg/ml for a pre-dose level causing a fatal error as the computer attempted to take the logarithm of zero.

This type of error was overcome in several ways:

1) Improving prompts and instructions; e.g. since the codons had

to be upper case for correct identification, and could not be converted from lower case or readily tested for quality, the instructions include specific statements to this effect).

2) Automatic correction, (e.g. serum levels of 0.0 were converted to 0.0001, rather than omit the point).

3) Warnings, (e.g. Volumes of distribution outside the normal range (as % body weight) caused a message to be output to the VDU and the printer).

4) Rejection of input, (e.g. attempts to use points not in the data set, as per section 6.4.3, resulted in a message to the VDU and the return of the program to a previous request for the number of points to be used).

.2 Typing or other Accidental Error

The procedure described above also covered many of these mistakes. The NOVA 3 automatically queried any input with too few items and stopped if too many items (or the wrong type of items) were input. Whilst this prevented further errors it did mean that those users without some knowledge of the program and the NOVA 3 found difficulty in continuing the program without restarting from the beginning. To avoid this,and yet maintain multiple input,it would have been necessary to take all input as string literals and convert in parts to the desired variables. This facility was not available in NOVA 3 BASIC.

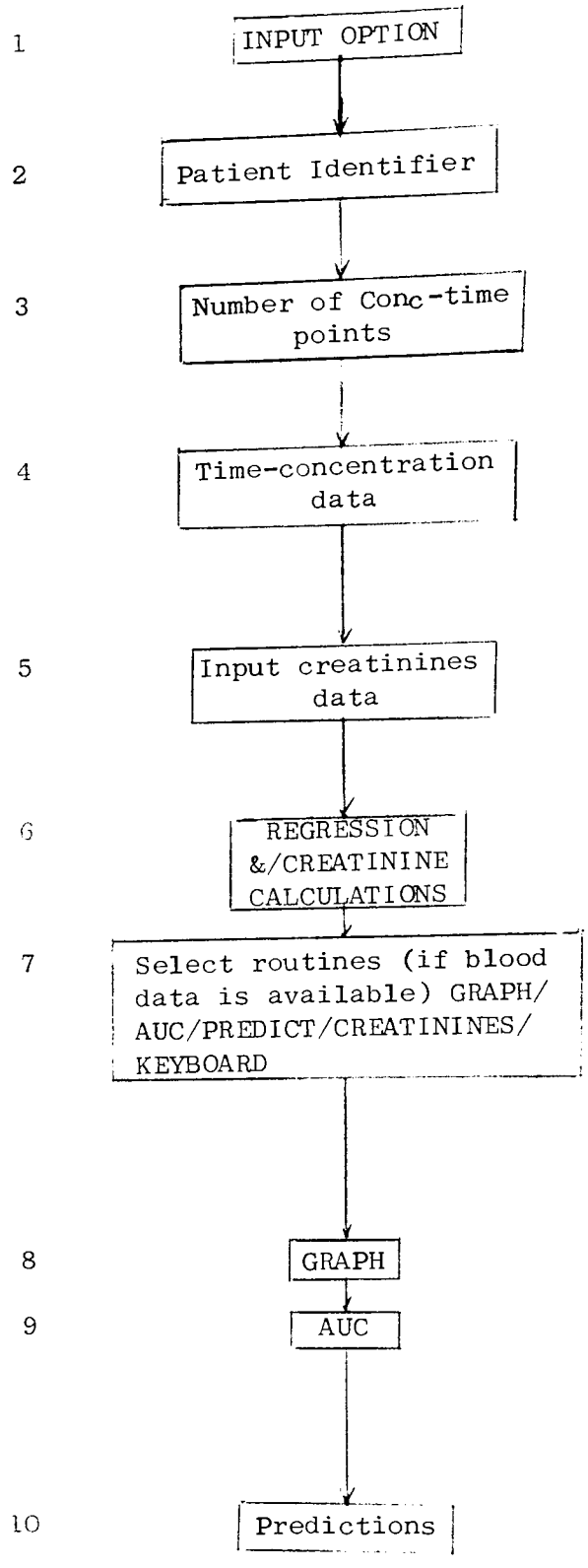
Mistakes noted by the operator before a fatal error occured, or before the input routine was concluded (e.g. as incorrect serum level), could be corrected by certain special input values. Entering 9999 as the time value for a sampling point would end the input and return to the request for the number of data points to be input. Similarly entering 0 for that number would return the program to the selection of options.

.3 Poor data or incorrect use of Data

The output contained warnings (e.g. above) and was expressed in terms

that should convey the limitations of the data. The regression statistics were quoted with a correlation coefficient (r) and a coefficient of variation (a hybrid statistic formed from the standard error of the estimate and the mean serum level (146)) as a guide to the quality of fit of the regression line. The graphs also provided a guide to the nature of the curve formed by the serum data points.

The interpretation of this information was, however, largely a matter of experience and little reliable advice could be given to novice users. An overall correlation coefficient was provided for multi-exponential data as well as for each regression. The Komologrov-Smirnov test (69) and the 'runs' test (302) were not used because of the risk of confusing users with unfamiliar statistics.



Input which is non-numeric stops program. Input which is <1 or >8 or not integer results in warning and repeated request.

If numeric input then data is read from file, if non-numeric then used as a title and output as such.

If zero, return to 1. If less than 3, issue warning about regression and repeat request.

If times are not in order, issue warning about incorrect AUC results. If first concentration is zero, correct to 0.0001 If time = 9999 return to 3.

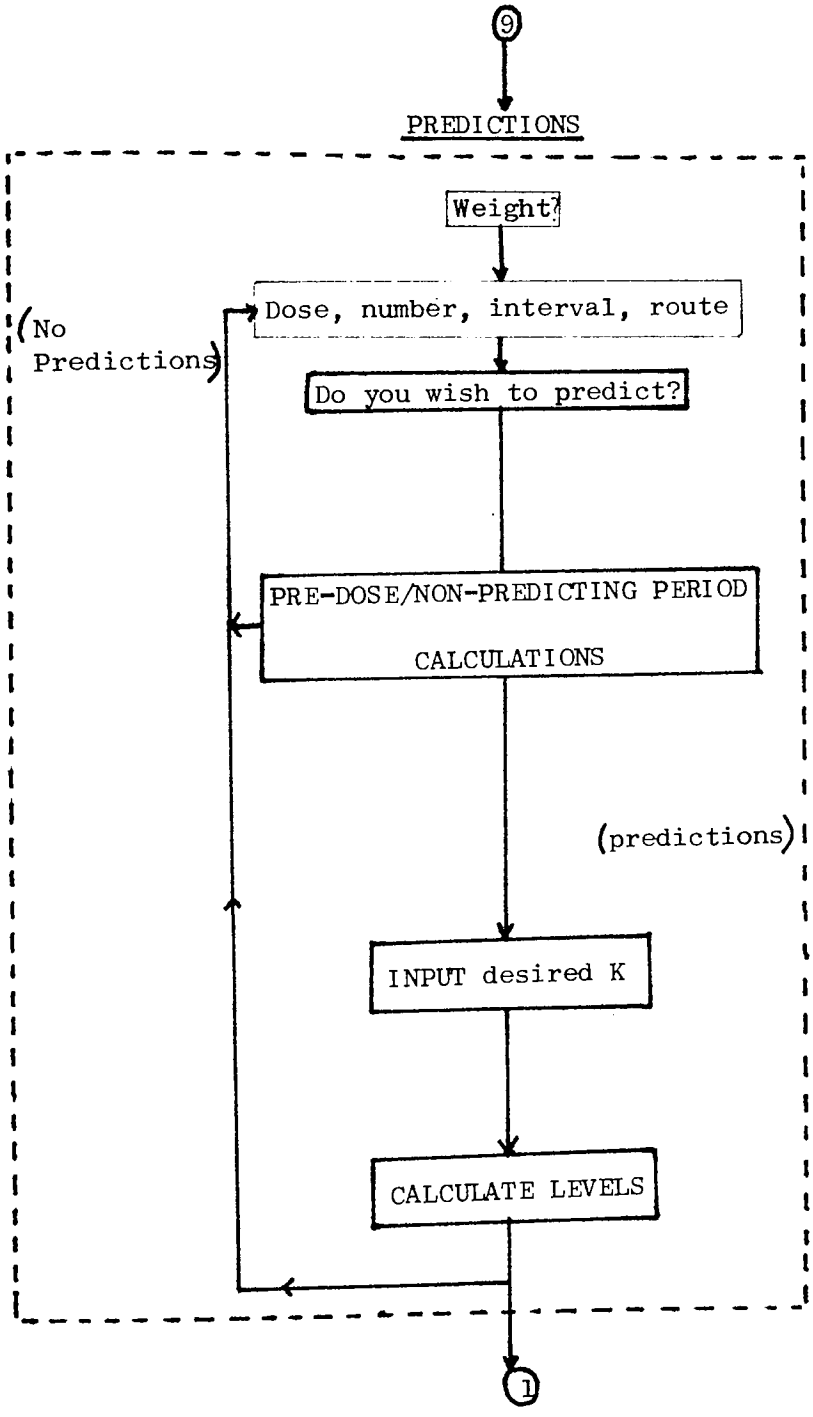
Zero creatinine returns to 1 or converts option 1 to option 5, warning and chance to correct if Weight/Age = 0.

If Y for predict from creatinines, but no creatinine calculations have been done, change Y to N. Issue warning. If coden is less than 5 characters, add N, to complete.

If no dose/interval/route, input
If no weight, input
If V outside normal range, warning
If lst level is at t>0, input new values

See separate chart.

Fig 6.12 Program flow, as seen by user, with controls and safeguards emphasised



Program flow, as seen by user, with controls and safeguards emphasised (II)

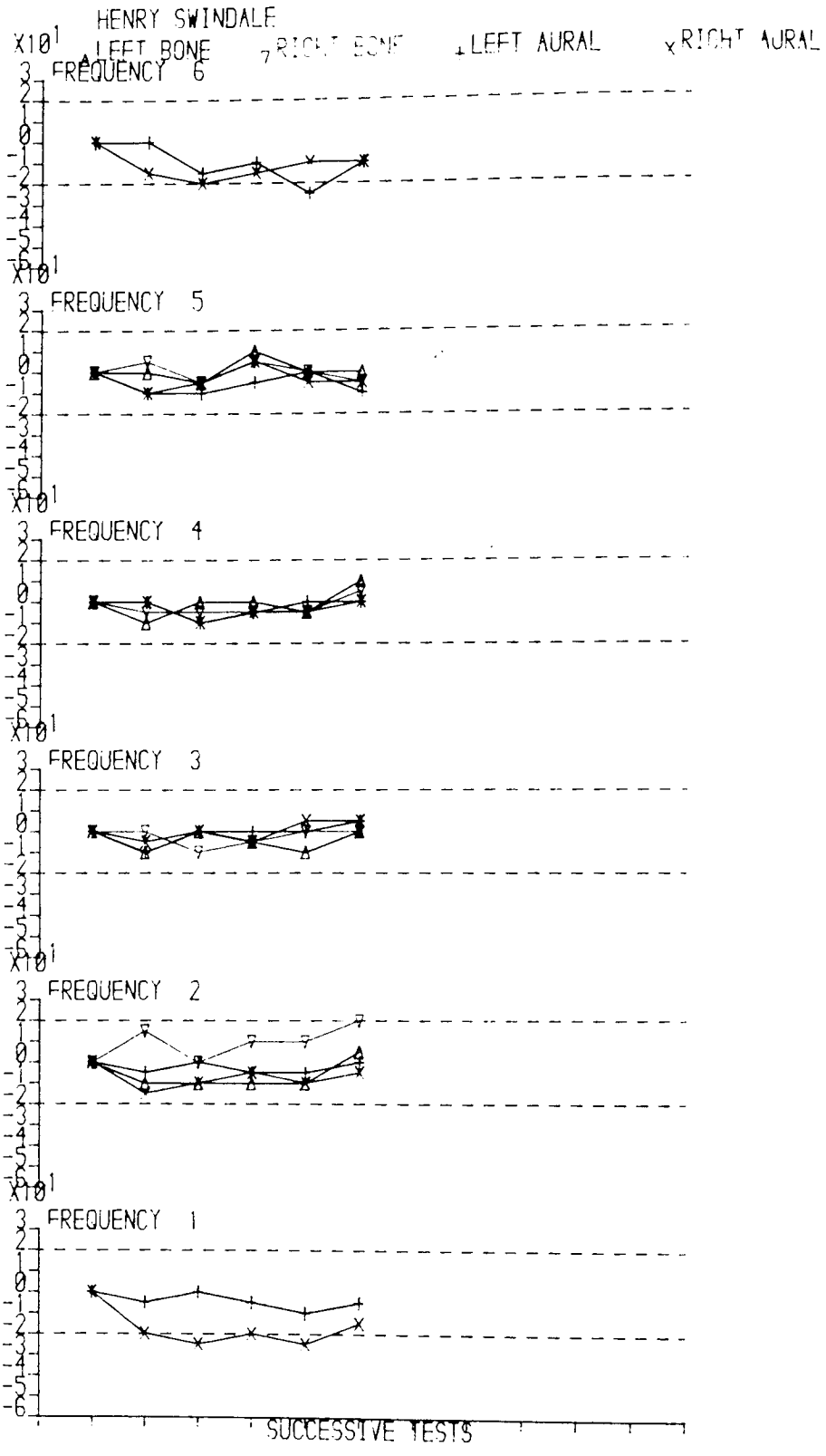


Fig 6.13

Output from MINIG 1

6.5 Other Programs

6.5.1 Ototoxicity

Two programs were written to assist the toxicity studies by plotting graphs of changes in a subject's auditory threshold. In each case data were taken from a file as threshold levels (dB) at each of 6 frequencies for aural testing, and four of those for bone conduction, for each ear. The data were converted to changes in threshold from the first test and plotted in four colours on sub-graphs with common X-axes, individual but repeated Y-axes and titling. Rasters were printed at +/- 20dB. In MINIG 1 (output illustrated in fig 6.12) the X-axis was the ordinal test number and the Y axes were the hearing changes, with the 6 frequencies plotted above each other. In MINIG 2 (not illustrated) the frequencies were plotted on the X-axis and successive tests were plotted above each other, using a second and third column as necessary.

Large-scale versions (30 cm high) were also available. The graph in fig 6.13 is a black and white photocopy of a black, red, green and blue plot. The routines used are from the GINO-F and GINO-GRAF series run on a Calcomp graph plotter via ICL 1904S. Program listings are in appendix 2.

6.5.2 HPLC

A program was written on HP2000 to calculate some chromatographic, reduced parameters using the methods of Bristow and Knox (156). A variety of inputs is available, leading to several complexities of output for standard or extempone solute-eluent systems. The program is listed in appendix 2.

6.5.3 Statistics

An interractive teaching program for statistics was written for use in the undergraduate analysis course. The program (appendix 2) was based on a least-mean squares linear regression routine (146) with two main purposes:

1) Routine data analysis for experimental work

2) Teaching.

In this latter mode each student-pair was allocated a set of data based on the regression

$$Y = 2 + 6 X$$

but with a random element added. Following instructions in a separate schedule, they used various points, selected semi-automatically from the data set and noted the resultant statistics (correlation coefficient, standard deviations of slope and intercept, standard error of the estimate, co-variance, F-statistic, confidence limits for slope, intercept and interpolated values). The quality of these statistics with regard to range, size and number of data points was noted. This program also had a monitor-file which kept a record of work done by the students and which was accessed by FILER, also listed in appendix 2.

Other statistics programs were based on this work, and other programs written for calculating standard deviations, t-statistics etc. Some of these are also listed in appendix 2, as are programs for file-handling and patient-parameter calculations, written on NOVA 3.

7 GENTAMICIN TRIAL DATA

7.1 Statistics

In this chapter statistical tests are quoted assuming a probability level of $p < 0.05$ to be 'significant' and $p < 0.01$ to be 'highly significant', using two-tailed tests unless stated otherwise. Linear regression and correlation on parametric data are performed by the method of Davis (146), and rank-correlation by the method of Spearman (143). t-tests are performed by the method of Hayslett (143) on paired data, if appropriate, or group data.

χ^2-tests are used for non-parametric comparisons of groups unless the Wilcoxon rank test is cited (143). Variance ratio (F) tests are also used and the significance of correlation coefficients is tested by the Fischer z_f transformation (143). Statistical tables were employed from two sources (204, 302).

7.2 Patient allocation and groups

30 patients had adequate serum level data for pharmacokinetic analysis and these were divided into two groups. Group A were judged clinically to have normal, or impaired-but-stable, renal function as indicated by serum creatinine at the start of the study. There were 20 such patients, of whom 19 had no other known disease that would affect gentamicin kinetics and had serum creatinine changes during the study of less than 50%. The 20th patient had inappropriate ADH secretion which lead to haemodilution in the middle of treatment (and lead to deafness amongst other consequences) but which responded later to appropriate therapy. The remaining 10 patients were allocated to

group B. Appendix 3 lists the measured parameters for each patient.

Analysis of the raw data for each patient resulted in a number of parameters (K, V, Cr, Clearance, each calculated in several ways) that were filed on magnetic disk and then analysed statistically by a suite of programs listed and described in appendix 2. Up to 31 parameters were input for each patient (from up to 3 doses per patient) and 140 other parameters generated from these. (Generated parameters are described in appendix 3 and include differences between several methods of calculating one parameter, differences in parameters between doses etc., each analysed using signed and un-signed data). The means and standard deviations of the general and first-dose parameters are given in table 7.1.

There was no significant difference between the groups with regard to age, weight, Body Surface Area (BSA) or sex distribution. There was no known history of nephrotoxicity or oto-toxicity (or significant pre-disposing factors) in any of the patients other than in those admitted for renal disease (all in group B). There was no significant difference between the groups with regard to past aminoglycoside therapy or concurrent administration of cytotoxics, diuretics or other potentially nephrotoxic or ototoxic drugs.

Since serum creatinine was used as the criterion for group allocation, there was a highly significant difference ($p < 0.01$) between groups for initial creatinine level and a significant difference ($p < 0.05$) for K. (All quoted statistical data are based on paired, or group, 2-tailed t-tests unless otherwise stated).

The volumes of distribution show a greater mean for group B, but also a much higher standard deviation (s.d.). Both these increases (not significantly different from group A) were largely due to one patient (VB) whose first dose gave extremely low serum levels, and thus a high V (99 litres, 211% BW). Since a subsequent dose gave a

Parameter	All	Group A	Group B	Group B, without VB
Sex	19M 11F	13M 7F	6M 4F	6M 3F
Age (yrs)	51.1(14.2)	50.2(13.5)	52.9(16.1)	50.3(14.76)
Weight (Kg)	65.1(10.6)	65.5(10.4)	64.4(11.4)	66.3(10.23)
BSA(m^2)	1.79(0.20)	1.80(0.21)	1.76(0.19)	1.81(0.13)
Cr(μM /1)	116.6(97.8)	84.25(17.61)	181.2(152.3)	191.5(157.7)
K(hr^{-1})	0.244(0.102)	0.277(0.079)	0.179(0.117)	0.195(0.112)
V(AUC)(1)	23.53(16.44) [20.92(8.27)]	20.67(6.06)	29.23(27.19)	21.45(12.32)
Clearance (ml/min)	83.3(41.4) [84.3(38.8)]	92.33(30.88)	65.8(54.7)	66.5(58.0)
Dose to give 2.5 μg/ml				
(mg/Kg)	1.59(0.91) [1.59(0.93)]	1.75(0.75)	1.28(1.15)	1.25(1.22)
(mg/m^2)	58.55(30.72) [58.8(31.2)]	62.51(26.38)	51.01(38.06)	50.97(40.37)
Volumes (%)				
of Weight	38.24(35.3) [32.28(13.73)]	31.93(10.0)	50.86(59.5)	33.0(20.5)
of BSA	1401.6(1201.4) [1196.8(485.0)]	1177.1(356.4)	1828.3(1981.9)	1238.5(710.7)
of $BSA^{3/2}$	1078.6(1029.1) [901.01(386.0)]	885.4(297.0)	1445.9(1699.9)	933.9(550.2)
Projected Mean Steady-State level				
(μg/ml)	3.45(2.59) [3.36]	2.74(0.84)	4.89(4.1)	5.12(1.27)
Initial dose administered				
as mg/8 hr	104.4(22.4)	111.6(21.5)	90.0(17.0)	91.1(17.6)
as mg/Kg/ 8hr	1.63(0.37)	1.73(0.37)	1.42(0.27)	1.39(0.27)

Square brackets indicate data without VB

Table 7.1 Mean values (s.d.) of general and 1st dose parameters

normal value of V (15.7 litres, 33.4% BW) and there was no evidence of any abnormal pathology or physiology, this high value was attributed to an incorrect, low, dose. This patient was one of two to receive an iv bolus, rather than a short infusion, whereby a significant proportion may have been 'lost' due to poor administration. It was for this reason that infusions were used routinely. The mean values for group B, without patient VB, are also quoted in table 7.1 and are not significantly different from group A (unless otherwise indicated, group B data excludes patient VB). The volumes, expressed as percentages of BW, BSA and $BSA^{3/2}$, were also not significantly different. The mean clearance for group B was much smaller than that for group A, but group B had a large s.d. and so the difference was not significant, and neither were the differences in standardised doses (mg/Kg and mg/m^2 to give steady-state mean levels of 2.5 μg/ml). This wide variation was partly due to one patient (VJ) who had a very large V throughout the study (45.5 litres, 78% BW). Since VB had a very low value of K, consistantly throughout the study, the mean clearance and dependant parameters are not greatly affected by the omission of their data, although if included with a normal value of V the mean clearance for the group would have been reduced by about 10%.

The volume of distribution (V), clearance and K did not correlate significantly with any other independant parameter apart from creatinine (see below). This is not surprising for elimination rate but is surprising for V, which might have been expected to be dependant upon BSA or weight.

Conflicting statements have been published (122, 123) with regard to the suitability of BW and BSA for calculating doses in terms of the levels obtained from a standarised dose. In this study there was no significant difference (Variance ratio test) in the variances

of the standardised doses calculated to give a steady-state mean level of 2.5 μg/ml (for BW cv = 58.5% overall, 42.8% for group A; for BSA cv = 53.1% overall, 42.2% for group A). There was no advantage in using an increased power of BSA, the cv of V as % BSA and % $BSA^{3/2}$ increased from 40.5% to 42.8% for all patients and from 30.3 to 33.5% for group A.

The use of harmonic mean volumes has been advocated by Chiou (224) for group mean calculations because V is the denominator in serum concentration calculations. Each of the four methods of calculating V (AUC, intercept, intercept minus trough, intercept minus calculated trough) was evaluated by arithmetic and harmonic means and compared by paired t-test. The harmonic means were highly significantly lower (group A mean difference in mean volumes = 2.2 litres, sd = 0.59, t = 6.5; overall mean difference in mean volumes = 2.83 litres, sd = 0.4, t = 12.2). Application of the F-test to the squared cvs for each set (the true variances being not strictly comparable because of the inversion of one set of values) showed a significant reduction ($p < 0.05$, 1-tailed test) in variance by harmonic calculations in group B and in A + B for V calculated by AUC, intercept minus trough and intercept minus calculated trough but the intercept method, although lower, was not significantly so. Group A showed a reduced cv by the harmonic method for all Vs but none were significantly different from the arithmetic method. The reduced curves (5 points) showed similar, but non-significant, trends for all methods of calculating V. Subsequent doses, with only 4-18 data sets evaluated by each method were insignificant with regard to differences in cv (although favouring the arithmetic method) but still gave significantly lower harmonic than arithmetic means.

The mean of V (expressed as a fraction of BW) was also lower for

PARAMETERS		GROUP A					GROUP B					A + B				
X	Y	n	r	sig	Sp	sig	n	r	sig	Sp	sig	n	r	sig	Sp	sig
Age	Creatinine	20	.11	NS	.10	NS	9	-.06	NS	.38	NS	29	-.02	NS	.23	NS
BW	"	20	.58	HS	.67	HS	9	-.27	NS	.73	S	29	-.03	NS	.66	HS
BSA	"	20	.58	HS	.59	S	9	.37	NS	.79	S	29	.24	NS	.55	S
Creatinine	K	20	-.78	HS	-.81	HS	9	-.79	S	-.30	NS	29	-.70	HS	-.73	HS
"	Gentamicin Clearance	20	-.52	S	-.28	NS	9	-.55	NS	-.23	NS	29	-.54	HS	-.47	S
Clearances (Jadrný)	"	20	.74	HS	.48	S	9	.81	HS	.27	NS	29	.75	HS	.48	S
(Cockcroft)	"	20	.34	NS	.28	NS	9	.81	HS	.35	NS	29	.59	HS	.44	S
(S-N)	"	20	.35	NS	.34	NS	9	.76	S	.53	NS	29	.58	HS	.47	S
(Jadrný)	K	20	.57	HS	.64	HS	9	.75	S	.45	NS	29	.71	HS	.51	S
(Cockcroft)	K	20	.23	NS	.38	NS	9	.83	HS	.64	NS	29	.57	HS	.48	S
(S-N)	K	20	.25	NS	.34	NS	9	.85	HS	.70	S	29	.59	HS	.46	S
Age	V (AUC)	20	.17	NS	.31	NS	9	-.45	NS	.37	NS	29	-.12	NS	.05	NS
BW	"	20	.28	NS	.30	NS	9	-.045	NS	.17	NS	29	-.12	NS	.16	NS
BSA	"	19	.22	NS	.27	NS	8	-.17	NS	.40	NS	27	-.08	NS	.12	NS
Creatinine	"	20	.26	NS	.40	NS	9	.08	NS	-.70	NS	29	-.10	NS	.20	NS
K	"	20	-.40	NS	-.41	NS	9	-.16	NS	-.07	NS	29	-.26	NS	-.28	NS
Age	K	20	-.26	NS	-.39	NS	9	-.31	NS	-.28	NS	29	-.25	NS	-.36	NS

r = linear correlation coefficient, Sp = rank correlation coefficient, sig = significance
(S = significant, HS = Highly significant, NS = Non-significant)

Table 7.2 Correlations of 1st-dose and independant parameters

harmonic means compared with arithmetic means in group A and overall, (harmonic means 29.7% and 28.3% respectively) but the difference was not significant. The cvs were not significantly reduced either (24.28% and 34.96% for group A and A + B respectively). The initial dose (maintained until the results of the first kinetic profile were known) is given in table 7.1, both as mg/8 hours and mg/Kg/8 hours. Although these are not significantly different between groups there was a large difference in the resultant mean levels. The range of doses necessary to maintain the patients at a mean level of 2.5 μg/ml was 1.04 to 4.1 mg/Kg/8 hours in the 16 patients in group A judged to have at least normal renal function on the basis of serum creatinine, age and sex.

Table 7.2 gives some parametric and rank correlations for measured serum parameters and patient characteristics. Serum creatinine correlated significantly with weight and BSA in all rank tests, but parametric tests on group B (and hence in A + B) were non-significant because of the over-riding effects of diminished renal function in some patients.

Age was not significantly correlated with creatinine, V, K or clearance.

Serum creatinine did not correlate significantly with V but did so with K (negative, $p < 0.01$ for A and A + B, $p < 0.05$ for B) and drug clearance ($p < 0.05$ for A and A + B).

The drug clearances and creatinine clearances calculated from serum creatinine by the methods of Jadrný, Cockcroft and Siersback-Nielsen varied in their correlations with measured clearance and K. Significant correlations were obtained overall, but in each group there were several non-significant correlations. Jadrný's method did significantly better for drug clearance in group A than the other two, but Siersback-Nielsen's method did better in group B (a non-significant trend). This may reflect differences in the populations used to derive the methods, or it may reflect the small size of groups in this study.

X	Y	Group A (n = 20)		Group B (n = 9)		A + B (n = 29)	
		intercept	slope	intercept	slope	intercept	slope
Cl (J)	Kf	.1003	.00194	.0467	.00292	.0771	.00222
Cl (CG)	Kf	.2147	.00077	.0058	.00373	.1089	.00200
Cl (SN)	Kf	.2041	.00085	1×10^{-6}	.00351	.0957	.00204

(N.B. correlation coefficients for K (x) vs Kf are the same as Cl (x) vs Kf).

Table 7.3 Regression data for correlations of clearance estimates and measured gentamicin half-life

The range of serum creatinine values in this study was smaller than those in most other studies and thus the linear correlations are not biased by out-lying values.

The effect of outliers is demonstrated by this trial in that if 31 full estimates of K are compared with serum creatinine by linear regression, a correlation of the form,

$$K = 0.145 + 0.031\ Cr \quad (r = 0.92)$$

is obtained. Only two of those creatinine values exceeded 200 μM/l and were 440 and 490 μM/l respectively. If they are removed from the data set,

$$K = 1.19 + 0.020\ Cr \quad (r = 0.53)$$

showing a highly significant difference in correlation.

The regression parameters for the first dose are given in table 7.3 for comparison with Jeliffe's method;

$$K = 0.012 + 0.0034\ (Cr\ clearance)$$

(The estimates of clearance and K from creatinine are linearly related and the correlations of these parameters with any other parameter are identical. These data, are not, therefore, duplicated in tables).

Although the parameters for the two groups are varied, and group B is the most similar to Jeliffe who used measured rather than predicted clearances, the overall values are very similar between these three methods.

Method	Signed Parameters				Absolute Parameters				
	mean (%)	cv	t	sig	mean (%)	cv	t	sig	Upper Confidence limit (95%)
				GROUP A (20 patients)					
I	-10.9	136	-3.19	HS	16.0	59.6	7.31	HS	34.7
I-CTr	3.0	695	.63	NS	15.3	94.9	4.59	HS	43.7
I-Tr	6.1	118	3.68	HS	6.5	106.6	4.09	HS	19.8
5 AUC	9.2	253	-1.72	NS	12.6	171.0	2.55	S	54.8
4 AUC	9.8	238	-1.83	NS	12.9	167.9	2.60	S	55.4
I3-Tr	-15.9	136	-3.19	HS	16.5	129.6	3.36	HS	58.4
				GROUP B (9 patients)					
I	-23.3	135	-2.1	NS	33.7	54.2	5.22	HS	69.5
I-CTr	15.1	257	1.1	NS	27.6	109.4	2.59	S	86.8
I-Tr	5.9	413	0.69	NS	11.0	200.5	1.41	NS	54.0
5 AUC	-0.5	9333	-0.03	NS	26.1	161.5	1.75	NS	115.6
4 AUC	1.9	2742	0.10	NS	29.1	143.9	1.97	NS	111.2
I3-Tr	0.5	11000	0.03	NS	29.4	139.9	2.02	NS	110.0
				A + B (29 patients)					
I	-14.81	142	-3.7	HS	21.5	69.1	7.94	HS	50.6
I-CTr	6.8	408	1.3	NS	19.1	109.3	4.84	HS	60.0
I-Tr	6.0	236	2.2	S	7.9	168.3	3.14	HS	33.9
5 AUC	-6.5	511	-1.0	NS	16.8	174.9	3.03	HS	57.6
4 AUC	-6.1	557	-.95	NS	17.9	165.4	3.20	HS	75.8
I3-Tr	-10.8	312	-1.7	NS	20.5	140.7	3.76	HS	77.1

Table 7.4 Standardised differences in estimate of V from that of AUC on first monitored dose

eg $(V_I - V)/V$

7.3 Alternative estimates of parameters

Three methods of estimating V, detailed in chapter 4 and referred to above, were compared to that from the Area Under a full Curve (AUC) (denoted Vf), as were three methods from reduced curves (AUC from 5 points, V5, AUC from 4 points, V4, and intercept minus trough from 4 points, VI3 - Tr).

The differences in estimates were expressed in standardised form (eg., (V5 - Vf)/Vf) for both signed and absolute differences. (In this thesis, 'standardised differences, also referred to as 'errors', are always calculated in this way, with the fuller method as denominator and the result expressed as a percentage). Table 7.4 gives these data and table 7.5 gives correlation data with Vf as the Y-vector. These results should be viewed in the light of the desirability of minimising invasive procedures, i.e. the number of blood samples. The full (10 or 11 point) method may be reduced to 6 or 7 (V_I - Tr) or to 5 or 6 (V_I and V_I - CTr) without affecting the estimate of K. As predicted in chapter 6, the V_I - Tr is superior to the other methods in terms both of correlation and of standardised differences. In group A the maximum likely error (95% confidence limit) is 19.8% whilst the correlation coefficient is 0.999. The maximum likely error is based upon unsigned data, the signed data serving mainly to show the presence of any bias in the error. The group A mean signed difference is 6.1%, highly significantly different from zero, showing an underestimate in the intercept or an overestimate in the trough. The former is highly likely, since the intercept is calculated from the α_2 phase and by definition α_1 phase levels will be higher than those prediced by α_2 regression. The AUC methods are affected much less than the intercept methods by use of an incorrect model.

Also as predicted in chapter 6, the V_I method gives estimates that are significantly lower than those of Vf and which correlate

PARAMETERS		Group A			Group B			A + B		
X	Y	n	r	sig	n	r	sig	n	r	sig
V_I	Vf	20	.85	HS	9	.72	S	29	.70	HS
V_I-CTr	Vf	20	.86	HS	9	.80	HS	29	.80	HS
V_I-Tr	Vf	20	1.0	HS	9	.98	HS	29	.97	HS
V_5	Vf	19	.92	HS	8	.95	HS	27	.93	HS
V_4	Vf	19	.92	HS	8	.95	HS	27	.92	HS
V_{I3}-Tr	Vf	19	.91	HS	8	.95	HS	27	.89	HS
Cl_I	Cl	20	.85	HS	9	.93	HS	29	.88	HS
Cl_I-CTr	Cl	20	.80	HS	9	.91	HS	29	.84	HS
Cl_I-Tr	Cl	20	.98	HS	9	.98	HS	29	.98	HS
Cl_5	Cl	19	.97	HS	8	.96	HS	27	.99	HS
Cl_4	Cl	19	.98	HS	8	.96	HS	27	.95	HS
Cl_{I3}-Tr	Cl	19	.97	HS	8	.95	HS	27	.96	HS
K3	Kf	19	.86	HS	8	.96	HS	27	.92	HS

Table 7.5 Correlations of various estimates of V, K and Cl

significantly less well with Vf than does V_I-Tr. The use of a calculated trough, rather than a measured one, resulted in a slightly improved correlation in all groups and smaller mean differences. There was a greater variability in the differences, however, which rendered the signed mean difference non-significant and the upper confidence limits greater than those of the other intercept methods.

Reducing the estimate of K to that using 3 points, and the whole curve to 3, 4 or 5 points, increased the variability of differences in estimates and consequently increased the upper confidence limits for V (54 - 59% for group A) whilst not significantly increasing the mean differences for signed or unsigned data. The correlations are all good but there is a non-significant trend towards poorer results (grouped t-test) as the sampling points are reduced from 5 to 4 to 3.

The estimate of K by 3 or by 5 or 6 points is similarly compared in tables 7.5 and 7.6. The 34 standardised differences have a signed mean of 9.9% and an unsigned mean of 13.4%, both highly significantly different from zero. The absolute mean differences from the first dose were greater than those from all doses, but not significantly so. The correlation coefficients were all highly significant. There was no significant correlation between K or V and the corresponding standardised differences in K for full and 5 point curves for any group or dose.

The estimates of clearance (Cl) from the six methods, compared with that of full AUC, all show good correlations, but methods I and I-CTr are significantly worse in group A and A + B than the other methods. Clearance is an important parameter because the dose (D mg) to attain a given steady-state mean level is calculated directly from it by means of

$$D = (\text{mean level}) \times Cl \times \tau$$

which, for τ = 8h and mean level = 2.5 μg/ml becomes,

Group	n	Signed Mean (%)	cv	t	sig	Absolute Mean (%)	cv	t	sig
A	19	8.5	371.0	1.14	NS	20.0	128.2	3.31	HS
B	8	-6.3	680.4	-.39	NS	25.0	135.4	1.95	NS
A + B	27	4.2	844.3	0.6	NS	21.5	129.1	3.95	NS
A	26	11.4	147.5	3.39	HS	13.3	115.1	4.43	HS
B	8	5.1	404.1	-.65	NS	13.7	111.7	2.37	NS
A + B	34	9.9	177.8	3.23	HS	13.4	112.5	5.10	HS

Standardised difference = (K3 - Kf)/Kf

Table 7.6 Standardised differences in estimates of K by 3 point and full (5 or 6) point methods

	GROUP A			GROUP B			A + B		
Parameter	Mean	cv	n	Mean	cv	n	Mean	cv	n
Full K	.272	30.5	31	.201	55.6	12	.252	38.1	4
3pt K	.286	31.7	38	.159	65.6	16	.249	44.5	5
full V	20.0	35.0	31	21.3	56.0	12	20.4	41.8	4
5pt V	18.9	29.4	34	22.0	50.2	16	19.9	39.1	5
Cl(AUC)	86.7	35.1	31	67.5	82.2	12	81.7	48.4	4
Cl(5pt)	88.4	36.5	34	62.3	108.6	16	80.8	59.1	5

$K(h^{-1})$, V(l), Cl (ml/min)

Table 7.7 Combined estimates of parameters from all doses

$$D = 20\ Cl$$

Table 7.8 lists the mean standardised differences in doses calculated by each of these methods compared with AUC. (These values, or their means, are a simple multiple of the difference in clearance, but are more relevant to clinical practice). The mean differences range from 8 to 20 mg (highly significant) but the signed means show no significant bias except for methods I and I-Tr. The latter has a lower cv than the other methods. The most relevant parameters, however, are the percentage of patients who would have received identical doses by AUC method and the method of choice. The discriminant levels given (5 mg and 10 mg) reflect the precision of dosing in optimum and typical circumstances respectively (using gentamicin injection, 80 mg/2ml). I-Tr proves to be the best method, in comparison to AUC, with 65.5% within 5 mg of the AUC-calculated dose and 82.7% within 10 mg in groups A + B.

Method I was the worst (13.8% and 31% respectively) but the other methods were very similar, with different rank-orders for 5mg and 10 mg. The differences, expressed as a fraction of the AUC-calculated dose, did not correlate significantly with V, K or clearance for any group or method.

The choice of a reduced-sampling method, assuming the full AUC to be standard, is not, therefore, clear. If 6 or 7 samples are acceptable, then I-Tr is definitely superior to the others (sampling once pre-dose and 5 or 6 times after 2 hours). Since, however, 6 or 7 samples are unlikely to be acceptable, both in terms of staff time and patient discomfort, the choice ranges from I3-Tr and 4AUC (4 points each) to I-CTr and 5AUC (five points), I having been discarded. It is useful to have a measured trough value, to give a clearer indication of the cause of abnormally high or low levels and to give

several options for calculations, and so I-CTr is eliminated. The remaining three methods do not show any clear differences and so 5AUC, using one more sampling point, may be discarded.

In comparison with creatinine methods, however, all the drug-level methods are good. Tables 7.9a and 7.9b give data comparable to that in table 7.8 and show that whilst using the Jeliffe value for K and V equal to 30% weight is an improvement over creatinine clearance, the best result (Cockcroft and Gault method, group B) has only 44% of patients within 10mg of the reference dose and group A has a maximum of 30% within 10mg. (As noted earlier, the method of Jadrný proves to be highly significantly worse than Siersback-Nielsen in group B.) Since the Jadrný formulae are much easier to use than those of Siersback-Nielsen, they would seem to be the methods of choice. Cockcroft's methods are arithmetically easier than either of the others, and achieve similar results to those of Siersback-Nielsen and so would also be preferred to Siersback-Nielsen.

Methods for V and K		SIGNED PARAMETERS Mean (mg)	cv (%)	t	sig	ABSOLUTE PARAMETERS Mean (mg)	cv (%)	t	sig	% within 5mg	% within 10mg
				GROUP A (20 patients)							
I	Kf	-11.5	179	2.44	S	18.37	79	5.36	HS	20	35
I-CTr	Kf	2.5	1047	0.42	NS	18.24	101	4.30	HS	35	55
I-Tr	Kf	8.0	158	2.76	S	8.36	148	2.94	HS	55	80
5AUC	K3	4.2	523	0.83	NS	12.88	142	3.08	HS	45	65
4AUC	K3	3.7	590	0.74	NS	12.41	148	2.94	HS	45	70
I3-Tr	K3	-5.1	378	-1.15	NS	10.05	169	2.57	HS	35	85
				GROUP B (9 patients)							
I	Kf	-17.5	165	-1.7	NS	22.7	108	2.62	S	0	22.2
I-CTr	Kf	-1.5	2190	-.13	NS	19.2	137	2.07	NS	22.2	77.8
I-Tr	Kf	2.2	574	.49	NS	6.7	163	1.73	NS	66.7	88.9
5AUC	K3	7.3	600	.47	NS	26.7	130	2.18	NS	55.5	55.5
4AUC	K3	9.4	487	.58	NS	26.5	142	1.99	NS	55.5	66.7
I3-Tr	K3	9.1	557	.51	NS	29.7	138	2.05	NS	55.5	55.5
				A + B (29 patients)							
I	Kf	-13.4	172	-3.02	HS	19.7	90	5.78	HS	13.8	31.0
I-CTr	Kf	1.26	2229	.24	NS	18.6	112	4.73	HS	31.0	62.0
I-Tr	Kf	6.21	205	2.58	S	7.8	150	3.52	HS	65.5	82.7
5AUC	K3	5.20	574	.92	NS	17.2	144	3.67	HS	48.3	62.1
4AUC	K3	5.50	557	.95	NS	16.8	155	3.41	HS	48.3	69.0
I3-Tr	K3	-.68	4727	-.11	NS	16.1	171	3.10	HS	41.4	75.9

Table 7.8 Differences between the required doses (mg) calculated by six methods and the dose calculated by AUC. (1st dose, assuming $\tau = 8$, $\bar{c}_{ss} = 2.5\ \mu g/ml$)

	Signed parameters			Absolute parameters				% with difference less than	
	Mean	t	sig	Mean	cv	t	sig	5mg	10mg
				GROUP A (20 patients)					
J	-1.8	-.32	NS	17.9	93.2	4.68	HS	15.0	30.0
CG	-14.3	-1.63	NS	32.1	75.3	5.79	HS	5.0	10.0
SN	-7.9	-.91	NS	30.5	75.4	5.78	HS	0.0	15.0
				GROUP B (9 patients)					
J	-18.7	-1.15	NS	31.5	119.3	2.37	S	0.0	33.3
CG	-18.9	-1.10	NS	28.8	148.0	1.91	NS	11.1	33.3
SN	-13.1	-.75	NS	29.8	136.4	2.07	NS	11.1	22.2
				A + B (29 patients)					
J	-7.1	-1.13	NS	22.2	113.8	4.65	HS	10.3	31.0
CG	-15.7	-2.04	S	31.1	97.5	5.43	HS	6.9	17.2
SN	-9.5	-1.23	NS	30.3	95.1	5.56	HS	3.4	17.2

Table 7.9(a) Differences between the required doses (mg) calculated by estimated creatine clearances and the dose calculated by AUC ((Cr-AUC), 1st dose, τ = 8, $\bar{c}_{ss}$ = 2.5 μg/ml)

				GROUP A					
J	13.9	1.95	NS	27.37	71.8	6.07	HS	20.0	30.0
CG	3.96	.32	NS	41.49	82.2	5.30	HS	5.0	15.0
SN	11.13	.88	NS	41.50	87.8	4.96	HS	5.0	20.0
				GROUP B					
J	-6.22	-.34	NS	34.17	108.7	2.60	S	11.1	33.3
CG	-6.34	-.34	NS	31.26	133.5	2.12	NS	33.3	44.4
SN	0.42	.02	NS	34.35	118.6	2.39	S	22.2	33.3
				A + B					
J	7.66	1.04	NS	29.48	87.5	6.04	HS	17.2	31.0
CG	0.76	0.08	NS	38.32	94.5	5.60	HS	13.8	24.1
SN	7.80	0.76	NS	39.28	94.8	5.58	HS	10.3	24.1

(b) Difference between required doses (mg) calculated from V = 30% BW and K derived from creatinine by means of Jeliffe formula (1st dose, as above)

Parameters		GROUP A n	Mean (cv)	t	Absolute Mean (cv)	t	GROUP B n	Mean (cv)	t	Absolute Mean (cv)	t	A + B n	Mean (cv)	t	Absolute Mean (cv)	t
								DOSE 2								
Kf	Kf	8	1.1 (2887)	.09	23.65 (75.5)	3.5	3	-16.4 (345)	-.41	40.9 (80.4)	1.76	11	-3.7 (1 005)	-.31	28.4 (79.3)	3.99
K3	Kf	15	-3.1 (926)	-.40	23.27 (70.5)	5.3	6	-27.3 (134)	-1.66	39.5 (48.1)	4.65	21	-10.0 (323)	-1.38	27.9 (65.6)	6.81
K3	K3	14	-12.2 (208.1)	-1.7	22.17 (74.6)	4.8	5	-20.2 (158)	-1.26	34.9 (8.4)	23.7	19	-14.3 (186)	-2.28	25.5 (59.8)	7.09
								DOSE 2 & 3								
Kf	Kf	11	-.26 (10500)	-.03	21.0 (77.7)	4.1	3	-16.4 (345)	-.41	40.9 (80.4)	1.76	14	-3.7 (8 99)	-.40	25.3 (83.3)	4.33
K3	Kf	19	-.5 (5940)	-.07	23.8 (70.6)	6.0	8	-37.1 (100)	-2.6	46.3 (48.4)	5.47	27	-11.3 (314)	-1.62	30.5 (68.8)	7.41
K3	K3	17	-12.3 (191)	-2.1	20.6 (78.5)	5.1	6	-24.7 (124)	-1.8	37.0 (15.5)	14.4	23	-15.5 (164)	-2.86	24.8 (63.8)	7.35
Vf	Vf	11	-.9 (5289)	-.06	29.3 (123.9)	2.5	3	14.3 (339)	.42	32.0 (105.4)	1.34	14	2.4 (19 66)	.18	29.9 (115.5)	3.12
V5	Vf	15	-5.1 (623)	-.60	22.0 (105.7)	3.5	8	11.6 (304)	.87	24.7 (108.7)	2.43	23	0.7 (47 86)	.10	22.9 (104.6)	4.49
V5	V5	13	-3.1 (1203)	-.29	28.7 (78.2)	4.4	6	-12.9 (179)	-1.25	17.8 (106)	2.11	19	-0.6 (5 34)	-.79	25.3 (85.1)	4.99
Cl	Cl	11	-10.7 (137)	-2.3	15.5 (56.7)	5.6	3	-19.3 (237)	-.6	40.1 (40.6)	3.48	14	-12.6 (179)	-2.02	20.8 (69.6)	5.18
C15	Cl	11	-26.9 (147)	-2.1	32.1 (109.6)	2.9	3	-21.2 (204)	-.7	38.4 (47.1)	3.00	14	-25.7 (151)	-2.39	33.4 (95.0)	3.80
C15	C15	13	-17.8 (79.4)	-4.4	19.4 (59.8)	5.8	6	-39.8 (14.6)	-15.3	39.8 (14.6)	15.3	19	-24.7 (64.3)	-6.6	25.8 (53.9)	7.87

Table 7.10 Comparison of parameter-estimates from 1st and subsequent doses by standardised differences (e.g. (V1 - V1)/V1%)

7.4 Between-dose comparisons

The parameter estimates in table 7.7 are the means of all doses. The estimates of K by full and 3-point methods in each group are not significantly different from each other or from the estimates based on the first monitored dose only. The same comments apply to estimates of V and clearance by full and 5pt methods.

The standardised differences, however, give a different impression (table 7.10). Results are quoted for all repeat measurements and for Ks estimated from the second dose only. (There were relatively few estimates of V and clearance that were repeated by the same method on the second dose and no significant information was obtained from them).

The means of the standardised absolute differences for all comparisons are significantly different from zero, but the signed differences are rarely significant, even in group B which was formed from those with unstable serum creatinine levels. The only consistant exceptions were the 5-point clearances which were significantly lower in all cases on repeat measurements. The decrease in magnitude of K approached significance in 3-point but not full calculations whilst there was no significant mean change in signed estimates of V by any method.

The mean absolute changes in K were smaller in group A (20-24%) than in group B (37-46%), as were the changes in clearance (15-32% and 38-40% respectively). The changes in volumes were similar (22-30% and 18-32% respectively). This is in accord with the criteria for group allocation. In group A the mean changes in clearance were less than the mean changes in either V or K, for comparable methods, but in group B the changes in V were smaller than those in K or clearance. This demonstrates the dependance of clearance upon K when creatinine is changing and probably also demonstrates the

Parameter	Increase (+) or decrease (-) by subsequent measurements (compared to 1st dose)		Agreement in sign of changes between comparable measurements	
	2nd	3rd	1st - 2nd & 1st - 3rd	1st - 2nd & 2nd - 3rd
Kf	7- 4+	2- 1+	1 of 1	1 of 1
K_3	14- 7+	4- 2+	5 of 6	5 of 6
Cl(AUC)	9- 2+	3- *	1 of 1	1 of 1
Cl(I-Tr)	8- 3+	3-	1 of 1	1 of 1
Cl(5AUC)	16- 3+	5- *	5 of 5	3 of 5
Cl(5I-Tr)	12- 2+	7- *	7 of 7	5 of 7
V(AUC)	8- 3+	2- 1+	1 of 1	1 of 1
V(5)	5- 11+	2- 3+	4 of 5	3 of 5
Cr	4- 10+	3+	3 of 3	2 of 3

* These values have a sign distribution significantly different from 1:1 for the sum of doses 2 and 3 (χ^2 test). Comparisons between 1st - 2nd and 2nd - 3rd doses were non-significant

Table 7.11 Sign of intra-individual changes in parameters between doses

negative correlation of V and K calculated by AUC methods (referred to in sections 5 and 6).

The signs of parameter changes between doses are given in table 7.11 and show a trend towards decreases in K and Cl, whilst volumes and creatinine tended to increase. Statistical significance was rarely obtained. In 8 out of 30 cases the change was at least partially reversed by the 3rd measurement.

The 2nd and 3rd values of K, and the changes from the first dose, did not correlate significantly with either initial creatinine levels, or changes in creatinine levels (parametric and non-parametric). There was no linear correlation with the starting value of K, although in group A rank correlation showed a significant association between initial and subsequent values of K and in A + B between initial values and the standardised difference between doses. This latter effect, including group B, may be because those with the best initial renal function had more to lose than those with poor function before reaching a common base-line. There was a highly significant association between changes in K and changes in V in groups A + B by linear and rank correlation. Changes in V were not significantly related to the initial value of K or to changes in creatinine. There was a significant linear association between 2nd and 3rd Vs and the initial value, overall, but not in group A. The change in volume was also related significantly to the initial value.

Changes in clearance were highly significantly related to changes in creatinine (overall) and to changes in K (group A) whilst being significantly related to changes in V (group A) but not to any initial values in any group. The values of subsequent clearances were, however, significantly related to initial values of K and Cl.

7.5 Bi-exponential curve fitting

Sufficient early data to consider curve-stripping was available in nineteen iv doses and five im doses. One of the latter had too much scatter in the data to give a reasonable fit. Of the iv doses, all had a 15 minute point that was clearly above the regression line, but nine had no other point that was clearly so. Of the remaining ten, only one (VJ) was not in group A (VJ had insufficient data to permit inclusion into the trial) and only five had significant correlation coefficients. (This is probably due to the small number of data points, 3 or 4 in nine cases and 5 points in one. The significant correlations were found only in the 4 and 5 point plots, although one 3 point plot was nearly significant at the 5% two-tailed level. The two worst correlations (VJ and NF, 20th dose) occurred where the α_2 correlations were also non-significant). One patient had two determinations of $alpha_1$ (1.0 and 0.73 h^{-1}) on doses between which the value of $alpha_1$ decreased by 40%, V increased by 25.5% and the clearance decreased by 25% (1-compartment calculations). This patient had inappropriate ADH secretion which complicated their management but which explains the observed changes in K, V and clearance. Values of $alpha_1$ were plotted against body weight, $alpha_2$ and clearance, without a significant correlation, and also against V and V as percentage of body weight. These latter correlations were significant, that against V being more so, and negative.

$$\text{Volume (\%BW)} = 80.69 - 31.14\alpha_1 \quad (r = -.724,\ p < 0.02)$$

$$\text{Volume (l)} = 47.16 - 16.93\alpha_1 \quad (r = -.861,\ p < 0.01)$$

The negative correlation indicates that patients with large volumes have a slower distribution rate constant but that the dependance of $alpha_1$ upon the percentage of body weight is

significantly weaker. Had it been otherwise, the result might have suggested that $alpha_1$ was a mixing rate constant dependant upon relative vascularity and/or cardiac function. As it is, however, the result suggests that the volume *per se* has at least some importance. It suggests that differences in blood volume (or cardiac function) may be compounded by differences in penetration of (or binding to) other tissues. Since the blood volume is only 4-5% of body weight (313) gentamicin must penetrate at least the extra-cellular fluid (ecf) to attain a volume of some 30% body weight. Variations between individuals in circulatory volume would only account for small percentage changes in V, by virtue of their ratio, but the effect of variation in ecf is less clear because of the similarity of values. (The plasma and ecf volumes typically total some 25% of body weight (318)). It is unlikely, however, that tissue binding accounts for a major portion of the variation since extensive tissue or protein binding would result in higher values of both $alpha_1$ and V. Extensive intra-vascular binding would result in a low volume (the bio-assay measures total drug) and a high alpha, conforming to the observed data, but whether protein-binding occurs at all is uncertain (as discussed in chapter 1) and the possibility of intra-, but not extra-, vascular binding is merely conjecture.

Evidence relevant to the inter-relation of volume, $alpha_1$ and ecf comes from group studies and from the patient quoted above (NF) with inappropriate ADH secretion. In this patient it was noted that at periods of high ADH secretion the gentamicin levels were much lower than anticipated, the elimination and clearance rates were reduced and the volume was increased. The serum levels were reversible upon treatment but K and V were only determined twice each. The decrease in $alpha_1$ corresponded to the increase in V to within 2% of the respective starting values (-27%, +25.5% respectively). Dividing

PATIENT	α_1	α_2	K_{12}	K_{21}	K_{10}	V_c	V (AUC)
MG	2.03	.311	.623	1.18	.532	8.47	15.4
LP	1.57	.222	.430	1.02	.342	12.2	22.3
Al Wh	1.32	.166	.403	.813	.269	14.6	24.1
SP	1.24	.232	.306	.813	.354	9.68	18.9
Al Wi	1.03	.188	.300	.591	.328	15.9	28.5
NF(1)	1.00	.347	.135	.760	.457	13.3	29.1
NF(2)	0.73	.207	.135	.499	.303	14.3	36.5
VJ	0.67	.236	.087	.509	.310	12.9	35.4
TD	0.552	.185	.085	.391	.261	20.5	33.6

rate constants (h^{-1}) volumes (l)

Table 7.12 Two-compartment characteristics of eight patients (nine doses)

the nineteen suitable patients into two groups, according to the demonstrability of the $alpha_1$ phase, showed that those in whom $alpha_1$ was measurable had significantly greater volumes than the others (mean differences 11.45 litres (18.5%), t = 3.6 (volume, $p < 0.01$), t = 2.7 (% BW, $p < 0.02$)). Thus small volumed patients had faster distribution phases, implying either a faster rate for a given process or differing relative importance of several processes. Thus if penetration of the ecf were slow, and small-volumed patients somehow had less accessible ecf the data would be consistant. (Similarly, if tissue-binding or intra-cellular absorption were a major part of the apparent volume the same observations would be predicted). None of the measurable $alpha_1$ phases lasted beyond one hour, and the causal relationships remain conjectural.

An alternative explanation for the failure to observe an α_1 phase is that A (Eq 7.1) is relatively small, rather than that α_1 is very large.

$$C = Ae^{-\alpha_1 t} + Be^{-\alpha_2 t} \qquad (7.1)$$

where $A = \frac{D}{Vc} * \frac{(\alpha - K_{21})}{(\alpha - \beta)}$ $\qquad B = \frac{D}{Vc} \frac{(K_{21} - \beta)}{(\alpha - \beta)}$

Thus for A to be much smaller than B,

$$\alpha - K_{21} \ll K_{21} - \beta$$

$$\text{and } \alpha + \beta \ll 2K_{21} \qquad (7.2)$$

Since $\alpha + \beta = K_{12} + K_{21} + K_{10}$ (126)

$$K_{12} + K_{21} + K_{10} \ll 2 K_{21}$$

and

$$K_{12} + K_{10} \ll K_{21} \qquad (7.3)$$

Table 7.12 shows that this is not true in observed cases where $K_{12} + K_{10} \simeq K_{21}$. Thus the effect is probably dependent upon α and not A. K_{10} correlated strongly with α_2 as did K_{12} with K_{21} and α_1.

Intramuscular administration, more rarely performed, yielded four measurable absorption rate constants, 2.3, 3.7, 2.1 and 1.4 h^{-1} respectively. The last two were based on two point slopes and only the first value had significant correlation coefficients. The absorption phase was very short and more, early, measurements would be needed to determine Ka accurately. The significance of false estimates of Ka is discussed in section 5 and shown to be of little consequence beyond the time of the peak serum level. A value of 2.16 h^{-1} for this work seems justified.

7.6 Prediction of subsequent levels

The predictive ability of the various methods was tested in group A patients with a possible 137 predictions. Group B was not used because changing renal function was outside the scope of the model. Of the 137 points, (Group C) 109 were one hour or more after the dose (Group D) and so avoided the distribution phase, where it was distinguishable.

Correlation and linear regression calculations were performed for each method and standardised differences determined between the prediction and the measured level. The methods used were:

(1) the full curve (5 or 6 levels for K, 10 or 11 for V)

(2) full K (5 levels for K, 30% body weight for V)

(3) five-point curve (3 levels for K, 5 for V)

(4) Intercept minus trough (3 levels for K, Intercept for V)

	Non-adjusted levels									Adjusted levels					
	137 points (C)			109 points (D)			101 points (E)			109 points (F)			86 points (G)		
thod	r	a	b	r	a	b	r	a	b	r	a	b	r	a	b
Full K & V	.802	.543	.724	.822	.378	.781	.839	.486	.736	.855	.327	.830	.862	.177	.875
Full K 30% V	.801	.892	.636	.758	.789	.647	.791	.903	.636	.780	.775	.679	.773	.741	.677
5 point K & V	.818	.526	.718	.817	.545	.707	.846	.660	.660	.841	.525	.743	.839	.488	.729
Intercept-trough, 4 pt	.807	.633	.652	.834	.449	.715	.853	.555	.672	.866	.407	.758	.876	.309	.784
3 point K 30% V	.840	.778	.601	.798	.769	.574	.808	.849	.583	.805	.797	.591	.795	.790	.570
Creatinine	.680	1.18	.561	.595	1.26	.512	.608	1.31	.479	.612	1.25	.538	.571	1.26	.468

= correlation coefficient, a = regression intercept, b = regression slope

oups C and E include levels less than one hour post-dose, groups D, F and G do not. Groups E and F include adjusted and non-justed levels. Detailed explanation of the groups is given in the text together with details of the methods denoted by numbers 1 to 6

Table 7.13 Correlation and regression data for predictions

(5) three-point curve (3 levels for K, 30% body weight for V)

(6) Cockcroft and Gault (creatinine for K, 30% body weight for V)

It was also found that in many cases the pre-dose level was greater than the post-dose trough (measured or calculated) by up to 3 μg/ml. Two possible explanations are an improvement in renal function (for which there was no evidence) or late administration of the previous dose. This latter event was known to occur and the consequences have been analysed in chapter 5. It was calculated that on at least two occasions the previous dose was given over 2 hours late. To account for this, in cases where the discrepancy was apparent (47 out of 137), the blood levels were adjusted by taking the difference in trough values and decayed it according to the time elapsed from the dose. (This effectively assumes that the system is otherwise at steady-state and that the post-dose trough is correct. These assumptions slightly under-estimate the discrepancy in serum levels, but the under-estimate is negligible in normal or near-normal renal function). Having done this there were 101 out of 137 points (Group E) (and 86 out of 109 points (Group G) greater than 1 hour post-dose) that had been so adjusted or required no adjustment. The remainder were from curves with insufficient blood levels to determine the pre-dose trough or the post-dose trough. (Group F in tables includes these points together with the others).

Table 7.13 lists correlation and regression parameters for each of the models under these various conditions. All correlation coefficients are highly significantly different from zero and all creatinine correlations are significantly poorer than equivalent blood level methods. Method (1) (the full curve) was significantly better than methods using body-weight to estimate V in groups D to G and the five point curve was similarly better although the

	GROUPS														
	C			D			E			F			G		
	% Mean Error		Conf.	% Mean error		Conf.	% Mean error		Conf.	% Mean error		Conf.	% Mean error		Conf.
THOD	signed	unsigned	limits	signed	unsigned	limits	signed	unsigned	limits	signed	unsigned	limits	signed	unsigned	limi
	-12.5	23.5	57.9	-10.4	22.0	54.3	-9.7	26.4	46.8	-6.0	18.8	50.2	-6.0	19.2	44.7
	-11.0	24.5	51.5	-10.0	25.0	53.0	-3.5	28.6	68.3	-3.9	27.8	67.5	-2.5	29.3	69.9
	-13.2	23.5	57.0	-12.1	22.9	54.7	-10.9	26.9	48.5	-7.7	19.8	51.0	-8.5	20.3	47.8
	-17.1	24.2	56.3	-14.7	22.1	52.0	-13.5	26.6	46.0	-10.4	19.0	46.9	-10.1	18.6	42.0
	-17.6	25.5	53.3	-17.7	25.9	55.1	-10.6	29.1	70.4	-11.9	28.4	67.7	-11.1	29.6	72.0
	-8.6	31.6	76.2	-6.8	32.6	79.9	-3.0	36.0	95.3	-0.5	46.1	105.2	-1.7	35.9	97.8

Groups and methods as in table 7.13

Errors are standardised as described in the text. Conf. limits = 95% confidence limit for standardised percentage error

Table 7.14 Standardised errors in prediction

probability of significance of the difference between methods 3 and 4 in group F was just less than 0.95. Group C, including all points in the distribution phase and un-adjusted levels, showed no significant differences between methods 1 to 4. The correlations for the blood-level methods generally improved (but not significantly) in the groups where adjusted levels were used. The regression slopes also increased and the intercepts decreased, demonstrating that errors of timing play a measurable part in the accuracy of predictive methods. The differences were not marked in methods using body-weight to estimate V and particularly so in the creatinine method where this trend was not seen.

The standardised differences, between predictions and measured levels, are summarised in table 7.14 as signed means (to indicate bias in a method), unsigned means (to indicate the precision of the method) and 95% confidence limits (based on the unsigned mean to indicate the maximum likely error in prediction). All unsigned means were significantly different from zero and the confidence limits ranged from 44.7% to 105%. All signed means were negative (indicating underprediction) and ranged from -0.50% to -17.7%. Those nearest to zero (the creatinine method in each group) did not indicate the best methodology since they also had the highest unsigned means and the widest confidence limits, together with the poorest correlation and regression parameters. The trends in parameters were similar to those in the regression analyses in that the data improved when adjusted blood levels were used. Using paired t-tests it is possible to compare the errors in predictions and assess their significance. Tables 7.15 and 7.16 shows some of these comparisons and indicates the mean differences between methods and the significance level attained. In all cases the creatinine method is significantly poorer than blood-

	Methods vs C & G (6)			Methods vs full KV (1)		
Method	C	F	G	C	F	G
1	9.2 (<.001)	16.3 (<.001)	16.7 (<.001)			
2	7.3 (<.005)	7.2 (<.02)	10.1 (<.001)	-1.8 (>.2)	-9.0 (<.001)	-10.1 (<.001)
3	8.5 (<.005)	15.3 (<.001)	6.6 (<.05)	-.7 (>.2)	-1.0 (>.2)	-1.1 (>.2)
4	7.4 (<.001)	16.1 (<.001)	17.3 (<.001)	-1.3 (>.2)	-0.1 (>.2)	.93 (>.2)
5	5.6 (<.05)	6.7 (<.05)	15.6 (<.001)	-3.5 (<.05)	-9.5 (<.001)	-10.4 (<.001)
6				-9.2 (<.001)	-16.2 (<.001)	-16.7 (<.001)

Data are presented as mean differences in standardised percentage errors (significance level by paired t-test). A positive sign indicates that the method in the left-hand column has lower errors.

Table 7.15 Accuracy of prediction by creatinine (6) and full curve (1) methods compared with other methods

Method	METHOD 2	3	4	5
2	-	-8.9 (< .001)	-10.7 (< .001)	.3 (> .2)
3	8.0 (< .001)	-	-1.72 (> .1)	9.3 (< .001)
4	8.9 (< .001)	0.83 (> .2)	-	11.0 (< .001)
5	0.5 (> .2)	-8.5 (< .001)	-9.4 (< .001)	-

Upper triangle is for group G, lower triangle for group F. (values defined as in table 7.15, positive values favour the left-hand method).

Table 7.16 Comparison of Errors between methods 2 - 5

level measurements, whilst the full curve is insignificantly better than the 5 point curve or the intercept method (4) but significantly better than any other. The 5-point and intercept methods are also significantly better than any method using body-weight to estimate volume whilst the full K and 3 point K methods (3 and 5) are not significantly different from each other and compare least favourably against the creatinine method.

7.7 Causes of predictive errors

Inappropriate times for prediction (under 1 hour) and nursing error in administration of drug were eliminated, so far as possible, in group G and, compared with the raw data of group C, there was a marked improvement in mean errors and confidence limits. Other causes of error include laboratory error and model error. Both are discussed in chapter 5 where it is demonstrated that the former should not cause bias (i.e. under-prediction) but scatter (i.e. wide confidence limits) and the latter should cause some under-prediction due to accumulation but that the extent will vary between patients due to the varying (unknown) β rate constant. That these are not the only sources of error is demonstrated by the lack of correlation between elapsed time changes (decreases) in K (a correlation should be seen if accumulation is the only operating factor) and indeed the increases in K in some individuals indicate other causes. Intra-individual variation, therefore, cannot be dismissed as a source of error in pharmacokinetics and yet it is rarely considered in text books or in research papers. Variation in other parameters

Time (Min)	Serum Concentration (μg/ml)	
	PGD	ESH
10	11.0	10.5
20	8.9	8.0
30	7.3	7.1
40	-	6.2
50	6.4	5.7
60	6.0	5.0
120	4.0	3.7
150	3.1	-
180	2.6	2.8
210	2.3	2.4
240	2.0	2.1
270	1.8	1.9
300	1.6	1.5
330	1.5	1.2
360	1.25	1.0
$\alpha_1 (h^{-1})$	2.77	4.35
$\alpha_2 (h^{-1})$	0.297	0.319
V AUC (l)	18.84 (26% BW)	18.93 (26.3% BW)
Cl (l/h)	5.60	6.04
Ke (h^{-1})	0.188	0.282

Urinary excretion data

Time (h)	PGD Concn. (μg/ml)	Total (mg)	Clearance (l/h)	ESH Concn. (μg/ml)	Total (mg)	Clearance (l/h)
0-2	90	72	5.86	86	68.8	5.9
2-4	116	17.4	3.35	48	16.8	3.0
4-6	21.6	10.8	3.37	26	10.1	3.35
6-8	8.6	6.9	3.95	13.8	5.2	3.35
8-10	6.8	3.4	3.5	-	-	-

Table 7.17 Volunteer trial data

(e.g. biochemical profile) is well known (346, 347) and causal links between illness and variation are also known (348) and so it is not surprising that pharmacokinetic parameters should also vary with time.

7.8 Volunteer Study

As described previously (section 4), two healthy volunteers were given 140 mg of gentamicin by iv bolus and monitored thoroughly over the next eight hours (until hearing loss forced the end of the study). Data are given in table 17.17.

An important feature is that urinary collections were made and calculated clearances are given. It is apparent that the urinary clearance in the first collection (0-2 hours) is significantly greater than that in subsequent collections, and is very close to the calculated plasma clearance. The means of subsequent measurements, however, are 63.2% and 88.3% of plasma clearance respectively. The calculated renal elimination rate constants (Ke) are given in table 7.17 and represent K_{10} in the Schentag 2-compartment model. From chapter 5 we find that $(\alpha - K_{10})/K_{10}$ is well correlated with accumulation of drug in the central compartment and since α is approximately equal to α_2 in this study (or K in the one compartment model) it should be possible to predict those patients who will accumulate much drug because of their peripheral compartment characteristics.

7.9 Recommendations for control of therapy

The four-point (Intercept minus trough) method for estimating clearance is almost as accurate as an 11-point curve and is superior to three-point and creatinine methods. This is, therefore, the method of choice, combining reliability with minimal sampling. Blood samples should be taken at least one hour after the dose (to avoid α_1 effects) but less than five hours afterwards (to reduce assay errors) and should be spread over at least two hours (to minimise regression error).

The accuracy of any method, however, depends upon accurate drug administration and close attention must be paid to nursing procedures, perhaps using im doses to ensure better timing.

Urinary clearances should be estimated to predict accumulation, and all measurements repeated every three days, or more frequently if there is evidence of changing renal function, altered volume of distribution or toxicity.

8. HPLC

High Performance Liquid Chromatography (HPLC) has been a theoretical possibility since 1941 when Martin and Synge (151) showed the advantages that would accrue from using very small particles as a stationary phase in a liquid mobile phase. For many years the theoretical advantages could not be realised because of technical problems in the production of uniform small particles and consistently high pressures to force liquid through the dense beds of stationary phase. Various workers, including Giddings (152) and Knox (153) contributed to the practical implementation of earlier theory and by the mid-1970's there were a number of commercial systems available that used (typically) 20μm particles and 1000 - 1500 p.s.i. pumps (154). Since then the refinement of chromatographic practice has lead to the out-dating of much of this equipment (discussed in subsequent sections) and has brought 3μm and 5μm particles into regular use. Typical pumps are now capable of 5 - 10,000 p.s.i. and there has been a vast increase in the literature available, represented mainly by a considerable increase in the number of papers published by the 'Journal of Chromatography' and in the introduction of a new journal 'Journal of Liquid Chromatography'. The change in emphasis is well illustrated by two editions of a book by J. J. Kirkland and L. R. Snyder, 'Modern Liquid Chromatography', (John Wiley (New York), 1974 and 1980). The second edition is much larger than the first and yet has lost much of the discussion of different types of pumping systems, packing materials and column construction. In its place are chapters on applications of HPLC and on practical problem-solving. It is perhaps also worth noting that the 'Journal of Chromatographic Science' published a special 'Introducing HPLC' issue in October 1977, showing just how recently HPLC has become a practical and commercial procedure.

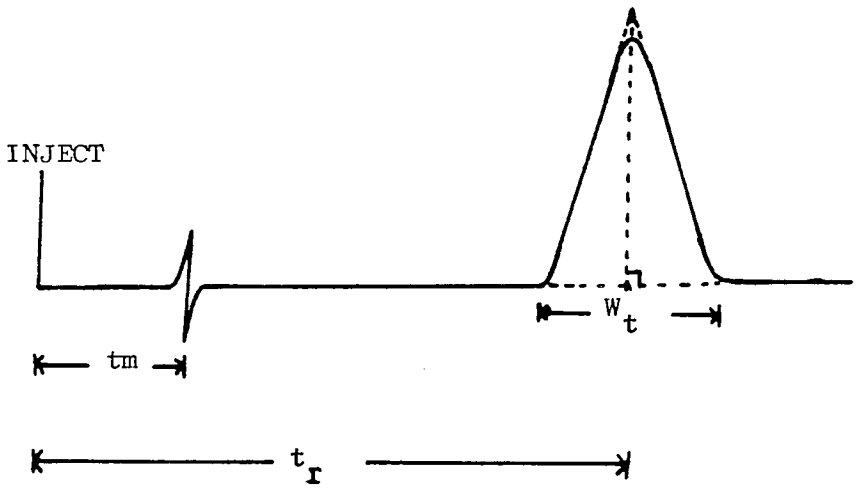


Fig 8.1 Detector output labelled in time units

8.1 Background Theory and Definitions

Some basic definitions are given here to facilitate further discussion. Fig 8.1 illustrates some of the parameter in terms of the detector output.

The elapsed time between injection and elution of a solute is called the retention (or elution) time (t_r) and the corresponding volume of eluent is called the retention (or elution) volume (V_r). These are clearly related by the volumetric flow rate (f_v)

$$v_r = t_r f_v \qquad (801)$$

If the column holds Vm mls of eluent and that volume is eluted in tm minutes then tm is the retention time of an un-retained solute. The capacity ratio (K') is related to the relative lengths of time that a solute spends in each phase and so

$$K' = \frac{tr - tm}{tm} \qquad (802)$$

If W_t is the width at the base of a peak in time units (W_v in volume units) it may be shown that W_t increases as the square root of the distance migrated (length of column) (155). A convenient measure of the peak dispersion is the height equivalent to the theoretical plate (H) (Martin and Synge) where

$$H = \frac{L}{16}\left[\frac{W_t}{t_r}\right]^2 \qquad (803)$$

where L is the length of the column and H is in the same units. Correspondingly, the number of theoretical plates to which a column is equivalent (N) is

$$N = L/H = 16\,(t_r / W_t)^2 \qquad (804)$$

(If the width at half-peak height is used instead of W_t, the figure 16 should be replaced by 5.54).

The resolution (R) between two peaks (A and B) is defined as

$$R = \frac{t_{rB} - t_{rA}}{\frac{1}{2}(W_{tB} + W_{tA})} = \frac{1}{4}\,\frac{(\alpha-1)}{\alpha+1}\,\frac{(\bar{K}')}{1+\bar{K}'}\,N^{\frac{1}{2}} \qquad (805)$$

where $\alpha = \frac{K'_B}{K'_A}$ and $\bar{K}' = \frac{1}{2}\,(K'_A + K'_B)$

K' is related to the equilibrium distribution coefficient (D) and the relative volumes of stationary (V_s) and mobile (V_m) phases by

$$K' = D\,\frac{V_s}{V_m}$$

Thus K' (and hence V_r and t_r) depends upon the amount of stationary phase. Although this is specifically true for partition processes, a similar relationship exists for adsorption (155).

From the Van't Hoff equation, the dependance of K' upon temperature (T^oK) is less for HPLC than GC since the heat of transfer of solute from mobile to stationary phases (ΔH) is much smaller,

$$\frac{d(\ln K')}{d\,T} = \frac{\Delta H}{RT^2} \qquad (806)$$

Thermostatting is not, therefore, essential in HPLC but is sometimes used, as discussed later. R. P. W. Scott (171) calculates that limits of $\pm$ 0.35°C should lead to retention volume precision of 1%.

Dispersion of a band of solute is caused by three processes:

(a) The variable path length of solute molecules due to the tortuosity of the route between particles.

(b) Axial molecular diffusion. This will depend upon the residence time of solute in the column.

(c) Equilibration between mobile and stationary phases. The

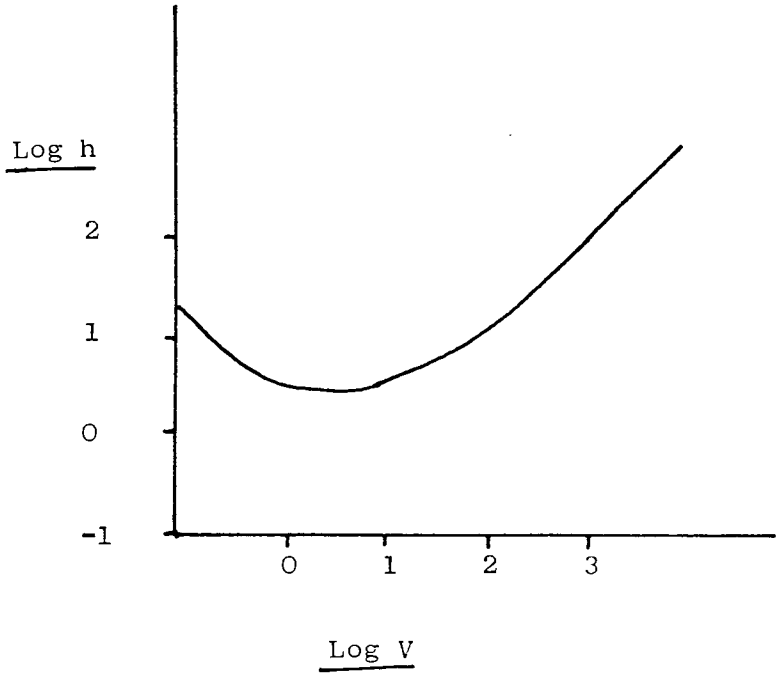


Fig 8.2 Chromatographic efficiency as a function of solvent velocity

slower the equilibration, the greater the despersion.

If h is the value of H in terms of stationary phase particle diameters, (the reduced plate height) and v is the reduced linear velocity (the linear velocity of the solvent in terms of particle diameters) (156), then

$$h = Av^{.33} + \frac{B}{v} + Cv \qquad (807)$$

The terms A, B and C are constants related to processes a, b and c above respectively. For good columns $A < 1$, $B \simeq 2$ and $C < 0.1$ giving a shallow parabola with a minimum value of h at $v \simeq 3$ (fig 8.2) but good values of h over a three to four-fold range of v. (155).

In practice such values of v may not be attainable because the flow rate required is too low to be of practical use. (Based on Bristow and Knox (156), the desired flow rate for a column 250 mm long, with 10 μ m particles and a typically aqueous solute diffusivity of 10^{-9} M^2S^{-1} is 0.16 ml/min, giving a value of 14 minutes for tm).

8.2 Modes of Chromatography

Chromatography has often been divided into adsorption, partition, ion-exchange and exclusion modes. Whilst this is not a universally agreed system (several authors have criticised it vigorously on theoretical grounds (155 157)) and a common theoretical basis for all modes has been proposed (155), it is often convenient to refer to such descriptions. The basis

for adsorption chromatography is a competition between solute and eluent molecules for active adsorption sites on the stationary phase. These active sites are generally hydroxyl groups attached to silica particles. Such systems are also called 'normal-phase' and tend to use non-polar eluents. Partition is often, but not necessarily, associated with reverse-phase chromatography and polar eluents. Retention depends upon the partition of a solute between stationary and mobile phases. It has been suggested, however, that reverse-phase methods are not partition but adsorption (157) or a hybrid (155). The stationary phase in the reverse-phase mode is generally formed by chemically bonding an alkyl chain (such as an octadecylsilyl group, ODS) to the silica particles. Other such chains, with terminal tertiary amine or sulphonic acid groups, form the basis of ion-exchange systems whereby ions of the opposite charge compete with eluent ions for these sites. Ion-pairing may be associated with normal-phase systems but is more commonly found in reverse phase. An ionised ion of the opposite charge to the solute (counter-ion) is included in the eluent to modify the adsorption or partition characteristics. A more detailed consideration of the mechanism is given in chapter II.

Exclusion chromatography (gel-permeation chromatography) utilises a porous matrix as stationary phase and retention depends upon the size of the solute molecule since the smallest molecules will penetrate the matrix most and elute last. This method is most commonly used for separation of polymers.

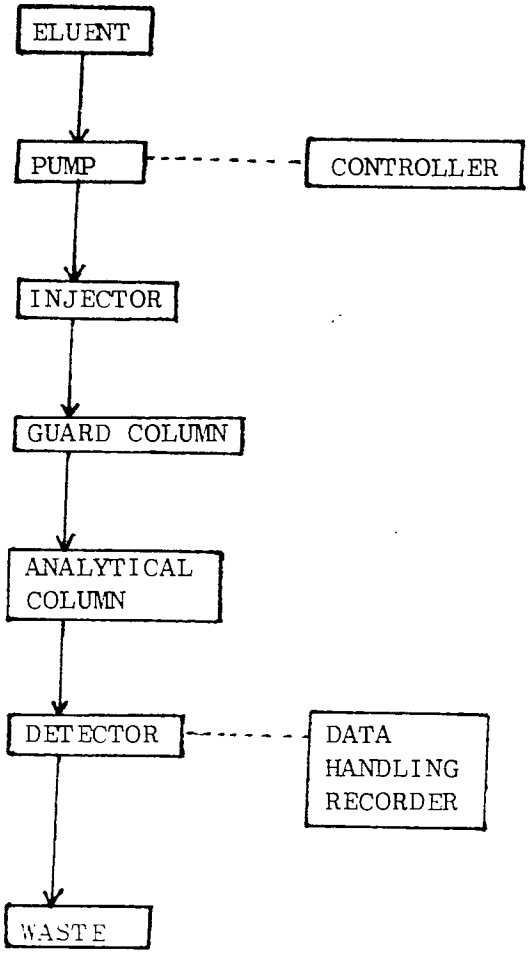


Fig 8.3 Typical Configuration of an HPLC System

8.3 HPLC

.1 Pump and Eluent

The most common pumps in use are now reciprocating-piston pumps, capable of very reproducible flow-rates at up to 10,000 p.s.i. Typically they have a small pumping chamber and a rapid piston action driven by a stepping-motor, the rate of which is virtually independant of back-pressure. In the more expensive systems the motor drives two pistons which are out of phase with each other and pump from two chambers into a mixing chamber and so on to the column. This reduces the pulsing in eluent flow and pressure that occurs in piston pumps. Some pumps have flexible helical tubes or Bouden gauges as pulse-dampers after the pumping chamber. (The Altex 110 pump, used in this study, is of the single-piston type without pulse-damping and is capable of up to 5000 p.s.i. or 11 mls/min. The Pye LC3, used for the majority of this study, is an older design and although a single-piston device, it is not driven by a stepping-motor. The flow-rate was, therefore, dependant upon the back-pressure of the system and the maximum pressure obtainable was 160 BAR ($\simeq$ 2400 p.s.i.)). Earlier designs, now largely discarded, included pneumatic pressure, pneumatic amplifiers (now used as high volume, low reproducibility, column-packing systems) and syringes. The pneumatic pumps operated at a constant pressure, rather than a constant flow rate, and the syringes had a very limited pumping cycle since they had to be re-charged with eluent at intervals, a procedure which interrupted the flow rate.

Gradient elution systems (comparable in function to GLC temperature-programming) whereby the eluent composition is changed throughout a run according to a pre-determined pattern are now finding greater usage and are essential for assays of some complex systems (e.g. serum amino acids). The most favoured configuration features a second pump and eluent reservoir in parallel to the pump in fig 8.3 with a mixing chamber prior to the

injector. A control unit (often a microprocessor) is linked to both pumps. Other systems employ low-pressure mixing prior to a single pump.

The eluent composition is critical to the success of the chromatographic process and a small variation may make a significant difference to the resolution, efficiency or retention of the solutes (171, and subsequent chapters of this thesis). Multi-component eluents are common and may typically contain:

1 or 2 liquids to govern the polarity of the eluent,

a pH modifier or ionic strength control,

a counter-ion for ion-pairing.

It is important to ensure that the eluent is fully miscible with that which preceeded it and that which succeeds it and that it does not damage the column. The pH should not exceed 7.4 for any length of time since silica dissolves above that pH. Similarly, precipitation of inorganic salts (e.g. phosphate) can occur if the eluent is changed to methanol. Ideal eluents should also be non-toxic, non-volatile, uv-transparent, of high purity and of low viscosity.

De-gassing the solvent used to be considered important to prevent out-gassing in the column or detector (167). Out-gassing could be caused by a compression-induced temperature rise or by a decrease in pressure at the end of the column. Aqueous solutions of alcohols are most prone to out-gassing and may be de-gassed by ultrasonics, vacuum or continuous helium-cover. The latter is the most efficient method, but also the most expensive. A vacuum will also remove part of the eluent by differential evaporation of its components, causing a change in composition. In addition to these disadvantages, work by Chamberlain (158) has shown that degassed eluent returns to 99% gaseous saturation in typically 30 minutes. It, therefore, seems to be of little value in comparison to the disadvantages. In practice, the only difficulties encountered with out-gassing in this work were with certain methanol-water mixtures at

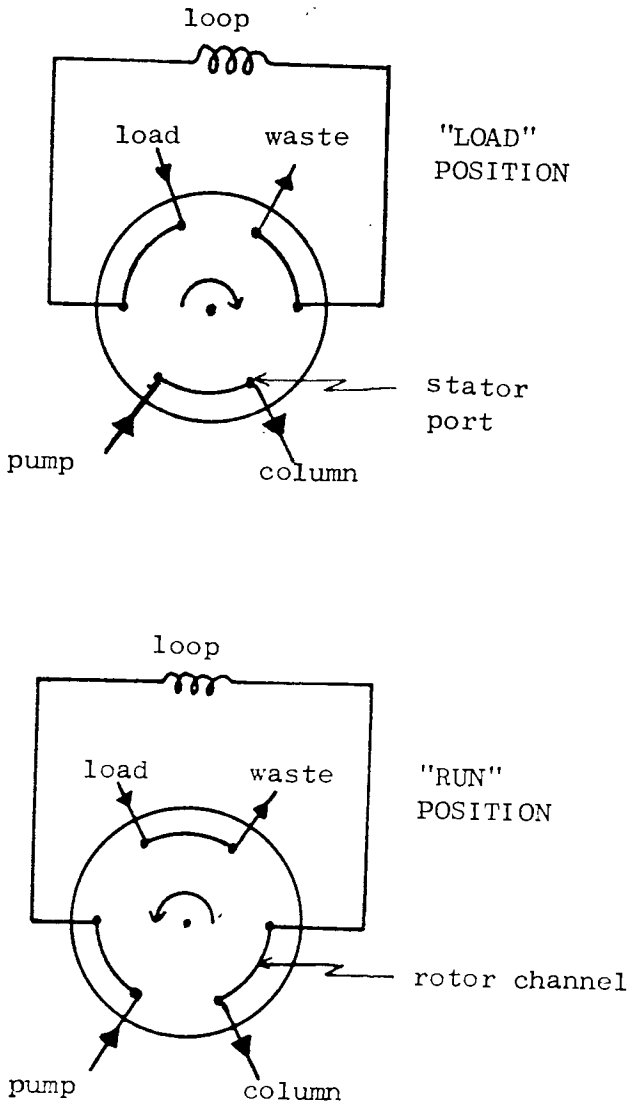


Fig 8.4 Configuration of 6-port loop injector

maximum detector sensitivity. Such conditions were rare, and degassing was, therefore, also rare.

The eluent may be filtered prior to the pump with a stainless-steel filter block to remove particulate debris.

.2 Injection Systems and Couplings

Two main types of sample-injector have been developed, the syringe-injector and the loop-injector. The former is very similar in principle to GLC injectors, the sample being injected directly into the liquid flow and the volume of the sample being controlled by the syringe. These are, at their best, more efficient than loop-injectors, causing less band-spreading, but are less reproducible and are difficult to use at higher pressures. Loop-injectors attain their good reproducibility because the sample is loaded (by syringe) at low pressures into a fixed-volume loop. If the loop is over-filled (i.e. excess goes to waste) then when the valve is actuated and the eluent is directed through the loop a precise volume of sample is swept on to the column. A typical, six-port system is illustrated in fig 8.4. Some systems such as the Rheodyne 7125 (used in this study) enable partial loop filling as well as complete filling. The size of loop is readily changed, typically from 10 to 100 μl. Coupling of components throughout the system is usually by means of stainless steel tubing, 1/16" O.D. and 0.005 to 0.02" I.D. The lower the dead volume the better for efficient separations and so the length and diameter of all couplings should be minimised. Zero dead-volume Swagelok-type couplings are normally used.

.3 The Column

The availability of closely-controlled microparticulate packing materials is a major factor in the improvement of HPLC efficiencies over the past decade (162). Most current packing materials are based upon porous silica with a controlled particle-size of 3-10 μ and a pore size of $\simeq$ 10nm. A specific surface area of 100 - 500 $m^2 g^{-1}$ is typical,

whilst the use of spherical particles allows operation of short columns at very low pressures (20-50 bar). There is choice of packing materials:

(i) Silica: This gives the traditional adsorption chromatography in which a relatively non-polar mobile phase desorbs components from the column in order of increasing adsorptivity (159).

(ii) Chemically-Bonded Silica: these stationary phases are prepared by reacting the surface silanol groups of the silica microparticles with usually a chlorosilane derivative (163). These stationary phases are generally of low-polarity and are used in a reverse-phase mode (160). Reverse-phase chromatography is useful for highly polar compounds which would not be readily eluted from silica columns. Indeed the technique is now so highly developed that most separations of pharmaceutical importance can be achieved in this mode. The partition processes may be modified by changing the mobile phase characteristics (polarity, pH etc) and it is here that the main selectivity is attained. This contrasts markedly with GC in which temperature variation and a wide range of stationary phases, but usually only one mobile phase, are available. Further selectivity is available by using processes such as ion-pairing (168). (See chapter II for a discussion of the mechanisms of ion-pairing).

(iii) Other Packing materials include ion-exchange phases which are most frequently used for components of biochemical origin and which may be based upon bonded silica or conventional cross-linked polymers. Gel permeation chromatography (particularly for polymers) and bio-affinity chromatography (useful for complex biochemicals such as enzyme systems) also have important applications.

Although the reverse-phase is now the system of choice, it has not always been so. Up to 1977 or 1978 the majority of published work utilised silica or ion-exchange columns, primarily because assays were developed from existing TLC or LC procedures (161) and reverse-phase is rare in these methods. In addition, pellicular and 20-40μm particles

were still in fairly common use (164). Ion-exchange columns have largely given way to ion-pairing, chiefly because of the comparative difficulty of stabilising ion-exchange columns and of changing the eluent composition.

10-25 cm columns are typical (25 cm in this study) with internal diameters of 3 - 5 mm for analytical work (4.6 mm in this study). The incorporation of a short (2-3 cm) column between the injector and the analytical column is often a necessity in biological work. This removes colloidal material, polar contaminants and debris not removed by solvent filtration. The guard column, which can be replaced more cheaply than the expensive analytical column, lengthens the life of the main column and has only a small adverse effect upon the chromatography. The most common contaminants from biological materials are proteins and lipids.

Thermostatting of the columns is sometimes used to raise the temperature and give more efficient transfer of solutes between phases. It is also, but less importantly, used to minimise the effects of variation in ambient conditions. Temperatures up to 50^{o}C have been reported (166).

.4 Detectors

Detectors for HPLC (167), require to be generally applicable to a wide range of substances in a wide range of mobile phases, rapid in response and sensitive (20μ l of injected sample may be diluted to as much as 1 or 2 ml by the time it reaches the detector). The earliest detectors were based on refractive index changes, but whilst having wide applicability they were extremely susceptible to changes in temperature and mobile phase composition and were not very sensitive. In many applications they have been replaced by variable wave length UV detectors with very small flowcells (typically 8 μl) and detection from 210-400 nm in most mobile phases. They are sensitive enough for most, but not all, pharmaceuticals, even down to typical blood levels of 1 μg ml^{-1} or so.

For compounds with suitable absorption and emission spectra,

fluorescence detectors are available and have permitted quantification of some drugs down to a few nanograms ml^{-1} of serum. It may be necessary to derivatise compounds before detection to obtain adequate absorption or fluorescence and a variety of reagents are available (169). The derivatisation may be performed on the sample prior to injection ("pre-column") or post-column by means of a T-junction allowing mixing of the eluate with reagent prior to detection. The geometry and flow-rates are critical to maintain resolution and the kinetics of such reactions are demanding. Nevertheless several efficient systems have been described (170). The use of scanning spectrometers or dual wavelength measurements (see section 16) offers some assistance in the identification of unknown components but perhaps the greatest potential is in the field of on-line HPLC-MS systems (171). Commercial systems based upon moving-wire transport interfaces are already available and the proven value of GC-MS-computer systems in medical and pharmaceutical applications indicates the vast scope available for the MS detector.

Parameter	Chemical	uv-visible Spectroscopy	TLC	GC	HPLC
Initial cost	*****	***	****	*	*
Running cost	****	*****	**	***	*
Selectivity/specificity	*	*	***	****	*****
Quantification	***†	***†	*	*****	*****
Ease of Sample preparation	***	***	*****	**	***
Ease of use by general lab-workers	***	***	*****	**	***
Range of applicability in pharmacy	***	***	****	***	*****
	requires suitable reacting functions	restricted by absorb-tion or by derivitisation		restricted by Mol. Wt. and volatility	

Table 8.1 Comparison of HPLC with Other Assay Methods

(The greater the number of stars the greater the advantage in using a particular method).

† variable depending upon specificity

8.4 The Role of Chromatography in Analysis

The purpose of every quantitative analytical procedure is to determine the quantity of a particular chemical entity (or entities) in the presence of any other substances with the greatest possible accuracy and precision. Thus the best procedures have a minimum of manipulation combined with the greatest specificity and sensitivity. Most detection systems in chemical or biological analyses are non-specific (e.g. titrimetry, colorimetry, spectrophotometry) and so potentially interfering substances must be removed prior to the assay or the analyte must be selectively altered. In either case the sample is generally not preserved for further use. Chromatography, however, enables the separation of such entities, generally intact, with a minimum of operation intervention and the ability to use a sensitive but non-specific detector (e.g. U V.).

HPLC is relatively costly to install and run, but is not difficult to use routinely and generally permits easy sample preparation. (GLC, by comparison, requires samples to be volatile and derivatisation is often required). HPLC also has a wide applicability in clinical and pharmaceutical fields that formerly complemented GLC but is now starting to replace it.

Table 8.1 gives comparisons of HPLC and other methods.

8.5 Sample Preparation

HPLC requires the removal from the sample of anything which would block the system, either due to its physical size (e.g. solid particles blocking the narrow-bore tubing, or increasing column back-pressure) or due to its chemical behaviour (e.g. lipids adsorbing onto reverse-phase columns). Given these requirements it is often possible to devise a procedure which combines speed with accuracy and repeatability.

Intravenous fluids and simple solutions can usually be injected directly or with dilution to adjust the concentration to a suitable range. Syrups are viscous and need dilution to prevent blockages, whilst soluble tablets and powders require dissolution. Non-dispersible ('ordinary') tablets and capsules require dissolution and filtration to remove insoluble excipients, a 0.22-0.47μm membrane filter is satisfactory for this purpose. Creams, ointments and emulsions contain large quantities of lipophilic substances which are best removed by a one-step partition process (say between acetonitrile and hexane) assuming the drug is at least moderately polar (172).

Blood, urine and other body fluids contain lipids and proteins which are likely to precipitate out onto column packings, especially in reverse-phase mode. Lipids can be cleaned off such columns by periodic purging with chloroform but proteins will permanently damage a column, or guard column (165).

Protein-injection may be prevented by a precipitation or filtration step. Filtration (or centrifugation) is time-consuming and requires special apparatus to achieve the low levels required. Precipitation may be achieved by means of trichloroacetic acid, perchloric acid, an alcohol, acetonitrile or dimethylformamide leaving a supernatant suitable for use in HPLC (165, 201, 203).

Other techniques have been described, including the use of proteolytic enzymes for analysis of tissues (173), but have specialised uses and are not generally applicable.

8.6 Application of HPLC to Pharmaceutical and Clinical Practice

In recent years HPLC has found an increasing role in the isolation, manufacture and distribution of drugs as well as in studies of their effects and fate. In addition to analytical studies, much work has been done on the preparative isolation of active principles from biological, especially plant, materials. A recent review has been published (174). The evaluation of the quality of such materials, especially mixed-alkaloids, is important and several such assays exist, including a number of forensic importance (e.g. 175). Preparative columns are wide-bore, expensive items that require high volumetric flow rates and very often require runs of 24 - 36 hours or more. They often remain contaminated by unwanted materials after the desirable principles have eluted but some firms find it cheaper to throw such columns away rather than purge with solvents or use a non-HPLC procedure.

Similarly, bacterial fermentation broths may be sampled in order to follow the reaction or may be purified preparatively (176, 177). Some drugs have a stereospecific action and only one isomer of an R-S or Z-E pair may be significantly active. Examples include the cephalosporin, moxalactam, and some thioxanthene tranquilisers which have been studied by HPLC (178).

The stability and identity of pharmaceuticals is an area ideally suited to HPLC and work in this area is described in this thesis (sections 14 and 16). Break-down products in a range of formulations (injections, tablets, ointments and others) have been described (e.g. 179, 180) and toxic impurities (from raw materials, packaging or excipients) identified (181). The analysis of multi-component mixtures where there are many active components (e.g. Bacitracin with 22 components, tetracycline mixtures, co-trimoxazole) is possible by HPLC (183, 184) whereas bio-assays may only give a total activity. This may be useful for clinical assessment or for quality control. Other quality control applications

include the monitoring of eye-drops (185), biological raw materials (182), the checking of packing procedures (179) and the long-term stability of dispensed products (186). Physico-chemical parameters, including lipophilicity indices and partition coefficients have been derived from HPLC for use in structure-activity relationships (187).

Bioavoilability and pharmacokinetics may be assessed, by in-vitro or in-vivo studies, for a wide range of compounds, including some that have no suitable biological index of activity (188, 189). There are now many publications in this area and bibliographies and text-books are available (190, 191, 192, 193). The application of HPLC to drugs relevant to this work is discussed in the appropriate chapters but the identification of active and non-active metabolites is possible in urine, serum and other fluids and tissues. The use of HPLC in related areas of clinical chemistry is also increasing and reviews have been published (194, 195, 202). Toxicology has also benefitted from HPLC (196, 197, 198) and other wide-ranging papers are available (199, 200).

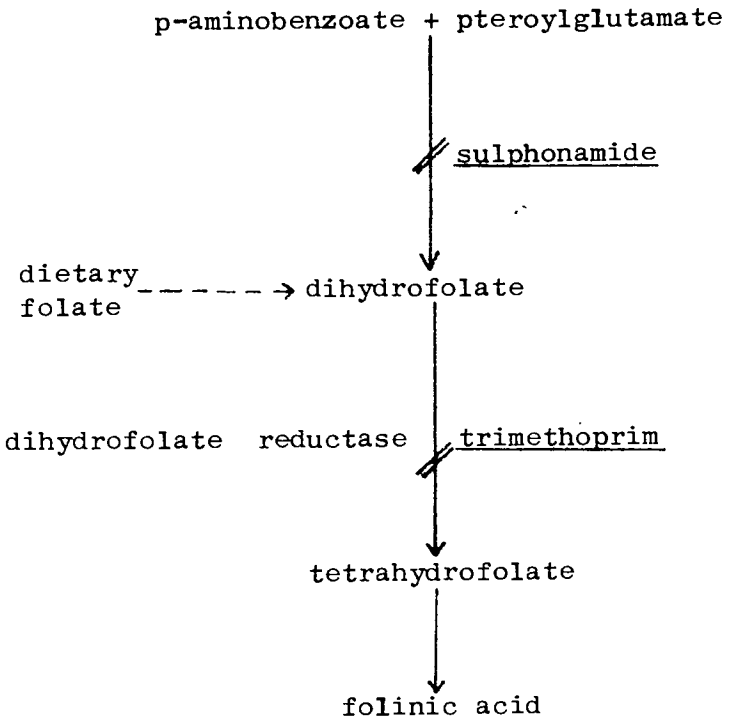


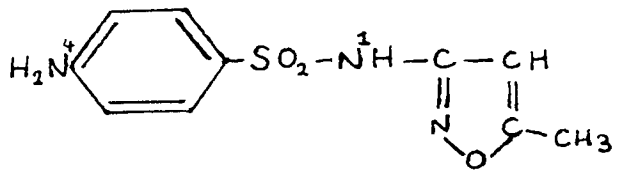
Fig 9.1 Biochemical Pathways and the Action of Co-trimoxazole

9. COTRIMOXAZOLE

9.1 Introduction, Mechanism of Action, Uses

Cotrimoxazole contains two antibacterial agents, sulphamethoxazole (SMZ) and trimethoprim (TMP) in a ratio of 5:1 by weight. It is a highly effective combination, having a wide anti-bacterial spectrum and relatively low toxicity (205). Both drugs act upon the synthesis of folinic acid, and hence upon the synthesis of purines and DNA, but at different stages (fig 9.1). Mammalian, but not bacterial, cells overcome the SMZ block by absorbing folate from the diet. TMP, however, is selective for bacterial (e.g. E. coli) or protozoal (e.g. Plasmodium berghei) dihydrofolate reductases compared with mammalian enzymes (e.g. rat liver), the relative doses for 50% reduction in activity being 5, 70 and 260,000 respectively (229). An in vitro synergism has been observed between SMZ and TMP but the in vivo value of this is debated (206). The hoped for prevention of resistance to both drugs, as a result of use of the combination, is not apparent and trials of TMP alone have shown it to be effective in many cases. This is especially so in urinary tract infections where the levels of SMZ are relatively low and the antibacterial activity of the combination is almost entirely due to the TMP (206).

In vivo studies show optimum synergy to occur at ratios of 10:1 to 40:1 (SMZ:TMP by weight). The ratio in cotrimoxazole (5:1) is designed to give such ratios in serum because of the larger volume of distribution of TMP and the similar half-lives of TMP and SMZ (half-lives 8 - 12 hours, Volumes: TMP 94-121, SMZ 12-18 litres) (207, 208). Both drugs are metabolised in vivo, although the extent of metabolism varies and is greater in animals than in humans. SMZ is acetylated at the N^4 position and this is the major non-conjugated metabolite (60% of urinary metabolites (210)), although several minor metabolites are known. None of the metabolites are active except by re-hydrolysis to SMZ (209, 210). TMP forms a number of metabolites, classified by Rieder (210), but the extent

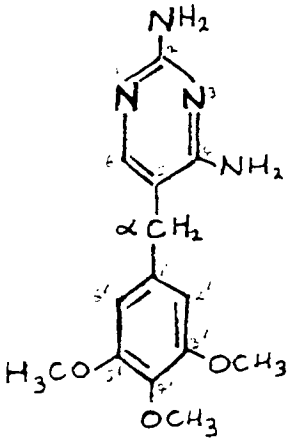


SULPHAMETHOXAZOLE (N^1 - (5-methylisoxazol-3-yl) sulphanilamide)

Major Metabolites N^4 - acetyl SMZ

N^1 - glucuronide

TRIMETHOPRIM (2, 4 - diamino-5-(3', 4', 5',-trimethoxy benzyl pyrimidine))



Metabolites (after Rieder)

I 4'-demethyl-TMP (plus glucuronide)

II α -hydroxy-TMP

III TMP-1-oxide

IV 3'-demethyl-TMP (plus glucuronide)

V α -carbonyl-TMP

Table 9.1 Cotrimoxazole,

Active Drugs and Metabolites

of metabolism and the relative importance of each one is not clear. Schwartz et al found 7.3% "readily extractable" metabolites and 13.9% conjugates in serum 4-6 hours after dosing. Rieder suggests that metabolites I-IV have low activity, but does not give details.

9.2 Previous Assays

SMZ has been assayed colorimetrically by forming a diazo dye (Bratton-Marshall reaction (211)). This is not specific for SMZ but includes all metabolites with a free N^4 amino group. Hydrolysis may be used in a differential assay between these compounds and those such as N^4-acetyl-SMZ which do not react in the unmodified method. TMP has been assayed fluorimetrically, by oxidation with permanganate to trimethoxy-benzoic acid but this is non specific (211). Specificity for both compounds has been achieved by the use of differential extraction techniques or TLC (212, 213). Microbiological assays for active components are known (211) but other active drugs interfere. Pulse polarography (214) and GLC (230) have been used for TMP. Prior to this study Helboe and Thomsen (215) used HPLC (RP8, phosphate buffer with acetonitile, 40°C) to separate TMP, SMZ and two other sulphonamides in pharmaceuticals but did not deal with biological material or metabolites

Elution Volumes (mls)

H_3PO4	SMZ	TMP	
0.15M	7.2	12.6	(30% acetonitrile)
0.075M	16.2	30.0	
0.012M	19.8	35.7	
0.178M	6.4	40.0	(44% methanol)

Table 9.2 Elution Volumes in 30% acetonitrile and 44% methanol (RP18, 5μm)

Elution Volumes (mls)

Ortho-phosphoric acid content

	1%	½%	¼%	1%
TMP 1	8.6	11.5	12.1	8.8
TMP 2	-9.2	-12.0	-14.4	-9.2
TMP 4				
SMZ	12.2	14.0	14.4	6.4
TMP	20.0	25.6	30.4	40
TMP 3	16.4	21.6	24.8	14.6
	acetronitrile 20%			methanol 44%

Table 9.3 Elution Volumes of SMZ, TMP and Metabolites

9.3 Reasons for Study of SMZ and TMP

Several requests were made, by physicians at East Birmingham Hospital and Selly Oak Hospital, for specific assays for SMZ or TMP but a number of these progressed no further. Nonetheless the assay was developed and two applications arose. Dr. M. W. McKendrick arranged a study of TMP by iv and oral routes in Asian typhoid patients and Dr. B. Mehta arranged a study of cotrimoxazole in peritoneal dialysis. Both these studies are continuing and initial results are described below. TMP has not previously been studied pharmacokinetically by the iv route. The only reports of TMP and SMZ in peritoneal dialysis are in French and German respectively and are vague in terms of the practical effect (see below).

9.4 HPLC

A silica column was found to give poor separation of SMZ and TMP over a wide range of mixtures of chloroform, ethylacetate and methanol. Ammonia sharpened the peaks and improved resolution but the retention times remained small and the solvent front interfered with the SMZ peak.

Using reverse phase (RP18, 5μm, Altex) it was found that methanol-water mixtures did not appear to elute TMP but SMZ was eluted in 14mls at 30% methanol. The addition of acetic or phosphoric acids greatly

Column	% CH_3CN	% H_3PO4	pH	Ret. vol. TMP (mls)	Ret. vol. SMZ (mls)
ODS-2	25	1.0	-	solvent front	8.2
	12.5	0.5	1.6	8.0	22.25
	8.3	0.33	-	22.6	51.6
ODS-1	20	N/A	6.95	23.8	4.4
	20	N/A	8.95	18.8	solvent front

Table 9.4 Elution Volumes of TMP and SMZ with respect to pH

% CH_3CN	pH
16.66	6.22
28.57	6.40
44.44	6.55
50.00	6.60
60.00	6.60
63.64	6.60
66.67	6.55
71.43	6.40
75.00	6.25

Table 9.5 pH of acetonitrile-buffer mixtures

(buffer = 0.2 M/l (KH_2PO_4:Na_2HPO_4, 8:1))

increased the speed of elution of SMZ and TMP was also observed as a broad hump. The use of acetonitrile gave earlier, sharper peaks and increasing acid concentration speeded up elution (tables 9.2 and 9.3).

Small quantities of TMP metabolites 1 to 4 (Reider 211, table 9.1) were received from Wellcome Laboratories and were tested in these mobile phases. It was found that the relative retention volumes of SMZ and the TMP metabolites varied with mobile phase.

Using an ODS-II column (Whatman) and acetonitrile-water-phosphoric acid eluents it was found that the elution order altered, such that SMZ eluted much later than TMP which only left the solvent front at low acetonitrile concentrations. Using phosphate buffers, with acetonitrile on ODS-I, it was found that pH affected the SMZ elution volume markedly and that at high pH SMZ eluted earlier. (Table 9.4). This study was complicated by changes in buffer ionic strength (see below) but the gross changes are clear.

The buffer salts used were potassium di-hydrogen orthophosphate (KH_2PO4) and di-sodium hydrogen phosphate (Na_2HPO_4). It was found that the addition of acetonitrile to these buffers changed the pH in an unpredictable pattern and so it was difficult to isolate the effects of changes in pH from those of changes in buffer strength. Without acetonitrile the buffers gave pHs which depended upon the ratio of the component salts and not upon buffer-strength, but with acetonitrile added there was a significant difference (e.g. with a molar ratio of 8:1 (KH_2 : Na_2H-PO4) aqueous solutions (0.025M-0.2M) had a pH of 5.90 whereas with 1.5 volumes of acetonitrile added, 0.025M buffer was pH 7.1 and 0.2M buffer pH 6.6). Table 9.5 shows a non-linear dependance of pH upon acetonitrile composition at a fixed buffer strength.

Table 9.6 lists the elution volumes of TMP, SMZ and some metabolites in various phosphate eluents. These were evaluated primarily for TMP since SMZ and the metabolites were of less importance in some of the

	% CH_3CN	KH_2PO4 (M/L)	Molar ratio	pH	TMP Efficiency	Retention volumes (mls) TMP	TMP2	TMP3	TMP4	SMZ
(1)	13	0.087	5:1	6.40	-	24.6	-	30.1	11.4	-
(2)	13	0.1	5:1	-	-	23.5	11.4	-	11.9	-
(3)	18	0.1	5:1	6.50	240	15.2	-	-	-	7.1
(4)	20	0.1	5:1	6.45	360	12.4	-	9.4	7.3	-
(5)	20	0.1	2:1	6.90	490	13.0	-	9.1	7.6	4.5
(6)	20	0.025	2:1	6.95	662	23.8	8.8	13.0	10.5	4.4
(7)	13	0.1	No Na_2HPO4	4.65	-	13.6	7.7	29.0	7.9	-
(8)	20	0.025	2:1	6.95	118	33.4	10.4	19.9	12.7	Solvent front

Table 9.6 Retention data for phosphate eluents

Molar ratio is the ratio of KH_2PO4:Na_2HPO4 in aqueous fraction of mobile phase.

The concentration of KH_2PO4 is also expressed relative to aqueous fraction.

proposed studies. The table shows that,

increasing acetonitrile decreases retention volume (lines 2, 3, 4)

increasing buffer strength decreases retention volume (lines 1,2,5,6)

Increasing pH increases retention volume (lines 1, 7)

Similar trends were seen with ODS-2, but retention volumes were greater (line 6 -ODS1, line 8 -ODS2). Metabolite 1 was degraded by the time of this experiment and generally gave four peaks, all eluting early, from the solvent front to about 1/3 retention volume of TMP. No fresh material was available from the company. Metabolite 3 also decomposed (into 2 peaks) but one of these appeared to be the parent drug, TMP and the other was assumed to be TMP3. No reference spectra were available to verify this assumption. Although the efficiency rose as the buffer was diluted the increase in retention volume reduced the peak height, such that the condition of line 5 (table 9.6) yielded a peak height (equivalent to 1 μg/ml at 0.01 AUFS) of 200 mm whereas line 6 gave 145 mm.

Fig 9.2 shows UV scans of TMP in various solvents and demonstrates that the peak used by others (217, 231) at 270 - 280 nm is much less sensitive than 220 - 230 nm used in this study. Scans were also performed with 12.5% acetonitrile in the same buffers and these were found to be similar to the aqueous solutions. SMZ has a peak at about 260 nm but is still suitably intense at 220 - 230 nm and peak heights from sera are usually comparable with TMP, assuming the standard cotrimoxazole dose ratio of 5:1. It was found that TMP base is poorly soluble in water or normal saline and that TMP lactate (Wellcome) gave UV absorptions 6.6 times as intense as the molar equivalent of base. TMP lactate was used for standards for HPLC and UV scans.

Based on the above data, two systems were used in the following studies:

(1) For TMP alone

ODS 1, 25.44% CH_3CN, 74.56% (0.02M KH_2PO4, 0.01M Na_2HPO4), pH7.18.

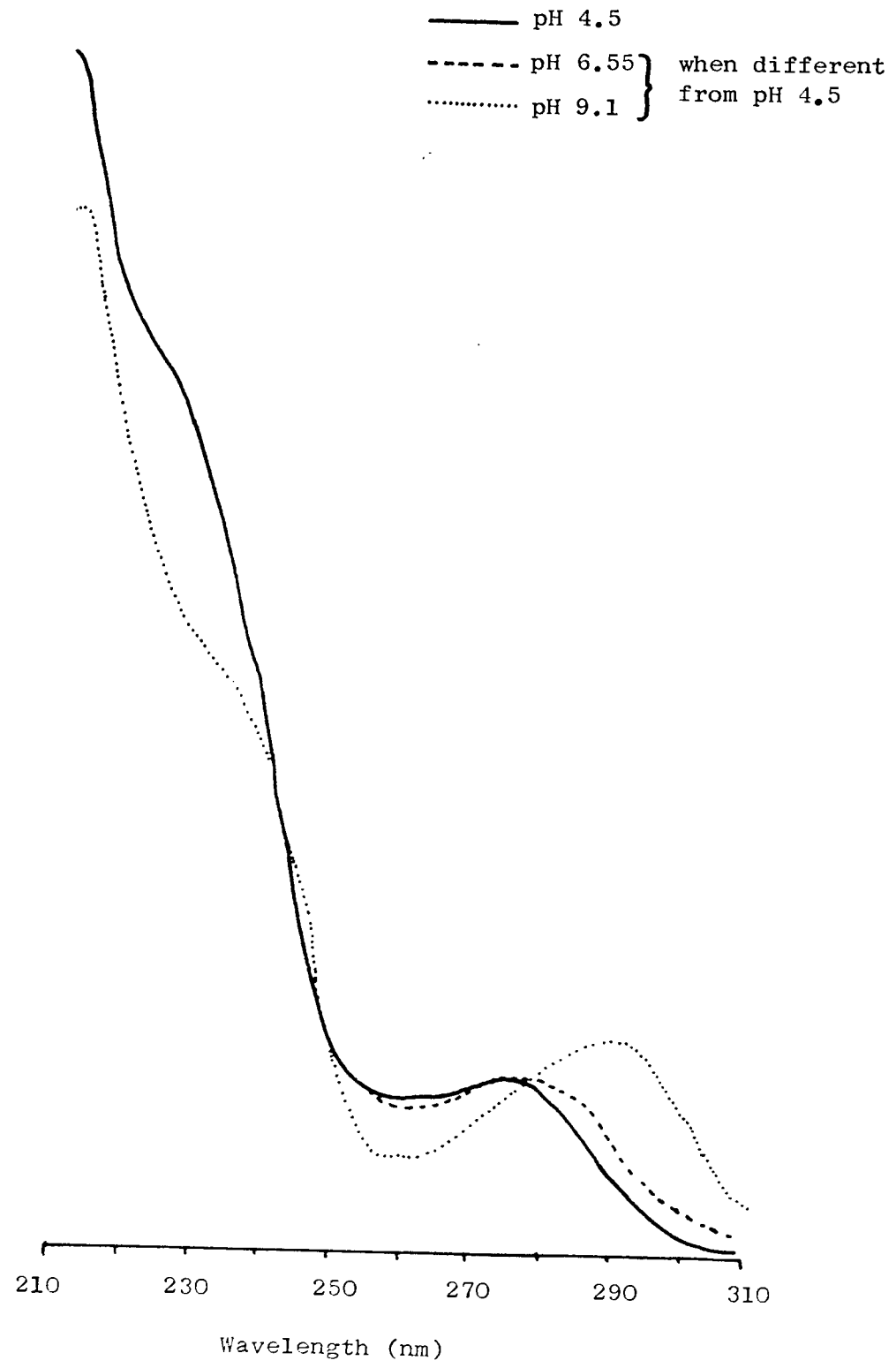


Fig 9.2 UV absorption of trimethoprim (210 - 230 nm).

(TMP retention volume 9.89 mls, n = 424 theoretical plates)

(2) For cotrimoxazole

ODS-2, 12.5% CH_3CN, 0.5% H_3PO4, pH1.6.

(TMP retention volume 8.0 mls, SMZ 22.2 mls).

During this study several HPLC methods have been published using fluorimetry for TMP alone (216), separate assays for each component, without TMP metabolites (217, 218), normal phase separation of SMZ, TMP and metabolites (219) and a combined assay without considering TMP metabolites (220). The two combined assays were both suitable for use in biological fluids but that of Weinfeld and Macasieb (219) required evaporation to dryness of large volumes of chloroform. That of Vree (220) was primarily concerned with SMZ and N^4-acetyl-SMZ and did not consider TMP metabolites. There is a general similarity in the method of Vree and this work in that both use phosphate buffers for pH control in a reverse-phase system but Vree used a different column (RP8) and organic modifier (methanol) and was not primarily concerned with TMP.

9.5 Extraction

Since TMP has a pKa of 7.2 and SMZ is amphoteric with pKas of 2 and 5.6 (206, 215) the textbook assays for these drugs separately (e.g. 230) recommend alkaline extraction into chloroform and acid extraction into ether respectively. Since neither method extracts both drugs well, and

chloroform extracts of blank serum were found to give messy chromatograms, new methods were investigated. Blank and spiked sera (cotrimoxazole, 32 μg/ml SMZ) were extracted with equal volumes of dimethyl formamide (DMF), butan-2-ol and butan-2-one.

Butan-2-ol yielded a relatively clean extract which was 42.8% efficient for SMZ and 67% for TMP. (Extracts from normal saline were 73% and 63% efficient respectively). DMF and butan-2-one gave reduced efficiencies and more interference. There was no interference from blank plasma in butan-2-ol extractions, although a late eluting peak (3 times retention volume of SMZ) was seen.

Acetonitrile, as a protein-precipitant (1 vol:1 vol serum) gave a 49.9% efficient extract of TMP (n = 5, cv = 3.7%) and 56.3% of SMZ (n = 4, cv = 2.0%) from spiked serum at 2 and 100 μg/ml respectively.

Other precipitants (for TMP only) with efficiencies at 5 μg/ml from serum were:

1:1 9.4% perchloric acid (46.0%)

5:1 70% trichloroacetic acid (26.3%)

5:1 35% trichloroacetic acid (50.5%)

10:1 70% trichloroacetic acid (61.8%)

20:1 70% trichloroacetic acid (61.8%)

(Ratio is that of serum volume to reagent volume)

No difference was found between a fresh (20:1 TCA) extract and a 4 hour old extract of the same serum. It was found, however, that blank serum yielded a significant peak at about 4 - 5 times the TMP elution volume that interfered with subsequent assays for TMP alone. The peak was not found in urine and its extraction varied with the reagent used. Relative peak heights were, in acetonitrile 1080 mm, ethanol 640 mm, 6% perchloric acid 48 mm, TCA (5:1) 7 mm. TCA was, therefore, used as the protein precipitant (1 ml serum, urine or saliva : 50 μl 70% TCA). Urine and PD fluid gave 100% yields, whilst saliva was assumed to be the same as

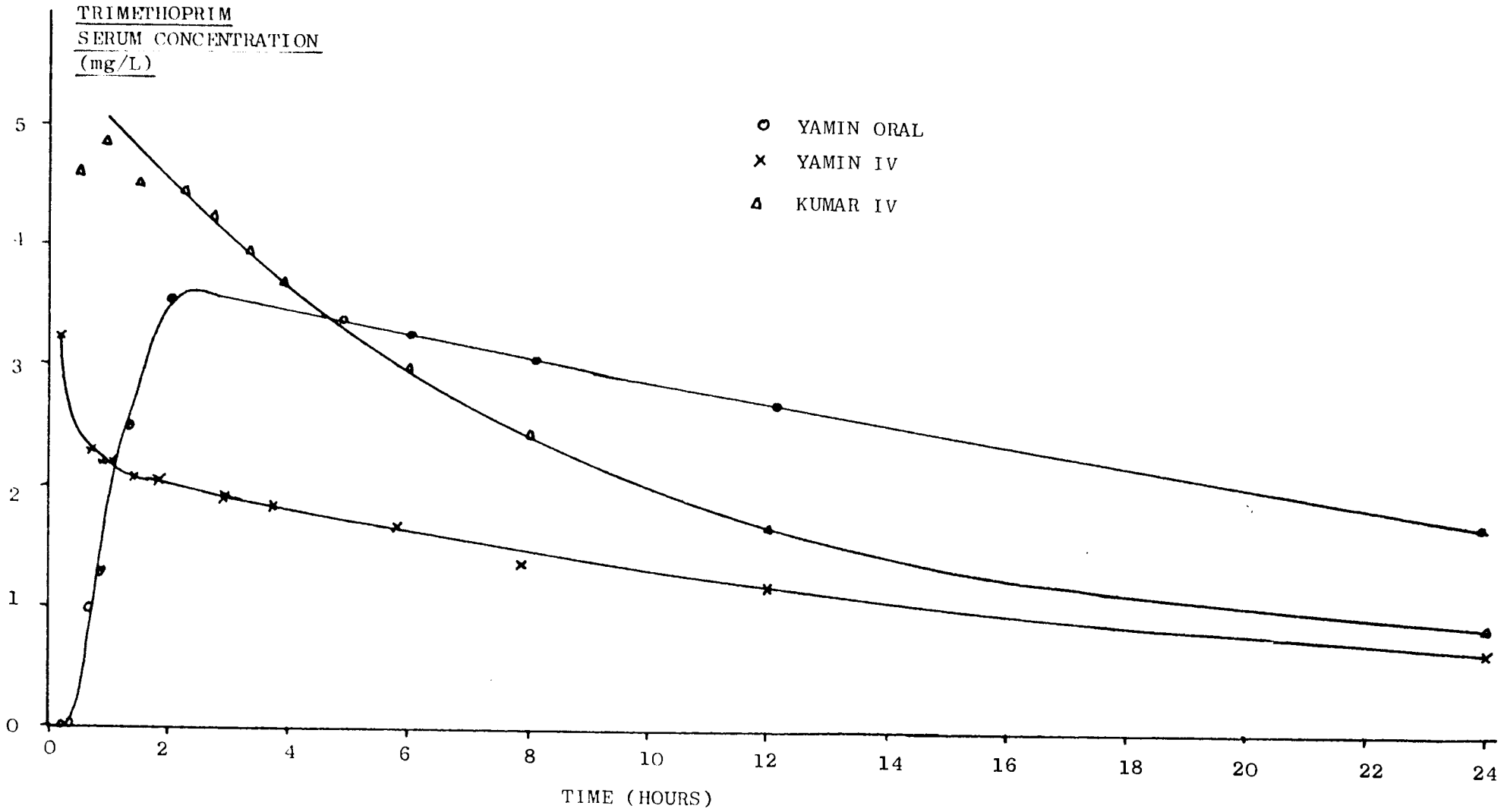


Fig 9.4 Serum concentration curves for trimethoprim

serum. (The viscosity of the saliva varied greatly between patients and no 'blank' saliva was available from either patient to assess extraction efficiency). Three blank sera (N.B.T.S.), a pooled serum (Clinical Chemistry D1) and seven volunteer urines were tested for further interference and none was found. Drugs known to be taken by patients in the study (methyldopa, minoxidil, gentamicin) were tested for co-elution with TMP or SMZ and found not to interfere. A blank specimen from each of the typhoid patients (see below) was found to be free from interfering peaks. The coefficient of variation (20:1 TCA) was found to be 2.6% (n = 8). The coefficient of variation of peak height from an aqueous solution of TMP (16.9 μg/ml) was 1.2% (n = 7) and was 1.6% from two solutions (n = 11). Four other solutions, each one normalised to its own mean, from 2.1 to 16.9 μg/ml gave cv = 1.8% (n = 11).

Weighing the dispensed volume of water from the Finnpipette used (1ml) gave a mean of 0.989 ml (cv = 0.351%, n = 16) and the Eppendorf pipette (50 μl) 49.4 μl (cv = 2.1%, n = 16).

Pyrex and poly-carbonate extraction tubes were used without any observable effect on the extraction. It was found, however, that on one day there was a considerable amount of interference in the serum samples. Tests were performed to isolate the cause and it was found that finely powdered debris from a previously shattered glass centrifuge tube was adversely affecting the result. (The tubes were uncapped in the centrifuge where the tube had broken). Capping the tubes with sellotape prevented recurrance of this problem.

	SERUM SAMPLES			
	F.C.		I.J.	
Timing	TMP (μg/ml)	SMZ (μg/ml)	TMP (μg/ml)	SMZ (μg/ml)
Pre-Oral Dose	2.35	3.4	0.13	3.0
3 hours Post Dose	2.43	5.9	0.12	5.61
6 hours Post Dose	2.58 (0.22)	2.6 (0.64)	0.10 (0.24)	3.16 (1.38)
9 hours Post Dose	1.64	4.34	0.18 (0.39)	4.21 (0.8)
2 hours Post Dose	-	-	- (1.55)	- (6.6)
Pre-IV Dose	1.62	7.44	0.17	3.19
60 mins Post Infusion	3.44	102.1	0.82	6.94

(Saliva levels in parantheses)

	DIALYSIS SOLUTIONS			
	F.C.		I.J.	
	TMP (μg/ml)	SMZ (μg/ml)	TMP (μg/ml)	SMZ (μg/ml)
	.38	3.7	.81	4.4
	.40	9.0	1.20	4.6
	.45	8.5	1.03	4.0
			1.08	4.4
Mean protein (g/l)	.347		.608	
pH	8.4		8.4	

Table 9.7 SMZ and TMP levels in peritoneal dialysis

9.6 Peritoneal dialysis

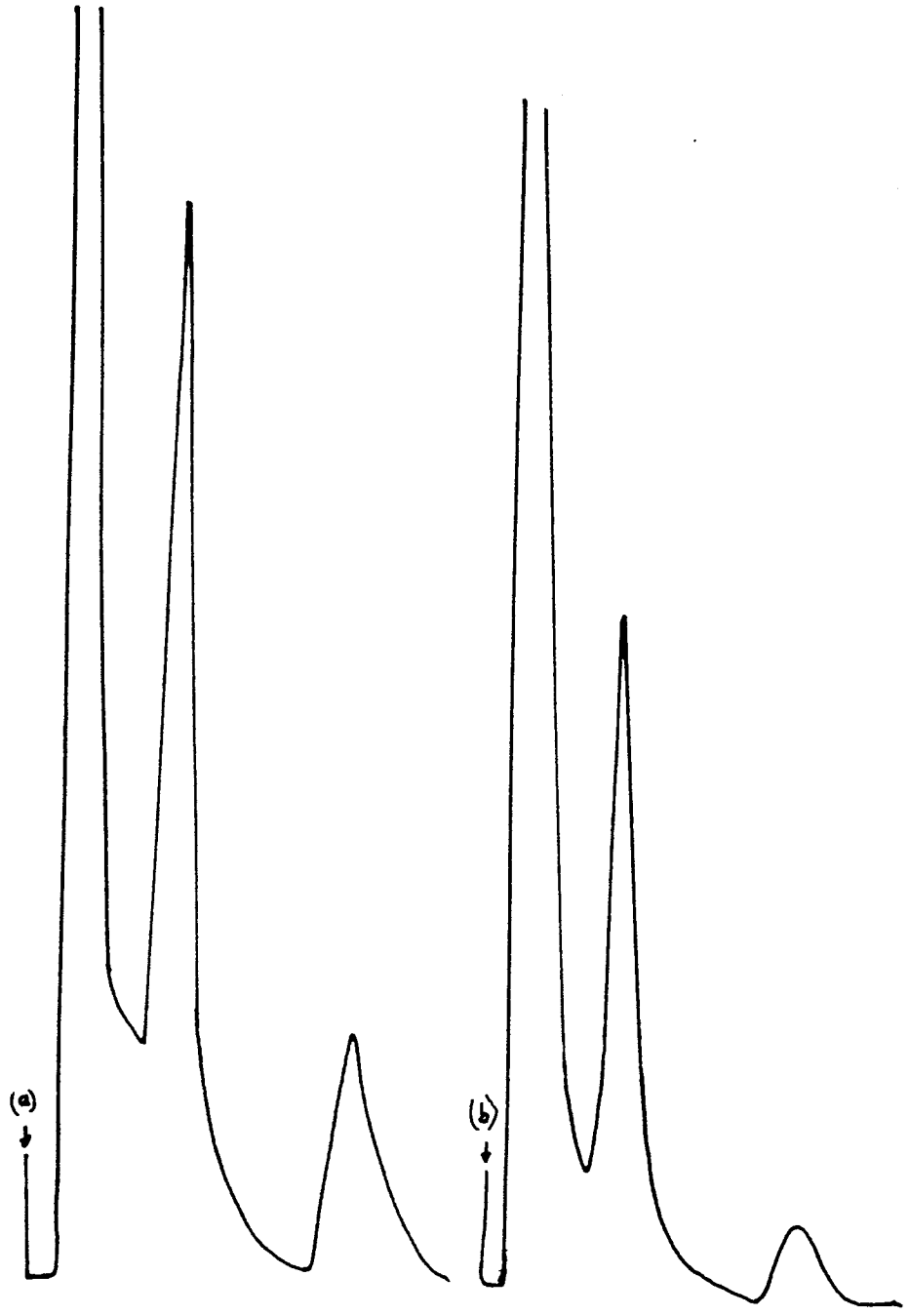
Two papers (221, 222) have been published purporting to show that neither TMP nor SMZ are removed from the body by peritoneal dialysis, and yet many patients undergoing peritoneal dialysis (PD) receive normal doses of cotrimoxazole by mouth without any reports of excessive accumulation. A request was received to investigate two such cases in the renal unit at East Birmingham Hospital. Both were males, one (I.J.) having received the drugs for a week and the other (F.C.) for 48 hours, each receiving two tablets twice daily and having two litres of dialysis fluid (Boots dialoflex No. 61) every four hours commencing two hours after the oral dose of cotrimoxazole.

Fig 9.3 shows the output resulting from chromatography of PD fluid. It was found that extraction of spiked PD fluid with acetonitrile or TCA resulted in a 100% yield of TMP and SMZ, presumably due to the low levels of protein and other insoluble matter. Total protein and albumin assays were performed by the Clinical Chemistry Department but the levels of albumin were found to be too low for the routine method. Total protein levels are given in table 9.7 together with the measured levels in serum, dialysate and saliva. Salivary levels are of interest because of the possibility of using saliva collection as a non-invasive technique for estimating serum levels. Non-invasive techniques are preferable for many reasons but especially in paediatrics and intensively-investigated patients. Saliva has been used for other drugs (221, 87) but the reproducibility, between individuals and within an individual, of serum:saliva drug ratios, and the lag-time for the salivary curve need to be established. Although collecting devices for isolating the outflow of specific salivary glands have been used (225) they require a good salivary flow to obtain sufficient sample without undue suction and mixed samples are widely available. No chemical stimulation was used to induce salivary flow. When it was found that the levels were so low in patient I.J. it was

Fig 9.3 HPLC Traces of cotrimoxazole in patients' fluids

(a) Serum (TMP 2.43 mg/L, SMX 5.88 mg/L)
(b) dialysate (TMP 1.68 mg/L, SMX 1.84 mg/L)

Conditions as in Text



decided to administer iv infusions over 30 minutes to eliminate the possibility of poor compliance. It became apparent, however, that some samples were not correctly labelled, in that F.C.'s 'post-dose' levels were very much lower than the 'pre-dose' levels. These results were reversed in table 9.7 so as to give the higher levels after the dose but other results were allowed to stand as labelled by nursing staff. It has been noted previously, however, that the considerable fluid dislocations in PD affect serum levels of drugs and so the minor discrepancies remaining may be a true record of events (222). For similar reasons the timing of the dialysate samples, relative to dosing, is uncertain. The SMZ levels were similar in both patients and were very low. Dialysate levels were similar to serum levels but saliva levels were rather lower. (This confirms the observations of Quayle et al (226) and Eatman et al (227) with respect to Saliva). I.J.'s TMP levels were low throughout the study, which probably explains his poor response to therapy, but the dialysate levels were higher than those of F.C. who had higher serum levels. Since TMP is significantly protein-bound the difference in dialysate levels may be due to the significantly higher protein levels in I.J. dialysate ($p < 0.05$, t-test). The pHs of the solutions were identical (8.4). Salivary TMP levels were higher than serum levels for I.J. but the one level available for F.C. was lower. Previous reports have shown higher salivary levels (226, 227, 228). Enzyme induction is reported to increase TMP metabolism and may, therefore, have caused I.J. to have lower TMP levels than F.C. An alternative is simply that I.J.'s volume of distribution is greater than that of F.C. It is proposed to test the hypothesis concerning enzyme induction by serial measurements of half-life in patients on high-dose TMP therapy.

9.7 Typhoid Infections

A study was commenced to investigate the comparative pharmacokinetics of oral and iv TMP in young, male, Asian, typhoid patients. TMP alone has not previously been investigated by iv dosing. To date, two patients (M.Y., 23 years, 65 kg and R.K., 21 years, 55 kg), having recently received a course of TMP to cure an infection of Salmonella typhi, have also received an iv bolus of 300 mg and later (5 days and 2 days respectively) an oral dose of 300 mg TMP. Urine collections and serum samples were taken and are detailed in tables 9.8 and 9.9. Only three of the four doses were analysed.

M.Y.'s half-life, volume of distribution and clearance of TMP all decreased between his two measured doses. The urinary output of TMP was slightly higher, as a percentage of dose, from the oral dose but the urine volume was lower. The urinary clearance, calculated by the method of Gibaldi and Perrier (128), was significantly lower after the oral dose ($t = 3.36$, $p < 0.02$) and was approximately 85% of the plasma clearance, whereas urinary clearance was 70% of the iv plasma clearance. The differences in Knr and Ke are not significantly different ($p > 0.2$ and $p > 0.15$ respectively) but the trend shows greater metabolism (or deposition) in the iv dose. This might be expected if the effect of the enzyme induction supposed to occur during longer therapy was reduced by the time of the second dose (12 days after cessation of anti-typhoid therapy). Urinary pHs were not greatly different (pH 5.6 $\pm$ 0.2 units range).

Some workers have reported an unquantified effect of pH on TMP and SMZ excretion, such that low pH promotes TMP excretion and reduces that of SMZ (345). Both this and the previous study showed no such effect because of the uniformity of the subjects. Since typhoid patients generally have acidotic urine, TMP would give good urinary concentrations.

SERUM TMP LEVELS (μg/ml)

Time (hr)	R.K. (iv)	M.Y. (iv)	M.Y. (po)
0.25	-	3.23	0
0.5	4.44	2.38	0
0.75	-	2.20	1.90
1	4.71	2.20	2.20
1.5	4.18	2.14	2.68
2	4.26	2.13	3.61
2.5	4.35	-	-
3	4.18	2.02	2.57
3.5	3.95	-	-
4	3.73	1.08	2.45
5	3.42	-	3.42
6	3.02	1.75	3.27
8	2.44	1.36	3.12
12	1.69	1.21	2.75
24	0.77	0.62	1.77
K (h^{-1})	0.101	0.0568	0.0345
$t_{\frac{1}{2}}$ (hr)	6.85	12.2	20.1
AUC (μghml^{-1})	54.7	42.8	115.5
V(1) [% body weight]	54.3 [98.7]	127.7 [196.5]	78.3 [120.5]
Cl (ml/min)	91.4	120.9	45.0
C_{ss} (μg/ml, daily dosing)	2.28	1.78	4.81
Ka (hr^{-1})			0.52

Table 9.8 Serum TMP Levels and Calculated Parameters

Time (Hours)	Volume (mls)	TMP (µg/ml)	TMP (mg)	Clearance (ml/min)	
		R.K. (iv)			
0-2	626	24.6	15.4	92.4	Mean clearance = 106.7 ml/min (116.7% plasma clearance)
2-4	534	26.6	14.2	88.7	
4-6	483	24.6	11.9	104.4	
6-8	570	19.9	11.4	125.3	
8-24	1560	23.6	36.9	122.6	Ke = 0.0327 hr^{-1}
	3772		89.7 (29.9% dose)		Knr = 0.0683 hr^{-1}
		M.Y. (iv)			
0-2	43	94.9	4.08	55.6	Mean clearance = 84.5 ml/min (85.5% plasma clearance)
2-4	64	75.6	4.84	71.9	
4-6	132	31.3	4.13	69.7	
6-8	360	20.1	7.22	131.0	
8-24	1140	21.3	24.26	94.4	Ke = 0.0397 hr^{-1}
	1739		44.53 (14.8% dose)		Knr = 0.0171 hr^{-1}
		M.Y. (p.o)			
0-2	175	17.8	3.12	42.5	Mean clearance = 38.5 ml/min (70% plasma clearance)
2-4	60	69.5	4.17	35.8	
4-6	302	18.9	5.71	50.1	
6-8	268	14.4	3.86	36.6	Ke = 0.0295 hr^{-1}
8-24	500	70.3	35.16	27.5	Knr = 0.005 hr^{-1}
	1305		52.02 (17.3% dose)		

Table 9.9 Urinary TMP levels and Calculated Parameters

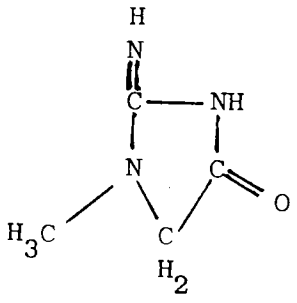
10. CREATININE ASSAYS

10.1 Introduction and Outline of Events

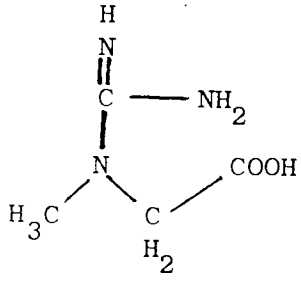
As indicated above (section 3.1) serum creatinine levels may be used as an index of renal function and were measured for this study. The assays were performed as routine upon a Clinical Chemistry continuous-flow analyser using the Jaffé reaction (described below). The chart recorder output had a range of 0 - 2000 μM/l, giving peaks of $\frac{1}{2}$" - 1" for most normal patients (say, 60 - 150 μM/l). This was imprecise, even when read with a proportioning arm, and values were often reported as round numbers (e.g. 80, 100). When the initial data were examined it was found that in several cases the gentamicin half-life and the serum creatinine in any one patient changed in opposite directions between measurements instead of the same direction and it was thought that poor assay technique was the cause. (An internal drift standard showed a coefficient of variation of 15% between assays over one month). The Jaffé reaction has a number of limitations and is liable to interference from a variety of serum components (see below). Manual methods and rate-analysis methods were reported to show less interference and so were investigated to evaluate the precision and selectivity obtainable. An LKB8600 rate analyser would be made available if the studies using SP1800 spectrophotometer showed promise of a satisfactory method.

These studies are reported below, but did not show any significant advantage over the possibility of using a modified version of the routine method whereby a larger sample was taken and the output scale expanded. The principal problems were those of obtaining reproducible temperatures for rate analysis and eliminating protein interference.

HPLC was evaluated as a means of obtaining a selective result. (The value of this selectivity is disputed in any case, since some authors maintain that the excess chromogens in serum counteract the tubular secretion of creatinine and the result reflects glomerular filtration more



CREATININE



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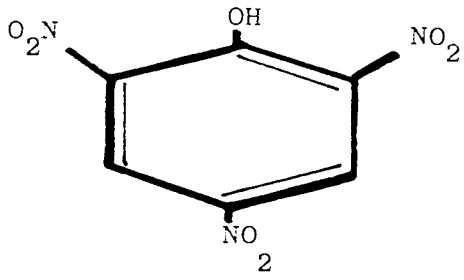


PICRIC ACID

Fig 10.1 Structures of Creatinine, Creatine and Picric Acid

accurately than does a true creatinine assay (232)). A selective assay was designed and was capable of dealing with one sample every ten minutes. A prolonged period of instrument failure, however, prevented immediate use of this method and it was decided to use a modified version of the routine method.

10.2 Jaffé Reaction Studies

The Jaffé reaction was first described in 1886 (233) and although there are many variations the reaction remains the most common means of assaying creatinine in body fluids. The assay depends upon the spectrophotometric determination of a complex formed between creatinine, picric acid (2, 4, 6 trinitrophenol) and an alkaline sodium buffer. The structure of the complex is not known, but many suggestions have been made (234, 235). Its composition and rate of formation are reported to vary with the concentration of the reactants, pH, ionic strength and temperature (235, 236, 244, 245). Other substances produce a similar complex, notably proteins, acetone, acetoacetic acid, cephalosporins and chlorpromazine. Glucose, ascorbate and L-dopa reduce picric acid to picramate, which also increases absorbance (236, 246). Although each one produces only a small amount of interference their sum may be significant and they are known collectively as 'Jaffé-positive chromogens'. Bilirubin, on the other hand, has a negative interference (236, 246). The specimen-collecting

container may increase the rate of complex-formation (249). The complex has a maximum absorbance at about 480-490 nm (depending upon composition) but the reagents have a maximum in the region of 470 nm, dropping rapidly above 500 nm to almost zero at 530 nm (own data). Many operators prefer, therefore, to use 505 nm as detection wavelength.

A typical reaction procedure (designed for use on an LKB8600 rate analyser) is summarised here (237):

Buffer: 50 ml 0.05M $NaHCO_3$
adjust to pH 11 (37°C) with approx. 24 ml 0.1M NaOH
dilute to 100 ml.

Picrate: equal volumes of saturated picric acid solution (≃13g/l) and 0.75M NaOH

Method: Mix 3 ml buffer and 0.6 ml sample.
Add 0.6 ml picrate.
Determine rate at 505 nm.

For use in a multi-channel analyser the picrate solution is made from a 70% saturated solution of picric acid rather than a saturated solution. This is thought to minimise the interference from chromogens since some are less reactive than creatinine. The rate of reaction is held to be proportional to the creatinine concentration (a first-order relationship) and this method of analysis is thought to eliminate some interference from the slower reacting species. A typical reaction run (say on an LKB8600) lasts 2 - 3 minutes. Omitting the first few seconds eliminates the very fast reacting species, e.g. acetoacetate. Differences in reaction rate at two different pH values have also been used to reduce some interferences (242).

The concentrations in the above method indicate a one hundred-fold excess of picrate over creatinine at 200 μM/l creatinine (over twice the normal serum value), assuming a 1:1 reaction product.

Creatinine Concentration (μm/l)	Mean Absorbances (A.U. at 500 nm) 80 mins	160 mins
75.88	.1070	.1060
94.85	.1303	.1317
113.83	.1590	.1620
151.77	.2180	.2200
189.71	.2723	.2755
284.6	.4150	.4163

Statistics (based on 18 points each):

80 mins Absorbance = -0.0088 + 0.00149 (Creatinine concentration)

r = 0.999 95% confidence limits at 100 μm/l = ± 1.9%

160 mins Absorbance = -0.0055 + 0.00148 (Creatinine concentration)

r = 0.999 95% confidence limits at 100 μm/l = ± 2.6%

Reaction mixture:

3 ml water, 0.5 ml NaOH, 0.6 ml creatinine, 0.6 ml picrate (saturated)

Table 10.1 Calibration Data for Jaffé Reaction

Modifications of this method include the use of Lloyds reagent (fullers earth) as a selective adsorbent for creatinine prior to a Jaffé reaction (240). Alternatively creatinine may be converted to methylguanidine and assayed by a Sakaguchi reaction (239). This is highly specific but is manipulatively complex and not suited for continuous flow analysers (238). Enzymatic analyses (241) have also been used.

.1 Non-Rate Studies

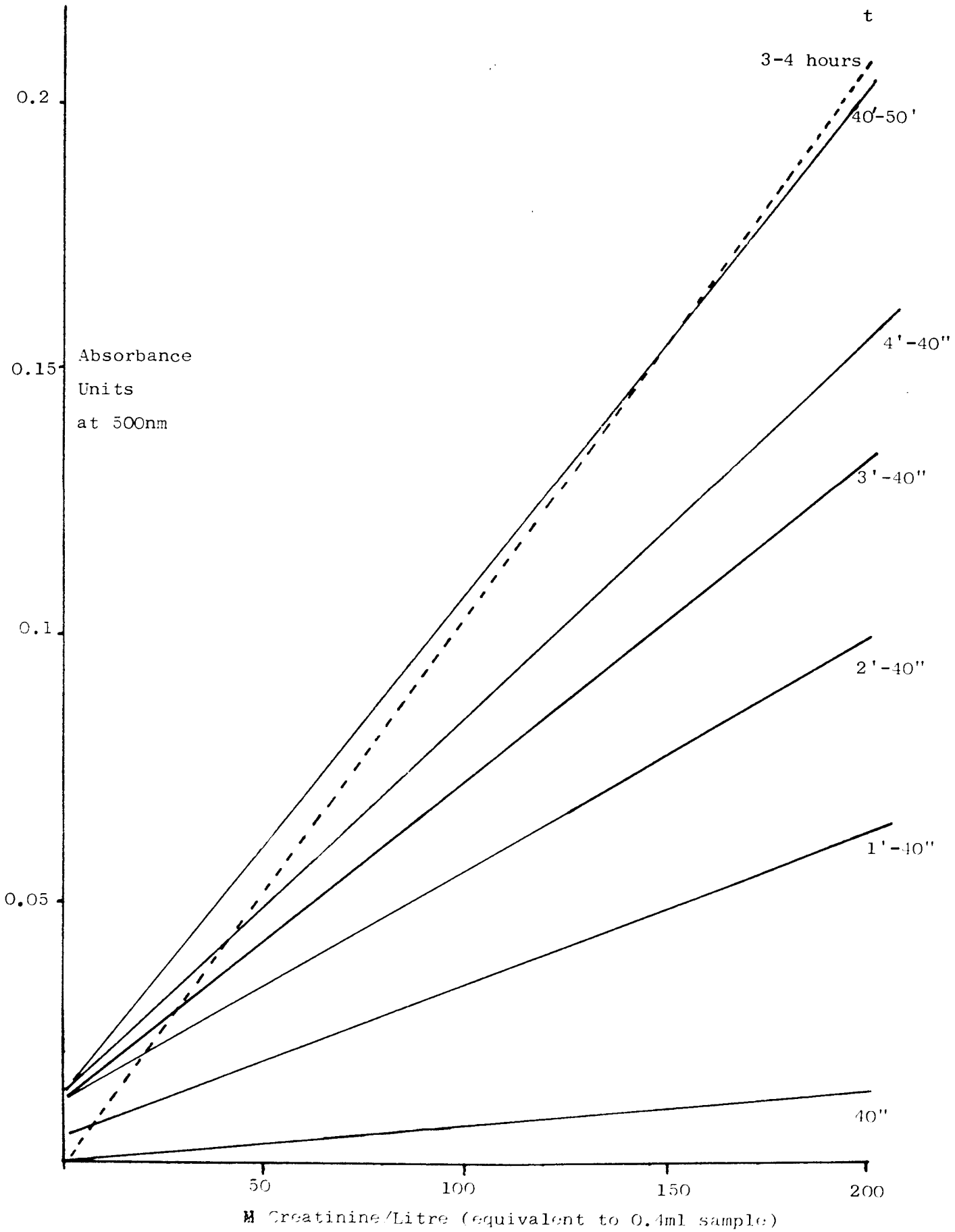
A non-thermostatted SP1800 (Pye Unicam, cf appendix 1) was used to evaluate the possibility of using a manual Jaffé reaction for serum analysis. The method of Hurn was modified to use sodium hydroxide (0.75m) instead of the bicarbonate buffer (this increased the intensity of the absorbance). Appropriate volumes, as given below, of alkali and creatinine were pipetted into a 1 cm quartz uv cell and the reaction initiated with the rapid addition of picrate by Finnpippette. The stock solution of saturated picric acid was calibrated against N/20 NaOH (phenolphthalein indicator) and found to be 54.2 mM/1. The sodium hydroxide solution was calibrated against potassium hydrogen phthalate (BDH standard).

Table 10.1 shows the results of a calibration run using aqueous solutions of creatinine (75.88 - 284.6μM/1). Triplicate determinations of each of six concentrations were performed, measuring the absorbance at 500 nm, 80 and 160 minutes after reaction at 23^{o}C (room temperature). The method shows good linearity and confidence limits.

A similar run, with 2.8 ml water and 0.4 ml creatinine, was performed on duplicate mixtures at each of six creatinine concentrations, measuring at 1 minute intervals from 40 seconds to 4 minutes 40 seconds and also at 12 minutes, 40 - 50 minutes and several hours after mixing.

Fig 10.2 shows the results, which are linear with respect to concentration from 1 minute 40 seconds onwards. The 1 minute 40 seconds

Fig 10.2 Absorbance at time (t) versus concentration of creatinine in sample



to 40 minute measurements exhibit a positive intercept, the 2 minutes 40 seconds - 40 minutes intercepts being almost identical. The 'several hours' line passed through the origin. A possible explanation is that the cells were zeroed with a warm reference solution and a cold sample solution and that as the sample mixtures warmed up the intercept rose due to the temperature-dependant rise in absorbance of reactants. The 'several hours' samples would all be cold when measured.

After 12 minutes there was very little change in absorbance with time, under this particular set of conditions.

The temperature-dependance of the absorbance of the mixture was investigated by leaving a reaction mixture (284 μM/l creatinine) in the UV spectrophotometer for an hour. At the end of this time the absorbance had been constant for 39 minutes and the cell was at 33^{o}C. The solution was removed from the spectrophotometer and allowed to cool down whilst being re-measured at intervals. The results are given in table 10.2 and confirm the temperature-dependance reported elsewhere (235, 245). It was also found that alkaline picrate alone showed temperature-dependant absorbance. It is important to note that this is a reversible change in absorbance. Spierto et al reported a 0.01 AU/^{o}C change in 8 mM picrate at 490 nm (245).

Increasing the concentration of hydroxide was found to decrease the net increase in absorbance due to a given quantity of creatinine. Vasiliades (235) observed a similar pattern of behaviour and stated that this could be explained by an increase in both forward and reverse rate constants with hydroxide concentration. The effect of increasing picrate concentration in non-thermostatted experiments was opposite to hydroxide but less marked. In thermostatted experiments (following) there was no trend with picrate concentration and so the non-thermostatted results may reflect random variation or diurnal temperature changes. (The series of experiments in table 10.3 took over five hours to complete and the

Temperature (^{o}C)	Absorbance (A.U.) at 484 nm
33.0	0.757
29.8	0.715
28.2	0.702
26.5	0.695

Table 10.2 Effect of Temperature on the Absorbance of a Creatinine-Picrate Reaction Mixture

ambient temperature rose by 1°C in that time (26°C - 27°C). The experiments with varying hydroxide were all at 26°C and so probably not affected significantly by temperature).

Several authors have absorbed creatinine onto fullers earth prior to reaction in order to reduce interference from other substances (e.g. creatine) that are not so absorbed. Oxalic acid is reported to improve the selectivity of the process (238). A calibration run was performed using 0.2 ml saturated oxalic acid solution, 0.6 ml creatinine, 1 ml water and approximately 20 mg of fullers earth (ICI). Having vortexed these together, allowed to stand for 20 minutes, centrifuged and decanted the supernatant, 0.5 ml sodium hydroxide, 3 ml water and 0.6 ml picrate were added and the mixture vortexed again. After standing for one hour, the mixture was centrifuged and the supernatant measured colorimetrically.

The results were compared with solutions reacted as before to assess the efficiency of recovery of creatinine from fullers earth.

Recovery was 96.2%, 94.2% and 93.3% at 75.88 μM/l, 113.83 μM/l and 151.77 μM/l creatinine respectively. (Each value is the mean of duplicate tests). It was found that fine particles of fullers earth suspended in the supernatant gave falsely high results and several minutes centrifugation was required (3,400 rpm) to prevent interference.

Further tests showed that doubling the amount of fullers earth made no significant difference at 189.71 μM/l creatinine (means of triplicate absorbance determination 0.294 and 0.296 AU respectively, $p > 0.10$, t-test). Replacing the oxalic acid solution with water, however, lead to a significant decrease in recovery (absorbances 0.112 and 0.041 AU respectively at 75.88 μM/l creatinine. $p < 0.05$).

.2 Rate Studies

Let us denote the concentration of hydroxide, picrate, creatinine and their coloured product by [OH], [P], [C] and [CP] respectively.

If the formation of the product proceeds in a first order fashion,

Final molar Concentrations of

Picrate	Sodium hydroxide	K_{obs}	Change in UV absorbance (A.U.)
0.00334	0.44	.00962	.094
0.00334	0.22	.00460	.163
0.00334	0.11	.00197	.415
0.00668	0.11	.00382	.451
0.01002	0.11	.00473	.458

Table 10.3 Non-thermostatted kinetic data

(1.67×10^{-5} M/l creatinine)

depending upon the product of the concentrations starting materials, then

$$\frac{d\,[CP]}{dt} = K\,[C]\,[OH]\,[P] \qquad (1001)$$

(The best evidence suggests a 1:1:1 complex, but for the purposes of creatinine assays this need not be so, provided there is only one molecule of creatinine per molecule of product as outlined below).

If [OH] and [P] are in great excess (as in the standard Jaffé conditions) throughout the reaction then their concentrations will remain effectively unchanged and

$$\frac{d\,[CP]}{dt} = K'\,[C] = \frac{-d\,[C]}{dt} \qquad (1002)$$

This is the familiar first-order relationship from which,

$$[CP] = Co(1-e^{-K't}) \qquad (1003)$$

where Co = [C] at time (t) = 0.

If the UV absorption of CP obeys the Beer-Lambert laws then

$$\text{UV absorbance} = K''\,Co\,(1-e^{-K't}) \qquad (1004)$$

where K" is a conversion constant.

It may be seen that at any given time t, the ratio of absorbances of two solutions which initially contained different values of Co (say, C_1 and C_2) will be the ratio of C_1 and C_2, irrespective of the dependance of the reaction upon species which are in large excess.

$$\text{Ratio} = \frac{K''C_1\,(1-e^{-K't})}{K''C_2\,(1-e^{-K't})} = \frac{C_1}{C_2} \qquad (1005)$$

To use the apparent rate constant, however, (1004) must be differentiated with respect to t.

Thus,

$$\frac{d\,(UV)}{dt} = \frac{d\,(K''Co)}{dt} - \frac{d\,(K''Coe^{-K't})}{dt} \qquad (1006)$$

$$= -K''Co\ \ d(e^{-K't})$$

$$= K''CoK'e^{-K't} \qquad (1007)$$

If $\frac{d\,(UV)}{dt}$ is approximated by $\frac{\Delta UV}{\Delta t}$ for a finite measurement

then $\ln \frac{\Delta UV}{\Delta t} = \ln (K'K''Co) - K't \qquad (1008)$

Thus a plot of $\ln \frac{\Delta UV}{\Delta t}$ versus t will give a slope of -K' and an intercept from which K'' or Co may be determined, provided one of these is known.

An alternative method of obtaining K' is to express (1002) as

$$[C] = Coe^{-K't}$$

$$= [CP]_\infty - [CP]$$

$$= \frac{UV_\infty - UV}{K''} \qquad (1009)$$

Thus

$$UV_\infty - UV = K''Coe^{-K't}$$

and $\ln (UV_\infty - UV) = \ln (K''Co) - K't \qquad (1010)$

A plot of $\ln (UV_\infty - UV)$ versus t will also give a slope of -K' but this does not depend upon the approximation of $\frac{\Delta UV}{\Delta t}$ to $\frac{dUV}{dt}$.

Once K' and K'' are known, then the concentration of an unknown creatinine solution may be determined from (1002) and (1004) provided that $\frac{\Delta UV}{\Delta t}$ approximate to $\frac{dUV}{dt}$.

Thus a typical procedure would measure the rise in UV absorption

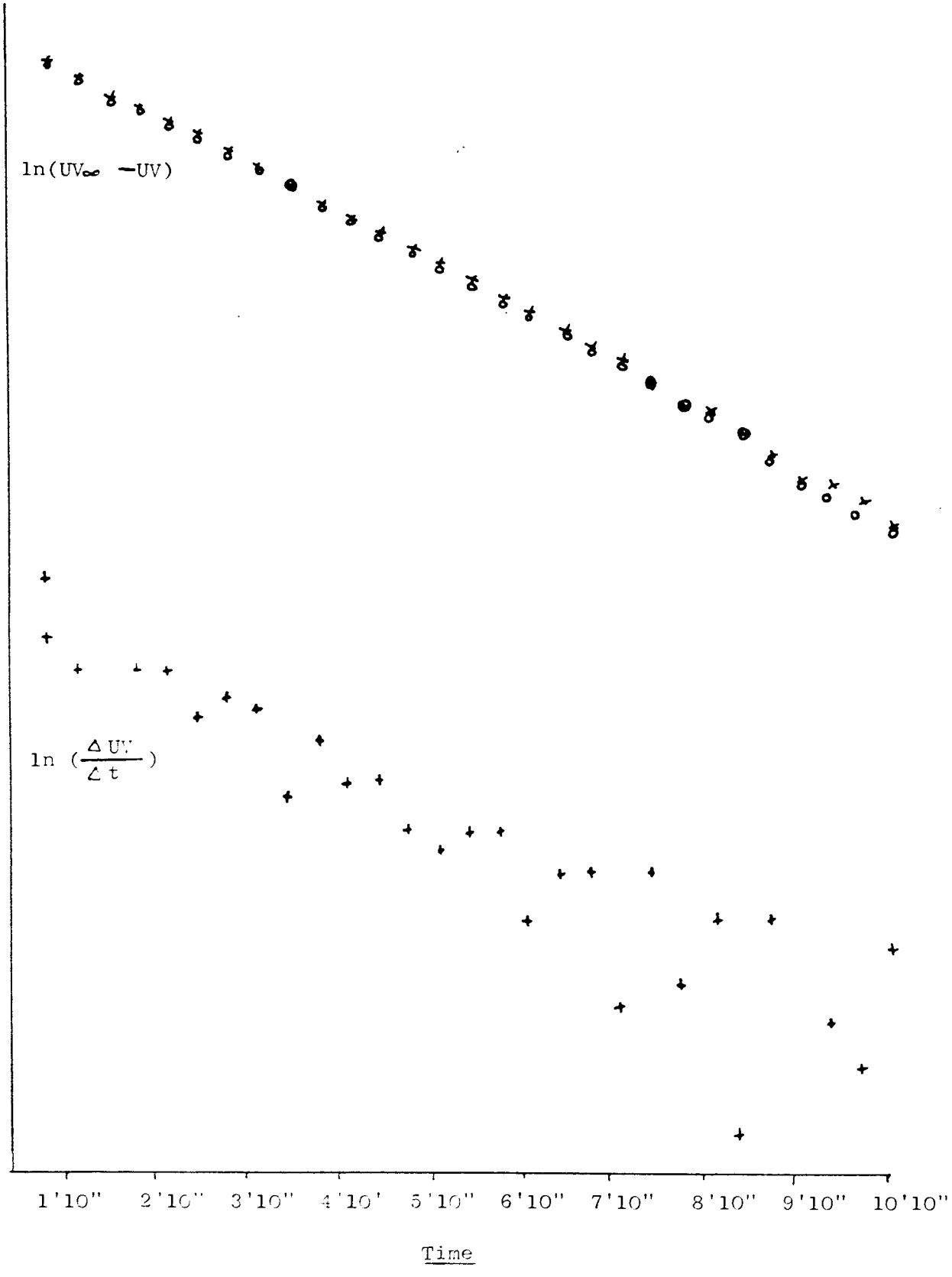


Fig 10.3 Rate of reaction data from replicate runs (+,o) analysed by methods 1010 (upper) and 1008 (lower)

(For conditions see text)

over a fixed time interval and compare it with that from standard solutions.

Eleven reaction mixtures, of five compositions, were monitored every 20 seconds from 40 seconds to 13 minutes and at 14, 15 and 21 minutes and about one hour. The ambient temperature was 27°C but the cell holder was at 33.5°C. The rate constants K' and K were calculated by three methods using 38 points for each:

(a) the method of (1010)

(b) the method of (1008) with 20 second intervals

(c) the method of (1008) with 40 second intervals

Fig 10.3 shows representative traces. The upper portion shows how closely two replicates were superimposed when analysed by method (a) whilst the lower portion shows the large scatter of points when one of those duplicates was analysed by method (b).

Reaction conditions and calculated Ks are given in table 10.4. Only method (a) was considered reliable, on no occasion was the correlation coefficient less than 0.98 for all 38 points.

K is not the same in all conditions, indicating that the hypothesised rate dependance upon [P] , [C] and [OH] is not valid. The value of K is not directly or inversely proportional to [P] or [OH] but does increase as [OH] increases and decrease as [P] increases.

A power relationship (i.e. ln K = a + b ln [P] etc) was not found for [P] or [OH]. Plots of K' versus [OH] and [P] (other components being constant) are inconclusive, showing a linear response for [OH] ($K' = -0.0005 + 0.02314\ [OH]$) and a linear response for [P] for the lower two concentrations only ($K' = 0.59\ [P]$). A linear relationship with picrate was shown on another occasion (6 mixtures at 4 concentrations of picrate (2.1-26.2m Mole/1), $K' = 0.0001 + 1.447\ [P]$) but this was on a thermostatted UV spectrophotometer at 30.3°C. Even so, it is apparent that K decreases as [P] increases and that the reproducibility between replicates is not good. (cv = 62.8%, each reading compared to

Molar Concentrations

Picrate	Sodium Hydroxide	K'	K	$UV_{\infty}-UV_{40''}$(AU)
0.00334	0.44	0.009624	6.547	0.094
0.00334	0.22	0.004605	6.267	0.163
0.00334	0.11	0.001971	5.365	0.415
0.00668	0.11	0.003821	5.200	0.455
0.01002	0.11	0.004728	4.290	0.457

(Creatinine = 1.67×10^{-5} M/l, all concentrations in final mixture, unthermostatted)

Molar Concentrations

Picrate	Sodium Hydroxide	K'	K
0.00210	0.1935	0.00192	4.72
		0.00209	5.14
0.00420	0.1935	0.00248	3.05
		0.00244	3.00
0.00525	0.1935	0.00373	3.67
		0.00395	3.89
0.01050	0.1935	0.00674	3.32
		0.00640	3.15
0.02100	0.1935	0.01219	3.00
		0.01032	2.54
0.02620	0.1935	0.01258	2.48
		0.01449	2.85

(Creatinine 9.16×10^{-6} M/l, thermostatted 30.7 - 30.35°C)

Table 10.4 Jaffé Rate Reaction Data

the mean for that picrate concentration). A likely explanation for the poor reproducibility is the difficulty of controlling the temperature of the UV cell. The controlling waterbath was remote from the spectrophotometer, with long tubes connecting it to the cell block and an erratic mixer. The range of temperature at one position in the waterbath was 0.6°C over the heating-cooling cycle. It was found in one case that measuring over 5 minutes gave a K' of 0.0092 but measuring over 12 minutes gave a K' of 0.0083. (All the results quoted in table 10.4 are for 5 minute periods commencing 40 seconds after mixing at 30.7°C. The temperature at 5 minutes ranged from 30.35 to 30.5°C). In the non-thermostatted case (fig 10.3) it may be seen that although the correlation coefficient is very high for a straight line relationship the line is a convex curve, the rate increasing with time. This suggests that the rate is increasing as the temperature increases from ambient to cell-block temperature.

Bowers and Wong (244) reported that a 5°C change in temperature changed the rate of reaction by 30% but also reported that the reaction is pseudo-first order for picrate concentrations up to 30mM/l and hydroxide concentrations greater than 0.5 mM/l. Shoucri and Pouliot (247), in uncontrolled ambient temperature experiments, reported rates varying from 0.0003 to 0.0084 s^{-1}. Bower (236) reported rate constants for creatinine (0.0022 s^{-1}), oxaloacetate (0.0027), α-ketoglutarate (0.0046), pyruvate (0.0049) and acetoacetate (> 0.5 s^{-1}) and concluded that rate reaction analysis based on two point determinations over a few minutes could not claim to be a specific technique for creatinine.

10.3 HPLC Assays

Lim et al (243) have reported a reverse-phase HPLC assay with an ammonium acetate (0.1M) eluent. Their sample preparation included adsorbing creatinine onto a specially prepared cation-exchange column and eluting first the interfering matter and then the creatinine before evaporating to dryness and re-dissolving in the HPLC eluent. Their HPLC separation was tested using pure creatinine solutions and found to elute creatinine in 9.6 mls on ODS-1. The concentration of ammonium acetate made no difference to the retention time or peak shape over the range 0.01 - 0.5 m/l. Using an SP1800 spectrophotometer it was found that creatinine absorbs UV light more strongly at lower concentrations of ammonium acetate and so 0.1M ammonium acetate was selected. It was found that acid (acetic or hydrochloric) decreased absorption and also shifted the absorption maximum to shorter wavelengths. (2 drops of dilute HCl (Ph. Eur.) in a 3 ml cuvette of neutral creatinine (90 μM/l) reduced the absorption by 70% and shifted the maximum from 236 nm to 226 nm). In water, creatinine was found to absorb maximally at 235 nm (log molar extinction coefficient ϵ = 3.83).

Modifying the pH of the mobile phase (1% orthophosphoric acid or 1% ammonia) showed that acid reduced the speed, symmetry and intensity of the peak whilst alkali did the opposite. It was not feasible to use ammonia, however, as the pH was too high for safe use of the column. The use of acetonitrile or methanol as modifiers resulted in broad, irregular peaks.

At 235 nm, 0.02 - 0.08 AUFS, 20 μl injections of duplicate creatinine solutions from 75.9 to 284.6 M/l in four strengths gave a correlation coefficient of r = 0.999

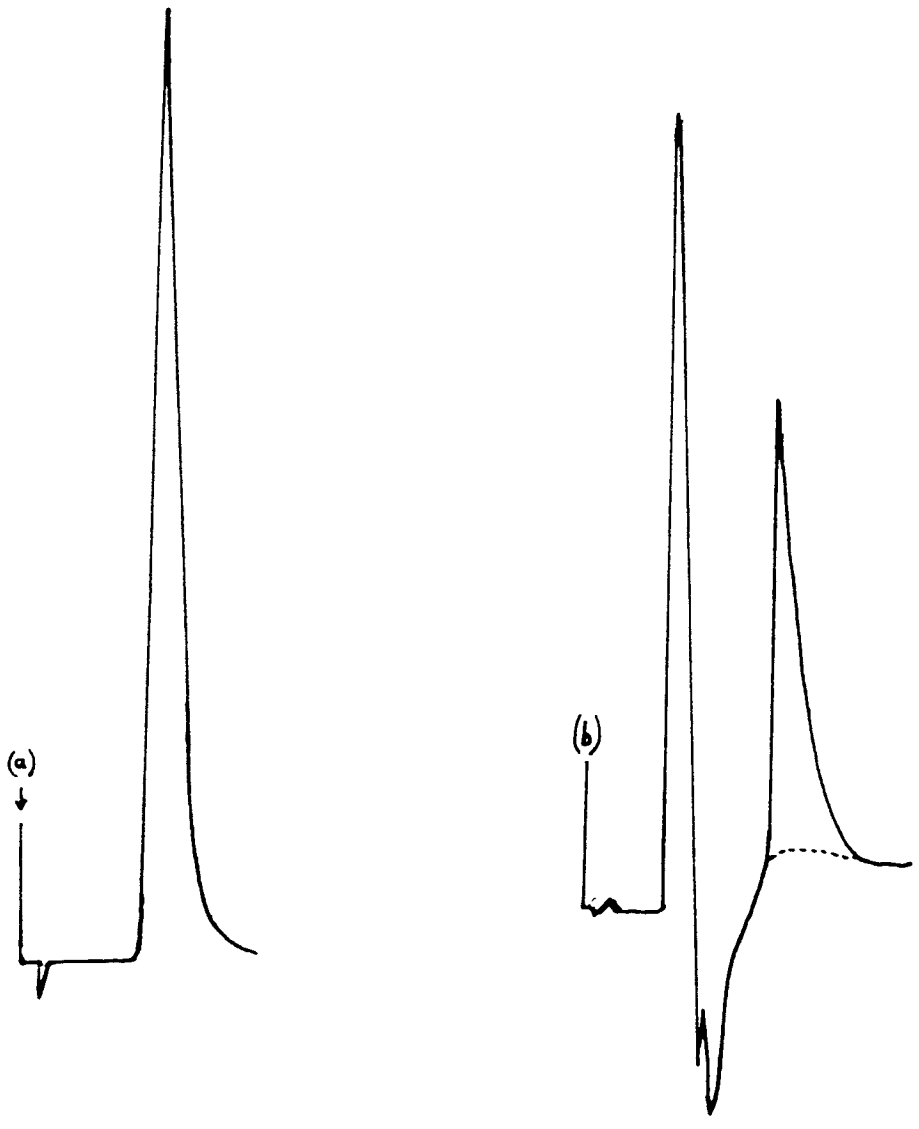
To check interference from other serum constituents, and to permit extraction studies, a solution of 38.0 g/l bovine serum albumin (BSA) was prepared. Concentrated solutions of amino acids and carbohydrates

Fig 10.4 HPLC of Creatinine in 0.1 M Ammonium acetate (230 nm)

(a) ODS2,1.25 ml/mm, Aqueous solution

(b) ODS1,0.4 ml/mm, Methanol extract of bovine serum albumin

- - - - blank ——— 93 mg/l creatinine



(Aminoplex 14, Vamin-glucose, Glucoplex 1000) were also used. None of the latter solutions interfered with the creatinine assay and a methanol extraction of BSA (3 vols: 1 vol) showed no interference. BSA plus creatinine showed an appropriate peak from methanolic extraction. Serum also showed a peak that proved absent when the serum was pre-treated with fullers earth and oxalic acid. BSA spiked with creatinine gave an extraction efficiency of 83%.

It was found that some serum components were eluting just prior to the creatinine and creating a poor baseline from which to measure the creatinine. It was decided to try an ODS-2 column to seek an improvement.

The same retention patterns were found on ODS-2 as on ODS-1 except that the retention volume increased to 27 mls in 0.1M ammonium acetate. The efficiency remained the same (about 230 theoretical plates) but there was less interference from serum components. Changing the wavelength (230 to 250 nm) did not improve selectivity and 235 nm was preferred. Acetonitrile, n-butanol and amyl alcohol gave more interference. Aminoplex 14, glucoplex and Vamin-glucose (containing glucose, malic acid, various vitamins and l-amino acids) showed no interference and neither did creatine or urea. The following drugs were also tested and found to be free from interference, as was a pooled serum standard from Clinical Chemistry Department, East Birmingham Hospital:

Ampicillin, talampicillin, amoxycillin, pivmecillinam, phenoxymethylpenicillin, benzyl penicillin, azlocillin, mezlocillin, fludoxacillin, clindamycin, gentamicin, chloramphenicol, oxytetracycline, chlortetracycline, tetracycline, demeclocycline, cephazolin, cephradine, cephalexin, erythromycin, trimethoprim, sulphamethoxazole, sulphathiazole, sulphadiazine, sulphamerazine, sulphaguanidine, metronidazole, aminophylline, digoxin, oxprenolol, frusemide, bumetanide, bendrofluazide, cyclopenthiazide, hydrochlorothiazide,

amiloride, disopyramide, aspirin and paracetamol.

The assay was, therefore, free from interference from drugs and endogenous materials, was sensitive enough for clinical use and was reasonably rapid. Subject to satisfactory reproducibility studies it should prove adequate for use.

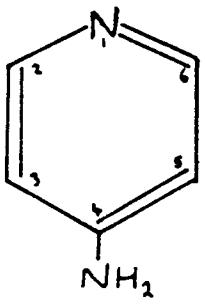


Fig 11.1 4-Aminopyridine and numbering convention

11. AMINOPYRIDINES

11.1 4-Aminopyridine and Botulism

.1 Introduction

4-Aminopyridine (4AP) is a low molecular weight heterocyclic amine (fig 11.1) used industrially as an analytical standard for titrating acids (251), as a bird-repellant (252), as a starting point for chemical syntheses (253) and in electrochemical plating (254). It is also known to affect the neuromuscular junction in animals where it promotes the release of acetyl choline probably by promoting potassium-channel conduction (253). It has been used in the treatment of human Eaton-Lambert Syndrome (257) and for the reversal of Amikacin muscle blockade (255) but remains an experimental drug. When an outbreak of botulism occurred in Birmingham (August 1978), it was suggested that this drug might be of value as an aid to the restoration of muscular function.

The nature of the outbreak, its causes and consequences, have been described elsewhere (256, appendix 5) but may be briefly summarised thus:

Botulism is caused by the endotoxins from Clostridium botulinum a spore-forming organism first characterised by Van Ermengem in 1896 and now classified into seven types (A-G) according to the endotoxin serotype. Type E toxin is a high molecular-weight protein, of incompletely determined structure, whose neurotoxicity is enhanced by the action of trypsin and abolished by cooking. The normal cause of illness is inefficient heat treatment of canned products but this outbreak seems to have been caused by contamination of a tin of John West salmon after processing at the False Pass Cannery, Alaska. Recovery from the disease is associated with proliferative regeneration of the nerve end plates, although no structural damage to the neural tissue is observed in acute intoxication. Two elderly, married Birmingham couples consumed a quantity of cold salmon from a tin contaminated with Clostridium botulinum type E and its endotoxin. They were admitted to East Birmingham Hospital over a

period 10 - 14 hours later and placed on artificial respirators. Doses of antitoxin were administered and iv feeding commenced due to the absence of gut, respiratory and voluntary muscle function and the low level of consciousness. Full supportive procedures were instituted but despite these one patient died soon after admission and one died some weeks later. The other two recovered and two years later remain well.

The details of administration of 4AP are given in Appendix 4 but although all four patients received a single bolus injection of 4AP, only two ('B' and 'C') received a further dose by infusion. Although there was an undoubted improvement in the muscle function of each patient, B and C (both female) had undesirable side-effects, apparently originating from the CNS.

Pharmacokinetic analysis of the serum and urinary levels was requested as a matter of urgency and an appropriate assay designed.

A survey of published work showed no assay for 4AP in body fluids, although spectroscopic and potentiometric methods were recorded (258, 265). No liquid chromatographic methods were available but paper, TLC and derivatisation-GC methods were found for aqueous solutions (259, 260, 261, 262). (Subsequently, an unpublished GC method for body fluids was received from the University of Groningen, Netherlands and is given in Appendix 4).

Since 4AP was found to have an intense UV absorbance (table 11.1) and be soluble in water (saturated solution, 0.11M) and alcoholic solutions, reverse-phase HPLC with UV detection was considered appropriate. It was found that 4AP was almost insoluble in non-polar mobile phases, which ruled out normal-phase chromatography. Ion-exchange systems were considered to be too unstable in view of the urgency of the assay. 4AP fluoresces weakly (263) and below the wavelength range of commercially-available instruments.

Using methanol-water mixtures with an octadecyl silane (5 μm RP18)

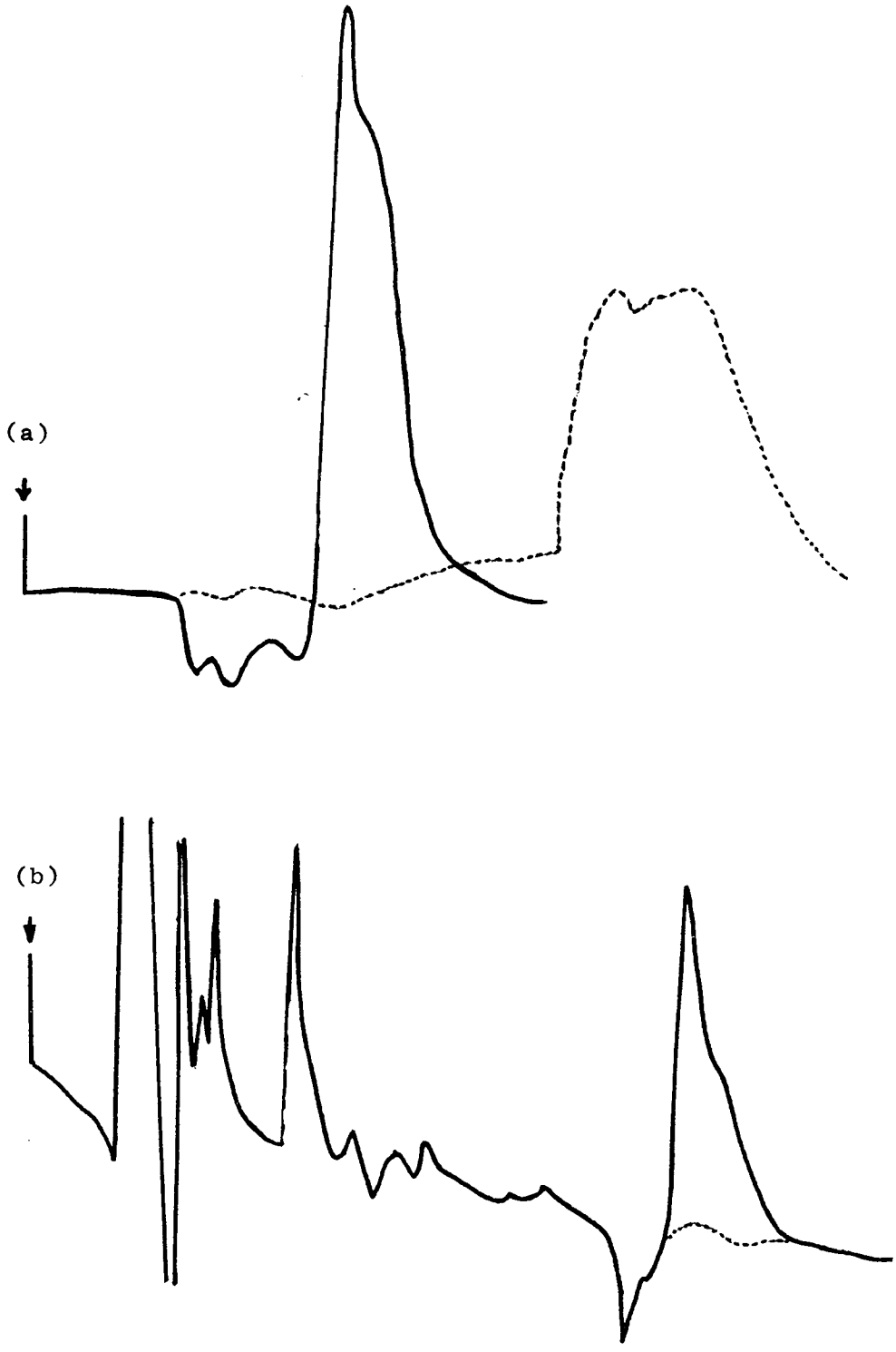
Subsitution Pattern	Literature Values pKa_{25^oC}	pKa_{20^oC}	Measured UV maxima (200-350 nm) and log molar absorptivities (log E)					
			nm	log E	nm	log E	nm	log E
2AP	6.71	6.82	232	3.95	302	3.81		
3AP	6.01	5.94	222	3.88	252	3.93	323	3.58
4AP	9.11	9.25	268	4.15				
2,3DAP	6.73	7.00	222	3.85	254	3.74	322	3.96
2,6DAP	6.00		244	3.97	335	4.14		
3,4DAP	9.19	9.14	228	4.09	293	3.71		
Pyridine	5.0							
NN 2 AP	6.99							

AP = amino-pyridine, DAP = diamino-pyridine, NN = N, N dimethyl-

UV maxima were determined in acetonitrile: 0.5% w/v sodium dodecyl sulphate: orthophosphoric acid (40:59:1) at 25^oC with solutions containing $1.5 - 2.3 \times 10^{-4}$ M/l AP.

pKas are from refs 251, 265, 268, 270, 271.

Table 11.1 pKa and UV data for aminopyridines



ODS 1 (25 x 0.46 cm id) 1 ml/cm

(a) aqueous solution of 4AP (10 μg/ml)

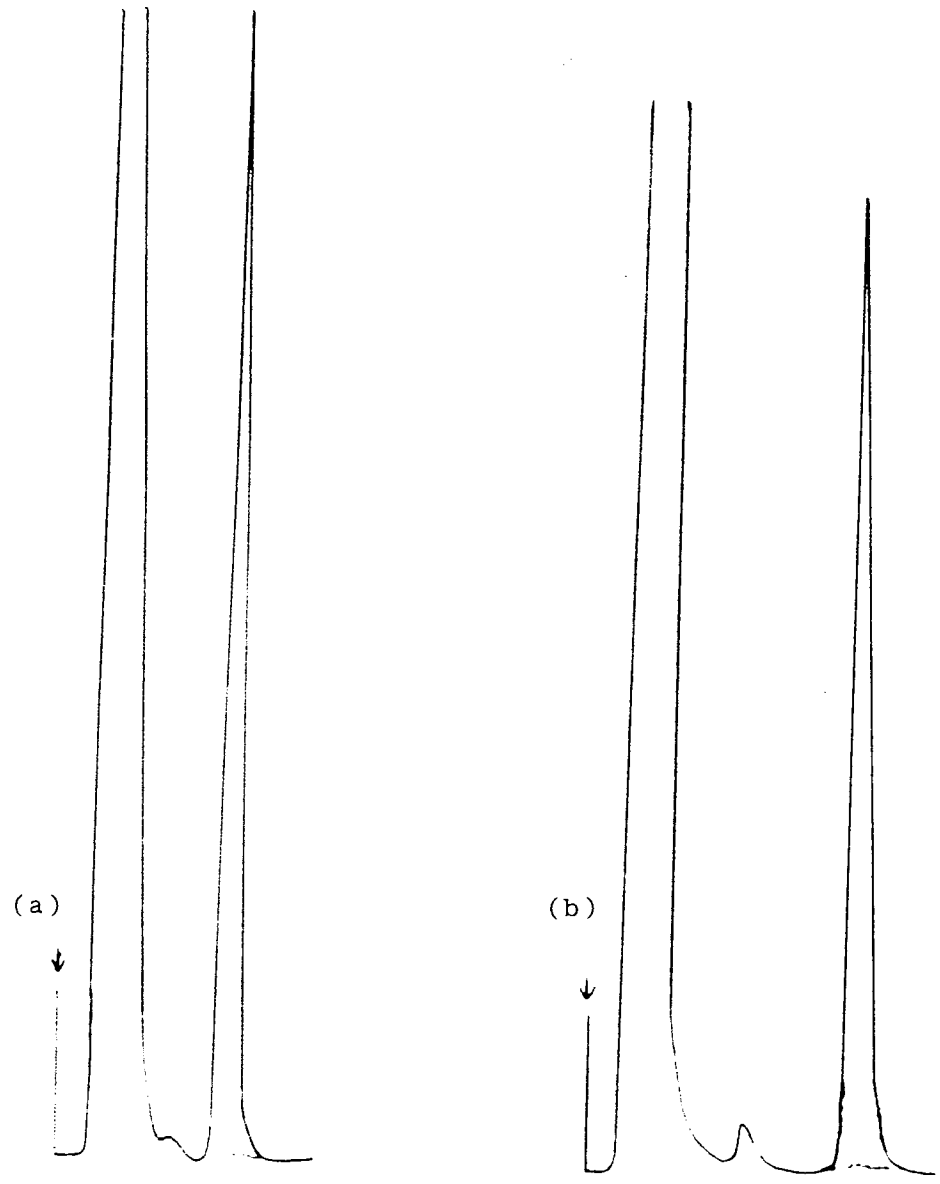
—— 0.04% SDS, 3% H_3PO_4, 40% CH_3CN

----0.04% SDS, 1% H_3PO_4, 40% CH_3CN

(b) DMF extracts of serum containing 12.5 μg/ml 4AP (——)

and a blank (....)

Fig 11.2 Early chromatograms of 4AP



ODS 1 4.46 ml/cm, 265nm, acetonitrile extracts of 4AP (——) and blanks (- - - -)

(a) pooled serum (6.25 μg/ml 4AP); 0.08 AUFS; 0.6% SDS, 40% CH_3CN

(b) urine (20 μg/ml 4AP); 0.32 AUFS; 0.67% SDS, 33% CH_3CN

Fig 11.3 Later chromatograms of 4AP

column (Anachem) 4AP eluted slowly in a broad band of low intensity. Addition of acetic or orthophosphoric acid (up to 4% v/v) to ensure complete ionisation of 4AP resulted in very rapid elution, close to, or at, the solvent front. 4AP having a reported pKa of 9.1, and the maximum recommended pH for operating HPLC columns being 7.4, it would be impossible to use pH control to suppress ionisation and so ion-pairing was attempted. Since 4AP may be rendered cationic by the addition of acid to the mobile phase, an anionic counter-ion was employed. Sodium dodecylsulphate (SDS, lauryl sulphate) was readily available and was used initially at 0.001M/l with orthophosphoric acid (1% v/v) in acetonitrile-water (40:60). The resultant peak was narrow, intense and eluted within ten minutes. Acetonitrile was preferred to methanol because of its lower viscosity and ease of preparation of aqueous mixtures. Lower SDS or acid concentrations reduced the intensity and symmetry of the peak, possibly due to incomplete ion-pairing, and higher concentrations showed no improvements (fig 11.2). The optimum composition was found to be 0.015M SDS, 1% v/v orthophosphoric acid, 40% v/v acetonitrile in water. The resultant peak (at 1ml/min) was equivalent to 1500 theoretical plates at 8.5 minutes.

.2 Extraction Studies

Since 4AP was found to be poorly soluble in non-polar liquids (chloroform, hexane, iso-octane) and evaporation of large volumes of such phases was not feasible, it was decided that extraction must be by protein-precipitation. 2-methyl_propan-1-ol and dimethyl_formamide (DMF) were tried, adding 2 volumes to 1 volume of spiked serum, mixing rapidly by repeated inversion and centrifuging at 3,400 rpm for 5 minutes. 100 μl of the supernatant was injected. Since DMF gave a 48% greater yield, as judged by peak height from spiked serum (50 μg/ml), and both methods showed no interference from serum constituents, DMF was preferred (fig 11.3). It was found that this gave a 46% efficient extract. Whilst

Time (Mins)	Concn. of 4AP in Serum (ng/ml) Patient B	Patient C
0	0	0
5	478	150
15	315	35
30	306	60
45	285	60
60	243	-
90	159	190
120	223	80
150	137	120
180	382,334	120
210	196	150
240	137	170

Urinary Excretion of 4AP

	Patient B			Patient C		
Time (Mins)	Urine Vol. (mls)	Concn 4AP (μg/ml)	Total 4AP Excreted (μg)	Urine Vol. (mls)	Concn 4AP (μg/ml)	Total 4AP Excreted (μg)
0 - 30	35	5.4	189	5	4.7	23.5
30 - 60	7	41.2	477.4	5	29.0	168.5
60 - 90	8	119.2	1431	5	Not Available	≃ 468
90 - 120	10	110.0	2531	6	133.3	1268
120 - 150	12	105.2	3793	8	298.7	3658
150 - 180	10	103.5	4828	10	247.2	6130
180 - 210	7.5	81.2	5437	15	288.0	10450
210 - 240	7.5	88.2	6099	15	253.3	14250

Table 11.2 4AP Levels in Two Patients

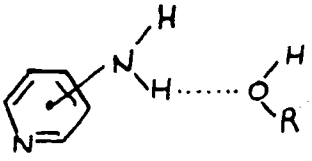
this was lower than might be desirable, it was consistant and was accepted in view of the time constraints upon the case.

After about fifty extracts the column back-pressure began to rise, presumably due to incomplete protein-precipitation. This caused the flow-rate to change, a problem inherent in the LC3 system, and so standards were run inbetween each pair of samples. Using 0.4 ml serum (the maximum available for each analysis assuming duplicate analysis) the minimum detectable concentration was 20 ng/ml at 0.01 AUFS and 268 nm. Back-pressure rose to 140-160 Bar at 1 ml/min flow rate.

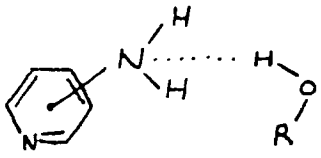
.3 Results of analyses of patients sera and urines

Table 11.2 gives the results from the four hour observation period. The serum levels were extremely low, especially in 'C' whose post-bolus levels were near the minimum quantifiable level. The urine collections (by catheter) were low, reflecting poor renal function. The data were too few, and too variable, to permit reasonable pharmacokinetic analysis, although both patients showed a large distribution volume. (This is evidenced by the low initial concentrations, the rapid fall off after iv bolus and the low initial urinary excretion). This is not surprising in a drug with neuromuscular and CNS effects (88).

Fig 11.4 Aminopyridine-solvent bonding

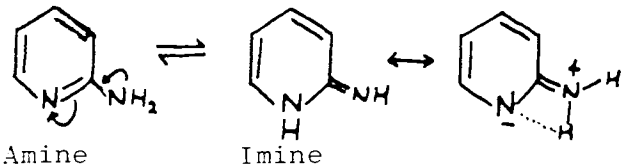


(a)



(b)

In water (pH11.5), bonding (b) dominates for 2-, 3- and 2, 6-di-AP but (a) dominates for 4AP and for all APs in ethanol



Amine Imine

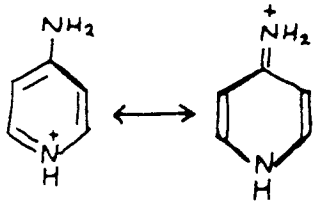


Fig 11.5 Major Tautomers and Canonical Forms of protonated and neutral aminopyridines (266, 268)

11.2 Further Studies on 4AP in body fluids

Following the initial botulism-oriented studies, some further work was done to evaluate and quantify other systems.

Extraction studies showed that acetonitrile was a better extraction solvent than DMF. Acetonitrile (1 ml serum to 1 ml acetonitrile) extracted 98.4% (coefficient of variation 0.57%, n = 5) at 6 μg/ml 4AP. The extract was much cleaner than that with DMF, was less viscous and less opaque to UV radiation. It also provided a solution one-and a half times more concentrated for analysis. (This, together with chromatographic changes outlined below, reduced the minimum detectable level from 20 ng/ml to 5 ng/ml).

.1 Chromatography of 4AP and other aminopyridines

Unpublished data (264) on aminopyridines in animals suggested that several such compounds may be active in muscles and these were investigated along with 4AP.

Several workers have studied the physical properties of aminopyridines in relation to basicity, charge density distribution and reactivity (e.g. 265, 266). Campese *et al* (267) related the structure to myorelaxant properties, finding that 4AP is a myocontractor whilst 3AP and 4-nitropyridine, amongst others, are myorelaxant. They suggest that the pyridine nitrogen acts an an electron acceptor in muscle relaxation.

The ring nitrogen protonates more readily than the amine (pKas; 9.11 and -6.55 for 4AP) but estimates of the pKa vary with temperature and methodology. Table 11.1 gives the most widely accepted values for aqueous solutions at 25^{o}C. Cumper and Singleton investigated the hydrogen bonding of APs and found that it varied with the solvent, see figure 11.4 (269). The same authors report that 4AP (pH $>$11) is stabilised by resonance between the amine and imine forms, but that 2AP is prevented from so doing by intra-molecular hydrogen bonding. This seems at variance, at least in emphasis, with Kalatzis, and others, who report

Retention volumes (mls) using

AP	Acetic Acid	Orthophosphoric Acid
2	8.17	5.52
3	8.42	5.41
4	8.12	5.51
2, 3	8.70	5.37
2, 6	9.47	5.56
3, 4	7.60	5.26
NN2	12.80	6.79
Solvent front	2.20	2.20
pH	3.3	2.0

Table 11.3 Retention volumes of APs in acid eluent

	% Organic Solvent	% SDS	Retention Volumes (mls) CH_3CN	MeOH
ODS2	40	0.173	17.2	41.0
ODS2	69	0.086	10.1	10.1
ODS1	60	0.6	39.5	42.5

Table 11.4 Retention volumes of 4AP

that the amine is strongly favoured in all mono-substituted amino and methylamino pyridines (266, 268).

These data suggested that the different APs might exhibit varied chromatographic properties. The 5μm RP18 column having been damaged by incomplete protein-precipitation, ODS-1 and ODS-2 columns (25 cm x 0.46 cm i.d., Whatman Inc.) were used. ODS-2 has a 14% carbon loading per unit mass of silica whereas ODS-1 has a 4% loading and so ODS-2 should exhibit greater selectivity than ODS-1. Both are 10 μm systems, thus having lower efficiencies and lower back-pressures than the RP18 column.

UV scans were made in a typical mobile phase (table 11.1) and HPLC observations made at the appropriate absorbance maximum using 10 μl injections of 10 μg/ml solutions. The density, viscosity, pH and temperature of the mobile phases were measured as described in appendix 1.

.2 Choice of Acid

The use of equivalent percentages (v/v) of acetic and orthophosphoric acids in 40% acetonitrile, 0.5% (w/v) SDS with an ODS 1 column showed that retention volumes decreased with phosphoric acid, as did selectivity (table 11.3).

Omission of the counter-ion resulted in very rapid elution. In CH_3CN 40%, H_3PO4 0.5, 1.0, 2.0 and 4.0% (pH 1.69-1.00) all APs eluted between 2.5 and 3.2 mls and all but NN2AP eluted in 2.5 to 2.7 mls (solvent front 2.2 mls).

Increasing acid concentration, in the presence of SDS, resulted in shorter retention times for all APs except 4AP. Table 11.5 (columns 1, 2) illustrate this, but the behaviour of 4AP was confirmed on ODS 2 with 33% CH_3CN, 0.2% SDS where 0.5% and 1.0% H_3PO4 yielded retention volumes for 4AP of 10.12 and 13.8 mls respectively.

Column Number	1	2	3	4	5	6	7	8	9	10
AP	1% H_3PO_4 1% SDS 40% CH_3CN	.5% H_3PO_4 1% SDS 40% CH_3CN	40% CH_3CN 0.6% SDS	50% CH_3CN 0.6% SDS	60% CH_3CN 0.6% SDS	80% CH_3CN 0.6% SDS	60% MeOH .6% SDS	40% CH_3CN 0.384% SPS	40% CH_3CN 0.6% H_3PO_4 0.6% SDS	40% CH_3CN 0.6% H_8PO_4 0.36% SPS
2	4.99	6.01	6.62	5.49	4.92	5.26	5.19	6.16	6.88	3.18
3	4.97	5.26	4.96	4.5	4.57	4.66	4.04	5.59	6.78	3.12
4	5.90	5.36	58.44	52.14	39.5	22.88	42.5	10.10	7.79	3.08
2, 3	5.23	5.69	6.48	5.73	5.21	-	5.8	5.66	7.21	3.12
2, 6	6.06	6.65	5.35	4.66	4.14	4.84	5.3	4.95	8.04	3.16
3, 4	4.28	5.02	43.74	39.19	31.5	20.4	42.5	7.95	6.26	3.03
(NN diMe)2	9.78	10.14 pH 1.98	10.00 pH 7.8	6.95 pH8.05	5.41 pH 8.3	4.72 pH 8.6	7.68 pH 7.55	9.53 pH 6.7	11.76 pH 1.9	3.95 pH 1.9

Table 11.5 Elution Volumes of aminopyridines in ten eluents on ODS-1

.3 Organic Phase

Acetonitrile was preferred to methanol primarily because of its lower viscosity, but also on account of methanol's exothermic mixing with water and its tendency to outgas. Increasing the mobile phase content of either reduced all retention times (table 11.4, fig 11.6) and elution was generally faster in acetonitrile but the ratio of retention times varied according to the content of organic phase. The difference (for 4AP) was more marked at lower organic concentrations. See also table 11.5 columns 5, 7 (choice of organic) and columns 3 - 6 (concentration of acetonitrile).

.4 Choice of counter-ion

Use of sodium pentane-1-sulphonate (SPS) instead of SDS had a variable effect. Table 11.5 columns 9, 10 shows that in the presence of phosphoric acid, retention times were decreased with SPS, but that without acid the effect is variable (columns 3, 8). The concentrations chosen were equimolar (3.48×10^{-2}M) but whereas SPS was below its aqueous critical micelle concentration (cmc), SDS was above. The acidified solutions were of the same pH, but the others were of different pHs and this may well have had a significant effect. (SDS is known to be impure, even in the 'specially purified' grade used, and contains alcohols related to dodecyl silane and homologues that will raise the pH). It could not have caused the great difference in retention volumes of 4AP and 3,4AP, however, since when the SPS mobile phase used in the column 8 experiment was alkalinised to pH 7.8 with sodium hydroxide the retention volumes decreased still further (7.1 and 5.8 mls respectively).

Experiments with 4AP on an ODS 2 column using SDS, SHS (sodium hexane sulphonate) and SPS in 33% acetonitrile showed that increasing counter-ion concentration reduced retention time, but comparisons between counter-ions can only be made subject to the proviso that SDS was above the aqueous cmc and SPS and SHS below (table 11.6).

Molar Concentration of counter-ion (33% CH_3CN)	4AP Retention Volumes (mls): ODS2		
	SPS	SHS	SDS
0.023	6.6	-	-
0.046	5.5	5.3	20.3
0.092	-	-	14.0
0.184	-	-	8.3

Table 11.6 Retention Volumes of 4AP with various Counter-Ions

11.3 Retention Mechanisms

The mechanisms by which retention occurs, and varies between APs, are not clear. Three principal entities may exist:

(a) unionised AP

(b) ionised AP

(c) ion-pair

That 4AP and 3,4DAP have markedly differing properties from the other APs and also have different pKas (> 9 cf 6 - 7) probably indicates some ionisation/pH phenomenon. It is possible that charge distribution is involved, rather than degree of ionisation, but the literature cited shows some disagreement and a general lack of awareness of the charge distribution in each AP. The 4-substituent should be capable of sustaining the highest positive charge and the 3 and 5 substituents the least.

Since amino groups are electron donators, on account of the lone pair of electrons available for interaction with the delocalised pi-bond system, they will be able to stabilise a positive charge on the molecule, but will do so more at the 2, 4 and 6 positions than the 3 and 5 positions (270).

This explains the rank order of pKas (table 11.7), assuming that the effects are additive for di-substituted compounds, except for the low value for 2, 6-DAP. This may, however, be due to steric hindrance reducing the access to the ring nitrogen.

The order of elution of the APs varies from eluent to eluent, 4AP and 3,4DAP elute very late at high pH and early at low PHs. Using Spearman rank-order analysis (143) of the groups of eluents, those with acid and those without, a significant difference was found ($p < 0.05$). The rank order of the non-acid group was similar to the rank order of pKa. (The only difference being in 3AP and 2,6DAP where the pKas were only 0.01 unit different). This would suggest that the degree of ionisation was important, and that 3,4DAP and 4AP are much more highly ionised than

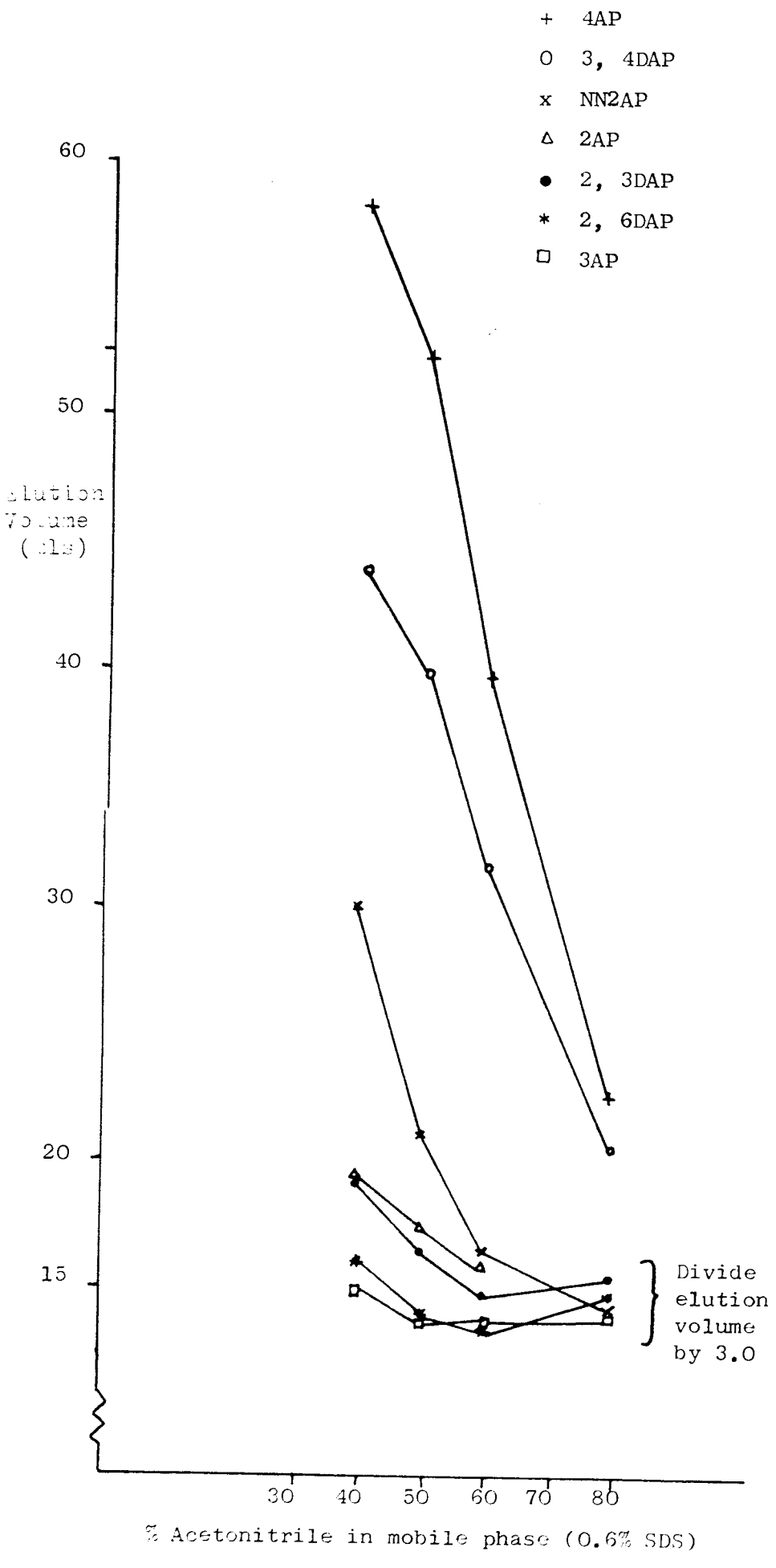


Fig 11.6 Elution volumes of aminopyridines versus acetonitrile content of mobile phase

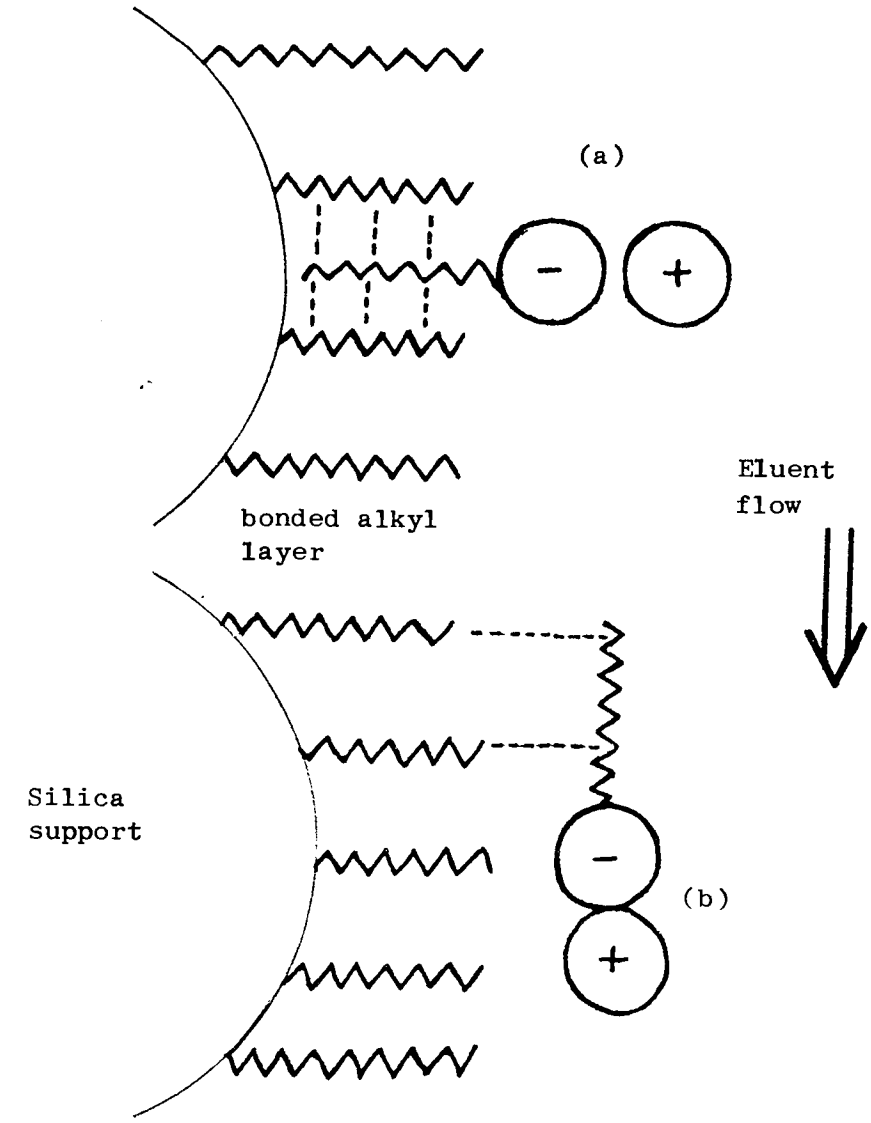


Fig 11.7 Putative mechanisms of ion-pairing between a positively-charged species and a negatively-charged alkyl-sulphate

	2AP	3AP	4AP	2, 3AP	2, 6AP	3, 4AP
λ max	4	3	6	2	1	5
pKa	4	5	2	3	6	1
Acid Retention	4	3	5	2	1	6
Non-Acid Retention	4	6	1	3	5	2

Spearman rank correlation coefficients (r_s) and probabilities (p) of null-hypothesis being correct (one-sided test. Null hypothesis: rank orders are unrelated. Alternative: correlation of appropriate sign).

pKa and acid retention $r_s = -0.828$ ($p=0.05$)

pKa and non-acid retention $r_s = 0.886$ ($p<0.05$)

Acid and non-acid retention $r_s = 0.643$ ($p>0.05$)

λ max and acid retention $r_s = 0.943$ ($p=0.01$)

λ max and non-acid retention $r_s = -0.714$ ($p>0.05$)

Table 11.7 Rank Orders of Aminopyridines

the others. At low pH (1-2 units) the whole group would be ionised and this effect would cancel out. (NN2AP was omitted from this series because of its different substituent). It is not surprising, therefore, that the rank orders do not correlate well for pKa and acid retention. (It is anticipated that the presence of acetonitrile would reduce ionisation, compared to water, and so the pKa values will reflect trends rather than absolute values).

Where greater ionisation leads to longer retention, however, it would indicate that the neutral AP is not well retained and that one (or both) of the other species are retained. It is unlikely that the ionised AP will be well retained by a non-polar stationary phase and so it would seem that the ion-pair must be the dominant factor. Two mechanisms have been postulated for ion-pair retention and are represented by fig 11.7(155).

Mechanism (a) assumes that the AP migrates as an ion and is attracted electrostatically to the counter ion which is held in position on the stationary phase by van der Waals forces. Mechanism (b) assumes that the AP migrates down the column as an ion-pair and partitions into the alkyl phase as a neutral entity. The latter mechanism is preferred in the literature but both methods depend upon the attraction between opposite signed charges, and thus on the ionisation of AP. Both mechanisms are, therefore, consistant with the observed data.

Another factor in the formation and permanence of the ion-pair is the charge distribution in the AP. A widely-dispersed charge will form a weaker bond than a concentrated charge which more nearly matches the size of the sulphate or sulphonate group in the counter-ion. Since high pKa is an index associated with charge dispersion, a negative correlation with pKa might be expected when ionisation is complete. This was observed for the acid eluent data.

Further colloaborative theory may be drawn from molecular orbital calculations (257) which predict that positive charges in heterocyclic

systems will result in greater charge densities upon the heteroatoms, including any exocyclic atoms adjacent to the ring,but little upon the carbon atoms (e.g. benzotriazinium compounds, appendix 4). Thus, 2, 6AP and 2, 3AP might be expected to have the most suitable charge distribution, whilst 3, 4AP and 4AP would have the least suitable distribution.

The case of ionisation is also indicated by the wavelength (263) of UV absorption. Long wavelengths indicate great charge dispersion and although the transitions involved are π,π^* and not those of bonding electrons, the ranks may be expected to be the same for ionisation and UV absorption. A highly significant correlation is found of λ max (longest wavelength absorption) and acid retention volume, potentially providing a simple test for retention characteristics of new compounds.

11.4 Reproducibility of replicates, retention and symmetry measurements

.1 Retention volume

Several studies were conducted to determine the repeatability of readings:

(a) 4AP was run eight times with two batches of eluent (three times on one batch, five times on the other). Approximate chart distance: 150 mm.

(b) NN2AP was run five times in the same eluent. (Chart distance: 170 mm).

(c) 2AP, 4AP, 3, 4AP and NN2AP were each run at four flow rates (0.246, 0.51, 1.12 and 1.89 ml/min) and the results compared by defining the mean for each AP as 1 and expressing each value in proportion to that mean. (Chart distance: 60 - 80 mm).

The results are expressed as coefficient of variation (%) in table 11.8.

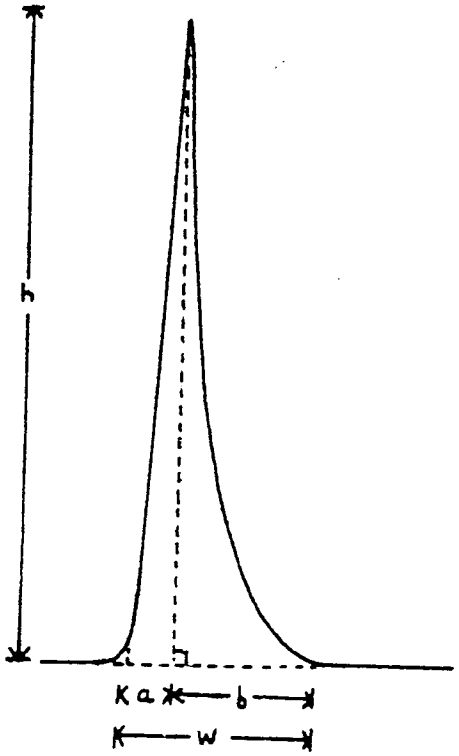


Fig 11.8 Symmetry Calculations

.2 Symmetry

Various measures of symmetry have been proposed (273, 274, 276). Using the notation of fig 11.8 , they may be summarised as

(i) $\frac{b}{a}$ at 0.1h or 0.15h (273, 276)

(ii) $\frac{a+b}{2a}$ at 0.05h (274)

The measurement of a, however, is usally difficult because of its small size (perhaps 1 - 2 mm). The measurement of 0.15h or 0.05h is similarly subject to error.

It was thought, therefore, that other measures of symmetry should be sought.

R. P. W. Scott (235) has shown that the theoretical output from a single component should correspond to a poisson distribution:

$$X(n) = \frac{X^o e^{-v} v^n}{n!}$$

where X(n) = solute concentration after n^{th} plate, X^o is the solute concentration at the origin and v is the eluent volume (in plate volumes).

This distribution has its origin at the injection point, but may be converted to a normal distribution having its origin at the maximum peak height:

$$X(n) = \frac{X^o e^{-\omega^2/2n}}{\sqrt{2\pi n}}$$

where ω is the distance from the origin and n is numerically equal to the variance of the distribution.

For convenience, these curves may be described as a triangle, with a minima loss of accuracy. Since asymmetry is rarely a problem at 0.5h, by observation, an index which included the total width at two fractions of the peak height would possibly suffice, and be easier to measure.

	a_1	a_2	b	c	d
n	8	5	4	16	18
Ret. Volume	1.64	1.16	0.43	.610	-
WO.05h	10.83	1.11	2.79	13.74	-
WO.1h	9.93	3.53	0.811	9.25	-
$\frac{WO.1h}{WO.5h}$	16.95	2.64	2.26	11.60	-
$\frac{b}{a}$	10.01	6.31	6.89	-	-
$\frac{b}{a+b}$	1.57	1.11	1.31	-	-
h	2.84	0.67	-	-	0.96
Approx. Retention (mm)	150	150	170	30-40	-
Approx. Width (mm at 0.1h)	21-28	21-23	28	7-12	-

Table 11.8 Coefficients of Variation (%) of Various Symmetry Parameters

Since 0.1h is less prone to mensuration error than 0.05h, the ratio

$$\frac{W\ 0.1h}{W\ 0.5h}$$

was tested.

A normalised index (i.e. one have a value of unity at some chosen level of symmetry, e.g. perfect symmetry) would be preferable and could be obtained by noting that the ratio for a perfect triangle is 1.8 and that in a normal distribution is 1.82. Thus an index,

$$\frac{W\ 0.1}{1.82\ W\ 0.5}$$

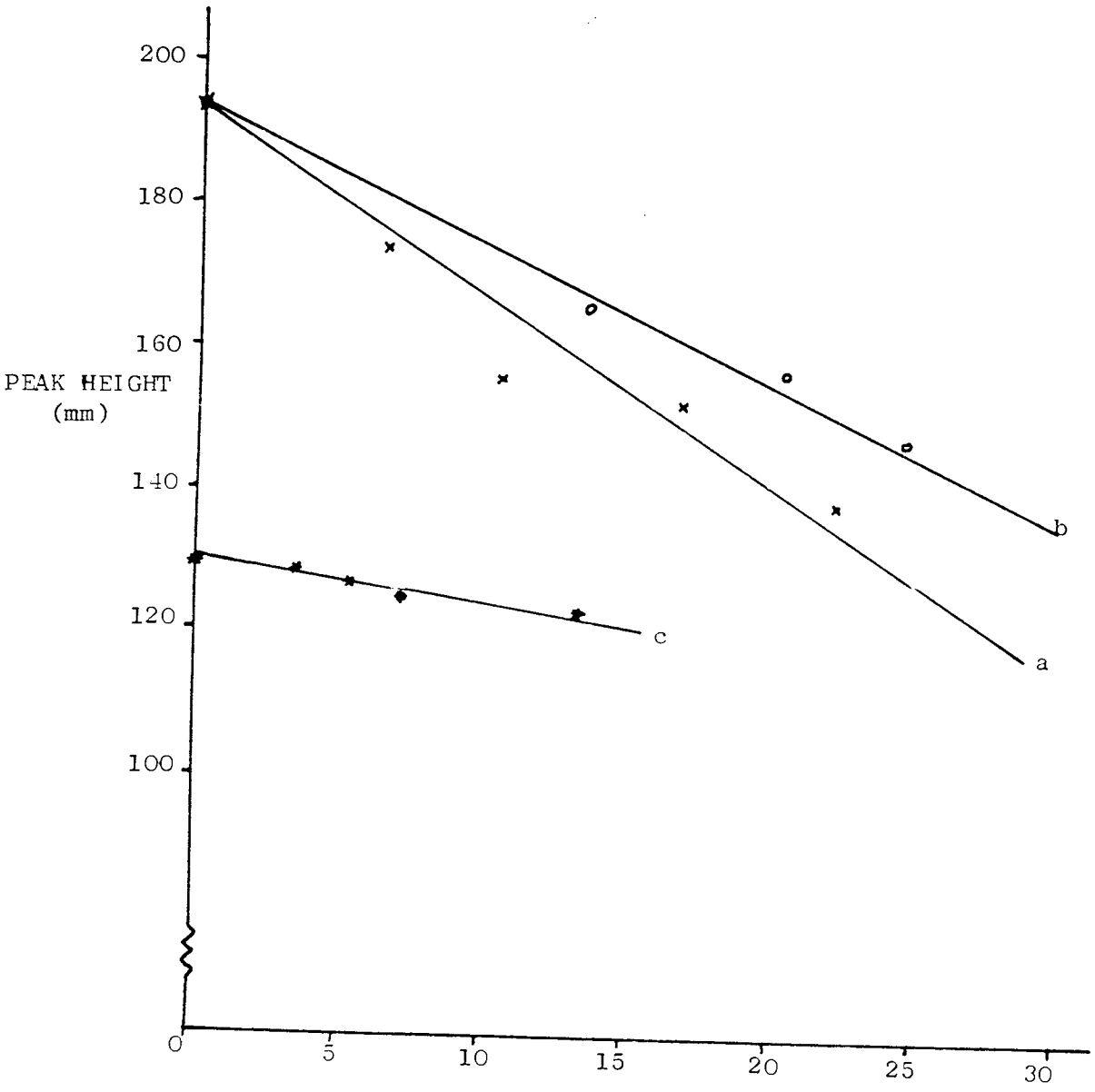
would equal 1 if the peak was perfectly symmetrical and represented a normal distribution.

The measurement of column efficiency is based upon the retention volume and a width (usually 0.5h, but may be 0.1h) and the error would thus be similar to the error for the appropriate width since these are always much greater than that for the retention volume.

The measure, b/(a + b), has some merit in that it combines the a and b parameters used in the literature but reduces mensuration errors.

Data for experiments a - c are listed in table 11.8 which shows the merits of using indices having low mensuration errors, at least as far as precision is concerned. It also confirms that the shorter measurements (e.g. c) exhibit the greatest errors and that even where distances are similar, the retention volumes are more reproducible than the peak widths.

Experiment a_1, compared with its subset a_2, shows that whilst different batches of eluent may yield very similar retention volumes, the peak symmetry can differ markedly. The peak heights were much more repeatable than widths, experiment d consisted of 18 samples of 4AP (10 μg/ml, 0.16 AUFS) giving 140 mm peak heights.



a 10 µg/ml 4AP in 0.0028% SPS

b solution a reloaded from stock container

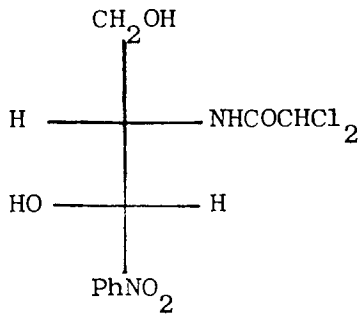
c 10 µg/ml 4AP in water

Fig 11.9 4AP concentration in solutions stored in loading syringe before HPLC analysis

Because of the need to use replicates and large amounts of chart paper the peak widths and symmetry were not routinely determined.

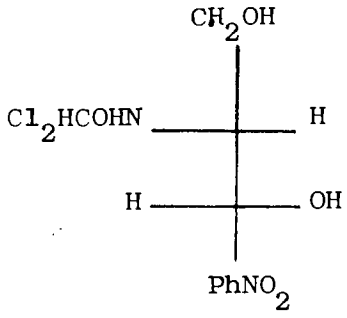
11.5 Adsorption onto Glass

Normally solutions were drawn into a glass syringe (with steel plunger) and injected immediately. It was noticed, however, that when a solution of 4AP was left in a syringe, and portions injected at intervals over 25 minutes, the peak heights reduced steadily. With SPS in the solution the effect was even more marked. Upon re-loading with an identical solution (from a large, well-equilibrated stock vessel) the peak height was restored to its original height and then decreased steadily, but at a lower rate. Fig 11.9 illustrates these effects. 4AP has been used as an adsorbance modifier for carbon (277) but extensive adsorption onto glass has not been reported. Addition of a saturated solution of 4AP (0.109 m/l by titration with HCl to bromocresol green indicator) to ten times an equivalent molar quantity of powdered charcoal (B.P.) resulted in no detectable loss of 4AP from solution.

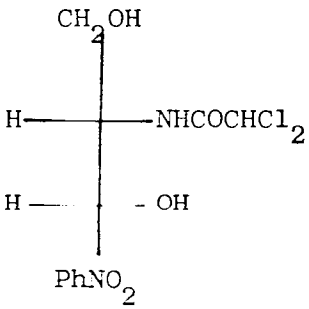


THREO

L (+) [0.5% activity]

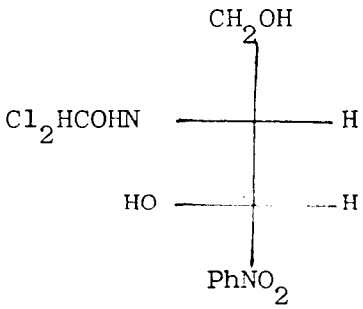


D (-) [100% activity]



ERYTHRO

D (-) [inactive]



L (+) [inactive]

Fig 12.1 Fischer projections of chloramphenicol isomers

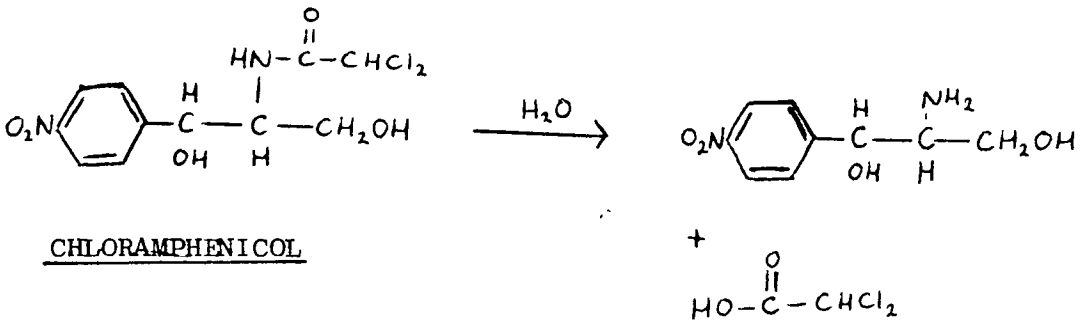
12 CHLORAMPHENICOL

12.1 Mode of Action, History and Uses

Chloramphenicol (D-threo-(-)-2,2-Dichloro-N-[β-hydroxy-α-(hydroxymethyl)-p-nitrophenethyl] acetamide) is produced by an organism, Steptomyces venezuelae, first isolated by Barkholder in 1947 from Venezuelan soil samples (278). It was one of the first antibiotics to be synthesised chemically and was very successful against typhus in South America and Asia (1947 et seq., 229). It is effective against a wide range of bacteria, is well absorbed from oral doses, penetrates into the cerebrospinal fluid and brain and crosses the placental barrier. It is convenient to use in ophthalmic and topical preparations (229).

Systemic administration is limited to life-threatening infections, such as typhoid fever and Haemophilus influenzae meningitis, because of the potentially fatal aplastic anaemia that is reported to occur in 1 in 20,000 patients and the 'gray' syndrome in neonates (4, 5). The mechanism of bone-marrow toxicity (other than as idiosyncratic response) is thought to be related to Chloramphenicol's action upon reticulocyte ribosomes by which the content of ATP is greatly reduced and protein and RNA synthesis inhibited. The human mitochondrial ribosomes and bacterial ribosomes are both 70S units, whereas human cytoplasmic ribosomes are 80S units, and chloramphenicol binds to the 50S sub-unit (of the 70S ribosome), suppressing the action of peptidyl transferase. Thus the ribosomes can still move along the m-RNA, but protein synthesis does not occur and the bacterium or reticulocyte fails to grow (280).

Chloramphenicol has four isomers, of which only the D(-) Threo form is significantly active against bacteria (fig 12.1). It is stable in solutions for several years, unless buffered to a low pH (281), Hydrolysis may then occur, however, as shown overleaf:

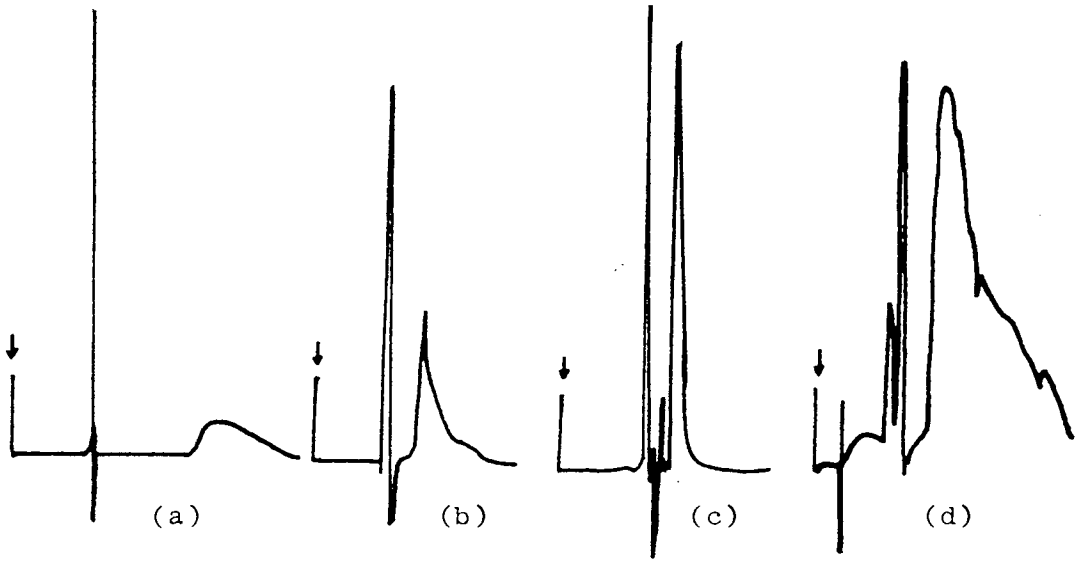


CHLORAMPHENICOL

12.2 Other Assays

Apart from microbiological assays several chemical techniques exist. Derivatisation and colorimetric determination of the derivative, has been employed by Glazko (282), Masterson (283) and Doulakas (284). Doulakas also developed a column chromatographic method that employed ethanol-ethyl acetate-ammonia (50:50:1) as eluent on an alumina column. The method of Glazko is only specific for nitro groups and all the methods are slow and ill-suited to clinical specimens.

TLC and GLC assays have also been developed, but GLC requires the silylation of CAP to increase volatility (285). Vigh and Inczedy have use anionic and pellicular column chromatography to separate chloramphenicol and its production intermediates (176).



25 cm silica column.

Eluent flow:3 ml/cm (sample loaded ↓)

Composition:

(a) $CHCl_3$: CH_3CN (90:10)

(b) $CHCl_3$: iPrOH (93.9:6.1)

(c) $CHCl_3$: MeOH (97:3)

(d) EtoAc

(e) EtoAc: MeOH (50:50)

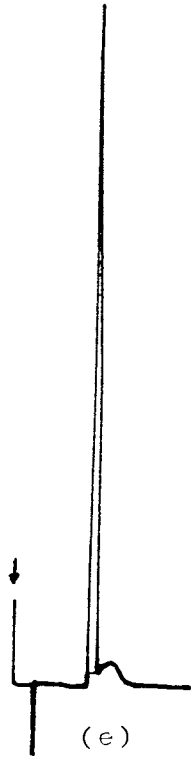


Fig 12.2 Normal-phase chromatography of chloramphenicol

12.3 Development of Assay

.1 Normal Phase

Clarke (230) describes a tlc assay using ethyl acetate (EtOAc) as eluent on a silica plate (chloramphenicol Rf=0.95), and so a silica column (Whatman, Partisil 10 μm) was used with ethyl acetate-based eluents. UV detection was employed at 275 nm because of a plateau in the UV absorption spectrum from 274 - 280 nm very near the maximum absorption of 270 nm.

EtOAc alone eluted CAP as a very irregular, low, broad hump with a retention volume of 4.8 mls at its peak. The addition of methanol to the eluent (50:50) produced a very sharp, narrow peak on the solvent front, increasing the methanol content to 75% showed no change. Chloroform and chloroform-EtOAc mixtures (up to 80% EtOAc) produced low broad humps for CAP after 4 - 6 mls but the addition of a small quantity of methanol greatly improved the symmetry and intensity of the peak, without greatly affecting the retention volume (fig 12.2). Chloroform-acetonitrile (10 - 40% acetonitrile) eluted a broad peak at 6 mls but isopropanol (6 - 12.5% in chloroform) produced a much sharper peak near the solvent front (lower isopropanol content moving the peak away from the solvent front but reducing the symmetry).

In view of these results, methanol-chloroform mixtures, with a low methanol content, were tried. It was found that the concentration of methanol was critical over the range 0 - 10%. At 10% and over, the CAP co-eluted with the solvent front, but at lower concentrations the symmetry and intensity worsened. The optimum was found to be 3% methanol.

.2 Extraction

Clarke (230) used buffered iso-amyl acetate to extract CAP from serum but a simpler method was sought. Ethyl acetate proved to be satisfactory, whilst chloroform was poor (approximately 25% efficient, 2 vols 40 μg/ml aqueous solution:3 vols extractant). EtOAc yielded 89.4%

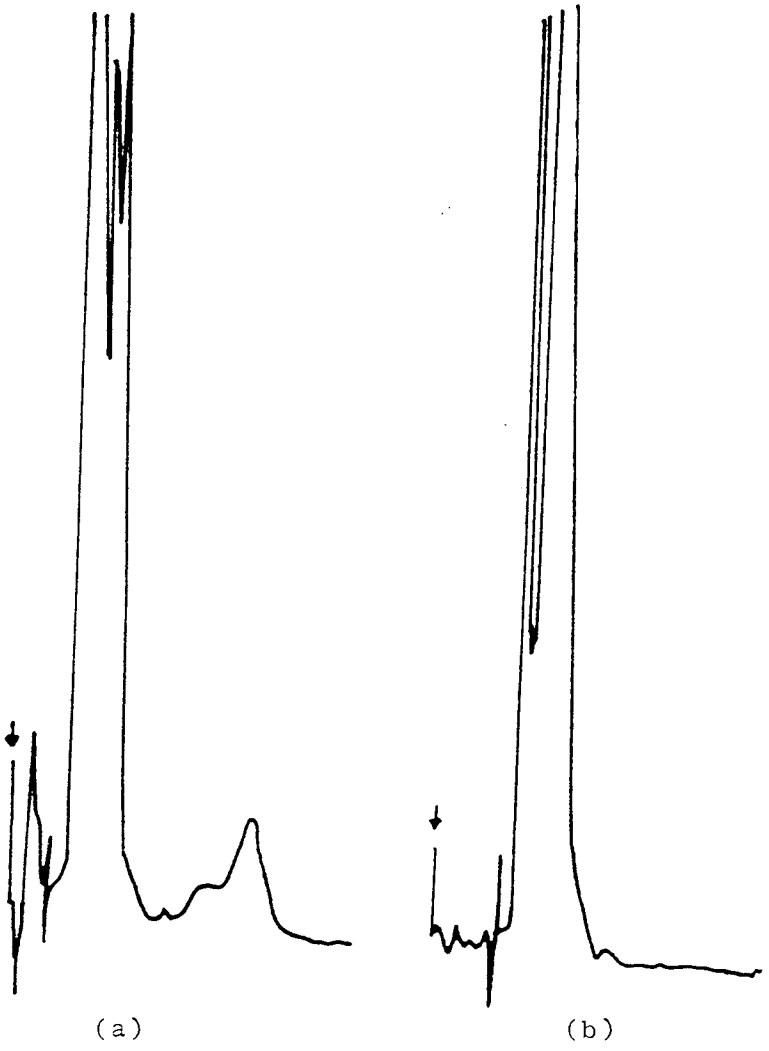


Fig 12.3 Chromatography of ethyl acetate

ODS 1 : 275 nm : 17% methanol in water.

100 μl ethyl acetate : (a) before distillation

(b) after distillation

of CAP under the same conditions, using 30 inversions to mix the solutions. Acidifying the aqueous layer with 0.1 ml acetic acid produced a visually clearer EtOAc layer but did not improve the extraction efficiency.

Two problems arose with EtOAc extractions, however. It was found that EtOAc of 'Laboratory Reagent' and 'Analar' grades contained impurities that interfered with the assay for CAP at low concentrations by producing small peaks in the chromatogram, including one that co-eluted with CAP. Distillation of the EtOAc was found to remove the co-eluting peak and, therefore, minimise the problem (fig 12.3). It was also found that dextrose co-eluted with CAP and was extracted by EtOAc. Since dextrose is a common anti-coagulant (as in the CPD mixture used by the National Blood Transfusion Service and others) it was thought to be too great a problem for routine use. Rather than change a good extraction method it was decided to attempt a reverse-phase separation.

.3 Reverse-phase

Using a partisil ODS-10 column (Whatman, 25 cm X 0.46 cm id) it was found that methanol eluted CAP on the solvent front as an irregular group of peaks but that methanol-water mixtures (10 - 50% methanol) produced a single symmetrical peak well clear of the solvent front. Within this range it was found that increasing the methanol content speeded up elution. 17% methanol was considered to be optimal and no interference was found from blank whole blood (NBTS), anticoagulant solutions or ampicillin, amoxycillin, cloxacillin, talampicillin, cephazolin, flucloxacillin, phenoxymethyl penicillin, oxytetracycline, demeclocycline, chlortetracycline, tetracycline, clindamycin, sulphamethoxazole, sulphathiazole, sulphadiazine, sulphamerazine, sulphaguaridine, metronidazole, aminophylline,trimethoprim, gentamicin, diazepam, stilboestrol. The retention volume was 9.9 mls and the efficiency equivalent to 340 theoretical plates and a peak height of 55 mm/ μg/ml

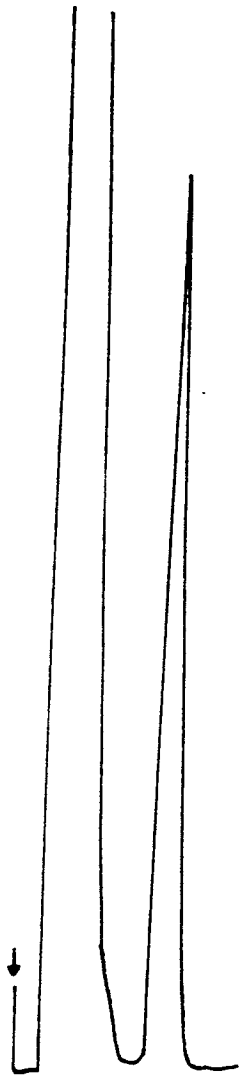


Fig 12.4 Serum Extract of Chloramphenicol (2.06 μg/ml)

ODS-1 : 17% methanol in water : 275 nm, 0.01 AUFS :

100 μl sample (in ethyl acetate)

from a serum extract at 0.01 AUFS (100 μl, 275 nm) (fig 12.4).

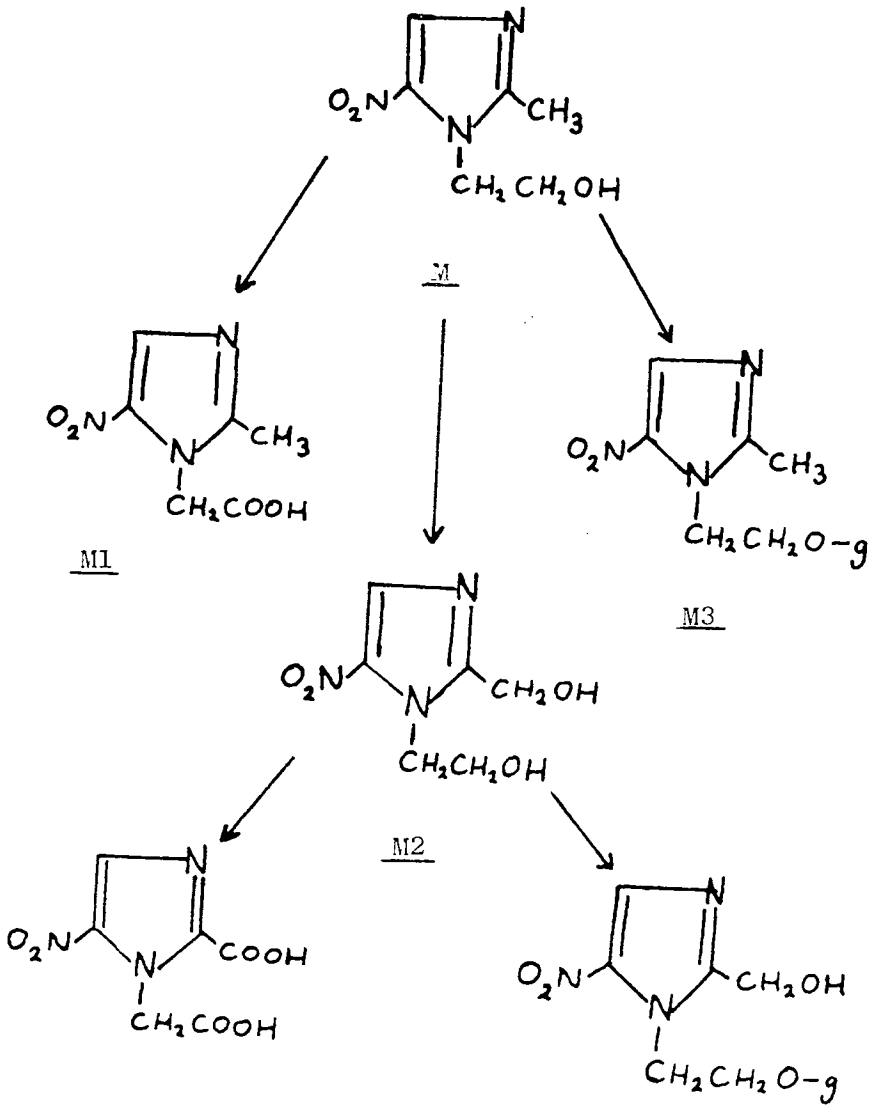
Later, on a new column (of identical manufacture) the same eluent gave a retention volume of 12.2 mls, an efficiency of 1086 theoretical plates and an equivalent peak height of 111 mm/ μg/ml.

Two calibration curves were made (with eluents of 10% and 17% methanol respectively) using duplicate extractions of blood containing 0, 0.35, 1.23, 4.32, 15.11 and 52.88 μg/ml CAP. Each extract was assayed twice, taking 100 μl of ethyl acetate filtered through a 0.8 μm regenerated cellulose filter. (The more common cellulose acetate and cellulose nitrate filters dissolved in ethyl acetate). In each case the correlation coefficient was greater than 0.99 and the 95% confidence limits for interpolated values (10 μg/ml) were 5.2 and 7.9% respectively. This compares with the estimated 30% for the standard bacteriological assay (estimate from antibiotic-assay department, East Birmingham Hospital).

12.4 Applications and published work

The restricted use of chloramphenicol does not obviate the need for assays since the remaining uses are critical ones, often in neonates. It would be an advantage to have a micro-sample technique for serum, csf or, preferably, saliva. Although there have not been any reports of the use of such assays, a number of HPLC assays have been reported (279, 286 - 288). Ali separated the parent drug and its hydrolysis products in

pharmaceutical preparations only (279). Nilsson-Ehle (288) and Peng (286) used one-step protein-precipitation as the extraction method whilst Thies (287) used a more elaborate technique involving Tris buffer. All used reverse-phase with acidified eluents, but Peng thermostatted the column at 30°C.



g = glucuronic acid

M = metronidazole

Fig 13.1 Metabolism of metronidazole (after Stambough et al (293))

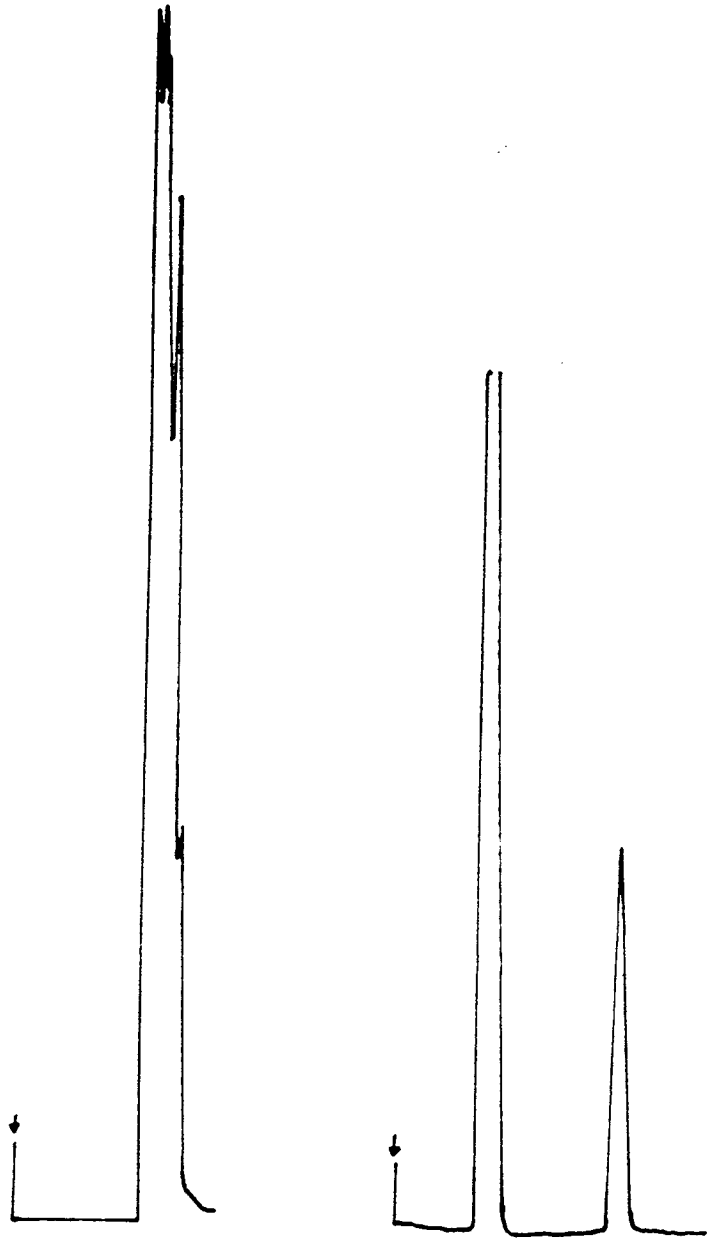
13 METRONIDAZOLE

13.1 Pharmacology and previously reported assays

Metronidazole (2(2-Methyl-5-nitroimidazol-1-yl) ethanol, 'Flagyl' has been used for some years as a trichomonacidal drug but has also been effective against a range of protozoal and vaginal infections. It has very few side-effects, the main one being an 'Antabuse'-type reaction with alcohol in some individuals (302). It is effective orally, intravenously and by rectum or vagina (289). In recent years it has been found to be highly effective against obligate anaerobic bacteria and has become the first choice antibiotic in many such infections (290, 295). Oral doses (200 mg) lead to peak levels of 5 - 7 μg/ml in serum and metronidazole has a half-life of about 6.5 hours (291). This is not greatly affected by renal function (292).

It is metabolised by oxidation and by glucuronidation, but at least 30 - 40% of excreted material is the unchanged drug (M) or the glucuronide (M3) (nomenclature from Stambough, (293), fig 13.1). The other principal metabolites are the 1-acetic acid (M1) and the 2-hydroxymethyl derivative (M2). M1 has very little activity against bacteria but M2 has about one third the activity of M against Bacteroides fragilis and one fifth against Trichomonas vaginalis (293). Metronidazole is known to be mutagenic but the significance in humans is not clear (294), although it does pass into milk and crosses the placental barrier (289). The mode of antibacterial action is not clear but may be related to the chromosomal mutagenicity of a biotransformation product (295). Reduction of the nitro group does not seem to occur in vivo (293, 295).

Reported assays include methods by microbiology (296), polarography (297), TLC (291) and GLC (298). Microbiological techniques give a combined result for all active compounds, including active metabolites (M2, M1) and other antibiotics. The polarographic method depends on the reduction of the nitro group and will be affected by any 5-nitroimidazole. The GLC technique is specific for the parent drug and its major metabolites.



Chloroform 20%
Methanol 80%

Chloroform 98%
Methanol 2%

1.5 ml/min, 320 nm, 0.04 AUFS, 12 μg/ml metronidazole

Fig 13.2 Normal-phase chromatography of metronidazole

13.2 Normal Phase and extraction studies

A silica column was selected, using ammoniated methanol as eluent, because Clarke (230) showed a tlc Rf value of 0.64 for metronidazole using this system.

It was found, however, that methanol (with and without 1% ammonia) caused an irregular peak on the solvent front and ethyl acetate was similarly poor. Mixtures of chloroform-methanol (10 - 50% chloroform) reduced the irregularity but still eluted metronidazole at the solvent front. 90% - 98% chloroform was a significant improvement, giving a reasonable peak clear of the solvent front (fig 13.2). It was relatively intense (16 mm/μ g/ml, 98% chloroform, 0.01AUFS, 10 μl) but reverse-phase systems were tried to improve the likelihood of extraction solvents being miscible with the mobile phase.

The optimum wavelength was found to be 320 nm, the absorption maximum, at which wavelength most solvents and interfering substances are transparent. (Fig 13.4 shows an extract of whole blood on reverse phase). Butanone, however, absorbed strongly and ethyl acetate had to be distilled prior to use (cf section 12.3). Ethyl acetate extraction of metronidazole in 0.9% sodium chloride (normal saline) (2 volumes ethyl acetate:1 volume saline) showed a 67% extraction efficiency but 3 volumes ethyl acetate:1 volume blood showed 58%. The addition of 0.8 volumes of solid ammonium chloride to the blood prior to the ethyl acetate improved the extraction to 74%.

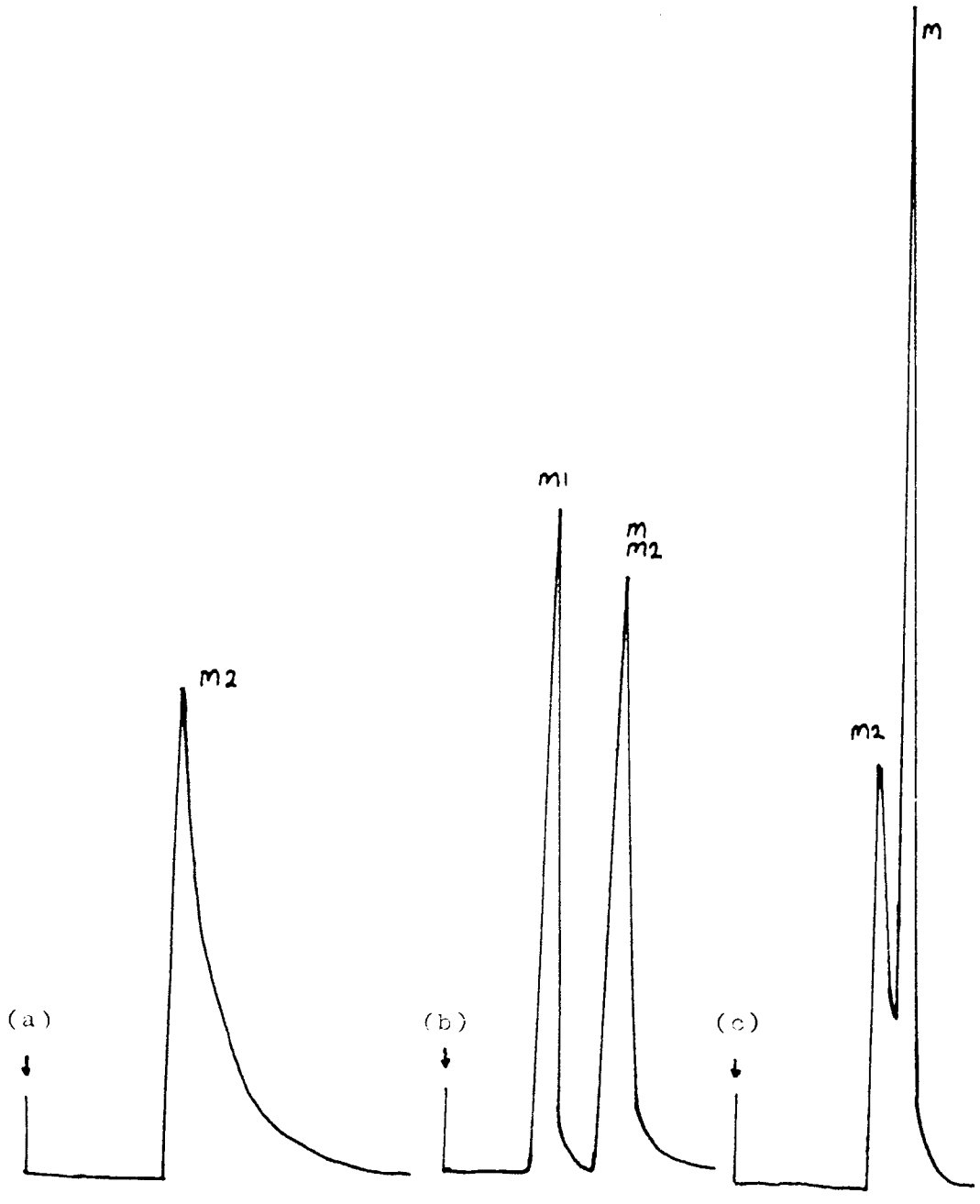
Preliminary experiments with acetonitrile (1:1v/v) showed good extraction ($>$ 94%) but a small interference from serum (say, equivalent to 3.5 μg/ml metronidazole). There was no interference from ampicillin, talampicillin, pivmecillinam, phenoxymethyl penicillin, amoxycillin, flucloxacillin, tetracycline, oxytetracycline, chlortetracycline, demeclocycline, clindamycin, chloramphenicol, cephazolin, benzyl penicillin, azlocillin, mezlocillin, cephalexin, cephradine, erythromycin,

Fig 13.3 Development of reverse-phase metronidazole chromatography

(a) methanol:ethyl acetate (4.5:95.5) 4.4 ml/cm

(b) isopropanol:acetic acid:ethyl acetate (5:10:85) 40 ml/cm

(c) methanol:acetic acid: ethyl acetate (4:1:95) 40 ml/cm



sulphamethoxazole, trimethoprim, aspirin or paracetamol

13.3 Reverse-phase chromatography

Ethyl acetate on an ODS column (Reeve Angel 25 cm x 0.46 cm id) produced broad tailing peaks for metronidazole (M) and especially so its 2-hydroxymethyl metabolite (M2). (Small quantities of metabolites M1 and M2 were obtained from May and Bakers Ltd., Dagenham, Essex.) These were improved by the addition of methanol or methanol and glacial acetic acid (fig 13.3) but neither mixture proved satisfactory (up to 25% methanol where both metronidazole and M2 eluted close to the solvent front and were not separated, and various ratios of methanol-acetic acid up to 10% of each showing partial separation but poor symmetry (fig 13.3)). In general, the lower concentrations of methanol produced the sharper peaks. Isopropanol was substituted for methanol but whilst this separated metabolite M1 from M and M2 the best separations of M and M2 showed M2 as a shoulder on the leading edge of the M peak and on occasions the two were indistinguishable.

The use of a 33% solution of acetic acid in water instead of glacial acetic acid in the mobile phase improved the symmetry and resolution of M, M1 and M2. The attainment of the correct ratio of the components was crucial, however, since small changes lead to decreased resolution (fig 13.4). The optimum mixture was found to be EtOAc:Methanol:33% Acetic Acid

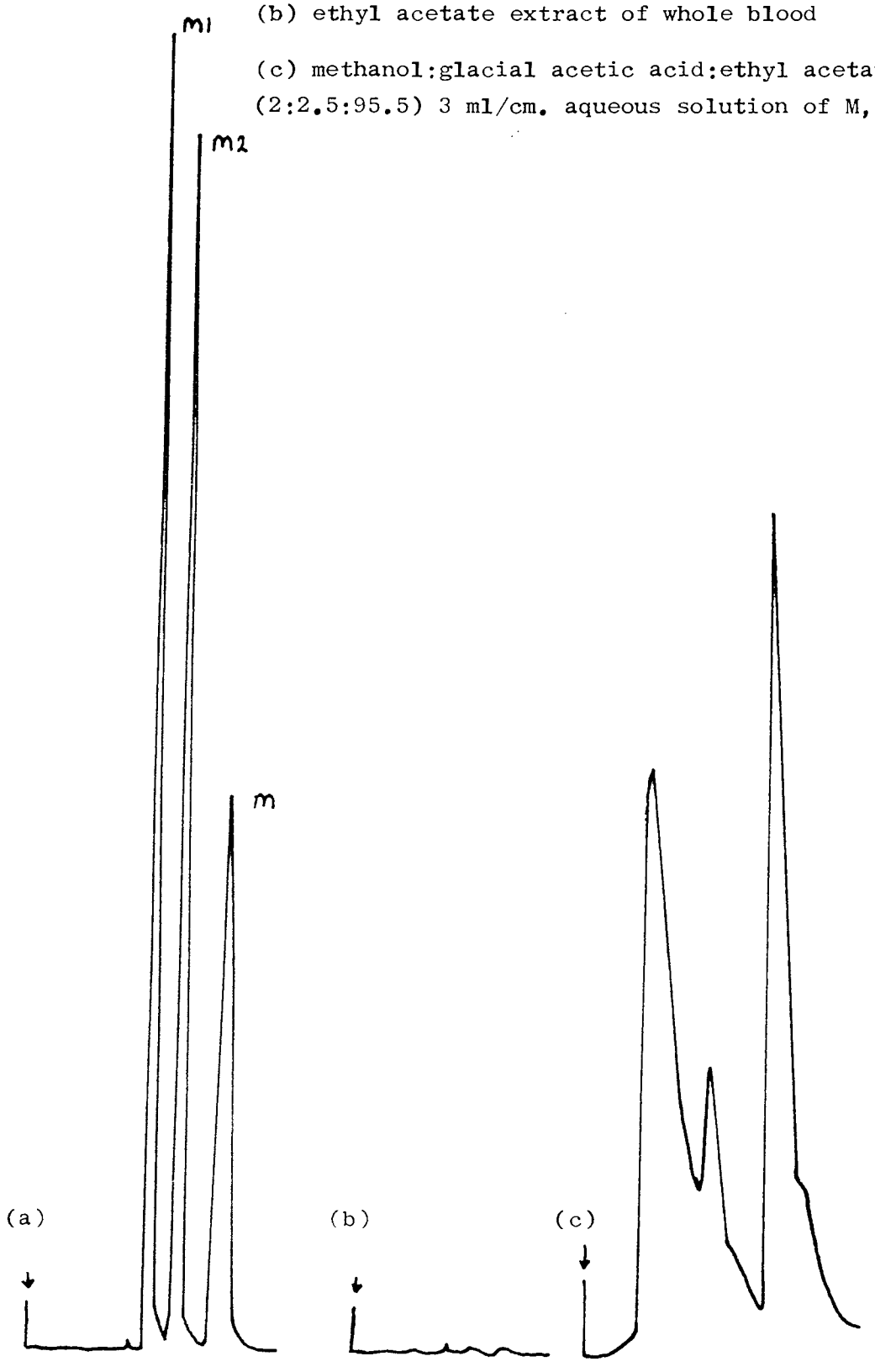
Fig 13.4 Metronidazole and metabolites

ODS 1 methanol: 33% Acetic acid:ethyl acetate (2:4.5:93.5) (a) 4 ml/cm (b) 3 ml/cm

(a) aqueous solution of M, M1, M2

(b) ethyl acetate extract of whole blood

(c) methanol:glacial acetic acid:ethyl acetate (2:2.5:95.5) 3 ml/cm. aqueous solution of M, M1, M2



(93.5:2:4.5). Retention volumes were: M1; 5.4 mls, M2; 6.75 mls, M; 8.55 mls. Approximate efficiencies (theoretical plates) were: M1; 1790, M2; 2804, M;1135.

No components of whole blood (extracted with EtOAc) interfered. It was found that ethyl acetate (5 vols: 3 vols blood + 1 vol NH_4Cl) did not extract the M1 metabolite, but extracted M (55.3%) and M2 (22.8%). Since the interested physician left East Birmingham Hospital at this point other work was pursued and this work was not resumed.

Since this work, three specific HPLC assays have been published. Gulaid *et al* (301) devised a very sensitive technique (20 ng/ml quantifiable) with a long extraction procedure and a reverse-phase separation using 0.1M diammonium hydrogen phosphate and methanol (5:1). Marques *et al* (299) used ethanolic protein precipitation and a pH4 buffered acetonitrile eluent but did not examine metabolite M1 and failed to separate metronidazole from misonidazole. Wheeler *et al* (300) used a similar reverse-phase system to separate M, M1 and M2. The extraction procedure involved a temperature-sensitive stage (mixtures stored in ice) and terminal filtration.

Compound	Volumes (mls) ODS (10μm)	RP18 (5μm)
Cephazolin	3.6	4.1
Ampicillin (& talampicillin)	5.0	7.0
Benzyl penicillin	13.1	15.5
Azlocillin	11.3	17.8
Mezlocillin	15.8	20.4
Amoxycillin	22.1	24.0
Talampicillin	15.1	22.9
Cloxacillin	28.1	40.8
Flucloxacillin	28.1	40.8
Cephalexin	-	47.3
Cephradine	-	69.7
Phenoxymethyl Penicillin	24.5	29.9
Efficiencies (theoretical plates)		
Azlocillin	1330	1696
Mezlocillin	934	2210
Test Compounds		
Phenol	4150	7930
m-Cresol	3870	7010

Table 14.1 Retention volumes for β-lactams

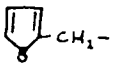
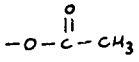
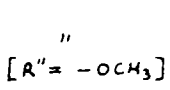
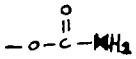
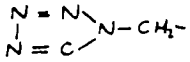
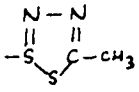
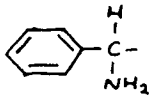
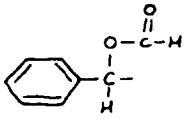
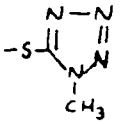
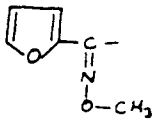
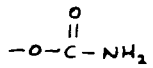
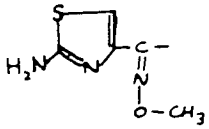
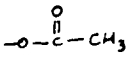
14. HPLC OF SOME β-LACTAM ANTIBIOTICS

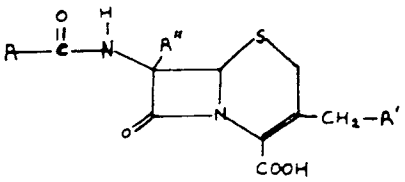
Penicillins and cephalosporins have long been a useful part of the antibacterial armamentarium (4). They, together with the more recent cephamycins and ureido-penicillins, interfere with bacterial cell-wall synthesis and are so selective that side-effects in humans are rare (5). Most have either no metabolites, being readily excreted by the kidney with half-lives typically less than 2 hours, or have only inactive products. An exception, however, is the new cephalosporin cefotaxime which has an active desacetyl metabolite.

Several assays have been published for β-lactams, mostly employing reverse-phase columns and simple acidified eluents. Diastereomers have been separated (303) from biological fluids as well as degradation products and synthetic pre-cursors in pharmaceuticals (304, 305). Other assays have also been published (306, 307, 308).

Enquiries were received about assays for mezlocillin and azlocillin (Bayer), two ureido-penicillins then on clinical trial. These, and other penicillins and cephalosporins, were tested under reverse-phase conditions. Solutions were made from oral capsules or injectable powder (azlocillin and mezlocillin). Using ODS (10 μm, Whatman) it was found that methanol-water mixtures gave asymmetrical peaks, but acidified mixtures (0.5 - 1% glacial acetic acid) gave more regular peaks. Lowering the proportion of methanol improved resolution between β-lactams but slowed elution. At 40% methanol (0.5% glacial acetic acid), azlocillin and mezlocillin were well resolved but talampicillin interfered with mezlocillin. Talampicillin also produced a peak that co-eluted with ampicillin, a known hydrolysis product. Cloxacillin and flucloxacillin co-eluted.

To resolve mezlocillin from talampicillin, a 5 μm reverse-phase column (Anachem, spherisorb RP18) was employed. The order of elution was similar, but not identical, to that for ODS with a similar eluent (40% methanol, 0.8% acetic acid). The retention times and efficiencies

Compound	Measured UV absorbances		R	R'
	max (Water) (nm)	max (40% MeOH, 1% acetic acid) (nm)		
Cephalothin	239	240		
Cefoxitin	238	239	 [R" = $-OCH_3$]	
Cefazolin	274	274		
Cephradine	264	264		H
Cefamandole nafate	270	272		
Cefuroxime	277	279		
Cefotaxime	239	258		



R" = H, except cefoxitin

Table 11.2 Structures and absorbance maxima of cephalosporins

were greater, however. Cloxocillin and flucloxacillin still co-eluted. Table 14.1 gives retention volumes for the available compounds. Sample chromatograms are given in fig 14.1. The test compounds, phenol and m-cresol, were chromatographed as part of the column-testing procedure given in appendix 4 and the efficiencies quoted demonstrate the expected increase in efficiency with a drop in particle size.

The optimum wavelength for mezlocillin (230 nm - 290 nm) was 240 nm, but azlocillin peak heights increased towards 230 nm. Wavelengths below 230 nm introduced much interference.

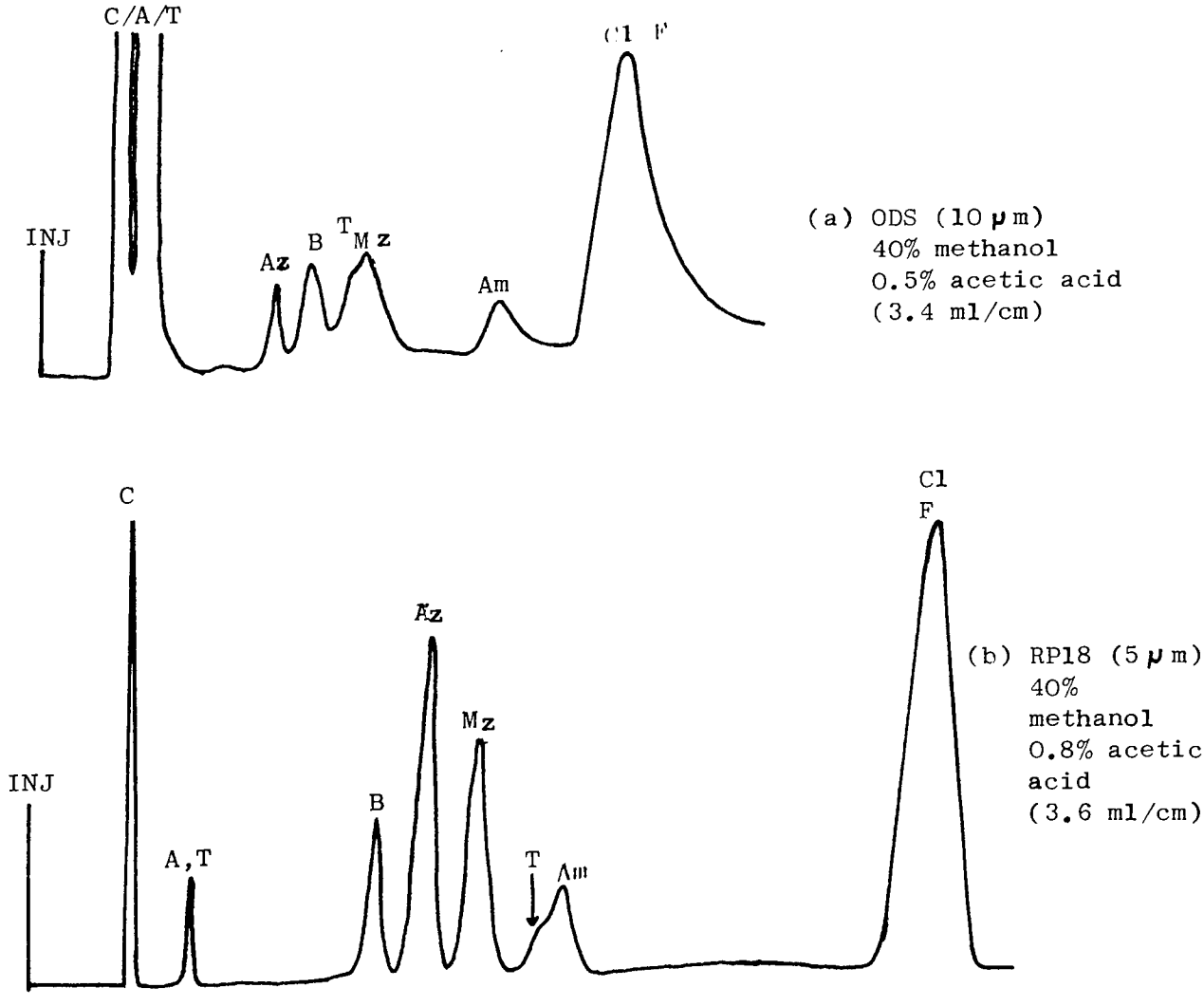
Extraction from serum was attempted using two volumes of extractant to one volume of spiked serum. It was found that chloroform did not extract either drug, whilst ethyl acetate extracted azlocillin very poorly ($\simeq$ 10%) and mezlocillin not at all. Butan-2-one had an intense absorption that obscured both drugs and the drugs were known to be poorly soluble in ethanol and acetone (316). Dimethylformamide extracted both drugs at approximately 35% efficiency (100 μg/ml).

The cessation of medical interest at this point lead to the discontinuation of this work but at a later date, the elution of a number of newer cephalosporins (table 14.2) was investigated with a view to possible stability studies in peritoneal dialysate. Many of these compounds co-eluted in 40% methanol and the content was reduced by stages to 20% (1% acetic acid). The sample solutions were made from injectable material at approximately 1/1600 normal strength. (This was intended to be comparable to peritoneal dialysis solutions). It was found that the eight compounds tested showed significant degradation within 24 hours at room temperature, some to as many as six peaks. Some data are given in table 14.3.

To develop any of these separations for clinical assay, a good extraction method is required. Acetonitrile, trichloro-acetic acid and perchloric acid have all been used for several β-lactams (303, 306, 307) and should be suitable for this application.

Drug	Time (hours)	Retention volumes of peaks (mls, size order)	Relative peak heights
Cephradine	0	20.0	
	48	20.0 17.1 13.3	63.3:31.9:4.8
Cephalexin	0	9.1	
Cephalothin	0	23.9 18.1	54.5:45.5
	48	81.5 17.7 23.9 62.5	35.6:28.8:25.0:10.5
Cefoxitin	0	26.0 21.5	97.3:2.7
	48	26.0 21.5	93.7:6.3
Cefazolin	0	11.8 5.5	94.4:1
	48	11.8 5.5	88.1:1
Cefamandole nafate	0	16.5 6.0	96:1
	48	62.7 8.2 16.5	81.8:10.7:7.5
Cefuroxime	0	26.8	
	48	26.8 22.2	65.5:34.5
Cefotaxime	0	20.4 3.6	92.3:7.7
	48	20.4 6.7 3.8 5.1 61.2 72.2	Not quantified, 20.4 very large

Table 14.3 Degradation of some cephalosporins over 48 hours



A = ampicillin, Am = amoxycillin, Az = azlocillin, B = benzyl penicillin,
C = cephazolin, Cl = cloxacillin, F = flucloxacillin, Mz = mezlocillin,
T = talampicillin

Fig 14.1 HPLC of β-lactams on reverse-phase columns

Method	Groups			
	1	2	3	Total
Plate, Gram - ve	7	15	9	31
Plate, Gram + ve	7	9	8	24
Broth Dilution	1	2	15	18
Overall	15	26	32	73

Groups are defined by 95% confidence limits for deviation of result from true value:

1	< 25%	('Good')
2	26 - 50%	('Poor')
3	> 50%	('Highly Misleading')

Table 15.1 Results of Reeves' survey of laboratory accuracy in gentamicin assays (314)

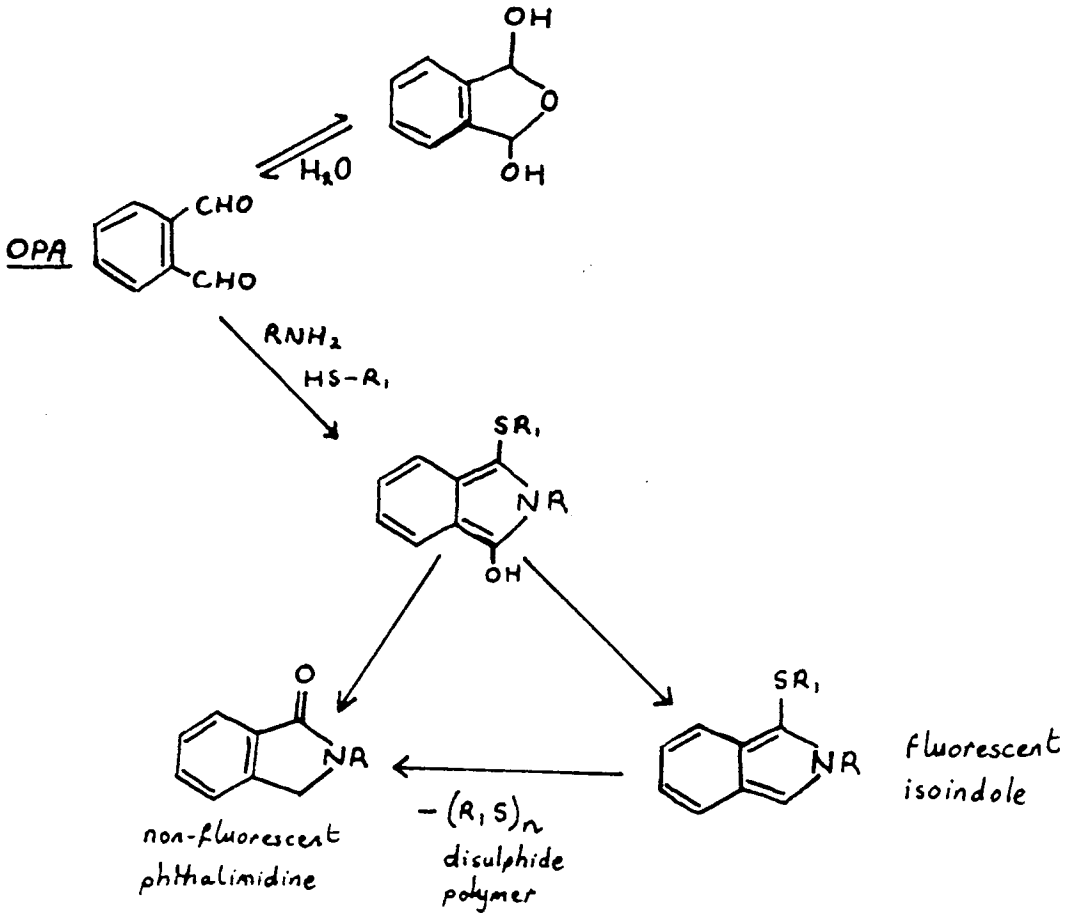
15. GENTAMICIN ASSAYS

15.1 Established assays and reported derivatisation techniques

Gentamicin has already been described (section 1) as a mixture of similar, polar amines with very low UV absorptivity. There are a number of standard assay techniques, reviewed elsewhere (309), including microbiological assays (cf section 4), enzymic acetylation (310), radio-immune labelling (311) and fluorescence-immune labelling (312). Standard kits are available for the latter two techniques and were evaluated by Dr. I. D. Farrell in parallel with the microbiological assay. They were found to be imprecise and labour-intensive in comparison with the established assay, although there was no interference from other antibiotics. They were also very expensive. Reeves has compared the use of the various methods in Britain and U.S.A. (Table 15.1, 314). Reeves has also instituted a national quality control scheme for gentamicin assays and has shown considerable discrepancies between laboratories (314).

In order to assay gentamicin in serum by HPLC the drug must be rendered detectable, gentamicin having no natural fluorescence and UV and RI being too insensitive. Derivatisation of the amino groups can be performed but although one recent paper has utilised UV detection for pharmaceuticals (315), for serum levels the sensitivity of fluorescence is generally required.

Derivatisation may be performed before or after HPLC separation (pre- and post- column). Pre-column procedures are better known since they resemble more closely derivatisation procedures for UV or GC analysis and require less specialised equipment (169, 317). Post-column procedures require a second pump and a reaction coil to mix the reagents prior to detection. Much has been written on the design of such systems and the variation in results that changing flow-rate, mixing geometry, reaction time, temperature and reagent composition can cause (e.g. 170, 319). Solid-bed reactors and open-coils have been proposed, as well as air-



Scheme taken from Simons et al (325) and Dominti et al (326)

Fig 15.1 Reaction of o-phthalaldehyde (OPA) with primary amines

segmentation to prevent band-spreading (170). The latter process resembles commercial multi-analysers in clinical chemistry laboratories. Apart from the technical problems of post-column reaction, it inevitably involves wasting much (expensive) reagent. Some workers, however, prefer to chromatograph known compounds rather than derivatives of sometimes uncertain structure from pre-column reaction (e.g. 320).

Three reagents have been commonly used for amine derivatisation, fluorescamine, o-phthalaldehyde (OPA) and 5-dimethyl_amino-1-naphthalene sulphonyl chloride (dansyl chloride). Biological amines, peptides and amino acids have been assayed with these compounds, with and without HPLC (321, 322). Benson and Hare published a major comparison between OPA and fluorescamine (323) in which they described the following advantages for OPA in comparison to fluorescamine:

1) cost

2) products are more stable (several days at room temperature)

3) water-soluble (unlike fluorescamine)

4) much greater quantum-yield (5-10 times)

5) immediate reaction

Both reagents react with primary amines but not secondary amines. Secondary amines can be oxidised to primary amines with N-chlorosuccinimide but OPA is itself oxidised by this procedure (323).

OPA requires a mercapto group (e.g. from mercapto-ethanol) in addition to the amine to form a fluorescent product and the mechanism of reaction has been described by two groups of workers (324, 325, 326) cf fig 15.1. Simons and Johnson showed that the products hydrolyse in a pseudo-first order fashion and do so faster at low pH. Ethanethiol, rather than mercapto-ethanol, leads to more stable products but the fluorescence intensity is less (a 35% decrease in aqueous buffers).

Dansyl chloride forms sulphonamides with primary and secondary amines

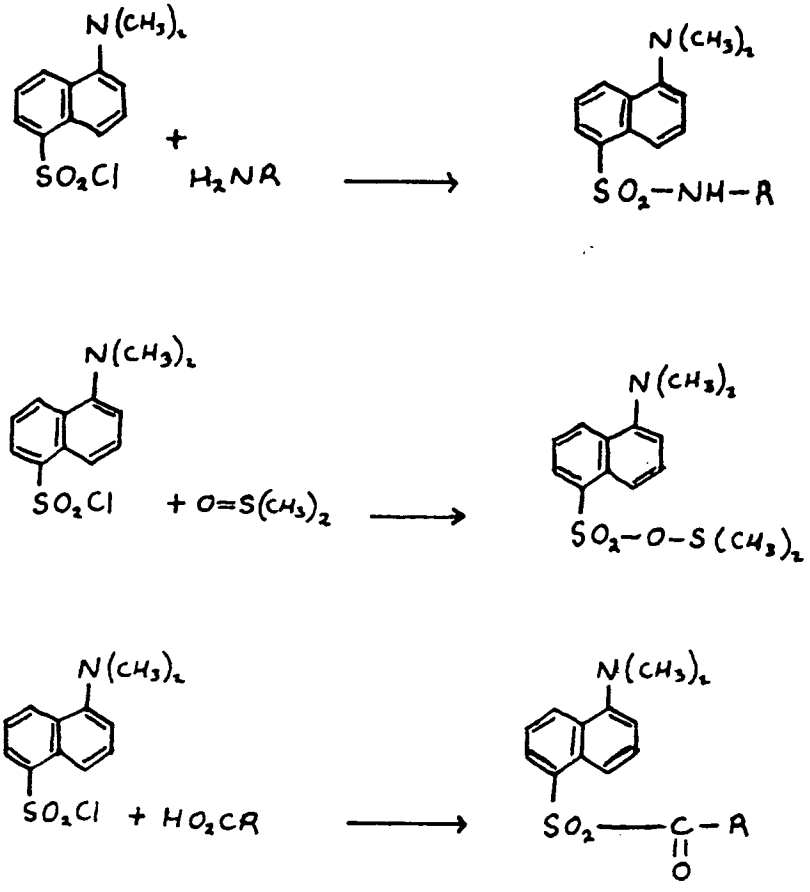


Fig 15.2 Typical Dansyl Chloride Reactions

and imidazoles. It also forms esters with phenols and reacts with thiols (321). Dansyl chloride is only slightly soluble in water and so mixed solvents, e.g. acetone-water, are commonly used. Increasing the acetone content decreases the rate of reaction, but also decreases the competing rate of reagent hydrolysis. The rate of reaction is also reduced at lower pHs (less than 8) because the amine groups are commonly ionised at these pHs. The rate of hydrolysis is not greatly diminished, however, and so reaction is usually performed at pH 9.5 - 10.5 (321). Typical reaction schemes are given in fig 15.2.

Three major methods for the derivatisation of aminoglycosides have been published. That of Peng et al (327, 328) uses dansyl chloride as a precolumn agent for gentamicin and netilmicin. The serum is alkalinised and then proteins precipitated with acetonitrile. The supernatant (after centrifuging) is added to methylene chloride and mixed. The aqueous portion is added to a dansyl chloride solution in acetonitrile and mixed in the dark at 75^{o}C for five minutes before cooling in ice. Ethyl acetate and alkaline buffer are added and a portion of the ethyl acetate chromatographed (95% acetonitrile in water, ODS column). Excitation is at 220 nm and emission at 470 nm. Mays et al (329) detected kanamycin in pure solutions, and Anhalt (320) gentamicin, netilmicin and sisomicin by post-column continuous-flow OPA reaction (340/418 nm fluorescence). The reagent was mixed with column eluent in a reaction coil by means of a direct pressure nitrogen gas pump. The serum samples were adsorbed and then eluted from a sephadex column prior to reverse-phase HPLC separation of the aminoglycosides (C18, 3% methanol, 0.1% acetic acid, 0.2M sodium sulphate, 0.02M sodium pentane sulphonate).

Maitra et al (330, 331) also used OPA but as a pre-column procedure. Serum was loaded onto a silicic acid column and washed off, utilising gentamicin's strong adsorption to silicic acid. The derivatisation reagent was added to the silicic acid and the eluent chromatographed;

Excitation Wavelength (nm)	Emmission intensity (arbitrary units) (λ max = 445 nm)
220	0.0
250	8.0
320	160.0
340	139.0
350	94.5
360	57.5

Table 15.2 Excitation wavelength and fluorescence intensity for Dansyl chloride reaction

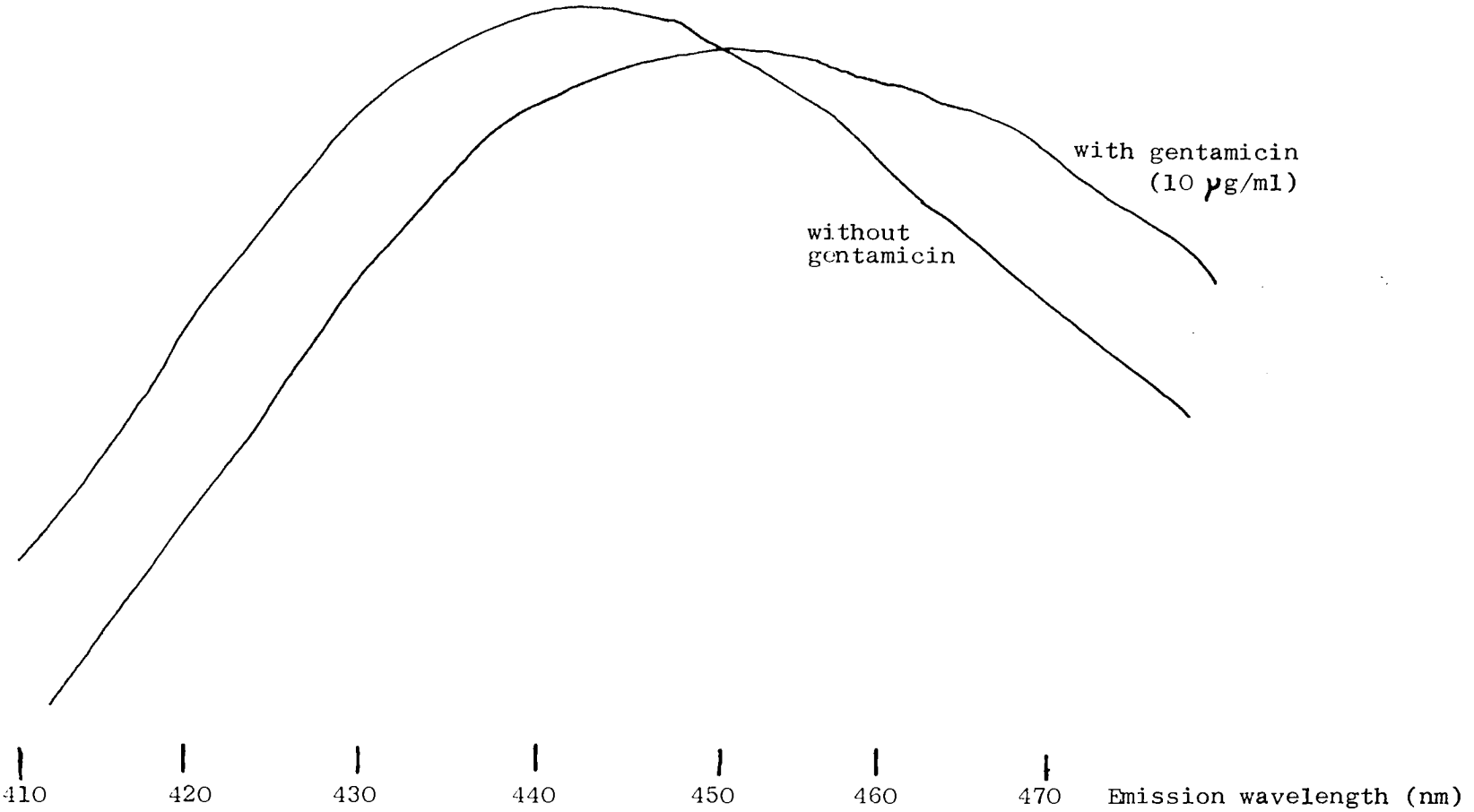
(C18, 79% methanol, 2 g/l tri-potassium EDTA, 360/430 nm). Maitra and Anhalt separated gentamicin C_1, C_{1a} and C_2 but Peng *et al* reported a single peak for C_{1a} and C_2. Nilsson-Ehle (332), a co-author with Maitra whilst on sabatical leave, used the derivatisation method of Anhalt followed by the separation method of Maitra to overcome incomplete extraction.

These reactions were tested using pure gentamicin solutions (Nicholas Laboratories) in place of serum. (Peng reported that aqueous solutions worked better than serum and Anhalt and Maitra used standard reactions reported elsewhere for pure solutions (323)).

15.2 Dansyl chloride derivatives

An Aminco-Bowman fluorimeter (cuvette model) and a Perkin-Elmer LC1000 fluorescence detector (loaded manually) were used to measure the fluorescence of the various solutions. A Pye-Unicam SP1800 was used to measure UV absorbances. Dansyl chloride was freshly dissolved in acetonitrile and a carbonate buffer (pH 9.6) prepared by the method of Peng (327). The method of Peng was carried out and fluorescence measured. The absorption/emission spectra are given in figure 15.3 (λ max. abs.= 320 nm, λ max. em. = 445 nm).

Fig 15.3 Emission spectra of dansyl derivatives, with and without gentamicin (Excitation 320 nm)



The individual components of the mixture were found to have negligible fluorescence but dansyl chloride and buffer, without gentamicin, fluoresced markedly. This fluorescence decreased upon inclusion of gentamicin and decreased with decreasing dansyl chloride. The fluorescence of the extract (without gentamicin) decreased as the buffer strength was decreased (0.5 - 0.125 M) as did the UV absorbance of the extract. The absorbance of the residue increased as that of the extract decreased. Lowering the pH of the buffer (9.6 to 7) reduced fluorescence of the extract but increased the absorbances of extract and residue (again, without gentamicin).

The extract from reacted gentamicin solutions had an absorption spectrum different from that of dansyl chloride and similar to that of reaction mixture without gentamicin (but more intense). The emission maximum for the blank mixture was 20 nm less than that of the gentamicin mixture.

Borate buffer (pH 8, Documenta Geigy) reduced the fluorescence of the extract but increased that of the residue. The extractant was varied to improve the sensitivity (table 15.3). The residue from the first extract was re-mixed with further extractant to assess the relative efficiency of extraction (% efficiency = 100 $(1 - I_1/I_2)$ where I is the intensity of emission, assuming immisible solvents).

Thus whilst chloroform showed no fluorescence at all, ethyl acetate (Peng's choice) was also relatively poor and butanol very good as non-quenching extractants. This may not be relevant to HPLC separation, however, since it is the eluent that will govern the intensity of fluorescence for any given extraction yield.

Peng et al reported the effects of reaction time, temperature, dansyl chloride concentration, and sodium hydroxide and buffer concentration (as % buffer-serum mixture). Their results show that in the appropriate ranges there is no significant difference for reaction time,

Extractant	Relative Intensities of fluorescence: 1st extract	2nd exract	% efficiency of extraction
Ethyl acetate	43	20	53.5
Methyl acetate	216	152	29.6
Butanol	548	-	-
n-pentanol	310	160	48.4
Chloroform	0	-	0
Methyl acetate extract diluted in butanol (1:1)		530	-

Table 15.3 Extraction efficiencies

temperature, dansyl chloride concentration or buffer content. Their results with regard to time and temperature were confirmed in this work, but increased dansyl chloride or buffer concentration leads to greater fluorescence (as reported above) although not to an improvement in the overall assay.

15.3 OPA derivatives

Anhalt modified the method of Benson and Hare by including 5% Brij (Polyoxyethylene lauryl ether) to prevent the precipitation of polysulphide in the detector. The OPA reagent was thus:

80 mg OPA dissolved in 1 ml 95% ethanol,

0.2 ml mercapto-ethanol

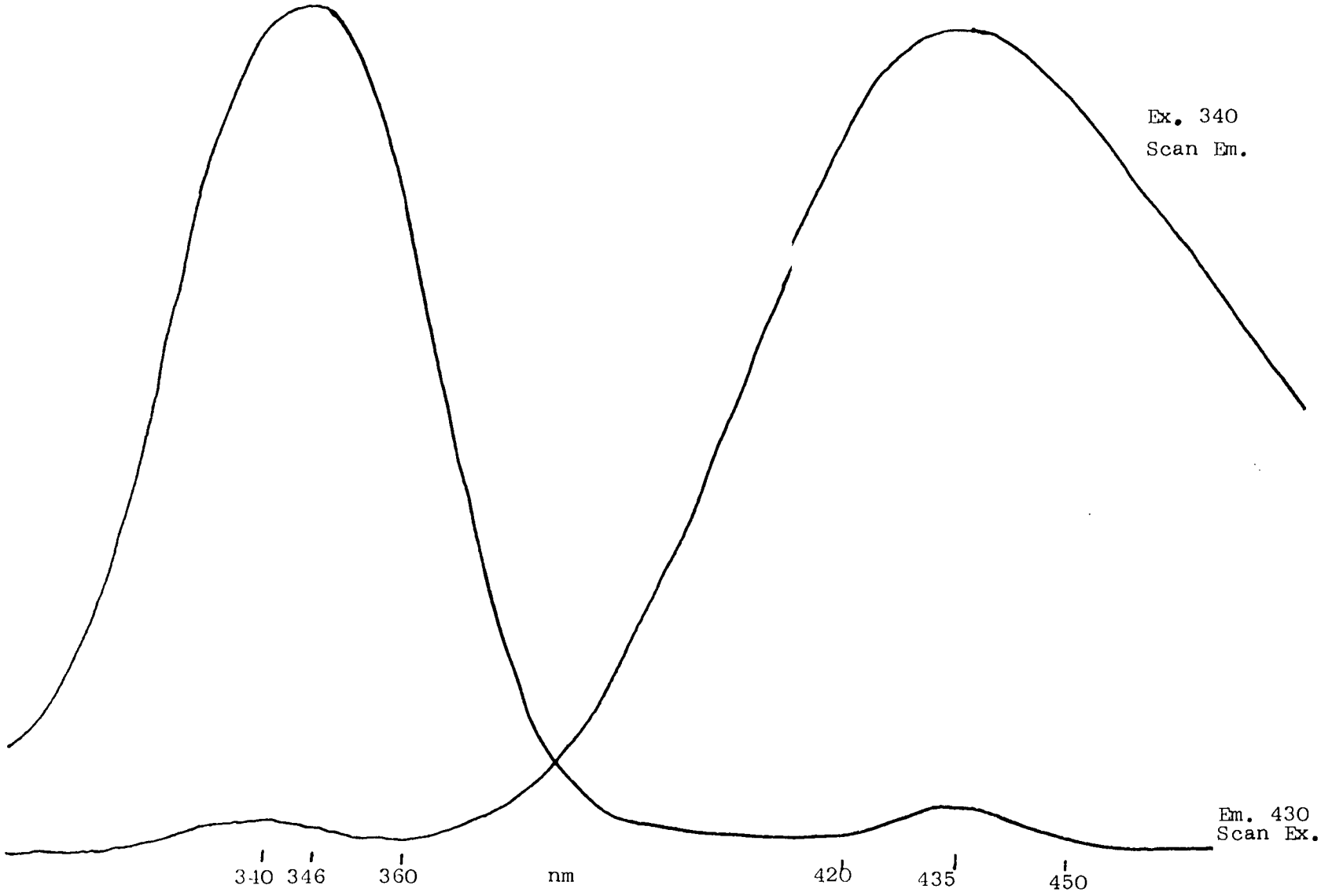
100 ml 0.4M borate buffer (pH 9.7 with KOH),

(with 5% Brij, further details unspecified)

To test this procedure the above reagent was used (pH 9.4 with 0.4 ml Brij (30% W/V solution) as per Benson and Hare) with an equal volume of gentamicin solution. (Unless otherwise stated, 10 μg/ml gentamicin was used). It was found that, without Brij, OPA precipitated from the reagent solution but redissolved when Brij was added.

The absorption maximum was 347 nm and the emission maximum 438 nm (fig 15.4). The reaction occured rapidly at room temperature, being

Fig 15.4 Excitation and emission spectra of OPA-gentamicin complex



complete in approximately 20 seconds (table 15.4). The fluorescence intensity was constant for several minutes (CV $<$ 3% typically up to 8 minutes) but then decayed at a rate which depended upon UV irradiation (and probably other factors). A solution left in a cuvette under constant irradiation (filter 340 nm) lost 76% of intensity in 105 minutes, whereas an identical solution irradiated six times in 120 minutes lost only 33.5% and one irradiated only once after 130 minutes lost only 20%.

The reproducibility of the procedure was assessed using solutions of 4 to 20 μg/ml gentamicin on several occasions. It was found that, whilst measurements of any one mixture over a period of say, 2 - 10 minutes had a CV $<$ 4%, reproducibility between replicates was poor, coefficients of variation being typically greater than 7%. E.g. at 10 μg/ml gentamicin (nitrogen flushed, dark reaction)

Solution A 5 measurements, mean 76.6 fluorescence units, CV = 1.69%

Solution B 6 measurements, mean 133.7 fluorescence units, CV = 2.45%

Solution C 8 measurements, mean 87.9 fluorescence units, CV = 1.17%

Overall 19 measurements, mean 99.4 fluorescence units, CV = 24.6%

Using darkened vessels, and saturating with nitrogen, improved the stability of solutions marginally but did not improve reproducibility.

To check experimental technique, solutions of quinine sulphate of equivalent fluorescence were made. Repeat measurements of the same solution gave a CV of 0.56% (n = 13) and measurements of nine separate dilutions of a stock solution (to mimic the OPA - gentamicin derivatisation) gave a CV of 1.1%.

The content of OPA (0.08 to 0.4 mg in 2 mls reaction mixture) and mercaptoethanol (0.05 to 0.2 % v/v final reaction mixture) caused no significant variation in intensity of fluorescence. This is to be expected, since both reagents were in great excess compared to gentamicin (approximately 80-fold and 200-fold respectively). The identity of the OPA was confirmed by IR spectoscopy which showed evidence of aldehydes but

Time (secs)	% of maximum intensity
1	76
5	88
10	94
15	98
20	100

Table 15.4 Rate of reaction of OPA and Gentamicin

none of the known degradation products, benzoic and phthalic acids (no 11 μm peak). The melting point was 60.0^{o}C (OPA value 58^{o}C, B.D.H.). Reagent solutions stored in a refrigerator (4^{o}C) for up to five days showed no difference in reaction to freshly-prepared solutions. This is consistent with Anhalt's work.

Attempts to separate the reaction products by HPLC, using the method of Maitra, and also a range of other eluents (5 - 45% acetonitrile in water, 30 - 70% methanol in water, 40% methanol, 3% acetic acid in water) on ODS-2 resulted only in irregular and irreproducible peaks that bore no relation to the content of gentamicin or OPA in the reaction mixture.

Other workers have found similar difficulty in implementing these methods. Communications to this effect have been received from Nottingham University Medical School Microbiology Department (H. Tupper, D. Greenwood), Southmead Hospital (Dr. L. R. O. White), Unilever Research Ltd., (E. C. Smith) and the London Hospital Microbiology Department (Prof. J. D. Williams et al). The author is unaware of any successful implementation of HPLC analysis, other than by the original workers, and is not aware of any subsequent publications from those workers demonstrating routine, or extensive, use of their methods.

16. OTHER WORK

16.1 Stability of Pharmaceuticals

A paper (Appendix 5) and a brief report follow on the use of HPLC in stability studies on pharmaceuticals. In each case the samples were analysed by the author who assisted in, but was not responsible for, the development of the assays. The aim of both studies was to assess the degree of breakdown of drug (aspirin and cysteamine respectively) to known metabolites (salicylic acid and cystamine respectively). The aspirin study was retrospective and found some considerable breakdown, but the cysteamine study was prospective and found no breakdown within two years.

.1 Cysteamine hydrochloride stability

The treatment of paracetamol poisoning has become increasingly important in recent years as that drug has assumed a greater popularity amongst the general public. All the drugs currently used (l-methionine, N-acetylcysteine, D-penicillamine and cysteamine) have toxicity associated with overdosage and may contribute to the hepatotoxicity in paracetamol overdosage (33, 334). Of these drugs, the most recent, and possibly safest, is N-acetylcysteine which has succeeded cysteamine as the drug of choice in Birmingham hospitals. It was not, however, widely used when a study was commenced on the stability of cysteamine hydrochloride solutions.

There being no officially recognised formulation, a variety of preparations have been used including freeze-dried material or aseptically filtered solutions (334) and autoclaved solutions packed under nitrogen (335, 336). Some preparations contain ascorbic acid as an anti-oxidant (336) whilst others contain Ethylene diamine tetra-acetic acid, sodium salt (EDTA) (337). Cysteamine degrades in aqueous solution to cystamine (337) which has been detected in cysteamine solutions by TLC (335, 336), HPLC (337) or GC (338). Cysteamine has also been assayed by iodine (339), iodocyanide (340) and iodoso-benzoic acid (341).

Batch Code	Excipients (g/l)	pH after manufacture	Final concentration (% of original)	Months from manufacture
U	Ascorbic Acid (14.72)	2.2	101.3	13
V	EDTA (0.1)	2.5	102.7	24
W		3.1	100.5	20
X		3.1	101.6	20
Y		-	99.5	23

(All batches nominally 100 mg/ml, autoclaved at 115°C for 30 minutes, packed under air)

Table 16.1 Stability of cysteamine solutions at 4°C in light-proof boxes

There are few published data on the stability of cysteamine although Prior found that a sterile, ascorbate-containing solution packed under nitrogen into glass ampoules contained no detectable cystamine after 30 days at room temperature whilst Purkis concluded that solutions were stabilised to autoclaving by the addition of ascorbate or EDTA or the removal of oxygen (i.e. packing under nitrogen).

Other than the work of Prior, however, no shelf-life has been suggested for the finished product. It was decided, therefore, to attempt to estimate a shelf-life for products available in three West Midlands hospitals.

The reported HPLC assay (Purkis) utilised a reverse-phase column with a methanol-borate eluent that was changed to methanol after the elution of cysteamine in order to elute cystamine. The published peak shape was poor and the non-isocratic eluent was inconvenient as well as being likely to precipitate the buffer salts on the column. It was found that this method did not separate the two components in any case and an isocratic system was devised.

Experimental Methods

Five batches of cysteamine HCL injection (equivalent to 100 mg/ml base) manufactured in three hospital pharmacies (table 16.1) were stored in light-proof boxes at 4^{o}C (following customary practice) and two ampoules were removed at a time for analysis by iodometric titration (for unchanged cysteamine) or HPLC (for cysteamine and cystamine) over one to two years.

The separation of ascorbate, cysteamine and cystamine was achieved using a Pye Unicam LC3 chromatograph with a 25 cm Partisil-10 silica column and detection at 225 nm. The mobile phase was a 0.015M aqueous solution of sodium dodecyl sulphate containing 1 ml glacial acetic acid and 0.2 Moles sodium sulphate per litre. A typical chromatogram is given in fig 16.2. Calibration curves were found to be linear over the range 0 to 10 mg/ml cysteamine base and solutions were diluted to be within this range. This method is faster than that previously reported

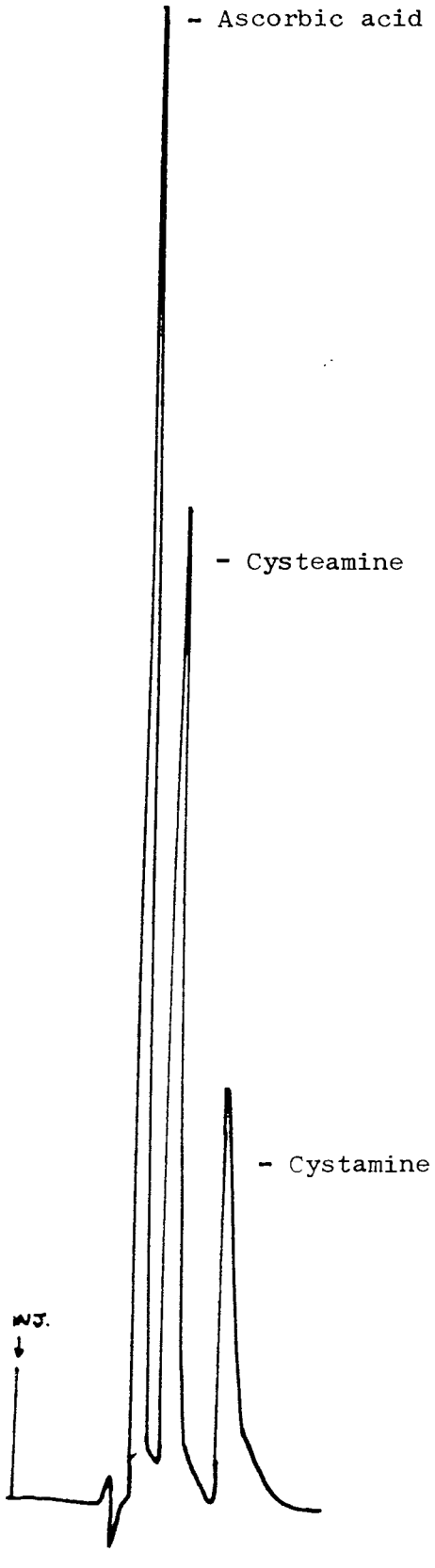


Fig 16.2 Chromatogram of batch Y with cystamine equivalent to 0.25% degradation

(337) without the need to change mobile phase in mid-run.

The iodometric titration (339) (in the presence of excess acetic acid) was used to standardise the reagents and also to assay the injectable material. It was verified that it is specific for cysteamine in the presence of cystamine and EDTA but is affected by the presence of ascorbic acid.

Standard samples of cysteamine and cystamine were provided by Robinson Brothers (West Bromwich). Iodine solutions were prepared from 'Volucon' reagents (M & B, Dagenham) and standardised against sodium thiosulphate.

Results

No significant quantity of cystamine was detected on any occasion ($< 0.5\%$ stated cysteamine content).

Iodine titrations were preferred for the later assays because of the greater simplicity in the absence of degradation. (HPLC standards were always standardised against iodine). The final assay result for each batch is listed, together with the composition of each batch in table 16.1. The final concentrations are not significantly different from the initial results ($p > 0.05$, paired t-test) and the overall coefficient of variation (31 assays, mean 99.6% of stated content) was 2.2% for cysteamine. It may be concluded that these preparations are stable in the dark at $4^{\circ}C$ for several months, and a shelf-life of at least a year is probably acceptable.

.2 HPLC analysis of white uncoded tablets

A paper is appended (Appendix 5) describing methods for identifying white, uncoded tablets. Many such tablets are used in hospital pharmacy because of the increasing number of generic products purchased by bulk contract. Items purchased in units of, say, 500 to 5000 must be sub-divided into units of 50 or 100 for use on the wards and such pre-packing is performed on a rotary tablet-counter. In order to ensure that the

Wavelength (nm)	230	240	250	260	270	280	290	300
Ascorbic Acid	0.61	1.00	1.3	1.37	1.35	1.30	1.15	0.42
Aspirin	2.5	1.00	0.54	0.51	0.49	0.42	-	0.63
Bendrofluazide	4.2	1.00	2.6	6.8	11.5	9.2	-	1.2
Chlormethiazole edysilate	0.66	1.00	1.09	0.80	0.13	0.0	0.0	0.0
Chlorpromazine	0.22	1.00	1.08	1.22	0.41	0.13	0.19	-
Codeine	0.99	1.00	0.87	0.32	0.40	0.63	0.53	-
Diazepam	1.19	1.00	0.79	0.62	0.34	0.22	0.16	-
Frusemide	1.33	1.00	0.49	0.73	1.14	1.14	0.49	0.24
Haloperidol	0.87	1.00	0.82	0.25	0.13	0.06	0.02	-
Isoniazid	0.88	1.00	1.11	1.23	1.20	0.88	0.51	-
Phenobarbitone	1.20	1.00	0.64	0.35	0.17	0.02	0.0	0.0
Phenytoin	2.5	1.00	0.81	0.62	0.02	0.02	-	0.01
Prednisolone	0.75	1.00	0.97	0.81	0.59	0.29	-	0.03
Prednisone	0.77	1.00	0.93	0.71	0.45	0.19	0.04	0.02
Stilboestrol	1.03	1.00	0.91	0.67	0.52	0.46	0.34	0.25
Sulphamethiazole	0.72	1.00	1.59	1.64	1.12	1.09	1.04	0.76
Sulthiame	0.97	1.00	0.95	0.93	0.65	0.27	0.10	0.0

Table 16.2 Relative absorbances of drugs in HPLC eluent (230 - 300 nm)

bottles are correctly labelled, and that the correct item has been supplied by the manufacturer, quality control procedures are instituted.

In 1977, 22 different types of white uncoded tablet were re-packed in West Midlands hospitals and a number of these were indistinguishable by physical appearance, weight or diameter. The systems described in appendix 5 were designed to overcome this problem. It was found that the two chromatographic systems were adequate for the resolution of all 22 drugs but were readily modifiable to give greater selectivity if required by the addition of further drugs. Quantification by peak height was satisfactory.

Apart from the use thus described the technique has been used to identify tablets in two clinical episodes.

In one, an over-dosed patient was brought to the Casualty Department (East Birmingham Hospital) with a supply of white uncoded tablets in an unlabelled container. By this method they were identified as phenobarbitone (30 mg).

In the second case a man was taking tablets of unknown origin and was experiencing fainting episodes and mood changes. The tablets were 'for indigestion'. One variety was pink, coded and identifiable as an old sample of Probanthine (propantheline bromide 15 mg). The other variety was white and uncoded. This was identified, by the method described, as carbachol and identity was confirmed by an external standard. In each case the procedure was completed within an hour.

A refinement of the published technique involves the use of several wavelengths to measure absorbance and, by comparison of the ratio of these absorbances with known data, prevent confusion between similarly-eluting drugs or between a suspected drug and another drug not included in this scheme. A stopped-flow technique may be used.

These data are given in table 16.2.

Baker et al have subsequently published a dual-wavelength absorbance

ratio that distinguished 95% of 101 drugs on three chromatographic systems whereas retention time alone distinguished only 9% (342).

16.2 Molecular orbital Calculations and Structure-Activity Relationships

Studies commenced as an undergraduate on the relationship between charge-distribution and conformation of some anti-arrhythmics were extended during the period of this study and published in conjunction with Drs. W. E. Hunt and C. H. Schwalbe (Appendix 5). Some of the data from this work are alluded to in connection with aminopyridine retention characteristics. Details of the background and calculations are contained in another thesis (343).

APPENDIX 1

Reagents and Materials

All reagents were BDH 'Analar' grades unless stated. Acetonitrile was 'laboratory reagent' grade from BDH or 'HPLC' grade from Fisons.

Sodium dodecyl sulphate was 'specially purified'. Aminopyridines were obtained from Aldrich or Sigma Chemicals. All were used as received except ethyl acetate and dimethyl formamide which were distilled before use.

Water was either freshly distilled or sterile Non-Injectable Water (Regional Sterile Supply Unit).

Blood and serum were obtained from the National Blood Transfusion Service.

Centrifuge

A MSE bench centrifuge with a 15 cm radius and a maximum speed of 3,400 rpm was used. Unless otherwise stated, centrifugation was performed at 3,400 rpm.

Viscometry

An Ostwald viscometer was used as described by Beckett and Stenlake (344) to determine the viscosity of some mobile phases. The procedure was carried out repeatedly until three successive readings had a range not greater than 1%. Temperature was determined by a mercury thermometer to the nearest $0.1^{o}C$. Densities were calculated by weighing known volumes of liquid.

pH

A Pye 290 pH meter with a combined glass electrode was employed. BDH buffer solutions were used for calibration at pH 2,00, 7.00 and 12.00. The scale permitted on accuracy of $\pm$ 0.025 pH unit.

UV

General scans, absorptivity determinations and certain kinetic determinations (chapter 10) were performed on a Pye Unican SP1800

double-beam, analogue scale spectrophotometer with a chart recorder. This had four ranges from 0.01 AUFS to 2.0 AUFS over the range 190 to 500 nm. The instrument was calibrated regularly and checked for linearity by means of dichromate solutions. 1 cm quartz cells were used at room temperature.

For temperature-controlled kinetic runs a similar machine, belonging to Clinical Chemistry, East Birmingham Hospital, with a waterbath and flow-through cell holder was used. The temperature of the waterbath varied by approximately $\pm$ 0.3°C at 30°C.

Chromatographic apparatus

The majority of experiments were performed upon a Pye-Unicam LC3 system located in the Pharmacy at East Birmingham Hospital. The basic system comprised a single-piston reciprocating pump capable of delivering a maximum of 11 ml/min or attaining a maximum pressure of 160 Bar (Approx. 2300 psi) together with a variable wavelength UV detector (190 - 350 nm, maximum sensitivity 0.01 Absorbance Unit per full scale deflection). The columns were stainless-steel (25 cm x 4.6 mm i.d.) fitted with $\frac{1}{4}$" - 1/6" ZDV reduction fittings and containing Partisil 10 μm support (Whatman Ltd.) or spherisorb (Anachem). Connections were made with stainless-steel tubing (0.0015" id) and output recorded on a Bryans Southern chart recorder. Initially a Specac loop injector was used, loaded via a standard glass luer-type insulin syringe. Later a Rheodyne 7125 injector was used, loaded by square-cut Hamilton-type needles which could be connected to either insulin syringes or low-volume chromatographic syringes. It was possible to use the Rheodyne injector in a partial-fill mode, thus enabling an infinite range of injection volumes up to the size of the loop fitted. 10 μl (Specac), 20 μl and 100 μl loops were used. It was found in several experiments, quoted elsewhere, that the reproducibility of injections in complete-fill mode was sufficiently good to obviate the need for an internal standard and so the partial-fill mode

was rarely used.

The system was varied on occasions to use an Altex 110 reciprocating pump (Aston) capable of pressures up to 6000 psi. This more powerful pump was preferred because it reduced analysis times, and in some cases made possible analyses that could not, on logistic grounds, be done with the LC3. It was not, however, available regularly. Both pumps were fitted with steel pre-filters (rated as non-porous above 2 μm), to remove particles from the mobile phase which was stored in glass vessels at room temperature. It was not found necessary to de-gas mobile phases, except for methanol-water mixtures where an in-house vacuum line was connected via a Buchner flask until shaking failed to release bubbles of gas. Components of mixtures were measured by the most accurate means available (glass pipettes, burette, measuring cylinders or a Class A electronic balance) prior to mixing to eliminate the effects of volume changes on mixing.

Flow rates were measured by weighing the outflow over a timed period and converting to ml/min. It was found that the flow from the LC3, at any given setting, varied with the column back-pressure, decreasing as the pressure rose. The Altex output, however, varied typically by less than $\pm$ 1% over a range of solvents throughout a period of several days.

Retention times and peak heights were measured with a ruler. Chart speed ranged from 2 to 30 mm/minute but was normally 5 mm/ minute.

APPENDIX 2

COMPUTER PROGRAMS

containing programs described in chapters 5 and 6:

1) DK1O, G1O } Interactive BASIC programs for handling patient data
2) Test data sets for these programs
3) Old prediction routine from these programs (DKFILE)
4) Simulated machine/operator exchanges using these programs
5) Program to calculate and file patient parameters (PARAMWRT)
6) Program to calculate standard deviations etc. on such parameters
7) Program to perform correlation and regression analyses on such parameters
8) Typical output from Programs 6 and 7
9) 3-program suite to perform general regression analyses
10) Test data set for 9
11) UMRCC FORTRAN program for Monte-Carlo analyses
12) MASTER AND PREDICTION segments from Aston FORTRAN programs for patient-data
13) BASIC program for calculation of HPLC parameters
14) BASIC program for teaching statistics
15) FORTRAN programs for graphical representation of audiological data, with output sample

DK10 PROGRAM FOR INPUT & REGRESSION ROUTINES

```
0020 CLOSE
0025 OPEN FILE (2,0),"GFL",1000
0030 OPEN FILE (3,0),"KUNDATA",2000
0040 OPEN FILE (1,1),"STT01"
0045 DIM W(20),Z(20),X(20),Y(20)
0060 DIM E(2,20),M(2,20)
0080 DIM M$(5),P$(60),Y$(8),B$(5),S$(80)
0082 LET KB=2.16
0083 LET TB=8.33333E-02
0089 RESET FILE (2)
0090 READ FILE (2,0),D0,E,N1,N,N3,X,W,C,S$,K,K1,K2,B$,T2,A0,B0
0092 READ FILE (2,4),V4,V5,V6,A1,A2,B1,B2,Z
0095 MAT READ FILE (2,1),W,Z,M
0097 REM**************************OPTION SELECTION AND FLOW CONTROL***************
0100 INPUT "SELECT THE OPTION YOU REQUIRE( /=info)",D0
0105 IF INT(D0)=D0 THEN IF D0>0 THEN IF D0<9 THEN GOTO 0110
0106 PRINT "INPUT AN INTEGER BETWEEN 1 AND 8. TRY AGAIN"
0107 GOTO 0100
0110 IF D0<2 THEN IF D0<5 THEN GOTO 0160
0120 IF D0<7 THEN INPUT "Input patient identifier",P$
0125 PRINT FILE (1),"<12><10><10><14>",P$;"<15><10><10>"
0160 ON D0 THEN GOTO 0230, 0230, 0232, 0268, 0230, 0230, 8000, 0225
0225 LET D0=0
0230 LET W=0
0232 LET C=0
0235 MAT W=ZER(20)
0240 MAT Z=ZER(20)
0262 LET N1=0
0264 LET N2=0
0268 LET N5=0
0270 LET N=0
0271 LET W0=0
0273 MAT M=ZER
0274 MAT X=ZER(20)
0275 MAT Y=ZER(20)
0276 LET Y$="QQQQQQQQ"
0277 MAT E=ZER
0282 ON ERR THEN GOTO 0300
0285 IF VAL(P$)<130 THEN GOTO 5000
0287 ON ERR THEN STOP
0300 IF D0=4 THEN PRINT FILE (1),"<10>Re-run for same patient<13>****** *** **** *******<13>"
```

```
0360 ON DO THEN GOTO 1030, 0900, 0910, 0520, 1030, 4100, 8000, 9999
0370 GOTO 9999
0520 INPUT " How many exponentials ?",Z
0522 IF Z=2 THEN GOTO 6320
0525 IF N1>3 THEN GOTO 0550
0530 LET N=3
0540 GOTO 0660
0545 REM************************REGRESSION-ARRAY FORMATION**********************
0550 INPUT "HOW MANY POINTS FOR K ELIMN. ? ",N
0570 IF N=N1 THEN GOTO 0660
0580 IF N=0 THEN GOTO 0100
0590 PRINT "Input code-numbers separated by CR"
0610 FOR I=1 TO N
0612   INPUT F(1,I);
0615   IF F(1,I)=0 THEN IF F(2,I)<>N1 THEN GOTO 0620
0617   PRINT "NUMBER OUT OF RANGE. INPUT CODE-NUMBERS AGAIN"
0618   LET I=0
0619   GOTO 0610
0620   LET X(I)=W(F(1,I))
0625   IF Z(F(1,I))<.7 THEN PRINT "WARNING : THIS MAY NOT BE SUITABLE FOR GENTAMICIN. POINTS AFTER 2 HOURS ARE BEST"
0630   LET Y(I)=Z(F(1,I))
0640 NEXT I
0650 GOTO 0692
0660 FOR I=1 TO N
0663   LET E(1,I)=1
0670   LET X(I)=W(I)
0680   LET Y(I)=Z(I)
0690 NEXT I
0692 REM
0695 IF N1>3 THEN GOTO 0700
0697 LET N3=3
0698 GOTO 0860
0699 REM************************AREA UNDER THE CURVE ARRAY FORMATION*************
0700 INPUT "<13>HOW MANY POINTS FOR AUC?",N3
0710 IF N3<N1 THEN GOTO 0860
0780 IF N3=0 THEN GOTO 0900
0790 PRINT "Input code-numbers separated by CR"
0810 FOR I=1 TO N3
0812   INPUT F(2,I);
0815   IF F(2,I)=0 THEN IF F(2,I)<>N1 THEN GOTO 0820
0817   PRINT "NUMBER OUT OF RANGE. INPUT CODE-NUMBERS AGAIN"
0818   LET I=0
```

```
0819   GOTO 0810
0820   LET M(1,I)=W(E(2,I))
0830   LET M(2,I)=EXP(Z(E(2,I)))
0840 NEXT I
0850 GOTO 0900
0860 FOR I=1 TO N3
0867   LET E(2,I)=I
0870   LET M(1,I)=W(I)
0880   LET M(2,I)=EXP(Z(I))
0890 NEXT I
0895 REM**********************FLOW CONTROL AND FILE-WRITING*********************
0900 IF Z<>2 THEN IF D0<>2 THEN IF D0<>3 THEN GOSUB 096(
0901 IF D0<4 THEN GOSUB 1500
0902 IF D0<>2 THEN IF D0<>3 THEN GOSUB 6000
0903 IF Z<>2 THEN IF D0<>2 THEN IF D0<>3 THEN GOSUB 182(
0904 IF D0<>1 THEN IF D0<>5 THEN GOTO 0920
0905 INPUT "DO YOU WISH TO RECORD THIS DATA IN FILE 'RUNDATA'?  ",Z$
0906 IF Z$(1,1)<>"Y" THEN GOTO 0920
0907 READ FILE (3,0),Z9
0909 LET Z9=Z9+1
0911 WRITE FILE (3,0),Z9
0912 MAT WRITE FILE (3,Z9*2),W,Z
0915 WRITE FILE (3,Z9*2-1),P$,E,N1,B$,X,W,S$,T2
0920 WRITE FILE (2,0),D0,E,N1,N,N3,X,W,C,S$,K,K1,K2,B$,P,A0,B0
0922 WRITE FILE (2,4),V4,V5,V6,A1,A2,B1,B2,Z
0930 MAT WRITE FILE (2,1),W,Z,M
0950 CHAIN "G10"
0955 REM**********************PRINT OUT ARRAY INFORMATIO**********************
0960 PRINT FILE (1),USING " ## DATA POINTS USED FOR REGRESSION WITH CODES:",N
0975 FOR I9=1 TO N
0980   PRINT FILE (1),E(1,I9);
0985 NEXT I9
0987 PRINT FILE (1),USING "<13> ## DATA POINTS USED FOR UC WITH CODES:",N3
0989 FOR I9=1 TO N3
0991   PRINT FILE (1),E(2,I9);
0993 NEXT I9
1000 RETURN
1030 REM**********************OPTION 1 INPUT SEGMENT**********************
1080 INPUT "Input dose, route, interval and number of data points ",E,B$(1,2),T2,N1
1100 IF N1=0 THEN GOTO 0100
1102 IF T2<4 THEN PRINT "COMMENT: The dose interval is very short (<4 hours)"
1103 IF T2>24 THEN PRINT "COMMENT: The dose interval is very long (>24 hours)"
```

```
1104 IF B$(1,2)<>"IM" THEN IF B$(1,2)<>"IV" THEN IF B$(1,2)<>"PO" THEN PRINT "COMMENT: Route unknown, results unpredictable"
1110 IF N1>2 THEN GOTO 1140
1120 PRINT "You need at least 3 points, try again"
1130 GOTO 1080
1140 PRINT " input pairs of x and y values, one pair to a line"
1145 PRINT FILE (1),USING "DATA SET OF ## POINTS",N1
1146 PRINT FILE (1),"CODE   TIME    CONC     TIME     LN(C)"
1150 FOR I=1 TO N1
1160   INPUT W(I),Z(I)
1170   IF W(I)<>9999 THEN GOTO 1200
1180   LET I=0
1190   GOTO 1030
1200   IF Z(I)>0 THEN GOTO 1230
1210   PRINT "this point is not possible, y must be positive"
1220   GOTO 1030
1230   PRINT FILE (1),USING "##    ##.##    ##.##       ",I,W(I),Z(I),
1231   IF I>1 THEN IF W(I)<W(I-1) THEN PRINT "POINTS OUT OF SEQUENCE ARE OK FOR REGRESSION BUT NOT AUC"
1232   LET Z(I)=LOG(Z(I))
1240   LET W(I)=INT(W(I))+(W(I)-INT(W(I)))/.6
1245   PRINT FILE (1),USING "##.##     -#.###",W(I),Z(I)
1250 NEXT I
1260 INPUT "1 OR 2 EXPONENTIAL CURVE?",Z
1265 IF Z=2 THEN GOTO 6320
1270 GOTO 0522
1450 REM***********************OPTION 2 INPUT AND CALCULATION*******************
1500 IF X*W=0 THEN INPUT "age, weight, creat., M/F  ?",X,W,C,S$
1505 IF D0<>2 THEN IF D0<>4 THEN IF C=0 THEN INPUT "Creatinine ?",C
1510 IF C=0 THEN RETURN
1520 LET C=C/84.8
1530 PRINT FILE (1),USING "  AGE ## : WEIGHT ###.# Kg  Creatinine ###.#",X,W,C*84.8
1600 IF S$(1,1)="F" THEN GOTO 1650
1610 LET H=1.251E-07*X^4-3.049E-05*X^3+.002553*X^2-.09888*X+2.9476
1620 LET J=95.99/(C^1.387)
1630 LET G=(140-X)/72
1640 GOTO 1680
1650 LET J=70.81/(C^1.307)
1660 LET H=-2.95E-07*X^4+7.25E-05*X^3-.00634*X^2+.217*X-1.091
1670 LET G=.85*(140-X)/72
1680 LET C2=G1*W/C
1690 LET G2=G*W/C
1700 LET K=.012+.00324*J
1770 LET K2=.012+.00324*C2
```

```
1780 LET K1=.012+.00324*G2
1790 RETURN
1810 REM**********************REGRESSION OUTPUT & DEPENDENT PARAMETERS***********
1820 PRINT "  Co=";EXP(A);"   calc. k =";B;"  half life =";ABS(.693/B)
1822 IF E*T2=0 THEN INPUT "Dose, route, interval ? ",E,B$(1,2),T2
1840 PRINT FILE (1),USING "<10><13>INTERCEPT **.** : CALC K .*** : HALF-LIFE **.**",EXP(A),B,.693/B
1845 PRINT FILE (1),USING "Correlation (r)=-*.*** : coeff var=***.*<10>",R0,S*100/X0
1850 IF B$(2,2)="M" THEN LET V4=E*K8/(K8-ABS(B))/EXP(A)
1855 IF B$(2,2)="V" THEN LET V4=E/T8/ABS(B)*(1-EXP(B*T8))/EXP(A)
1857 LET V5=V4*EXP(A)/(EXP(A)*(1-EXP(-ABS(B)*T2)))
1858 LET V6=V4*EXP(A)/(EXP(A)-EXP(Z(1)))
1860 PRINT FILE (1),"<13>Volumes from  (1)INTERCEPT     (2)INTERCEPT-TROUGH     (3)INTERCEPT-CALC TROUGH<13>"
1862 PRINT FILE (1),USING "                    ***.**          ***.**                    ***.**",V4,V6,V5
1880 RETURN
4000 REM ***************  OPTION 6 SEGMENT FOR KNOWN V,K,W ETC  ***************
4100 GOSUB 1500
4150 INPUT "Input volume if known",W0
4160 IF W0=0 THEN INPUT "What % of weight for volume?",W1
4180 IF W0=0 THEN LET W0=W*W1/100
4200 INPUT "Desired value of k, if any?",B0
4250 IF B0<>0 THEN LET Y$(3,3)="Y"
4260 IF C<>0 THEN LET Y$(4,4)="Y"
4270 LET Y$(2,2)="Y"
4280 LET Y$(5,5)="N"
4500 WRITE FILE (2,0),B0,0,0,0,0,0,W,C,"      ",K,K1,K2,"11   ",0,0,0
4510 WRITE FILE (2,3),W0,W1,B0,Y$(2,5)
4600 GOTO 1000
5000 REM  *************  FILE INPUT ROUTINE USING "RUNDATA" (FILE 3) ********
5050 ON ERR THEN STOP
5100 MAT READ FILE (3,VAL(F$)*2),W,Z
5200 READ FILE (3,VAL(F$)*2-1),P$,E,N1,B$,X,W,S$,T2
5210 PRINT P$;N1;"POINTS"
5230 PRINT FILE (1),"<14>";P$;"<13><10>"
5249 PRINT FILE (1)," N     Time    C(ug/ml)     Time      ln(C)<13>**     h.mm    ********     h.hh      ******"
5250 PRINT FILE (1)
5260 FOR I=1 TO N1
5268   DIM Q$(50)
5269   LET Q$="**    **.**     **.**      **.**     -*.***"
5270   PRINT FILE (1),USING Q$,I,W(I)*.6+INT(W(I))*.4,EXP(Z(I)),W(I),Z(I)
5320 NEXT I
5330 IF W=0 THEN INPUT "weight (for AUC & predictions) ?",W
5400 GOTO 0520
```

```
5450 REM**********************LINEAR REGRESSION AND CORRELATION******************
6000 LET P=0
6010 LET X1=0
6020 LET X2=0
6030 LET Y1=0
6040 LET D2=0
6043 LET D1=0
6046 LET F1=0
6050 LET F2=0
6060 FOR M=1 TO N
6070   LET P=(X(M)*Y(M))+P
6080   LET X1=X1+X(M)
6090   LET X2=X2+X(M)*X(M)
6100   LET Y1=Y1+Y(M)
6110 NEXT M
6120 LET X0=X1/N
6130 LET Y0=Y1/N
6140 FOR I=1 TO N
6150   LET D1=D1+(X(I)-X0)^2
6160   LET D2=D2+(Y(I)-Y0)^2
6170 NEXT I
6180 LET B=(N*P-X1*Y1)/(N*X2-X1*X1)
6190 LET A=(Y1-B*X1)/N
6200   LET E0=0
6210   LET E1=0
6220 FOR I=1 TO N
6230   LET E1=E1+(Y(I)-(A+B*X(I)))^2
6240   LET E2=E2+(Y(I)-(A+B*X(I)))^2
6250 NEXT I
6260 LET S=(E1/(N-2))^.5
6270 LET T=S*SQR(1/D1)
6280 LET U=T*SQR(X2/N)
6290 LET V=(P-N*X0*Y0)/(N-2)
6300 LET R0=(B/ABS(B))*SQR(E2/D2)
6310 RETURN
6315 REM**********************BIEXPONENTIAL CURVE-STRIPPING**********************
6320 INPUT "NO. OF POINTS FOR EXP1,EXP2, 1ST POINT FOR EXP2",N2,N4,N5
6330 FOR I=1 TO N4
6340   LET X(I)=W(N5-1+I)-T8
6350   LET Y(I)=Z(N5-1+I)
6360 NEXT I
6370 LET N=N4
```

```
6380 GOSUB 6000
6390 LET A2=EXP(A)
6400 LET B2=B
6410 LET R2=R0
6420 FOR I=1 TO N2
6430   IF B$(2,2)="V" THEN LET X(I)=W(I)-T8
6435   IF B$(2,2)<>"V" THEN LET X(I)=W(I)
6440   LET Y(I)=EXP(Z(I))-EXP(A)*EXP(B*W(I))
6450   IF B$(2,2)="V" THEN IF Y(I)>0 THEN GOTO 6510
6455   IF B$(2,2)<>"V" THEN IF Y(I)<0 THEN GOTO 6510
6460   PRINT "POINT ";I;" IS EXPLAINED BY 2ND EXPONENTIAL ONLY AND WILL BE LEFT OUT"
6470   LET N2=N2-1
6480   IF N2<3 THEN PRINT "THIS LEAVES TOO FEW POINTS. REGRESSION ABANDONED."
6490   IF N2<3 THEN PRINT "   Ln(C) = Ln(";A2;") ";B2;"*t"
6500   IF N2<3 THEN GOTO 6740
6510   LET Y(I)=LOG(ABS(Y(I)))
6520 NEXT I
6530 LET N=N2
6540 GOSUB 6000
6550 LET A1=EXP(A)
6560 LET B1=B
6565 IF B$(2,2)<>"V" THEN LET B1=-B1
6570 LET R1=R0
6580 LET N8=0
6590 LET N9=0
6600 FOR I=2 TO N1
6610   LET X(I-1)=EXP(Z(I))
6615   IF B$(2,2)="V" THEN LET W(I)=W(I)-T8
6620   LET Y(I-1)=A1*EXP(B1*W(I))+A2*EXP(B2*W(I))
6630   IF B$(2,2)="V" THEN LET W(I)=W(I)+T8
6690   PRINT FILE (1),"TERM     COEFFICIENT     RATE CONST.     CORRELN(R)     POINTS"
6700   LET S$="  #         -###.###       -##.####        -#.###          #"
6710   PRINT FILE (1),USING S$,1,A1,B1,R1,N2
6720   PRINT FILE (1),USING S$,2,A2,B2,R2,N4
6730   PRINT FILE (1),USING S$,"*","*********","*********",R0,N1
6733   LET A0=LOG(A2*EXP(ABS(B2)*T8))
6734   LET B0=B2
6740   GOTO 0695
8000   REM**********************INFO SECTION
8010   PRINT "<13>            PHARMACOKINETICS PROGRAMME<13>            **************************"
8020   PRINT "THE OPTIONS AVAILABLE INCLUDE:<13><13>1 Analysis of serum levels and serum creatinines"
8030   PRINT "2 Serum creatinines only<13>3 Repeat creatinine for same patient"
```

```
8040    PRINT "4 Repeat calculations on serum levels from options 1 or 5<13>5 Serum levels only"
8050    PRINT "6 Predictions from input values of K,V,Weight,Creatinine etc"
8060    PRINT "8 Finish<13>"
8070    PRINT "THESE NOTES ARE ONLY AN AIDE-MEMOIRE, YOU MUST READ THE MANUAL!"
8090    PRINT "<13>Please ensure you type in CAPITALS, answer Y for yes and N for no"
8100    PRINT "Acceptable routes of administration are IV, IM, PO"
8120    PRINT "Analysis of serum data may use 1 or 2 exponential models"
8130    PRINT "Predictions use 1-compartment only with 2nd exponential +/- fitted Ka"
8140    PRINT "Predictions may be made from any serum data assuming constant renal function"
8150    PRINT
8500    GOTO 0100
```

G10 PROGRAM TO DO OUTPUT, AUC & PREDICTIVE ROUTINES

```
0010 REM    G10 CHAINED TO DK10 FOR START AND FINISH
0012 DIM T(7,5),N6(9)
0025 LET K8=2.16
0030 LET T8=.0833
0041 RESET FILE (2)
0045 DIM X(20),Y(20),W(20),Z(20)
0050 DIM V$(120),S(60),S$(5)
0060 DIM Z$(50),D(10)
0080 DIM M$(5),P$(60),Y$(8),B$(5)
0100 DIM C(20),M(2,20)
0110 LET Z$="BLOOD DATAJADRNY    COCKCROFT SIERSBAEK KEYBOARD  "
0120 READ FILE (2,0),D0,E,N1,N,N3,X,W,C,S$,K,K1,K2,B$,T2,A0,B0
0122 READ FILE (2,4),V4,V5,V6,A1,A2,B1,B2,Z
0124 LET Y$="QQQQQQ"
0125 IF D0=6 THEN READ FILE (2,3),W0,W1,B0,Y$(2,5)
0130 IF D0<>6 THEN MAT READ FILE (2,1),W,Z,M
0135 LET B=B0
0136 LET A=A0
0140 IF B$(2,2)<>"V" THEN IF Z=2 THEN INPUT "Do you want Ka=calc.value? (Default=2.16). Y/N?",Y$(1,1)
0145 IF Y$(1,1)="Y" THEN LET K8=ABS(B1)
0160 ON D0 THEN GOTO 1762, 1762, 1762, 2220, 2220, 1700, 9999
1700 IF D0=6 THEN IF C=0 THEN GOTO 1803
1720 REM************************OUTPUT SEGMENT*******************************
1762 PRINT FILE (1),"<13><10>"; TAB(20);"k elimn."; TAB(34);"half-life"; TAB(48);"doses at 8 hour intervals to give levels"
1764 PRINT FILE (1), TAB(20);"********"; TAB(34);"**********"; TAB(48);"of  2.5ug/ml      4.0ug/ml"
1800 PRINT FILE (1),"Jadrny            ";K; TAB(35);.693/K; TAB(50);6*W*K; TAB(65);9.6*W*K
1801 PRINT FILE (1),"Cockcroft & Gault  ";K1; TAB(35);.693/K1; TAB(50);6*W*K1; TAB(65);9.6*W*K1
1802 PRINT FILE (1),"Siersbaek-Nielsen ";K2; TAB(35);.693/K2; TAB(50);6*W*K2; TAB(65);9.6*W*K2
1803 IF D0<>6 THEN GOTO 2220
1805 PRINT FILE (1),"Option 6 input values were :"
1806 IF B0<>0 THEN PRINT FILE (1),"elimn. rate const.(k):";B0,"half-life";.693/B0;"hours",
1807 IF W1<>0 THEN PRINT FILE (1),"volume=";W0;"litres based on";W1;"% of body weight (";W;"kg)"
1808 IF W1=0 THEN PRINT FILE (1),"volume:";W0;"litres"
1809 GOTO 5000
2000 REM************************MAIN CONTROL SEGMENT************************
2220 INPUT "graph/ AUC/ predict/ with Cr./ from input  ? ",Y$
2230 LET Y$(LEN(Y$)+1)="NNNNN"
2260 IF Y$(2,2)="Y" THEN GOSUB 3590
2270 IF Y$(1,1)="Y" THEN GOSUB 2600
2280 IF Y$(4,4)="Y" THEN IF C=0 THEN PRINT "CARELESS! NO CREATININE HAS BEEN GIVEN SO THOSE PREDICTIONS WON'T BE DONE"
2285 IF C=0 THEN LET Y$(4,4)="N"
2290 IF Y$(3,5)<>"NNN" THEN GOTO 5000
```

```
2310 LET B=B0
2311 LET A=A0
2400 IF D0=6 THEN GOTO 2520
2500 WRITE FILE (2,0),D0,E,N1,N,N3,X,W,C,S$,K,K1,K2,B$,T2,A0,B0
2502 WRITE FILE (2,4),V4,V5,V6,A1,A2,B1,B2,Z
2510 MAT WRITE FILE (2,1),W,Z,M
2520 CHAIN "DK10" THEN GOTO 0045
2600 REM************************GRAPH SUBROUTINE*****************************
2610 DIM V$(120)
2620 FOR I=1 TO N1
2630   LET X(I)=INT(W(I)*6+.5)
2640   IF EXP(Z(1))>1 THEN LET Y(1)=INT(Z(I)*19+.5)
2650 NEXT I
2690 FOR I=60 TO 3 STEP -1
2700   LET V$="          I  "
2710   IF I=60 THEN LET V$="  SERUM LEVEL"
2715   IF I=60 THEN LET V$(35)="* * * *     SERUM LEVELS"
2720   IF I=59 THEN LET V$="  (UG/ML) "
2725   IF I=59 THEN LET V$(35)="+ + + +      REGRESSION LINE"
2780   FOR J=1 TO N1
2783     IF INT(.5+19*(A+B*J))=I-3 THEN LET V$(J*6+10,J*6+10)="+"
2789     IF EXP(Z(J))<1 THEN GOTO 2800
2790     IF Y(J)=I-3 THEN LET V$(X(J)+10,X(J)+10)="*"
2800     IF INT(.5+19*LOG(J))=I-3 THEN LET V$(7,8)=STR$(J)
2810   NEXT J
2815   IF I=60 THEN PRINT FILE (1),"<12>"
2820   PRINT FILE (1),V$
2830 NEXT I
2840 LET V$="-----------------------------------------"
2850 PRINT FILE (1),V$(1,30);V$(1,30);V$(1,30);V$(1,30)
2855 PRINT FILE (1),"          ";
2870 PRINT FILE (1),USING "##    ",0,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
2880 PRINT FILE (1),"         HOURS AFTER DOSE"
2910 RETURN
3570 REM************************AUC TRAPEZOIDAL ROUTINES*********************
3580 REM   b is negative in this segment. m(1,-)=time  m(2,-)=level
3590 IF W=0 THEN INPUT "Weight ? ",W
3620 LET C1=0
3630 LET C0=0
3640 FOR I3=2 TO N3
3642   LET M(2,I3)=M(2,I3)-M(2,1)*EXP(B*M(1,I3))
3644   IF I3<3 THEN GOTO 3660
```

```
3650   LET C1=(M(2,I3)+M(2,I3-1))/2*(M(1,I3)-M(1,I3-1))
3655   LET C0=C0+C1
3660 NEXT I3
3690 IF B$(2,2)="M" THEN GOTO 3750
3710 LET C1=E/T8/W/.3/ABS(B)*(T8-(1-EXP(B*T8))/ABS(B))
3715 LET C0=C0+C1
3720 LET M5=EXP(A-ABS(B)*T8)-M(2,1)*EXP(B*T8)
3721 IF Z=2 THEN LET M5=EXP(A1-ABS(B1)*T8)+EXP(A2-ABS(B2)*T8)-M(2,1)*EXP(B2*T8)
3722 LET C1=(M5+M(2,2))*(M(1,2)-T8)/2
3740 GOTO 3755
3750 LET C1=K8*E/.3/W/(K8-ABS(B))*((EXP(-K8*M(1,2))-1)/K8-(EXP(B*M(1,2))-1)/ABS(B))
3755 LET C0=C0+C1
3810 LET C0=C0+(EXP(A+B*M(1,N3))-EXP(LOG(M(2,1))+B*M(1,N3)))/ABS(B)
3840 LET W0=E/C0/ABS(B)
3845 IF B$(2,2)="V" THEN GOTO 3855
3850 LET C0=C0-C1+C1*.3*W/W0
3852 LET W1=W0
3853 LET W0=E/C0/ABS(B)
3854 LET W0=E/(C0-C1+C1*W1/W0)/ABS(B)
3855 PRINT FILE (1),USING "<10>WEIGHT ###.# : INTERVAL ##.# : DOSE ### ##<10><13>AUC ###.# :",W,T2,E,B$,C0,
3857 LET D1=C0/T2
3860 PRINT FILE (1),USING " MEAN LEVEL ##.##   VOLUME OF DISTRIBUTION ###.## : (###.# % BODY WEIGHT)",D1,W0,W0/W*100
3870 PRINT "VOL DISTRIB ",E/C0/ABS(B)," (";E/C0/ABS(B)/W*100;"% BODY WEIGHT)"
3885 IF W0/W>.17 THEN IF W0/W<.35 THEN GOTO 3891
3887 PRINT FILE (1),"<10><14>WARNING !<15> VOLUME OUTSIDE GENTAMICIN NORMAL RANGE. CHECK TROUGH."
3891 PRINT FILE (1),"DOSES REQUIRED TO ACHIEVE STEADY-STATE MEAN LEVEL OF 2.5UG/ML:"
3892 PRINT FILE (1),USING "6 HOURS: ###,   8 HOURS: ###,   12 HOURS: ###,",2.5/C0*E*6,2.5/C0*E*8,2.5/C0*E*12
3893 PRINT FILE (1),"<10>DOSES REQUIRED TO ACHIEVE STEADY-STATE MEAN LEVEL OF 4 UG/ML:"
3894 PRINT FILE (1),USING "8 HOURS: ###,   12 HOURS: ###,   24HOURS: ###<10>",4/C0*E*8,4/C0*E*12,4/C0*E*24
3930 RETURN
5000 REM************************PREDICTIVE ROUTINE*******************************
5002 MAT T=ZER(7,5)
5003 MAT N6=ZER(9)
5004 IF W=0 THEN IF Y$(2,2)<>"Y" THEN INPUT "WEIGHT? ",W
5006 IF Y$(2,2)<>"Y" THEN LET W0=.3*W
5008 LET W8=W0
5010 FOR I4=1 TO 9
5011   PRINT "DOSING PERIOD ";I4
5012   INPUT "Dose, Number, Interval, Route, Predict ? ",E,N6,T2,B$(1,2),Y$(6,6)
5013   IF E=0 THEN IF N6=0 THEN GOTO 2310
5014   IF I4=1 THEN LET N6(1)=N6
5015   IF I4>1 THEN LET N6(I4)=N6(I4-1)+N6
```

```
5016   LET D7=T2
5017   LET M=N6
5018   LET W0=W8
5020   PRINT FILE (1),USING "<14>                    DOSING PERIOD #<15><13>",I4
5022   PRINT FILE (1),USING "## DOSES OF ###MG (##) EVERY ## HOURS<13>  PREDICTIONS FOR DOSE ##",N6,E,B$(1,2),T2,N6(I4)
5023   IF Y$(6,6)<>"Y" THEN GOTO 5030
5025   INPUT "Input 5 times (not peak/trough) for prediction ",D(2),D(3),D(4),D(5),D(6)
5026   FOR I=2 TO 6
5027     LET D(I)=INT(D(I))+(D(I)-INT(D(I)))/.6
5028   NEXT I
5029   LET D(7)=T2
5030   FOR I5=1 TO 5
5040     IF I5=1 THEN IF Y$(3,3)<>"Y" THEN LET I5=2
5050     IF I5=2 THEN IF Y$(4,4)<>"Y" THEN LET I5=5
5060     IF I5=5 THEN IF Y$(5,5)<>"Y" THEN GOTO 5590
5065     IF I5>1 THEN LET W0=.3*W
5067     IF I5=5 THEN LET W0=W8
5070     IF I5=2 THEN LET B=ABS(K)
5080     IF I5=3 THEN LET B=ABS(K1)
5090     IF I5=4 THEN LET B=ABS(K2)
5100     IF I5=5 THEN INPUT "INPUT K ",B
5110     IF I5=1 THEN LET B=ABS(B0)
5120     LET D(1)=LOG(K8/B)/(K8-B)
5160     IF N6>0 THEN LET T5=T(7,I5)
5245     IF Y$(6,6)<>"Y" THEN GOSUB 5610
5255     LET T(7,I5)=T5
5260     IF Y$(6,6)<>"Y" THEN GOTO 5719
5475     IF I5<>2 THEN IF I5<>3 THEN GOSUB 5690
5490     FOR I=1 TO 7
5500       IF B$(2,2)="V" THEN IF I=1 THEN GOTO 5515
5505       LET D7=D(I)
5510       GOSUB 5608
5512       LET T(I,I5)=T5
5513       LET T5=T(7,I5)
5514       IF I5<>2 THEN IF I5<>3 THEN GOSUB 5800
5515     NEXT I
5516     GOTO 5719
5590   NEXT I5
5600 NEXT I4
5608 IF M<0 THEN GOTO 5640
5610 LET T3=K8*E/W0/(K8-B)*(1-EXP(-M*B*T2))/(1-EXP(-B*T2))*EXP(-B*D7)
5620 LET T4=K8*E/W0/(K8-B)*(1-EXP(-M*K8*T2))/(1-EXP(-K8*T2))*EXP(-K8*D7)
```

```
5624 IF B$(2,2)="V" THEN LET T3=E/T8/W0/B*(1-EXP(-B*T8))*(1-EXP(-B*M*T2))/(1-EXP(-B*T2))*EXP(-B*(D7-T8))
5625 IF B$(2,2)="V" THEN LET T4=0
5630 LET T5=T3-T4+T5*EXP(-B*((M-1)*T2+D7))
5640 RETURN
5680 REM**********************SHORT OUTPUT ROUTINES ETC**********************
5690 IF I5<>4 THEN GOTO 5700
5692 PRINT FILE (1),"TIME      JADRNY      COCKCROFT      SIERSBAEK"
5693 PRINT FILE (1),USING "      K=    .###          .###          .###",K,K1,K2
5696 GOTO 5703
5700 PRINT FILE (1),USING "<10>##########   K=.###<13>##########",Z$(10*I5-9,10*I5),B
5701 IF I5=1 THEN PRINT FILE (1)," TIME     PREDICTIONS (UG/ML) FOR VOLUMES:<13>(HOURS)      V         V(1)      V(2)      V(3)"
5702 IF I5=5 THEN PRINT FILE (1)," TIME      PREDICTIONS<13>(HOURS)    (UG/ML)"
5703 RETURN
5719 LET D8=LOG(K8/B*(1-EXP(-B*T2))/(1-EXP(-K8*T2)))/(K8-B)
5720 LET C7=K8*E/W0/(K8-B)*((1/(1-EXP(-B*T2))*EXP(-B*D8))-(1/(1-EXP(-K8*T2))*EXP(-2.16*D8)))
5730 IF Y$(6,6)<>"Y" THEN PRINT FILE (1),USING "########## K=.###   trough= #.#",Z$(10*I5-9,10*I5),B,T5;
5735 IF Y$(6,6)="Y" THEN IF I5<>1 THEN IF I5<>5 THEN GOTO 5590
5740 PRINT FILE (1),USING "   Steady-state IM max.= ##.# at #.# hours",C7,D8
5750 GOTO 5590
5800 IF I5=4 THEN PRINT FILE (1),USING "##.##      ##.##         ##.##         ##.##",D(I),T(I,2),T(I,3),T(I,4)
5804 IF V4=0 THEN IF I5<>4 THEN PRINT FILE (1),USING "##.##       ##.##",D(I),T(I,I5)
5806 IF I5=5 THEN PRINT FILE (1),USING "##.##      ##.##",D(1),T(I,I5)
5810 IF V4<>0 THEN IF I5=1 THEN PRINT FILE (1),USING "##.##      ",D(I),T(I,I5),T(I,I5)*W0/V4,T(I,I5)*W0/V5,T(I,I5)*W0/V6
5820 RETURN
9999 CLOSE
```

BI-EXPONENTIAL TEST SET OF IM DATA 1981

DATA SET OF 8 POINTS

CODE	TIME	CONC	TIME	LN(C)
1	0.00	0.79	0.00	-0.236
2	0.30	6.64	0.50	1.893
3	1.00	7.61	1.00	2.029
4	3.00	4.47	3.00	1.497
5	5.00	2.25	5.00	0.811
6	7.00	1.12	7.00	0.113
7	9.00	0.56	9.00	-0.580
8	11.00	0.28	11.00	-1.273

TERM	COEFFICIENT	RATE CONST.	CORRELN(R)	POINTS
1	11.694	2.3504	-1.000	3
2	12.316	- 0.5466	-1.000	5
*	********	********	-1.000	8

THESIS TEST SET OF IM DATA 1981

DATA SET OF 8 POINTS

CODE	TIME	CONC	TIME	LN(C)
1	0.00	0.79	0.00	-0.236
2	0.30	6.64	0.50	1.893
3	1.00	7.61	1.00	2.029
4	3.00	4.47	3.00	1.497
5	5.00	2.25	5.00	0.811
6	7.00	1.12	7.00	0.113
7	9.00	0.56	9.00	-0.580
8	11.00	0.28	11.00	-1.273

5 DATA POINTS USED FOR REGRESSION WITH CODES:
4 5 6 7 8
8 DATA POINTS USED FOR AUC WITH CODES:
1 2 3 4 5 6 7 8
AGE 50 : WEIGHT 50.0 Kg Creatinine 92.8

INTERCEPT 12.68 : CALC K .347 : HALF-LIFE 2.00
Correlation (r)=-1.000 : coeff var= 0.0

Volumes from (1)INTERCEPT (2)INTERCEPT-TROUGH (3INTERCEPT-CALC TROUGH

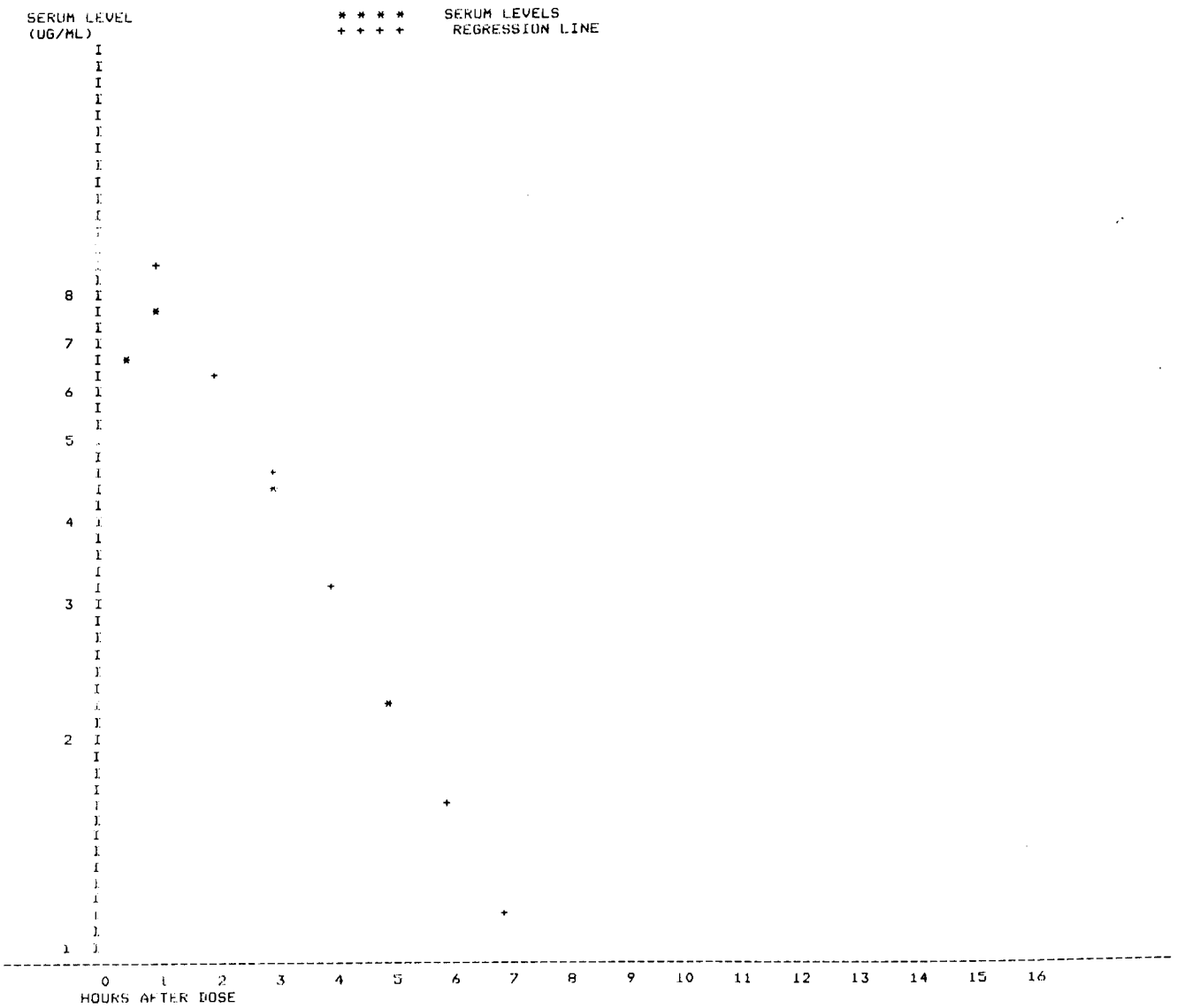
9.40 10.02 9.55

	k elimn.	half-life	doses at 8hour intervals to give levels of 2.5ug/ml	4.0ug/ml
	********	**********		
Jadrny	.286308	2.42047	85.895	137.428
Cockcroft & Gault	.197045	3.517	59.117	94.5807
Sierpsaek-Nielsen	.217842	3.25593	63.855	102.164

WEIGHT 50.0 : INTERVAL 12.0 : DOSE 100 IM

AUC 28.9 : MEAN LEVEL 2.41 VOLUME OF DISTRIBUTION 7.99 : (20.0 % BODY WEIGHT)
DOSES REQUIRED TO ACHIEVE STEADY-STATE MEAN LEVEL OF 2.UG/ML:
6 HOURS: 52, 8 HOURS: 69, 12 HOURS: 104,

DOSES REQUIRED TO ACHIEVE STEADY-STATE MEAN LEVEL OF 4 G/ML:
8 HOURS: 111, 12 HOURS: 166, 24HOURS: 332


SERUM LEVEL
(UG/ML)
* * * * SERUM LEVELS
+ + + + REGRESSION LINE
8
7
6
5
4
3
2
1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
HOURS AFTER DOSE

DOSING PERIOD 1

2 DOSES OF 100MG (IM) EVERY 8 HOURS
PREDICTIONS FOR DOSE 2

BLOOD DATA	K=.347	trough= 0.8	Steady-state IM max.= 7.6 at 1.0 hours
JADRNY	K=.286	trough= 0.9	Steady-state IM max.= 5.5 at 1.0 hours
COCKCROFT	K=.197	trough= 1.8	Steady-state IM max.= 6.8 at 1.1 hours
SIERSBAEK	K=.213	trough= 1.6	Steady-state IM max.= 6.5 at 1.1 hours
KEYBOARD	K=.346	trough= 0.8	Steady-state IM max.= 7.6 at 1.0 hours

DOSING PERIOD 2

3 DOSES OF 100MG (IM) EVERY 8 HOURS
PREDICTIONS FOR DOSE 5

BLOOD DATA K=.347
****

TIME (HOURS)	PREDICTIONS (UG/ML) FOR VOLUMES: V	V(1)	V(2)	V(3)
1.01	7.62	8.10	7.97	7.59
2.00	6.20	6.59	6.49	6.18
3.00	4.48	4.76	4.69	4.46
4.00	3.18	3.38	3.33	3.17
5.00	2.25	2.39	2.35	2.24
6.00	1.59	1.69	1.66	1.58
8.00	0.80	0.85	0.83	0.79

Steady-state IM max.= 7.6 at 1.0 hours

TIME	JADRNY	COCKCROFT	SIERSBAEK
K=	.286	.197	.213
1.19	5.53	6.74	6.45
2.00	4.72	6.14	5.81
3.00	3.61	5.11	4.76
4.00	2.72	4.20	3.86
5.00	2.04	3.45	3.12
6.00	1.53	2.83	2.52
8.00	0.87	1.91	1.65

KEYBOARD K=.346
****

TIME (HOURS)	PREDICTIONS (UG/ML)
1.01	7.63
2.00	6.21
3.00	4.49
4.00	3.19
5.00	2.26
6.00	1.60
8.00	0.80

Steady-state IM max.= 7.6 at 1.0 hours

DOSING PERIOD 3

6 DOSES OF 80MG (IV) EVERY 8 HOURS
PREDICTIONS FOR DOSE 11

BLOOD DATA K=.347
****

TIME (HOURS)	PREDICTIONS (UG/ML) FOR VOLUMES: V	V(1)	V(2)	V(3)
2.00	4.34	4.61	4.54	4.32
3.00	3.07	3.26	3.21	3.05
4.00	2.17	2.30	2.27	2.16
5.00	1.53	1.63	1.60	1.53
6.00	1.08	1.15	1.13	1.08
8.00	0.54	0.58	0.57	0.54

Steady-state IM max.= 6.1 at 1.0 hours

TIME	JAORNY	COCKCROFT	SIERSBAEK
K=	.286	.197	.213
2.00	3.39	4.57	4.30
3.00	2.54	3.75	3.47
4.00	1.91	3.08	2.81
5.00	1.43	2.53	2.27
6.00	1.08	2.08	1.83
8.00	0.61	1.40	1.20

KEYBOARD K=.346
****

TIME (HOURS)	PREDICTIONS (UG/ML)
2.00	4.34
3.00	3.07
4.00	2.17
5.00	1.54
6.00	1.09
8.00	0.54

Steady-state IM max.= 6.1 at 1.0 hours

```
RUN
SELECT THE OPTION YOU REQUIRE(7=INFO)? 1
INPUT PATIENT IDENTIFIER THESIS TEST SET OF IM DATA 1981
INPUT DOSE,ROUTE,INTERVAL AND NO. OF DATA POINTS 100,IM,12,8
INPUT PAIRS OF DATA POINTS, ONE PAIR TO A LINE
? 0,.79
? .3,6.64
? 1,7.61
? 3,4.47
? 5,2.25
? 7,1.12
? 9,.56
? 11,.28
1 OR 2 EXPONENTIAL CURVE? 1
HOW MANY POINTS FOR K- ELIMN. ? 5
INPUT CODE-NUMBERS SEPARATED BY CR
 4  ? 5  ? 6  ? 7  ? 8
HOW MANY POINTS FOR AUC? 8
AGE,WEIGHT,CREAT.,M/F  ? 50,50,92.8,M
CO=12.6581     CALC. K =-.346089      HALF-LIFE=2.00238
DO YOU WANT TO RECORD THIS DATA IN FILE 'RUNDATA' ? N
GRAPH/AUC/PREDICT/WITH CR./FROM INPUT ? YYYYY
VOL. DISTRIB = 10.0064   (20.0129% BODY WEIGHT)

DOSING PERIOD 1
DOSE,NUMBER,INTERVAL,ROUTE,PREDICT  ? 100,2,8,IM,N
INPUT K .346
```

```
DOSING PERIOD 2

DOSE,NUMBER,INTERVAL,ROUTE,PREDICT ? 100,3,8,IM,Y

INPUT 5 TIMES (NOT PEAK/TROUGH) FOR PREDICTION  2,3,4,5,6

INPUT K .346
 DOSING PERIOD 3

DOSE,NUMBER,INTERVAL,ROUTE,PREDICT ? 80,6,12,IV,Y

INPUT 5 TIMES (NOT PEAK/TROUGH) FOR PREDICTION 2,3,4,5,6

INPUT K .346

DOSING PERIOD 4

DOSE,NUMBER,INTERVAL,ROUTE,PREDICT ?  0,0,0,0,0

SELECT THE OPTION YOU REQUIRE (?=INFO) ?
```

[illegible] FILE

```
5000 REM**************************************************************
5010 INPUT "HOW MANY DOSING PERIODS ? ",I3
5020 IF I3=0 THEN GOTO 2310
5030 FOR I5=1 TO 5
5040   IF I5=1 THEN IF Y$(3,3)<>"Y" THEN LET I5=2
5050   IF I5=2 THEN IF Y$(4,4)<>"Y" THEN LET I5=5
5060   IF I5=5 THEN IF Y$(5,5)<>"Y" THEN IF Y$(7,7)<>"Y" THEN GOTO 5750
5070   IF I5=2 THEN LET B=ABS(K)
5080   IF I5=3 THEN LET B=ABS(K1)
5090   IF I5=4 THEN LET B=ABS(K2)
5110   IF I5=1 THEN LET B=ABS(B0)
5160   LET T5=0
5170   IF Y$(6,6)="Y" THEN LET T5=Z(1)
5180   IF Y$(8,8)="R" THEN LET T5=T(I5)
5190   FOR I4=1 TO I3
5200     IF Y$(2,2)="Y" THEN GOTO 5270
5220     IF W0<>0 THEN GOTO 5280
5230     INPUT "WEIGHT,DOSE,NUMBER,INTERVAL,ROUTE",W,E,NO,T2,B$
5250     LET W0=.3*W
5260     GOTO 5310
5270     LET W0=E/C0/ABS(B)
5280     PRINT V$(10*I5-9,10*I5)
5285     IF I4=1 THEN IF Y$(7,7)="Y" THEN GOTO 5310
5290     INPUT "DOSE, NUMBER, INTERVAL, ROUTE ? ",E,NO,T2,B$
5300     IF NO=0 THEN GOTO 5750
5310     LET D(7)=T2
5312     PRINT FILE (1),USING "<10><14>DOSING PERIOD # (##########  K)<15>",I4,V$(10*I5-9,10*I5)
5315     IF I5=5 THEN IF Y$(7,7)<>"Y" THEN INPUT "INPUT DESIRED K ",B
5317     PRINT FILE (1),USING "## ####mg ** doses of # hour intervals. K=-.###",NO,E,B$,T2,B
5320     IF Y$(7,7)<>"Y" THEN GOTO 5380
5330     INPUT "K1,K2,LIN/EXP ? ",B1,B2,B$(3,5)
5340     LET B3=(B2-B1)/(NO-1)
5350     IF B$(3,5)="EXP" THEN LET B3=(B2/B1)^(1/(NO-1))
5355     FOR I6=1 TO NO-1
5360       LET B=B1+(I6-1)*B3
5365       IF B$(3,5)="EXP" THEN LET B=B1*B3^(I6-1)
5370       LET T0=K8*E/W0/(K8-B)*(EXP(-B*T2)-EXP(-K8*T2))+T5*(EXP(-B*T2))
5375       LET T5=T5+T0     ,
5377     NEXT I6
5380     LET N5=N6(I5)+NO
5382     IF Y$(7,7)="Y" THEN LET NO=1
5385     IF I4=I3 THEN GOTO 5410
```

```
5390      LET I=7
5400      GOTO 5610
5410      PRINT "Input 5 times,(not peak/trough) for prediction"
5420      INPUT D(2),D(3),D(4),D(5),D(6)
5430      FOR I=2 TO 6
5440        LET D(I)=INT(D(I))+(D(I)-INT(D(I)))/.6
5450      NEXT I
5460      LET D(1)=LOG(2.16/ABS(B))/(2.16-B)
5470      LET D(8)=LOG(2.16/ABS(B)*(1-EXP(-B*T2))/(1-EXP(-2.16*T2)))/(2.16-ABS(B))
5475      PRINT FILE (1),
5480      PRINT FILE (1),USING "PREDICTIONS FOR DOSE ## USING K=.### FROM ##########",N5,B,V$(10*I5-9,10*I5)
5490      IF B$(2,2)="M" THEN GOTO 5600
5500      LET N0=N0-1
5510      LET I=7
5520      GOTO 5610
5530      FOR I=2 TO 7
5540        REM iv single dose level
5550        LET J1=E/.0833/W0/ABS(B)*(1-EXP(-B*.0833))*EXP(-B*(D(I)-.0833))
5560        LET J2=T0*EXP(-B*D(I))
5570        PRINT FILE (1),USING "##.## HOURS       ##.## UG/ML",D(I),J1+J2
5580      NEXT I
5590      GOTO 5710
5600      FOR I=1 TO 7
5610        LET T3=K8*E/W0/(K8-B)*(1-EXP(-N0*B*T2))/(1-EXP(-B*T2))*EXP(-B*D(I))
5620        LET T4=K8*E/W0/(K8-B)*(1-EXP(-N0*2.16*T2))/(1-EXP(-2.16*T2))*EXP(-2.16*D(I))
5630        LET T0=T3-T4+T5*EXP(-ABS(B)*((N0-1)*T2+D(I)))
5640        IF B$(2,2)="V" THEN IF I3=14 THEN GOTO 5530
5650        IF I3=14 THEN GOTO 5690
5660        PRINT FILE (1),USING "TROUGH AT END OF ##TH DOSE= #.#",N5,T0
5670        LET T5=T0
5680        GOTO 5710
5690        PRINT FILE (1),USING "##.## HOURS       ##.## UG/ML",D(I),T0
5700      NEXT I
5710      LET T(I5)=T0
5711      LET N6(I5)=N5
5715    NEXT I4
5720    LET C7=K8*E/W0/(K8-B)*((1/(1-EXP(-B*T2))*EXP(-B*D(8)))-(1/(1-EXP(-K8*T2))*EXP(-2.16*D(8))))
5730    PRINT FILE (1),USING "STEADY STATE MAXIMUM (IM): ##.# AT #.# HOURS",C7,D(8)
5750 NEXT I5
5760 LET Y$(8,8)="R"
5770 GOTO 5010
9999 CLOSE
```

PROGRAM TO CALCULATE AND FILE PATIENT PARAMETERS

```
0010 REM PARAMWRT  TO WRITE PARAMETERS TO FILE PARAMFL
0015 CLOSE
0017 DIM A(200)
0020 OPEN FILE (2,0),"P2FILE",2000
0050 INPUT "HOW MANY PARAMETERS PER PATIENT? ",N
0055 PRINT "SPECIFY CODE NUMBERS FOR PARAMETERS TO BE INPUT"
0057 MAT INPUT C(N);
0060 READ FILE (2,0),K
0063 PRINT "THE LAST RECORD IN THE FILE IS";K
0065 INPUT "SPECIFY RECORD TO BE USED",K1
0067 IF K1=0 THEN GOTO 9999
0095 PRINT "INPUT DATA ON ONE LINE IN SET ORDER"
0100 FOR I=K1 TO K1+30
0110   MAT READ FILE (2,I),A
0140   FOR J=1 TO N
0144     ON ERR THEN PRINT "<7><7><7><7><7><7>"
0150     INPUT A(C(J));
0151     IF A(C(J))=9999 THEN GOTO 0065
0152     IF J=N THEN PRINT "RECORD COMPLETED"
0153   NEXT J
0155   ON ERR THEN STOP
0160   LET A(12)=A(7)*A(8)
0165   LET A(13)=A(7)*A(9)
0170   LET A(14)=A(7)*A(10)
0175   LET A(15)=A(7)*A(11)
0180   IF A(8)<>0 THEN LET A(16)=1/A(8)
0185   IF A(9)<>0 THEN LET A(17)=1/A(9)
0190   IF A(10)<>0 THEN LET A(18)=1/A(10)
0195   IF A(11)<>0 THEN LET A(19)=1/A(11)
0196   IF A(4)<>0 THEN LET A(20)=20*A(12)/A(4)
0197   IF A(5)<>0 THEN LET A(21)=20*A(12)/A(5)
0200   LET A(26)=A(22)*A(23)
0205   LET A(27)=A(22)*A(24)
0210   LET A(28)=A(22)*A(25)
0215   IF A(23)<>0 THEN LET A(29)=1/A(23)
0220   IF A(24)<>0 THEN LET A(30)=1/A(24)
0225   IF A(25)<>0 THEN LET A(31)=1/A(25)
0230   LET A2=A(20)*A(4)
0231   IF A(12)=0 THEN GOTO 0270
0235   LET A3=A2/A(12)
0240   LET A(32)=A3*A(13)-A2
0245   LET A(33)=A3*A(14)-A2
```

```
0250   LET A(34)=A3*A(15)-A2
0255   LET A(35)=A3*A(26)-A2
0260   LET A(36)=A3*A(27)-A2
0265   LET A(37)=A3*A(28)-A2
0270   IF A(4)<>0 THEN LET A(38)=A(8)/A(4)*100
0275   IF A(5)<>0 THEN LET A(39)=A(8)/A(5)*100
0280   IF A(5)<>0 THEN LET A(40)=A(8)/A(5)^1.5*100
0285   IF A(22)*A(7)<>0 THEN LET A(41)=(A(22)-A(7))/A(7)
0289   IF A(6)=0 THEN GOTO 0348
0290   LET X=A(3)
0292   LET W=A(4)
0293   GOSUB 0295
0294   GOTO 0348
0295   LET A(42)=95.94/((A(6)/84.4)^1.387)
0297   IF A(2)=2 THEN LET A(42)=/0.31/((A(6)/84.4)^1.307)
0300   LET A(43)=((140-X)/72)*W/A(6)*84.4
0302   IF A(2)=2 THEN LET A(43)=A(43)*.85
0305   LET A(44)=1.251E-07*X^4-3.049E-05*X^3+.002553*X^2-.09888*X+2.9476
0307   IF A(2)=2 THEN LET A(44)=-2.93E-07*X^4+7.25E-05*X^3-.00634*X^2+.217*X-1.091
0310   LET A(44)=A(44)*W/A(6)*84.4
0315   LET A(45)=(A(42)*.06-A(12))/A(12)
0316   LET A(46)=(A(43)*.06-A(12))/A(12)
0317   LET A(47)=(A(44)*.06-A(12))/A(12)
0320   RETURN
0348   IF A(8)=0 THEN GOTO 0360
0350   LET A8=A(8)
0351   LET A(48)=(A(9)-A8)/A8
0352   LET A(49)=(A(10)-A8)/A8
0353   LET A(50)=(A(11)-A8)/A8
0354   LET A(51)=(A(23)-A8)/A8
0355   LET A(52)=(A(24)-A8)/A8
0356   LET A(53)=(A(25)-A8)/A8
0360   IF A(57)<>0 THEN LET A(62)=1/A(57)
0362   IF A(58)<>0 THEN LET A(63)=1/A(58)
0364   IF A(60)<>0 THEN LET A(64)=1/A(60)
0366   IF A(61)<>0 THEN LET A(65)=1/A(61)
0370   LET A(66)=A(57)*A(56)
0372   LET A(67)=A(58)*A(56)
0374   LET A(68)=A(60)*A(59)
0376   LET A(69)=A(61)*A(59)
0380   IF A(73)<>0 THEN LET A(78)=1/A(73)
0382   IF A(74)<>0 THEN LET A(79)=1/A(74)
```

```
0384   IF A(76)<>0 THEN LET A(80)=1/A(76)
0385   IF A(77)<>0 THEN LET A(81)=1/A(77)
0387   LET A(82)=A(73)*A(72)
0389   LET A(83)=A(74)*A(72)
0391   LET A(84)=A(76)*A(75)
0393   LET A(85)=A(77)*A(75)
0400   IF A(58)*A(57)<>0 THEN LET A(86)=(A(58)-A(57))/A(57)
0402   IF A(60)*A(57)<>0 THEN LET A(87)=(A(60)-A(57))/A(57)
0404   IF A(61)*A(57)<>0 THEN LET A(88)=(A(61)-A(57))/A(57)
0406   IF A(73)*A(74)<>0 THEN LET A(89)=(A(74)-A(73))/A(73)
0408   IF A(73)*A(76)<>0 THEN LET A(90)=(A(76)-A(73))/A(73)
0410   IF A(73)*A(77)<>0 THEN LET A(91)=(A(77)-A(73))/A(73)
0412   IF A(56)*A(59)<>0 THEN LET A(92)=(A(59)-A(56))/A(56)
0413   IF A(72)*A(75)<>0 THEN LET A(93)=(A(75)-A(72))/A(72)
0415   IF A(66)<>0 THEN LET A(94)=A(66)*A3-A2
0416   IF A(67)<>0 THEN LET A(95)=A(67)*A3-A2
0417   IF A(68)<>0 THEN LET A(96)=A(68)*A3-A2
0418   IF A(69)<>0 THEN LET A(97)=A(69)*A3-A2
0419   IF A(82)<>0 THEN LET A(98)=A(82)*A3-A2
0420   IF A(83)<>0 THEN LET A(99)=A(83)*A3-A2
0421   IF A(84)<>0 THEN LET A(100)=A(84)*A3-A2
0422   IF A(85)<>0 THEN LET A(101)=A(85)*A3-A2
0423   IF A(56)<>0 THEN LET A(102)=(A(56)-A(7))/A(7)
0425   IF A(59)<>0 THEN LET A(103)=(A(59)-A(7))/A(7)
0427   IF A(72)<>0 THEN LET A(104)=(A(72)-A(7))/A(7)
0428   IF A(75)<>0 THEN LET A(105)=(A(75)-A(7))/A(7)
0429   IF A(57)<>0 THEN LET A(106)=(A(57)-A(8))/A(8)
0430   IF A(73)<>0 THEN LET A(107)=(A(73)-A(8))/A(8)
0479   IF A(56)=0 THEN GOTO 0483
0480   LET A(108)=(A(66)-A(12))/A(12)
0482   LET A(109)=(A(68)-A(12))/A(12)
0483   IF A(72)=0 THEN GOTO 0487
0484   LET A(110)=(A(82)-A(12))/A(12)
0486   LET A(123)=(A(84)-A(12))/A(12)
0487   IF A(22)=0 THEN GOTO 0497
0488   IF A(68)<>0 THEN LET A(111)=(A(68)-A(26))/A(26)
0489   IF A(84)<>0 THEN LET A(112)=(A(84)-A(26))/A(26)
0490   IF A(59)<>0 THEN LET A(113)=(A(59)-A(22))/A(22)
0492   IF A(75)<>0 THEN LET A(114)=(A(75)-A(22))/A(22)
0494   IF A(60)<>0 THEN LET A(115)=(A(60)-A(23))/A(23)
0496   IF A(76)<>0 THEN LET A(116)=(A(76)-A(23))/A(23)
0497   IF A(66)=0 THEN GOTO 0502
```

```
0498   LET A(117)=20*(A(67)-A(66))
0500   LET A(118)=20*(A(68)-A(66))
0501   LET A(119)=20*(A(69)-A(66))
0502   IF A(82)=0 THEN GOTO 0510
0503   LET A(120)=20*(A(83)-A(82))
0505   LET A(121)=20*(A(84)-A(82))
0507   LET A(122)=20*(A(85)-A(82))
0510   IF A(12)*A(42)<>0 THEN LET A(124)=20*(A(42)*.06-A(12))
0512   IF A(43)*A(12)<>0 THEN LET A(125)=20*(A(43)*.06-A(12))
0513   IF A(44)*A(12)<>0 THEN LET A(126)=20*(A(44)*.06-A(12))
0520   IF A(60)*A(8)<>0 THEN LET A(127)=(A(60)-A(8))/A(8)
0522   IF A(76)*A(8)<>0 THEN LET A(128)=(A(76)-A(8))/A(8)
0524   IF A(6)=0 THEN GOTO 0534
0526   LET A(129)=.012+.0034*A(42)
0527   LET A(130)=.012+.0034*A(43)
0528   LET A(131)=.012+.0034*A(44)
0530   IF A(12)=0 THEN GOTO 0534
0531   LET A(132)=20*(A(129)*.3*A(4)-A(12))
0532   LET A(133)=20*(A(130)*.3*A(4)-A(12))
0533   LET A(134)=20*(A(131)*.3*A(4)-A(12))
0534   LET A1=A(12)
0535   LET A6=A(6)
0536   LET A(12)=A(66)
0537   LET A(6)=A(55)
0538   IF A(55)*A(66)=0 THEN GOTO 0570
0540   GOSUB 0295
0541   FOR I9=42 TO 47
         LET A(I9+93)=A(I9)
         IF I9<45 THEN LET A(I9+105)=.012+.0034*A(I9+93)
         IF I9<45 THEN LET A(I9+111)=20*(A(I9+93)*.06-A(66))
0548   NEXT I9
0570   IF A(71)*A(82)=0 THEN GOTO 0600
0573   LET A(6)=A(71)
0574   LET A(12)=A(82)
0576   GOSUB 0295
0580   FOR I9=42 TO 47
         LET A(I9+99)=A(I9)
         IF I9<45 THEN LET A(I9+108)=.012+.0034*A(I9+99)
         IF I9<45 THEN LET A(I9+114)=20*(A(I9+99)*.06-A(82))
0589   NEXT I9
0600   LET A(6)=A6
0602   LET A(12)=A1
```

```
0605   IF A(55)*A(6)=0 THEN GOTO 0620
0607   LET A(159)=(A(55)-A(6))/A(6)
0609   LET A(160)=(A(135)-A(42))/A(42)
0610   LET A(161)=(A(136)-A(43))/A(43)
0611   LET A(162)=(A(137)-A(44))/A(44)
0620   IF A(71)*A(6)=0 THEN GOTO 0640
0622   LET A(163)=(A(71)-A(6))/A(6)
0624   LET A(164)=(A(141)-A(42))/A(42)
0626   LET A(165)=(A(142)-A(43))/A(43)
0628   LET A(166)=(A(143)-A(44))/A(44)
0640   IF A(8)*A(61)<>0 THEN LET A(167)=(A(61)-A(8))/A8)
0642   IF A(8)*A(77)<>0 THEN LET A(168)=(A(77)-A(8))/A8)
0644   IF A(11)*A(61)<>0 THEN LET A(169)=(A(61)-A(11))A(11)
0646   IF A(11)*A(77)<>0 THEN LET A(170)=(A(77)-A(11))A(11)
0648   IF A(4)<>0 THEN LET A(173)=A(171)/A(4)
0650   LET A(174)=A(173)*A(54)
0700   MAT WRITE FILE (2,I),A
0750   IF I>K THEN WRITE FILE (2,0),I
0800 NEXT I
9999 STOP
```

PROGRAM TO CALCULATE STD. DEVIATIONS FROM FILED DATA

```
0010 REM  PARAMSD   19/12/80  (COPY OF PARAMSD2)
0020 CLOSE
0030 MAT A=ZER(200)
0040 MAT C=ZER(200)
0050 MAT M=ZER(200)
0060 MAT D=ZER(200)
0070 MAT M1=ZER(200)
0080 MAT N1=ZER(200)
0090 DIM S5$(60),S6$(42)
0100 LET S5$="###     ##  -----.####   ###.#  ---.##   ####.###  #####.###"
0110 LET S6$="   ####.####   ###.#   ---.##   ####.###"
0120 OPEN FILE (1,1),"$TT01"
0130 OPEN FILE (2,0),"P2FILE",2000
0140 INPUT "CODES FOR 1ST AND LAST PATIENTS ? ",N2,N3
0150 INPUT "1ST AND LAST PARAMETERS ? ",P1,P
0160 IF P1*P=0 THEN GOTO 0550
0170 FOR I=N2 TO N3
0180   MAT READ FILE (2,I),B(P)
0190   FOR J=P1 TO P
0200     LET T=0
0210     IF I=N2 THEN LET N1(J)=N3-N2+1
0220     IF B(J)=0 THEN GOTO 0270
0230     LET A(J)=A(J)+B(J)
0240     LET C(J)=C(J)+B(J)*B(J)
0250     LET D(J)=D(J)+ABS(B(J))
0260     GOTO 0280
0270     LET N1(J)=N1(J)-1
0280     IF I<N1(J) THEN GOTO 0530
0290     IF N1(J)<>0 THEN LET M(J)=A(J)/N1(J)
0300     IF N1(J)>0 THEN LET M1(J)=D(J)/N1(J)
0310     IF N1(J)>1 THEN GOTO 0320
0312     LET S=0
0313     LET S4=0
0314     GOTO 0400
0320     LET S2=1/(N1(J)-1)*(C(J)-A(J)*A(J)/N1(J))
0330     LET S3=1/(N1(J)-1)*(C(J)-D(J)*D(J)/N1(J))
0340     LET S=SQR(S2)
0350     LET S4=SQR(S3)
0360     IF S<>0 THEN IF N1(J)>0 THEN LET T=M(J)/S*SQR(N1(J)-1)
0370     IF S4<>0 THEN IF N1(J)>0 THEN LET T1=M1(J)/S4*SQR(N1(J)-1)
0380     IF S*M(J)<>0 THEN LET C0=S/M(J)*100
0390     IF S4*M1(J)<>0 THEN LET C1=S4/M1(J)*100
```

```
0400      IF J<15 THEN IF J<20 THEN IF M(J)<>0 THEN LET M(J)=1/M(J)
0410      IF J<20 THEN IF J<32 THEN IF M(J)<>0 THEN LET M(J)=1/M(J)
0420      IF J<77 THEN IF J<82 THEN IF M(J)<>0 THEN LET M(J)=1/M(J)
0430      IF J<61 THEN IF J<66 THEN IF M(J)<>0 THEN LET M(J)=1/M(J)
0440      IF I<>N3 THEN GOTO 0530
0450      IF J<>P1 THEN GOTO 0490
0460      PRINT FILE (1),"<12>";SYS(1);SYS(2);SYS(3),"PATIENTS";N2;" TO";N3
0470      PRINT FILE (1),"PARAM     N       MEAN      CO.VAR.    I       ";
0480      PRINT FILE (1)," STD.DEV    SUM SQ.     ABS. MEAN      CV         I      STD. DEV"
0490      PRINT FILE (1),USING 555,J,N1(J),M(J),C0,I,S,C(J);
0500      PRINT FILE (1),USING 565,M1(J),C1,I1,S4
0510      IF J<>P THEN PRINT FILE (1),"(PARAMETERS ";P3;P4;P5;")"
0520      IF J<>P THEN GOTO 0700
0530    NEXT J
0540 NEXT I
0550 REM
0560 PRINT "JOINT CALCULATIONS CAN BE DONE ON UP TO 3 ARRAYS. "
0570 IF P1*P5<>0 THEN GOTO 0700
0580 PRINT " DATA IS BEING RETRIEVED AND ARRAYED FOR USE"
0590 FOR I=N2 TO N3
0600    MAT READ FILE (2,I),B(200)
0610    FOR J=1 TO 180
0620      IF B(J)<>0 THEN LET N1(J)=N1(J)+1
0630      LET A(J)=A(J)+B(J)
0640      LET C(J)=C(J)+B(J)*B(J)
0645      LET D(J)=D(J)+ABS(B(J))
0650    NEXT J
0660 NEXT I
0670 PRINT FILE (1),"<12>";SYS(1);SYS(2);SYS(3),"PATIENTS";N2;" TO";N3;"  JOINT CALCULATIONS"
0680 PRINT FILE (1),"PARAM     N       MEAN      CO.VAR.    I       ";
0690 PRINT FILE (1)," STD.DEV    SUM SQ.     ABS. MEAN      CV         I      STD. DEV"
0700 INPUT "WHICH ARRAYS ? ",P3,P4,P5
0710 IF P3=0 THEN GOTO 0840
0720 LET I=N3
0730 LET J=J+1
0740 IF P3<>0 THEN LET N1(J)=N1(P3)
0750 IF P4<>0 THEN LET N1(J)=N1(J)+N1(P4)
0760 IF P5<>0 THEN LET N1(J)=N1(J)+N1(P5)
0770 IF P3<>0 THEN LET A(J)=A(P3)
0780 IF P4<>0 THEN LET A(J)=A(J)+A(P4)
0790 IF P5<>0 THEN LET A(J)=A(J)+A(P5)
0800 IF P3<>0 THEN LET C(J)=C(P3)
```

```
0810 IF P4<>0 THEN LET C(J)=C(J)+C(P4)
0820 IF P5<>0 THEN LET C(J)=C(J)+C(P5)
0821 LET D(J)=D(P3)
0822 IF P4<>0 THEN LET D(J)=D(P3)+D(P4)
0830 GOTO 0290
0840 STOP
```

PROGRAM FOR CORRELATION AND REGRESSION ON FILED PARAMETERS

```
0005 REM     PARAMREG
0010 CLOSE
0020 MAT A=ZER(200)
0023 DIM C(200),D(200)
0027 MAT X=ZER(100)
0028 MAT Y=ZER(100)
0050 OPEN FILE (1,1),"STT01"
0100 OPEN FILE (2,0),"P2FILE",2000
0135 PRINT "REGRESSION OF X-VECTOR AND Y-VECTOR."
0136 PRINT "Each vector may contain upto 3 arrays, taken in sequence"
0140 INPUT "CODES FOR 1ST AND LAST PATIENTS ? ",N2,N3
0900 INPUT "X VECTORS ? ",P1,P3,P5;
0902 IF P1<>0 THEN INPUT "Y VECTORS ? ",P2,P4,P6
0905 IF P1*P2=0 THEN GOTO 9999
0945 LET N=0
0950 FOR I=N2 TO N3
0960   MAT READ FILE (2,I),A
0965   IF A(P1)*A(P2)=0 THEN GOTO 0978
0970   LET N=N+1
0975   LET X(N)=A(P1)
0977   LET Y(N)=A(P2)
0978   IF P3*P4=0 THEN GOTO 0990
0979   IF A(P3)*A(P4)=0 THEN GOTO 0983
0980   LET N=N+1
0981   LET X(N)=A(P3)
0982   LET Y(N)=A(P4)
0983   IF P5*P6=0 THEN GOTO 0990
0984   IF A(P5)*A(P6)=0 THEN GOTO 0990
0985   LET N=N+1
0986   LET X(N)=A(P5)
0987   LET Y(N)=A(P6)
0990 NEXT I
0995 IF N>2 THEN GOSUB 7000
0999 IF N>=3 THEN GOSUB 6000
1000 IF N<3 THEN PRINT FILE (1),"INSUFFICIENT DATA FOR REGRESSION OF";P1;" &";P2,
1001 IF N<3 THEN IF P3*P4<>0 THEN PRINT FILE (1),P3;" &";P4,
1002 IF N<3 THEN IF P5*P6<>0 THEN PRINT FILE (1),P5;" &";P6,
1003 IF N<3 THEN PRINT FILE (1)
1004 IF N<3 THEN GOTO 0900
1005 IF Z<>0 THEN GOTO 1050
1010 PRINT FILE (1),"<13><13>   REGRESSION CALCULATIONS FOR PATIENTS";N2;" TO";N3
1011 PRINT FILE (1),"           **************************************************"
```

```
1020 PRINT FILE (1),"<13><13>X VECTORS       Y VECTORS          N   INTERCEPT   SLOPE   CORRELN.";
1021 PRINT FILE (1),"     SD(A)   SD(B)    STD.ERR.    SPEARMAN"
1030 LET Z=999999
1050 PRINT FILE (1),USING "  ###",P1;
1053 IF P3*P4<>0 THEN PRINT FILE (1),USING " *** ***  ",P3,P5;
1055 IF P3*P4=0 THEN PRINT FILE (1),"          ";
1058 PRINT FILE (1),USING " ###",P2;
1060 IF P3*P4<>0 THEN PRINT FILE (1),USING " *** ***   ",P4,P6;
1061 IF P3*P4=0 THEN PRINT FILE (1),"          ";
1065 PRINT FILE (1),USING " **  --**.***   --**.***   --.** ",N,A,B,R0;
1070 PRINT FILE (1),USING "     **.***  ***.***   ***.***",T,U,S;
1075 PRINT FILE (1),USING "      ---.***",Z1
1090 PRINT "A,B,R,SPEARMAN",A;B;R;Z1
1100 GOTO 0900
6000 LET P9=0
6010 LET X1=0
6020 LET X2=0
6030 LET Y1=0
6040 LET D2=0
6043 LET D1=0
6046 LET F1=0
6050 LET F2=0
6060 FOR M=1 TO N
6070   LET P9=(X(M)*Y(M))+P9
6080   LET X1=X1+X(M)
6090   LET X2=X2+X(M)*X(M)
6100   LET Y1=Y1+Y(M)
6110 NEXT M
6120 LET X0=X1/N
6130 LET Y0=Y1/N
6140 FOR I=1 TO N
6150   LET D1=D1+(X(I)-X0)^2
6160   LET D2=D2+(Y(I)-Y0)^2
6170 NEXT I
6180 LET B=(N*P9-X1*Y1)/(N*X2-X1*X1)
6190 LET A=(Y1-B*X1)/N
6220 FOR I=1 TO N
6230   LET F1=F1+((Y(I)-(A+B*X(I)))^2)
6240   LET F2=F2+((Y0-(A+B*X(I)))^2)
6250 NEXT I
6260 LET S=(F1/(N-2))^.5
6270 LET T=S*SQR(1/D1)
```

```
6280 LET U=T*SQR(X2/N)
6290 LET V=(P9-N*X0*Y0)/(N-2)
6300 LET R0=(B/ABS(B))*SQR(F2/D2)
6310 RETURN
7000 REM    SPEARMAN ROUTINE FOR NON-PARAMETRIC RANK REGRESSION
7090 LET Z1=0
7100 FOR I=1 TO N
7110   LET C(I)=1
7120   LET D(I)=1
7130   FOR J=1 TO N
7150     IF X(J)>X(I) THEN LET C(I)=C(I)+1
7155     IF J<>I THEN IF X(J)=X(I) THEN LET C(I)=C(I)+.5
7160     IF Y(J)>Y(I) THEN LET D(I)=D(I)+1
7165     IF J<>I THEN IF Y(J)=Y(I) THEN LET D(I)=D(I)+.5
7200   NEXT J
7250   LET Z1=Z1+(C(I)-D(I))^2
7300 NEXT I
7320 LET Z1=1-(6*Z1/N/(N*N-1))
7500 RETURN
9999 STOP
```

17 2 1981 PATIENTS 1 TO 21

PARAM	N	MEAN	CO.VAR.	T	STD.DEV	SUM SQ.	ABS. MEAN	CV	T	STD. DEV
8	20	20.6735	29.3	14.86	6.064	9246.470	20.6735	29.3	14.86	6.064
9	20	18.5490	38.9	11.20	7.217	7870.950	18.5490	38.9	11.20	7.217
10	20	21.6960	42.7	10.22	9.255	11041.700	21.6960	42.7	10.22	9.255
12	40	19.6113	34.0	18.37	6.667	17117.400	19.6113	34.0	18.37	6.667
(PARAMETERS 8 9 0)										
13	40	21.1848	36.5	17.09	7.740	20288.200	21.1848	36.5	17.09	7.740
(PARAMETERS 8 10 0)										
14	60	20.3062	37.5	20.49	7.612	28159.100	13.0742	133.2	5.77	17.420
(PARAMETERS 8 9 10)										

REGRESSION CALCULATIONS FOR PATIENTS 1 TO 21

****

X VECTORS	Y VECTORS	N	INTERCEPT	SLOPE	CORRELN.	SD(A)	SD(B)	STD.ERR.	SPEARMAN
8	9	20	-02.413	01.014	.85	0.147	3.159	3.883	.826
8	10	20	-05.335	01.308	.86	0.186	3.990	4.905	.876
8 8 0	9 10 0	40	-03.875	01.161	.83	0.125	2.694	4.684	.830
8 8 8	9 10 11	60	-04.086	01.205	.87	0.088	1.886	4.015	.867

REGRESSION PROGRAMS FOR 200 DATA PAIRS FROM FILES

```
0003 REM STAT200 CHAINS STATMID200 THEN STATCAL200 AND RETURNS.  VERIFIED 15.5.80
0004 CLOSE
0005 OPEN FILE (1,0),"STATFL",20000
0007 OPEN FILE (2,1),"$TT01"
0050 DIM W(200),Z(200),X(200),Y(200),E(200)
0055 DIM A$(120)
0060 INPUT "SELECT THE OPTION YOU REQUIRE (7= info) ",D0
0070 IF D0<>7 THEN GOTO 0850
0540 PRINT "1  Takes LINEAR data and logs the Y values."
0550 PRINT "2  Reads linear data from DATA statements, all Xs then all Ys"
0600 PRINT "3  Asks for pairs of data and uses them as they are"
0630 PRINT "4  Enables the selection of different points from your original data set"
0640 PRINT "5  Reads linear data from specified file, (codes optionally from file)"
0690 PRINT "6  Accepts X-values with 3 y-values for each X"
0700 PRINT "7  Lists this information set"
0730 PRINT "8  Finishes the program."
0840 INPUT "SELECT THE OPTION YOU REQUIRE ",D0
0850 IF D0=INT(D0) THEN IF D0<9 THEN IF D0>0 THEN GOTO 1050
0860 PRINT "OPTION MUST BE INTEGER, >0, <9"
0870 GOTO 0840
0999 REM STATCAL200  RETURNS TO 1000
1000 RESET FILE (1)
1010 READ FILE (1,0),D0,N,N1,J
1015 MAT READ FILE (1,3),W,Z
1020 MAT X=ZER
1030 MAT Y=ZER
1050 ON D0 THEN GOTO 3130, 3590, 3590, 1130, 3900, 4040, 0540, 9999
1110 REM ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1120 REM            option 4 segment
1130 PRINT " HOW MANY POINTS WILL YOU USE IN THIS RUN?"
1140 INPUT N
1150 IF N=INT(N) THEN GOTO 1180
1160 PRINT " Use integers only ! Try again."
1170 GOTO 1140
1180 IF N=0 THEN GOTO 0840
1190 IF D0<>6 THEN IF 00<>6 THEN GOTO 1250
1200 IF 00=6 THEN IF D0<>4 THEN IF D0<>6 THEN GOTO 1250
1210 IF N>J THEN IF N<=N1 THEN GOTO 1250
1240 GOTO 1140
1250 IF N>2 THEN GOTO 1280
1260 PRINT " You need at least 3 points, try again"
1270 GOTO 1140
```

```
1280 IF N>N1 THEN GOTO 1300
1290 GOTO 1330
1300 PRINT USING "YOU HAVE ONLY ## POINTS, PLEASE TRY AGAIN.",N1
1320 GOTO 1140
1330 IF N=N1 THEN GOTO 1660
1331 IF D0=5 THEN INPUT "Give record number for codes to be used or 0 to use terminal",N8
1333 IF D0=5 THEN IF N8>0 THEN MAT READ FILE (3,N8),E(N)
1340 IF D0<>5 THEN PRINT "Input code-numbers for points required, 1 per line <13>(negative to delete 1 point)"
1370 FOR I=1 TO N
1380   IF D0<>5 THEN INPUT E(I)
1390   IF E(I)=INT(E(I)) THEN GOTO 1420
1400   PRINT " Input CODE as an integer. Try again."
1410   GOTO 1380
1420   IF E(I)<>9999 THEN GOTO 1450
1430   LET I=0
1440   GOTO 1130
1450   IF E(I)>N1 THEN GOTO 1510
1470   IF E(I)<0 THEN GOTO 1580
1480   LET X(I)=W(E(I))
1490   LET Y(I)=Z(E(I))
1500   GOTO 1560
1510   PRINT "This is not part of your data set, please start again"
1520   LET I=0
1530   GOTO 1560
1540   LET X(I)=W(I)
1550   LET Y(I)=Z(I)
1560 NEXT I
1570 GOTO 1700
1580 FOR J=1 TO N
1590   IF J=-E(I) THEN GOTO 1640
1600   LET X(J)=W(J)
1610   LET Y(J)=Z(J)
1620 NEXT J
1630 GOTO 1700
1640 LET J=J+1
1650 GOTO 1620
1660 FOR I=1 TO N
1670   LET X(I)=W(I)
1680   LET Y(I)=Z(I)
1690 NEXT I
1700 GOTO 3315
3130 REM          option 1 segment
```

```
3140 PRINT " HOW MANY DATA POINTS DO YOU WANT TO US?"
3150 INPUT N1
3160 IF N1=0 THEN GOTO 0780
3170 IF N1>2 THEN GOTO 3200
3180 PRINT "You need at least 3 points, try again"
3190 GOTO 3150
3200 PRINT " input pairs of x and y values, one pai to a line"
3210 FOR I=1 TO N1
3220   INPUT W(I),Z(I)
3230   IF W(I)<>9999 THEN GOTO 3260
3240   LET I=0
3250   GOTO 3140
3260   IF Z(I)>0 THEN GOTO 3290
3270   PRINT "this point is not possible, y must bepositive"
3280   GOTO 3140
3290   LET Z(I)=LOG(Z(I))
3300 NEXT I
3310 LET N=N1
3312 GOTO 1110
3315 RESET FILE (1)
3317 MAT WRITE FILE (1,1),X,Y
3318 WRITE FILE (1,0),D0,N,N1,J
3319 MAT WRITE FILE (1,3),W,Z
3320 INPUT "INPUT TITLE  ",A$
3322 PRINT FILE (2),"<13><10><10>",A$
3340 CHAIN "STATM(0200"
3570 REM ****************************************************************
3580 REM            option 3 segment
3590 PRINT " HOW MANY DATA POINTS DO YOU HAVE ALL TGETHER ?"
3600 INPUT N1
3610 IF N1=0 THEN GOTO 0840
3620 IF N1>2 THEN GOTO 3645
3630 PRINT "You need at least 3 points, try again"
3640 GOTO 3600
3645 IF D0=2 THEN GOTO 3800
3650 PRINT " input pairs of x and y values, one pai per line, separated by comma"
3660 FOR I=1 TO N1
3670   INPUT W(I),Z(I)
3680   IF W(I)<>9999 THEN GOTO 3710
3690   LET I=0
3700   GOTO 3590
3710 NEXT I
```

```
3715 GOTO 1110
3798 REM ----------------OPTION 2-------------------------
3800 FOR I=1 TO N1
3810   READ W(I)
3815 NEXT I
3820 FOR I=1 TO N1
3830   READ Z(I)
3840 NEXT I
3850 GOTO 1110
3900 REM--------------------OPTION 5 SEGMENT-----------------
3910 INPUT "FILENAME,RECORD FOR X, RECORD FOR Y",N$,N6,N7
3920 IF N6*N7=0 THEN GOTO 4000
3930 OPEN FILE (3,0),N$,1000
3950 MAT READ FILE (3,N6),W
3960 MAT READ FILE (3,N7),Z
3970 INPUT "HOW MANY POINTS ALL TOGETHER ? ",N1
3973 IF N1<3 THEN IF N1>0 THEN INPUT "You need at least 3 points. Try again, 0 to change option",N1
3975 IF N1>2 THEN GOTO 1110
4000 GOTO 0060
4030 REM  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4040 REM                         option 6 segment
4050 INPUT "How many X-values ?  You may use upto 66 with 3 Y-values for each ",J
4080 IF J>66 THEN GOTO 4050
4110 IF J=0 THEN GOTO 0780
4120 PRINT "Input data as x value and replicate y values"
4130 PRINT " IE.,    X,Y1,Y2,Y3 "
4140 FOR I=1 TO 3*J STEP 3
4150   INPUT X(I),Y(I),Y(I+1),Y(I+2)
4160   IF X(I)=9999 THEN GOTO 4050
4170   LET X(I+1)=X(I)
4180   LET X(I+2)=X(I)
4190 NEXT I
4200 FOR I=1 TO 3*J
4210   LET W(I)=X(I)
4220   LET Z(I)=Y(I)
4230 NEXT I
4240 LET N1=3*J
4245 GOTO 1110
```

```
0005 REM STATMID200
0010 REM                    T DATA
0020 REM  data assumes values for .975 to give 5% levels
0030 DATA 0,0,12.7,4.3,3.18,2.78,2.57,2.45,2.36
0040 DATA 2.31,2.26,2.23,2.2,2.18,2.16,2.14,2.13,2.12
0050 DATA 2.11,2.1,2.09,2.09,2.08,2.07,2.07,2.06,2.06,2.06,2.05,2.05
0060 REM          F DATA (2,N-2) alpha/2 5%
0070 DATA 0,0,199.5,19,9.55,6.94,5.79,5.14,4.74,4.46,4.26,4.1,3.98,3.88,3.81
0080 DATA 3.74,3.68,3.63,3.59,3.55,3.52,3.49,3.47,3.44,3.42,3.4,3.39
0090 DATA 3.35,3.35,3.34
0100 REM           F DATA (1,n-2) alpha/2  1%
0110 DATA 0,0,16211,198.5,55.55,31.33,22.78,18.63,16.24,14.69,13.61,12.83,12.23
0120 DATA 11.75,11.37,11.06,10.8,10.58,10.38,10.22,10.07,9.94,9.83,9.73,9.63
0130 DATA 9.55,9.48,9.41,9.34,9.28
0140 REM           F DATA (1,N-2)  alpha/2  5%
0150 DATA 0,0,647.8,38.51,17.44,12.22,10.01,8.81,8.07,7.57,7.21,6.94,6.72
0160 DATA 6.55,6.41,6.3,6.2,6.12,6.04,5.98,5.92,5.87,5.83,5.79,5.75
0170 DATA 5.72,5.69,5.66,5.63,5.61
0240 DIM X(200),Y(200),H(30),F(30)
0260 DIM K(10,3),A(30),B(30)
0300 FOR I=1 TO 30
0310   READ H(I)
0320 NEXT I
0330 FOR I=1 TO 30
0340   READ F(I)
0350 NEXT I
0360 FOR I=1 TO 30
0370   READ A(I)
0380 NEXT I
0390 FOR I=1 TO 30
0400   READ B(I)
0410 NEXT I
0415 RESET FILE (1)
0420 READ FILE (1,0),D0,N,N1,J
0430 MAT READ FILE (1,1),X,Y
3730 REM ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3740 REM                          option 7 segment
3750 PRINT FILE (2),"<13>                                          ***** DATA SET *****<13>"
3800 PRINT FILE (2),"CODE          X              Y     ",
3802 PRINT FILE (2),"CODE          X              Y     ",
3803 PRINT FILE (2),"CODE          X              Y"
3805 PRINT FILE (2),"****      *********      *********",
```

```
1710   LET P=(X(M)*Y(M))+P
1720   LET X1=X1+X(M)
1730   LET X2=X2+X(M)*X(M)
1740   LET Y1=Y1+Y(M)
1753   IF X(M)<L1 THEN LET L1=X(M)
1757   IF X(M)>L THEN LET L=X(M)
1760 NEXT M
1770 LET X0=X1/N
1780 LET Y0=Y1/N
1790 FOR I=1 TO N
1800   LET D1=D1+(X(I)-X0)^2
1810   LET D2=D2+(Y(I)-Y0)^2
1820 NEXT I
1830 REM p=$xy,x1=$x,x2=$x*x,d=$ of diffs. squared, x0 =mean,s= std error estimate, t=std dev. slope
1840 LET B=(N*P-X1*Y1)/(N*X2-X1*X1)
1850 LET A=(Y1-B*X1)/N
1870 FOR I=1 TO N
1880   LET F1=F1+((Y(I)-(A+B*X(I)))^2)
1890   LET F2=F2+((Y0-(A+B*X(I)))^2)
1900 NEXT I
1910 LET S=(F1/(N-2))^.5
1920 LET T=S*((1/D1)^.5)
1930 LET U=T*((X2/N)^.5)
1940 LET V=(P-N*X0*Y0)/(N-2)
1950 LET R0=(B/ABS(B))*(F2/D2)^.5
1960 LET R1=-X0/SQR(X2/N)
1970 LET F0=(N-2)*R0*R0/(1-R0*R0)
1975 LET P$="(Not significantly different from zero. p>0.05)"
1980 IF ABS(F0)>B9 THEN LET P$="(Significantly different from zero. p<0.05)"
1990 IF ABS(F0)>A9 THEN LET P$="(Highly significant. p<0.01)"
2000 IF D0=1 THEN PRINT FILE (2),USING "<13><13>log(e) Y =----.#### +###.##### X",A,B
2010 IF D0<>1 THEN PRINT FILE (2),USING "<13><13>Y =----.#### +###.##### X",A,B
2020 PRINT FILE (2),"<10>number of points =";N;"  range of X =";ABS(L-L1);"  correlation coefficient (r)=";R0
2030 PRINT FILE (2),"F ratio = ";F0;P$
2040 PRINT FILE (2),"standard error of estimate =",S
2050 PRINT FILE (2),"standard deviations : slope=";T;"  intercept =";U
2060 PRINT FILE (2),"<13>Correlation of slope and intercept =";-X0/SQR(X2/N)
2070 PRINT FILE (2),"       independantly-calculated limits (95%):"
2080 PRINT FILE (2),"          slope:";H9*T;"  intercept:";H9*U
2090 PRINT FILE (2),"       combinded-calculation of limits (95%):"
2100 PRINT FILE (2),"          slope:";T*SQR(2*F9*(1-R1*R1));"  intercept:";U*SQR(2*F9*(1-R1*R1))
2130 PRINT " input suitable y values to determine corresponding x values"
```

```
2130 PRINT " input suitable y values to determine corresponding x values"
2140 PRINT "Use upto 9 values, one per line, zero to finish"
2210 FOR I=1 TO 10
2220   INPUT Q(I)
2230   LET V2=I-1
2250   IF Q(I)>0 THEN IF D0=1 THEN LET Q(I)=LOG(Q(I))
2260   IF Q(1)=0 THEN LET I=10
2270   IF Q(I)=0 THEN GOTO 2430
2380   LET V(I)=(Q(I)-A)/B
2390   FOR M=1 TO 3
2400     LET K(I,M)=(S^2/B^2)*(1/M+1/N+(Q(I)-Y0)^2/(B^2*D1))
2410     LET K(I,M)=K(I,M)^.5*H9
2420   NEXT M
2421   IF D0<>1 THEN PRINT FILE (2),"<13>FOR Y=";Q(I);"   X=";V(I);"<13>"
2422   IF D0=1 THEN PRINT FILE (2),"<13>FOR INTERPOLATION ";EXP(Q(I));"  Y=";Q(I);"   X=";V(I);"<13>"
2423   PRINT FILE (2),"95% confidence limits for X (1,2,3 replicates):<13>";K(I,1),K(I,2),K(I,3)
2430 NEXT I
3110 INPUT "SELECT THE OPTION YOU REQUIRE ",D0
3113 WRITE FILE (1,0),D0,N,N1,J
3114 CHAIN "STAT200" THEN GOTO 1000
```

TEST SET FOR REGRESSION

***** DATA SET *****

CODE	X	Y	CODE	X	Y	CODE	X	Y
1	0.0000	0.0000	2	0.0000	0.0000	3	75.8800	155.5000
4	75.8800	154.5000	5	94.8500	190.3000	6	94.8500	190.3000
7	151.7700	302.0000	8	151.7700	307.0000	9	284.6000	568.0000
10	284.6000	582.0000						

Y = -.0676 + 2.01802 X

number of points = 10 range of X = 284.6 correlation coefficient (r)= .999826
F ratio = 22978.4 (Highly significant. p<0.01)
standard error of estimate = 3.99421
standard deviations : slope= 1.33035E-02 intercept = 2.05051

Correlation of slope and intercept =-.787759
independantly-calculated limits (95%):
slope: 3.07311E-02 intercept: 4.73669
combinded-calculation of limits (95%):
slope: 2.44747E-02 intercept: 3.77237

FOR Y= 150 X= 74.3639

95% confidence limits for X (1,2,3 replicates):
4.84853 3.61332 3.09387

FOR Y= 300 X= 148.694

95% confidence limits for X (1,2,3 replicates):
4.81324 3.56583 3.03827

FOR Y= 600 X= 297.355

95% confidence limits for X (1,2,3 replicates):
5.49298 4.4408 4.02947

```
      PROGRAM GENT(INPUT,OUTPUT,TAPE6,TAPE2=OUTPUT,TAPE1=INPUT)
      DIMENSION REGR(40),A(40,6),B(40,6),VD(40,6),REGX(40),PX(40)
      DIMENSION PY(40),CER(40,40),C(40,40),AUCX(40),AUCY(40),T(40)
      DIMENSION XSM(8),XSQ(8),TITLE(15),COL(12)
      COMMON YMN(16),YVAR(16),YSD(16),YCV(16),YSQ(16)
      REAL I,K,VB,PREX,TINF,C,CER,PER,SIG,TIMF,SAMI
      INTEGER TAU
      CALL GOSCRF(6)
C GENTER PRODUCES CONCENTRATIONS AT TIME INTERVALS SAMI (HOURS) DURING
C A NUMER (NOD) OF INFUSIONS OF DURATION TINF (MIN, GIVEN AT TIME
C INTERVALS TAU(INTEGER HOURS).THE DOSE PER INFUSION (MG/KG),VOLUME OF
C DISTRIBUTION VB (L/KG),ELIMINATION RATE CONSTANT K (1/HR) AND
C PERCENTAGE ASSAY ERROR (PER) MUST BE ALSO SUPPLIED BY THE USER AS
C FOLLOWS
      READ(1,15)(TITLE(I),I=1,15)
      WRITE(2,16)(TITLE(I),I=1,15)
      WRITE(6,16)(TITLE(I),I=1,15)
   15 FORMAT(15A4)
   16 FORMAT(1X,15A4//)
      READ(1,*)NRUNS
      READ(1,1)NOD,TINF,TAU,DOSE,VB,RANGE,PER,SAMI
C MAKE SURE TINF < SAMI  & TAU>TINF
    1 FORMAT(I3/F5.2,I3/F5.2,F5.2/F7.4,F5.2/F5.2)
      TIMF=TINF/60.0
      NOS=TAU/SAMI
      IF(NOS.GT.40) GOTO 999
      WRITE(2,2)DOSE,VB,NOD,TIMF,TAU,PER,SAMI,RANGE
      WRITE(6,2)DOSE,VB,NOD,TIMF,TAU,PER,SAMI,RANGE
    2 FORMAT(8X,'MONTE CARLO SIMULATION WITH PARAMETERS: '//
     2 3X,'DOSE(MG/KG)=  ',F8.4/3X,'VOLUME OF DISTRIBUTION (L/KG) = ',
     2F9.4/3X/3X,'TOTAL NO. OF DOSES = '
     2 ,I3/3X,'LENGTH OF INFUSION (HR) = ',F9.6/3X,'DOSE',
     2' INTERVAL (HRS)= ',I3/3X,'ASSAY ERROR(%) = ',F5.3/
     2' MEASUREMENTS AT ',F5.2, 'HOUR INTERVALS','  RANGE ',F7.4//)
      IF(TIMF.GE.SAMI.OR.TINF.GE.TAU) GOTO 999
      DO 10 KK=1,4
      K=KK*0.05
      WRITE(2,22)K
      WRITE(6,22)K
   22 FORMAT(//20X,'K ELIM. = ',F6.4/20X,17('*')//)
      DO 300 I=1,16
  300 YSQ(I)=YMN(I)=0.0
      DO 10 II=1,NRUNS
      DO 170 I=1,NOD
      PREX=DOSE*(EXP(K*TIMF)-1)*(1-EXP(-I*K*TAU))/
     1     (TIMF*VB*K*(1-EXP(-K*TAU)))
```

```
      WRITE(2,3)I
    3 FORMAT(//5X,'DOSE NUMBER ',I3//)
      WRITE(2,100)
  100 FORMAT(3X,'TIME(HRS)',3X,'CONCENTRATION(MG/ML)',8X,'EXACT CONC'
     3//)
      DO 20 J=1,NOS
      T(J)=J*SAMI
      C(J,I)=PREX*EXP(-K*T(J))
      SIG=0.0100*PER*C(J,I)
      CER(J,I)=G05DDF(C(J,I),SIG)
      WRITE(2,4)T(J),CER(J,I),C(J,I)
    4 FORMAT(4X,F6.2,8X,F5.1,18X,F9.5)
   20 CONTINUE
      IK1=3
      IK2=6
      DO 170 IK=IK1,IK2
      WRITE(2,9)IK
    9 FORMAT(1X,I4,' POINTS FOR REGRESSION '//)
      DO 110 M=1,IK
      REGX(M)=T(4+IFIX(RANGE/IK/SAMI*M+0.5))
  110 REGR(M)=ALOG(CER(4+IFIX(RANGE/IK/SAMI*M+0.5),I))
      CALL REG(REGX,REGR,IK,A(I,IK),B(I,IK),R,S,TT,U)
      WRITE(2,40)(REGX(L),L=1,IK),(REGR(L),L=1,IK)
   40 FORMAT(1X,12F9.3/)
      A(I,IK)=EXP(A(I,IK))
  695 WRITE(2,5)B(I,IK),A(I,IK)
    5 FORMAT(1X,/'  CALCULATED SLOPE IS ',F7.4,6X,'INTERCEPT IS ',F7.3)
      IF(I.LT.13)GOTO 170
      JJ=3
      BB=B(I-JJ,IK)
      VV=DOSE/TIMF/K*(EXP(K*TIMF)-1)*(1-EXP(-(I-JJ)*K*TAU))/
     1(1-EXP(-K*TAU))/PREX
      DO 410 J=1,NOS
      IF(I.EQ.13.AND.J.EQ.1)XSM(IK)=XSQ(IK)=0.0
      PR=DOSE*(EXP(-BB*TIMF)-1)*(1-EXP(I*BB*TAU))
     1/(-TIMF*VV*BB*(1-EXP(BB*TAU)))
      PR=PR*EXP(BB*T(J))
      ER=(PR-CER(J,I))/CER(J,I)
      XSM(IK)=XSM(IK)+ER
  410 XSQ(IK)=XSQ(IK)+ER*ER
  400 CONTINUE
  170 CONTINUE
   10 CALL SDEV(NRUNS,II,XSM,XSQ)
      GOTO 9999
  999 WRITE(2,13)
   13 FORMAT(1X,'INPUT ERROR. DATA INCOMPATIBLE. CHECK!!!')
 9999  STOP
      END
```

```
C  THREE SUBROUTINES TO ANALYSE GENERATED DATA
      SUBROUTINE SDEV(NPTS,II,XSM,XSQ)
      DIMENSION XSM(8),XSQ(8)
      DIMENSION X(20,16),XMN(16),XVAR(16),XSD(16),CV(16)
      COMMON YMN(16),YVAR(16),YSD(16),YCV(16),YSQ(16)
      DATA DESC1/'PATIENT '/,DESC2/'  TOTAL '/
      DO 700 K=3,6
      XMN(K)=XSM(K)/256
      YMN(K)=YMN(K)+XMN(K)/NPTS
      YSQ(K)=YSQ(K)+XSQ(K)
      XVAR(K)=(XSQ(K)-256*XMN(K)*XMN(K))/18
      XSD(K)=SQRT(XVAR(K))
  700 CV(K)= XSD(K)/XMN(K)*100
      WRITE(6,740)DESC1,II
  740 FORMAT(////1X,1A8,'NUMBER ',I4/1X,19('*')//)
      DO 750 K=3,6
  750 WRITE(6,4)K,XMN(K),XVAR(K),XSD(K),CV(K)
    4 FORMAT(1X,I4,' POINTS USED FOR REGRESSION'
     1/1X,' MEAN ERROR,VAR,SDEV,CV ',4F10.4)
      IF(II.LT.NPTS)RETURN
      DO 900 K=3,6
      YVAR(K)=(YSQ(K)-NPTS*256*YMN(K)*YMN(K))/(NPTS*256-1)
      YSD(K)=SQRT(YVAR(K))
  900 YCV(K)=YSD(K)/YMN(K)*100
      ITOT=NPTS*256
      WRITE(6,740)DESC2,ITOT
      DO 760 K=3,6
  760 WRITE(6,4)K,YMN(K),YVAR(K),YSD(K),YCV(K)
  999 RETURN
      END
```

```
      SUBROUTINE REG(X,Y,N,A,B,R,S,T,U)
      DIMENSION X(N),Y(N)
C     THIS PERFORMS LINEAR REGRESSION ON DATA SUPPLIED
      XSUM=YSUM=XY=XSQ=YSQ=DFX=DFY=YHAT=YDIF=0.0
      DO 10 I=1,N
      XSUM=XSUM+X(I)
      YSUM = YSUM +  Y(I)
      XY = XY + X(I)*Y(I)
      XSQ=XSQ+X(I)*X(I)
      YSQ= Y(I)*Y(I)+YSQ
   10 CONTINUE
      XBAR = XSUM/N
      YBAR = YSUM/N
      B = (N*XY-XSUM*YSUM)/(N*XSQ-XSUM**2)
      A = (YSUM-B*XSUM)/N
      DO 20 I=1,N
      DFX = DFX +(X(I)-XBAR)**2
      DFY = DFY +(Y(I)-YBAR)**2
      YHAT = YHAT + ((Y(I)-(A+B*X(I)))**2)
      YDIF = YDIF + ((YBAR-(A+B*X(I)))**2)
   20 CONTINUE
      S = (YHAT/(N-2))**.5
      T = S*((1/DFX)**.5)
       U=T*((XSQ/N)**.5)
      V=(XY-N*XBAR*YBAR)/(N-2)
      R=(B/ABS(B))*(YDIF/DFY)**.5
      RETURN
      END
```

```
      SUBROUTINE AUC(X,Y,N,B,A,WT,DOSE,TAU,VD)
C  AREA UNDER CURVE BY TRAPEZOIDAL RLE
      INTEGER TAU
      DIMENSION  QQ(40),X(N),Y(N)
      WRITE(2,209)TAU,DOSE,WT,B,N
  209 FORMAT(1X,I10,3F10.4,I10)
      WRITE(2,211)(X(K),K=1,N),(Y(K)(=1,N)
  211 FORMAT(1X,12F10.3/12F10.3/12F10.3)
      QQ(2)=(Y(2)-Y(1)*EXP(B*X(2)))
      IF(VD.EQ.0)VD=.20*WT
      T3=5.0/60.0
      SEG=DOSE/T3/.3/WT/ABS(B)*(T3-(-EXP(B*T3))/ABS(B))
      SUM=SEG
      AM5=EXP(ALOG(A-Y(1))+B*T3)
      SEG=(QQ(2)+AM5)/2*(X(2)-T3)
      SUM=SUM+SEG
      DO 210 I=3,N
      QQ(I)=(Y(I)-Y(1)*EXP(B*X(I)))
      SEG= (QQ(I)+QQ(I-1))/2*(X(I)-X(I-1))
  210 SUM=SUM+SEG
      SEG=EXP(ALOG(AM5)+B*(X(N)-T3))/BS(B)
      SUM=SUM+SEG
      PSS=SUM/TAU
      VD=DOSE/SUM/ABS(B)
      WRITE(2,240)SUM,PSS
  240 FORMAT(1X,' TOTAL AREA = ',F7.2'  MEAN SS LEVEL = ',F5.2)
      RETURN
      END
```

TYPICAL OUTPUT

```
MONTE CARLO FIXED RANGE CV=2% VARIOUS K & N

        MONTE CARLO SIMULATION WITH PARAMETERS:

  DOSE(MG/KG)=     2.0000
  VOLUME OF DISTRIBUTION (L/KG) =      .2000

  TOTAL NO. OF DOSES =  20
  LENGTH OF INFUSION (HR) =     .083333
  DOSE INTERVAL (HRS)=  48
  ASSAY ERROR(%) = 2.000
MEASUREMENTS AT  1.50HOUR INTERVALS  RANGE 15.0000


                  K ELIM. =  .0500
                  ****************


PATIENT NUMBER     1
*******************

   3 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV       .0020      .0964      .3104 5304.0679
   4 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV       .0002      .0722      .2687**********
   5 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV      -.0107      .0795      .2820-2623.5451
   6 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV       .0016      .0797      .2823 7668.0327


PATIENT NUMBER     2
*******************

   3 POINTS USED FOR REGRESSION
MEAN ERROR,VAR,SDEV,CV      -.0719      .0902      .3003 -417.7159
   4 POINTS USED FOR REGRESSION
MEAN ERROR,VAR,SDEV,CV      -.0695      .0824      .2871 -412.9912
   5 POINTS USED FOR REGRESSION
MEAN ERROR,VAR,SDEV,CV      -.0729      .0465      .2156 -295.7473
   6 POINTS USED FOR REGRESSION
MEAN ERROR,VAR,SDEV,CV      -.0737      .0443      .2106 -285.6977
```

PATIENT NUMBER 3
****

3 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV .0053 .0542 .2329 4431.5258
4 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV .0260 .0502 .2240 862.9316
5 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV .0028 .0220 .1483 5238.6366
6 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV .0067 .0225 .1501 2247.0948

TOTAL NUMBER 764
****

3 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV -.0215 .0069 .0832 -386.3031
4 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV -.0144 .0064 .0802 -555.2236
5 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV -.0269 .0046 .0675 -250.7131
6 POINTS USED FOR REGRESSION
MEAN ERROR,VAR, DEV,CV -.0218 .0048 .0692 -317.3819

SEGMENTS OF ICL 1904S

PROGRAMS (FORTRAN)

```
      MASTER GENTAM
      COMMON/ZA/TAU(5),VD,DOSE(5),NDO(6,5),NDOSES(5),INJ(6,5),NLEV(5)
      COMMON/ZB/W(12,6,5),Z(12,6,5),XL(12,6,5),YLOG(12,6,5),
     1NPTS(6,5),LPTS(6,5)
      COMMON/C/NAGE,SEX,CR(6,5),WT,SNK(6,5),RJK(6,5),CGK(6,5)
      COMMON/ZD/IB,IA,M/E/NTRL,NPER,NAME(15)
      COMMON/AB/ A,B,BL
      DATA INT/2HIM/,T3/0.08333/
      REAL KA/2.16/
      DO 500 II=1,60
      CALL READIN
      IF(M.EQ.999)STOP
      A,B,BL,VD=0.0
      DO 500 IA=1,NPER
      BL=B
      WRITE(2,600)IA
  600 FORMAT(1X///'      DOSING PERIOD ',I2/6X,16('*')///)
      DO 520 IB=1,NLEV(IA)
      WRITE(2,610)NDO(IB,IA),INJ(IB,IA),NPTS(IB,IA)
  610 FORMAT(1H0,'DOSE NO.',I4,A4,' WITH',I4,' POINTS')
      IF(NPTS(IB,IA).EQ.0)GOTO 530
      WRITE(2,615)
  615 FORMAT(1X,'TIME(HH.HH)    CONC.         LN(CONC.)')
      WRITE(2,620)(W(K,IB,IA),Z(K,IB,IA),YLOG(K,IB,IA), K=1,NPTS(IB,IA
))
  620 FORMAT(1X,F7.2,8X,F5.2,8X,F8.4)
      IF(LPTS(IB,IA).EQ.0)GOTO 530
      NPT=NPTS(IB,IA)
      LPT=LPTS(IB,IA)
      CALL REG(W(NPT-LPT+1,IB,IA),YLOG(NPT-LPT+1,IB,IA),LPTS(IB,IA),
     1A,B)
      CO=EXP(A)
      THALF=-0.693/B
      WRITE(2,625)B,THALF,CO,LPTS(IB,IA)
  625 FORMAT(1H0,4X,'K.ELIM.=',F5.3,6X,'HALF-LIFE(HOURS)=',F5.2,
     1'  EXTRAP. CO=',F6.3,' USING',I3,' POINTS')
      M=2
      CALL COMP(M,INJ(IB,IA),3,INT,1)
      VCO=DOSE(IA)/T3/B*(1-EXP(B*T3))/(CO-Z(1,IB,IA))/(B*T3)
      IF(M.EQ.2)VCO=KA*DOSE(IA)/(KA-ABS(B))/(CO-Z(1,IB,IA))
      CALL AUC(W(1,IB,IA),Z(1,IB,IA),NPTS(IB,IA),B,CO,WT)
  530 IF(CR(IB,IA).NE.0)CALL CREATIN
  550 IF(NPTS(IB,IA).LT.5)GOTO 520
      CALL GRAF(W(1,IB,IA),YLOG(1,IB,IA),NPTS(IB,IA),A,B)
  520 CONTINUE
  500 CALL PREDICTOR
 9000 STOP
```

```
      SUBROUTINE PREDICTOR
      COMMON/ZA/TAU(5),VD,DOSE(5),NDO(6,5),NDOSES(5),INJ(6,5),LEV(5)
      COMMON/ZB/W(12,6,5),Z(12,6,5),XL(12,6,5),YLOG(12,6,5),
     1NPTS(6,5),LPTS(6,5)
      COMMON/C/NAGE,SEX,CR(6,5),WT,SNK(6,5),RJK(6,5),CGK(6,5)
      COMMON/ZD/IB,IA,M/E/NTRL,NPER,NAME(15)
      COMMON/AB/ A,B,BL
      REAL C(8),P(8),TR(8),SM(8),ASM(8),SQ(8),WSM(8),ZSM(8),WSQ(8)
      DATA INT/2HIM/
      REAL KA/2.16/
      I=IA
      NN=0
      IF(VD.EQ.0.0)VD=0.3*WT
      DO 801 JJ=1,8
  801 SM(JJ),ASM(JJ),SQ(JJ)=0.0
      WRITE(2,752)I,TAU(I),DOSE(I),NDOSES(I)
  752 FORMAT(1H1,//' PREDICTIONS FOR DOSING PERIOD ',I2,/33(1H*)///'  I
NT
     1ERVAL : ',F4.1,'HOURS      DOSE : ',F5.1,'MG',7X,I4,'DOSE GIVEN
')
      IF(NLEV(I).EQ.0)GOTO 760
      DO 740 J=1,NLEV(I)
      IF(SNK(J,1).NE.0.0.OR.J+I.EQ.2)GOTO 133
      IF(J.EQ.1)GOTO 753
      SNK(J,I)=SNK(J-1,I)
      CGK(J,I)=CGK(J-1,I)
      RJK(J,I)=RJK(J-1,I)
      GOTO 133
  753 IF(NLEV(I-1).EQ.0)GOTO 133
      SNK(J,I)=SNK(NLEV(I-1),I-1)
      CGK(J,I)=CGK(NLEV(I-1),I-1)
      RJK(J,I)=RJK(NLEV(I-1),I-1)
  133 WRITE(2,744)NDO(J,I),INJ(J,I),VD,B,CR(J,I),
     1SNK(J,I),RJK(J,I),CGK(J,I)
  744 FORMAT(1X,///' DOSE NUMBER ',I2,A4,6X,'VOLUME = ',F6.2,3X,ELIM.
KA
     1TE=',F6.3,/,' CREATININE OF ',F6.2,'   RATES USED WERE : ',F5.3,
     2'(S-N) ',F5.3,' (J)    ',F5.3,' (C&G)')
      IF(B.EQ.0)GOTO 780
      IF(NPTS(J,I).EQ.0)GOTO760
      WRITE(2,745)
  745 FORMAT(1X,//' TIME',8X,'LEVEL',6X,'BLOOD',15X,'C&G',15X,'JDRNY',
     415X,'S-N'/21X,4('LEVEL',1X,' %ERROR',5X))
      DO 740 K=1,NPTS(J,I)
      X=W(K,J,I)
      Y=Z(K,J,I)
      IF(X.NE.0.0)GOTO 726
      X,XMARK=TAU(I)
      NDO(J,I)=NDO(J,I)-1
```

```
      726 DO 730 L=1,4
          C(L),C(L+4),P(L),P(L+4)=0.0
          GOTO(721,722,723,724),L
      721 BB=ABS(B)
          IF(NLEV(I).EQ.1.AND.BL.NE.0.0)BB=ABS(BL)
          GOTO 720
      722 BB=ABS(SNK(J,I))
          GOTO 720
      723 BB=ABS(RJK(J,I))
          GOTO 720
      724 BB=ABS(CGK(J,I))
      720 M=2
          IF(BB.EQ.0.0.AND.L.GT.1)GOTO 731
          CALL COMP(M,INJ(J,I),3,INT,1)
          C(L)=DOSE(I)/VD*((1-EXP(-BB*TAU(I)*NDO(J,I)))/(1-EXP(-BB*TAU(I))
         1))*EXP(-BB*X)
          IF(M.NE.2)GOTO725
      729 C(L)=KA/(KA-BB)*(C(L)-DOSE(I)/VD*((1-EXP(-NDO(J,I)*KA*TAU(I)))
         1/(1-EXP(-KA*TAU(I)))*EXP(-KA*X)))
      725 IF(I.EQ.1)C(L)=C(L)+Z(1,J,I)*EXP(-BB*X)
          IF(I.EQ.1)GOTO727
          C(L)=C(L)+TR(I-1)*EXP(-BB*(X+(NDO(J,I)-1)*TAU(I)))
          IF(TR(I-1).EQ.0.0)C(L)=C(L)+Z(1,J,I)*EXP(-BB*X)
      727 C(L+4)=C(L)*VD/.3/WT
          P(L+4)=(C(L+4)-Y)/Y*100
          P(L)=(C(L)-Y)/Y*100
      730 CONTINUE
      731 IF(XMARK.EQ.0.0)GOTO 741
          X,XMARK=0.0
          NDO(J,I)=NDO(J,I)+1
      741 WRITE(2,732)X,Y,(C(JJ),P(JJ),JJ=1,8)
          NN=NN+1
          DO 800 JJ=1,8
          SM(JJ)=SM(JJ)+P(JJ)
          ASM(JJ)=ASM(JJ)+ABS(P(JJ))
      800 SQ(JJ)=SQ(JJ)+P(JJ)*P(JJ)
      740 CONTINUE
      732 FORMAT(1X,2(F5.2,5X),4(F5.2,1X,F7.2,5X),/,20X,4('(',F5.2,F8.2,')
     ',
         13X))
      760 TR(I)=DOSE(I)/VD*((1-EXP(-B*TAU(I)*NDOSES(I)))/(1-EXP(-B*TAU(I))
     ))
         1*EXP(-B*TAU(I))
      770 WRITE(2,768)TR(I)
      768 FORMAT(1X,/,' TROUGH AT END OF PERIOD =',F5.2)
      780 IF(IA.EQ.NPER)Q=1.0
          IF(NLEV(I).EQ.0)RETURN
          IF(B.NE.0.0)CALL SDEV(NN,SM,ASM,SQ,WSM,ZSM,WSQ,Q,ZT)
          RETURN
```

PROGRAM TO CALCULATE

HPLC PARAMETERS

```
10  REM HPLC PARAMETER CALCULATIONS
20  REM DKS 30.3.78 BASED ON BRISTOW,KNOX CHROMATOGR. 10,279,1977
30  REM modified 9/8/79
40  REM DIFF. COEFF. 20DEG. WILKIE-CHANG
50  REM SILICA TESTING
60  REM TOLUENE 3.12 NITROBENZ 3.2 ACETOPHEN 2.95 DINITROTOL 2.83
70  REM ODS ACETONE .55 PHENOL .49 M-CRESOL .44 ANISOLE .43 PHENA.39
80  REM ASSUMING MEOH/WATER 40:60:
90  DIM F$[20],G$[10],H$[40],I$[40],J$[40]
100  DIM K$[40],L$[40],M$[40],N$[40],P$[40],Q$[40]
110  DIM A$[72],E$[72],R$[40],S$[40],T$[40],U$[40]
120  DIM D$[40],C$[50],B$[40]
130  DIM Z$[80],Z0$[40],Z1$[10]
140  FILES SCRTT1
150  REM                    DATA REQUEST
160  PRINT "temp.,date & place etc"
170  INPUT T1,A$
180  PRINT " COLUMN AND PACKING"
190  INPUT E$
200  PRINT " L,B,(MM),PARTICLE SIZE(UM) "
210  L=250
220  B=4.6
230  D=10
240  PRINT " CHART(MM/MIN), INJ DET SENS "
250  INPUT C,C$
260  PRINT " info/col/colods/dat/allmult/mult"
270  INPUT F$
280  F$=UPS$(F$)
290  IF F$[1,4]="INFO" THEN 3410
300  IF F$[1,6]="COLODS" THEN 3180
310  PRINT " Copies ?"
320  U=1
330  IF F$[1,3]="MUL" THEN 2270
340  IF F$[1,4]="ALLM" THEN 2270
350  PRINT "P,F,SOLV. FRONT(MM),VISC.,ELUENT "
360  INPUT P,F,S0,V0,D$
370  PRINT " TIME,WIDTH(0.5H),DIFFUSIVITY,SOLUTE & STRENGTH "
380  FOR I=1 TO 9
390  INPUT T[I],W[I],D[I],B$[I]
400  IF T[I]=0 THEN 450
410  GOSUB 1660
420  NEXT I
430  REM    SOLUTE-INDEPENDANT CALCULATIONS
440  REM          ------------------------
450  S1=S0/C*F
```

```
460  U=L*C/S0
470  U1=U*D/D[I]/60
480  L1=L/D*1000
490  C1=U*V0*L/P/6.E+15
500  F1=D*D/C1*1.E-12
510  E=21.22*F*S0/C/(B ** 2*L)*60
520  K1=B*B/(L*D/1000)
530  REM
540  REM     SOLUTE-SPECIFIC CALCULATIONS
550  REM          -------------------------
560  REM I IH. VARIABLES ARE ZERO TO LIMIT INPUT,HENCE Z=I-1
570  X=I-1
580  FOR I=1 TO X
590  R[I]=T[I]/C*F
600  V[I]=W[I]/C*F*1.7
610  N[I]=((T[I]/W[I]) ** 2)*5.54
620  H[I]=L/N[I]/D*1000
630  K[I]=(T[I]-S0)/S0
640  A[I]=N[I]*N[I]/(T[I]/C*60*P)
650  Z[I]=H[I]*H[I]*F1
660  NEXT I
670  FOR H=1 TO 0
680  GOSUB 710
690  GOSUB 860
700  GOTO 960
710  REM OUTPUT STATEMENTS
720  REM          ----------------------------
730  PRINT
740  PRINT
750  PRINT "   COLUMN PARAMETER CALCULATIONS "
760  PRINT
770  PRINT #1;A$
780  PRINT
790  PRINT "  ********************************************************** "
800  PRINT
810  PRINT #1;E$
820  PRINT
830  PRINT "                    ********* "
840  PRINT #1;L;"mm. by";B;"mm.        particle diameter";D;" um."
850  RETURN
860  PRINT #1
870  PRINT #1;D$;F;"ml/min at";P;"bar"
880  PRINT #1;"Viscosity";V0;"centipoise"
890  PRINT #1
```

```
900  PRINT #1;"chart";C;"mm/min. ";C$
910  PRINT
920  PRINT "           GENERAL PARAMETERS "
930  PRINT "           ****************** "
940  PRINT #1;"Eluent volume";S1;"mls.     Eluent time ";S1/F;"min"
950  RETURN
960  PRINT #1;"Eluent linear velocity";U"mm/min"
970  PRINT #1;"Reduced eluent velocity";U1
980  IF F$[1,3]="DAI" THEN 1030
990  PRINT #1;"Column permeability";C1
1000  PRINT #1;"Dimensionless flow resistance";F1
1010  PRINT #1;"Knox-Parcher ratio";K1
1020  PRINT #1;"Total column porosity";E
1030  PRINT #1;LIN(2)
1040  PRINT
1050  PRINT "           SOLUTE-SPECIFIC PARAMETERS "
1060  PRINT "           *************************** "
1070  PRINT
1080  PRINT "  SOLUTE NAME                RETENTION      PEAK       THEORETICAL "
1090  PRINT TAB(28);"volume          vol.       no. of"
1100  PRINT "                              (MLS)          MLS       PLATES"
1110  PRINT "  ******************          *********   ********* ************"
1120  FOR I=1 TO X
1130  GOSUB 1970
1140  PRINT B$[I];TAB(28);R[I];TAB(40);V[I];TAB(52);N[I]
1150  NEXT I
1160  PRINT
1170  IF F$[1,3]="DAI" THEN 1440
1180  REM         COL OUTPUT ONLY
1190  PRINT "SOLUTE NAME";TAB(26);"REDUCED        CAPACITY      IF."
1200  PRINT TAB(26)"HEIGHT";TAB(40);"RATIO         DIAM. POSS.?"
1210  PRINT "  ***********"TAB(26);"********         ******        ******"
1220  FOR I=1 TO X
1230  REM IS INFINITE DIAM. MODE POSSIBLE?
1240  IF (B-2) ** 2/(L*D)*1000>(2.4+32/U1) THEN 1290
1250  GOSUB 1970
1260  PRINT B$[I];TAB(27);H[I];K[I];"       NO"
1270  NEXT I
1280  GOTO 1310
1290  PRINT B$[I];TAB(27);H[I];K[I];"      YES"
1300  GOTO 1270
1310  PRINT
1320  PRINT " SOLUTE NAME";TAB(30);"PERFORM.   SEPARATION"
1330  PRINT TAB(30);"INDEX       IMPEDANCE"
```

```
1340  PRINT " ************";TAB(27);"*******      *********"
1350  FOR I=1 TO X
1360  GOSUB 1970
1370  PRINT B$[I];TAB(30);A[I];TAB(40);Z[I]
1380  NEXT I
1390  PRINT
1400  GOTO 1540
1410  REM              DAT OUTPUT ONLY
1420  REM          -------------------------
1430  PRINT
1440  PRINT " SOLUTE NAME";TAB(26);"CAPACITY RATIO  RESOLUTION"
1450  PRINT " ***********";TAB(26);"**************  **********"
1460  FOR I=1 TO X-1
1470  Y[I]=2/1.7*(T[I+1]-T[I])/(W[I]+W[I+1])
1480  GOSUB 1970
1490  PRINT B$;TAB(26);K[I];TAB(43);Y[I]
1500  NEXT I
1510  I=X
1520  GOSUB 1970
1530  PRINT B$[I];TAB(26);K[I];TAB(43)
1540  NEXT H
1550  PRINT " NEW COL,ELU,SOLUTE ?"
1560  INPUT G$
1570  G$=UPS$(G$)
1580  IF G$[1,3]="COL" THEN 160
1590  IF G$[1,3]="ELU" THEN 350
1600  IF G$[1,3]="SOL" THEN 370
1610  GOTO 3170
1620  REM
1630  REM     STRING HANDLING FOR SOLUTE INPUT
1640  REM          ---------------------------
1650  GOTO G OF 1670,1690,1710,1730,1750,1770,1790,1810,1830
1660  GOTO I OF 1670,1690,1710,1730,1750,1860,1880,1900,1920
1670  H$=B$
1680  RETURN
1690  I$=B$
1700  RETURN
1710  J$=B$
1720  RETURN
1730  K$=B$
1740  RETURN
1750  L$=B$
1760  RETURN
1770  R$=B$
```

```
1780  RETURN
1790  S$=B$
1800  RETURN
1810  T$=B$
1820  RETURN
1830  U$=B$
1840  RETURN
1850  GOTO M OF 1860,1880,1900,1920
1860  M$=B$
1870  RETURN
1880  N$=B$
1890  RETURN
1900  P$=B$
1910  RETURN
1920  Q$=B$
1930  RETURN
1940  REM
1950  REM      SUBROUTINE FOR STRING OUTPUT
1960  REM      ---------------------------
1970  GOTO I OF 1990,2010,2030,2050,2070,2180,2200,2220,2240
1980  GOTO Q OF 1990,2010,2030,2050,2070,2090,2110,2130,2150
1990  B$=H$
2000  RETURN
2010  B$=I$
2020  RETURN
2030  B$=J$
2040  RETURN
2050  B$=K$
2060  RETURN
2070  B$=L$
2080  RETURN
2090  B$=R$
2100  RETURN
2110  B$=S$
2120  RETURN
2130  B$=T$
2140  RETURN
2150  B$=U$
2160  RETURN
2170  GOTO M OF 2180,2200,2220,2240
2180  B$=M$
2190  RETURN
2200  B$=N$
2210  RETURN
```

```
2220  B$=P$
2230  RETURN
2240  B$=Q$
2250  RETURN
2260  REM            ------------------------
2270  REM INPUT REQUEST FOR MULTI-ELUENT DATA
2280  PRINT "No. of eluents,solutes (max. 4,9)"
2290  READ X,G
2300  PRINT "visc.& eluent name then (flow,pressure,sol.front(mm)) in turn"
2310  READ V[1]
2320  INPUT B$
2330  FOR M=1 TO X
2340  V[M]=V[1]
2350  READ D[M],P[M],S[M]
2360  NEXT M
2370  H$="2-aminopyridine"
2380  I$="3 aminopyridine"
2390  J$="4-aminopyridine"
2400  K$="3,4-aminopyridine"
2410  L$="2,3-aminopyridine"
2420  M$="2,6-aminopyridine"
2430  S$="N,N dimethyl aminopyridine"
2440  REM    d(1)=molecular volume (leBas)
2450  D[1]=D[2]=D[3]=107.3
2460  D[4]=D[5]=D[6]=121.5
2470  D[7]=150.8
2480  PRINT " TIME,WIDTH(0.5H), (MM)?"
2490  FOR M=1 TO X
2500  FOR G=1 TO G
2510  READ T[M,G],U[M,G]
2520  NEXT G
2530  NEXT M
2540  FOR M=1 TO 4
2550  FOR G=1 TO 9
2560  B[M,G]=L[M,G]=G[M,G]=0
2570  T[M,G]=L[M,G]=X[M,G]=P[M,G]=0
2580  NEXT G
2590  NEXT M
2600  REM         CALCULATIONS
2610  REM           ------------------
2620  FOR M=1 TO X
2630  FOR G=1 TO G
2640  B[M,G]=T[M,G]/C*U[M]
2650  L[M,G]=U[M,G]/C*U[M]*1.7
```

```
2660  G[M,Q]=((J[M,Q]/O[M,Q]) ** 2)*5.54
2670  I[M,Q]=(J[M,Q]-S[M])/S[M]
2680  IF F$[1,7]#"ALLMULT" THEN 2730
2690  F[M,Q]=LOG(L/D*1000/G[M,Q])
2700  REM i(m+5,q)=diffusivity,v(m)=visc,d(q)=mol. vol.
2710  I[M+5,Q]=3.8E-11*(T+273)/V[M]/D[Q] ** .6
2720  X[M,Q]=LOG((L*C/S[M]*D/I[M+5,Q]/60)*1.E-09)
2730  Q1=Q+1
2740  IF Q=G THEN 2770
2750  L[M,Q]=2/1.7*(J[M,Q1]-J[M,Q])/(O[M,Q]+O[M,Q1])
2760  GOTO 2790
2770  L[M,Q]=1
2780  L[M,Q]=0
2790  NEXT Q
2800  NEXT M
2810  REM              OUTPUT
2820  REM              ------
2830  FOR M=1 TO D
2840  GOSUB 730
2850  FOR M=1 TO X
2860  PRINT #1;"[";M;"]    ";B$;"     ";O[M];"ml/min at";P[M];"bar"
2870  NEXT M
2880  PRINT #1;""
2890  PRINT #1;""
2900  PRINT #1;"solute/parameter ";TAB(26);"[1]";TAB(38);"[2]";TAB(50);"[3]";TAB(62);"[4]"
2910  PRINT #1;"****************";TAB(26);"***";TAB(38);"***";TAB(50);"***";TAB(62);"***"
2920  PRINT #1;"                 "
2930  FOR Q=1 TO G
2940  GOSUB 1980
2950  PRINT #1;B$
2960  PRINT #1;"************************"
2970  Z$="Retention vol.(mls) Peak volume          Capacity ratio       Resolution           "
2980  PRINT #1; USING 2990;Z$[1,20],B[1,Q],B[2,Q],B[3,Q],B[4,Q]
2990  IMAGE 20a,5x,4(4x,2d.3d)
3000  PRINT #1; USING 2990;Z$[21,40],C[1,Q],C[2,Q],C[3,Q],C[4,Q]
3010  PRINT #1; USING 3020;G[1,Q],G[2,Q],G[3,Q],G[4,Q]
3020  IMAGE "No. of plates           ",4(5x,5d)
3030  PRINT #1; USING 2990;Z$[41,60],I[1,Q],I[2,Q],I[3,Q],I[4,Q]
3040  PRINT #1; USING 2990;Z$[61,80],L[1,Q],L[2,Q],L[3,Q],L[4,Q]
3050  IF F$[1,4]="MULT" THEN 3100
3060  Z0$="ln reduced velocity ln reduced height   "
3070  PRINT #1; USING 3080;Z0$[1,20],X[1,Q],X[2,Q],X[3,Q],X[4,Q]
3080  IMAGE 20a,5x,4(5x,2d.2d)
3090  PRINT #1; USING 3080;Z0$[21,40],F[1,Q],F[2,Q],F[3,Q],F[4,Q]
3100  PRINT #1;""
```

```
3110  NEXT G
3120  NEXT H
3130  PRINT "Do you wish to do another run?"
3140  INPUT Z1$
3150  Z1$=UPS$(Z1$)
3160  IF Z1$[1,3]="YES" THEN 2280
3170  STOP
3180  REM     ODS INFO. SUBROUTINE
3190  REM      ------------------
3200  H$="ACETONE"
3210  I$=" PHENOL"
3220  J$="  M-CRESOL"
3230  K$="   ANISOLE"
3240  L$="    PHENETOLE"
3250  D[1]=.55
3260  D[2]=.49
3270  D[3]=.44
3280  D[4]=.43
3290  D[5]=.39
3300  V0=1.83
3310  D$=" METHANOL/WATER (40:60)"
3320  PRINT " PRESSURE(BAR),FLOW(ML/MIN),SOLV. FRONT(MM)"
3330  INPUT P,F,S0
3340  PRINT " SOLUTE TIME, WIDTH(0.5H), [MM]"
3350  FOR I=1 TO 5
3360  INPUT T[I],W[I]
3370  NEXT I
3380  PRINT "COPIES"
3390  INPUT U
3400  GOTO 440
3410  PRINT "  HPLC PARAMETERS  DKS APRIL 1978"
3420  PRINT " OPTIONS AVAILABLE INCLUDE :-"
3430  PRINT " COL    THIS GIVES FULL OUTPUT BUT NEEDS VISCOSITY + DIFFUSIVITY"
3440  PRINT " COLUDS  THIS ASSUMES STANDARD REVERSE-PHASE TESTING (DKS)"
3450  PRINT " DAT REDUCED OUTPUT FOR FILES, DOESN'T NEED VISC. OR DIFF."
3460  PRINT "MULT  UPTO 5 SOLUTES AND 4 MOBILE PHASES, SPECIAL FORMAT"
3470  PRINT " DIMENSIONS IN MM. EXCEPT PARTICLE DIAM.(UM)"
3480  PRINT " FLOW ML/MIN   PRESSURE BAR   VISCOSITY CENTIPOISE"
3490  PRINT " DIFFUSIVITY MM.MS-1"
3500  PRINT " USE DECIMAL POINT IN INPUT, SELECT 1 OR 2 COPIES"
3510  GOTO 260
3520  DATA 4,7,.89
3530  REM SDS1  H3PO4 .5
3540  DATA .4602,26,58.5
```

PROGRAM TO TEACH REGRESSION ANALYSIS

```
10  REM                    F DATA
20  REM  data assumes values for .975 to give 5% levels
30  DATA 0,0,12.7,4.3,3.18,2.78,2.57,2.45,2.36
40  DATA 2.31,2.26,2.23,2.2,2.18,2.16,2.14,2.13,2.12
50  DATA 2.11,2.1,2.09,2.09,2.08,2.07,2.07,2.06,2.06,2.06,2.5,2.05
60  REM          F DATA (2,N-2) alpha/2 5%
70  DATA 0,0,199.5,19,9.55,6.94,5.79,5.14,4.74,4.46,4.26,4.1,3.98,3.88,3.81
80  DATA 3.74,3.68,3.63,3.59,3.55,3.52,3.49,3.47,3.44,3.42,3.4,3.39
90  DATA 3.35,3.35,3.34
100  REM          F DATA (1,N-2) alpha/2  1%
110  DATA 0,0,16211,198.5,55.55,31.33,22.78,18.63,16.24,14.6,13.61,12.83,12.23
120  DATA 11.75,11.37,11.06,10.8,10.58,10.38,10.22,10.07,9.9,9.83,9.73,9.63
130  DATA 9.55,9.48,9.41,9.34,9.28
140  REM          F DATA (1,N-2)  alpha/2  5%
150  DATA 0,0,647.8,38.51,17.44,12.22,10.01,8.81,8.07,7.57,7.21,6.94,6.72
160  DATA 6.55,6.41,6.3,6.2,6.12,6.04,5.98,5.92,5.87,5.83,5.9,5.75
170  DATA 5.72,5.69,5.66,5.63,5.61
180  REM ------------------------------------------------------------
190  REM Aston University HP2000 Access System
200  REM Copyright D.R.Scott September 1978
210  REM Validation label is in line 4390
220  REM ------------------------------------------------------------
230  REM Least-squares program - this program disables break
240  DIM W[50],Z[50],X[50],Y[50],H[50],F[50],E[50]
250  DIM N$[40],F$[11],S$[15]
260  DIM G[10,5],K[10,5]
270  DIM A[50],B[50],S[50]
280  DIM M$[5],P$[70],A$[5],V$[72]
290  DIM V[50],Q[50],R[50],U[50]
300  FOR I=1 TO 30
310  READ H[I]
320  NEXT I
330  FOR I=1 TO 30
340  READ F[I]
350  NEXT I
360  FOR I=1 TO 30
370  READ A[I]
380  NEXT I
390  FOR I=1 TO 30
400  READ B[I]
410  NEXT I
420  REM       CONTROL SEGMENT
```

```
430  PRINT
440  PRINT "THIS IS A LEAST-SQUARES REGRESSION PROGRAM DESIGNED"
450  PRINT "FOR USE IN SECTION F PRACTICALS."
460  PRINT " DO YOU REQUIRE FURTHER INFORMATION?"
470  PRINT " type yes or no"
480  INPUT M$
490  M$=UPS$(M$)
500  IF M$[1,2]="dK" THEN 840
510  IF M$[1,2] <> "YE" THEN 760
520  PRINT "IT HAS THE FOLLOWING OPTIONS:-"
530  PRINT
540  PRINT "1  A REGRESSION USING LINEAR INPUT VALUES OF A LOGARITHMIC"
550  PRINT "FUNCTION  (EG. CHLORAMPHENICOL DEGRADATION DATA)"
560  PRINT
570  PRINT " 2   A LINEAR REGRESSION USING AUTOMATICALLY-GENERATED"
580  PRINT " DATA UNIQUE TO EACH STUDENT (STATISTICS EXERCISE)"
590  PRINT
600  PRINT " 3  A LINEAR REGRESSION BASED ON DATA FROM AN EXTERNAL"
610  PRINT " SOURCE (ANY OTHER EXPERIMENT)"
620  PRINT
630  PRINT " 4 THIS USES THE SAME DATA AS YOUR PREVIOUS RUN"
640  PRINT "   BUT ALLOWS YOU TO CHANGE THE POINTS USED FOR REGRESSION"
650  PRINT
660  PRINT " 5  THIS  ALLOWS YOU TO RETURN TO OPTION 2 AT SOME LATER DATE"
670  PRINT "    PROVIDED YOU CAN REMEMBER YOUR DATA POINTS"
680  PRINT
690  PRINT " 6 THIS ACCEPTS UPTO 50 POINTS WITH REPLICATE X-VALUES. YOU MUST"
700  PRINT "    HAVE THE SAME NUMBER OF Y-VALUES FOR EACH X."
710  PRINT
720  PRINT " 7 THIS GIVES A LIST OF YOUR DATA SET AND THE LAST OPTION USED"
730  PRINT
740  PRINT " 8 THIS FINISHES THE EXERCISE"
750  PRINT
760  PRINT "  TYPE THE NAMES OF STUDENTS USING THIS RUN ON A SINGLE LINE"
770  INPUT N$
780  CONVERT N$[1,2] TO N4,810
790  PRINT "Do not input a number. Input your name or initials."
800  GOTO 770
810  IF LEN(N$)>2 THEN 840
820  PRINT "Please input your proper name or initials using at least 3 letters"
830  GOTO 770
840  DO=DO+0
```

```
850  PRINT " SELECT THE OPTION YOU REQUIRE"
860  IF DO <> 2 AND DO <> 2 THEN 890
870  IF M$(1,2) <> "YE" THEN 890
880  PRINT " ( Option 4 allows you to change the points used for regression)"
890  IF DO=4 OR DO=7 THEN 910
900  DO=DO
910  INPUT DO
920  IF DO <> INT(DO) OR DO>8 OR DO<1 THEN 1080
930  IF DO=7 OR DO=8 THEN 1070
940  IF DO=4 THEN 1010
950  PO=BRK(1)
960  FOR I=1 TO 30
970  X(I)=Y(I)=Z(I)=W(I)=0
980  V(I)=U(I)=0
990  NEXT I
1000  N1=N2=J0=0
1010  N=F1=F2=D1=D2=X1=X2=Y1=Y2=P=P1=0
1020  L=L1=0
1030  IF DO <> 6 OR DO <> 4 THEN 1050
1040  J=N1/J0
1050  MAT G=ZER
1060  MAT K=ZER
1070  GOTO DO OF 3220,3410,3670,1200,1100,5150,3830,5460
1080  PRINT " only options 1 to 8 are available. Try again"
1090  GOTO 910
1100  PRINT " input x and y values separated by a comma, on pair per line"
1110  FOR I=1 TO 25
1120  INPUT W(I),Z(I)
1130  IF W(I) <> 9999 THEN 1160
1140  I=0
1150  GOTO 1100
1160  NEXT I
1170  N1=25
1180  REM ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1190  REM            option 4 segment
1200  PRINT " HOW MANY POINTS WILL YOU USE IN THIS RUN?"
1210  INPUT N
1220  IF N=INT(N) THEN 1250
1230  PRINT " Use integers only ! Try again."
1240  GOTO 1210
1250  IF N=0 THEN 850
1260  IF DO <> 6 AND DO <> 6 THEN 1320
1270  IF DO=6 AND DO <> 4 AND DO <> 6 THEN 1320
1280  IF N>J AND N <= N1 THEN 1320
1290  PRINT  USING 1300;J,N1
1300  IMAGE "You have ",d,"x-values but ",dd,"Points. Please try again"
1310  GOTO 1210
1320  IF N>2 THEN 1350
```

```
1330  PRINT " You need at least 3 points, try again"
1340  GOTO 1210
1350  IF N>N1 THEN 1370
1360  GOTO 1400
1370  PRINT  USING 1380;N1
1380  IMAGE "You have only",dd,"points. Try again"
1390  GOTO 1210
1400  IF N=N1 THEN 1730
1410  PRINT "INPUT CODE-NUMBERS FOR POINTS REQUIRED"
1420  PRINT "-ONE PER LINE"
1430  PRINT "A NEGATIVE VALUE WILL DELETE THAT POINT"
1440  FOR I=1 TO N
1450  INPUT E[I]
1460  IF E[I]=INT(E[I]) THEN 1490
1470  PRINT " Input CODE as an integer. Try again."
1480  GOTO 1450
1490  IF E[I] <> 9999 THEN 1520
1500  I=0
1510  GOTO 1200
1520  IF E[I]>30 THEN 1580
1530  IF E[I]>N1 THEN 1580
1540  IF E[I]<0 THEN 1650
1550  X[I]=W[E[I]]
1560  Y[I]=Z[E[I]]
1570  GOTO 1630
1580  PRINT "This is not part of your data set, Please start again"
1590  I=0
1600  GOTO 1630
1610  X[I]=W[I]
1620  Y[I]=Z[I]
1630  NEXT I
1640  GOTO 1770
1650  FOR J=1 TO N
1660  IF J=E[I] THEN 1710
1670  X[J]=W[J]
1680  Y[J]=Z[J]
1690  NEXT J
1700  GOTO 1770
1710  J=J+1
1720  GOTO 1690
1730  FOR I=1 TO N
1740  X[I]=W[I]
```

```
1750  Y[I]=Z[I]
1760  NEXT I
1770  FOR M=1 TO N
1780  P=(X[M]*Y[M])+P
1790  X1=X1+X[M]
1800  X2=X2+X[M]*X[M]
1810  Y1=Y1+Y[M]
1820  Y2=Y2+Y[M]*Y[M]
1830  NEXT M
1840  X0=X1/N
1850  Y0=Y1/N
1860  FOR I=1 TO N
1870  D1=D1+(X[I]-X0) ** 2
1880  D2=D2+(Y[I]-Y0) ** 2
1890  NEXT I
1900  REM P=$P,X1=$X1,X2=$X*X,d=$ of diffs. squared, X0 =mean,s= std error estimate, t=std dev. slope
1910  B=(N*P-X1*Y1)/(N*X2-X1*X1)
1920  A=(Y1-B*X1)/N
1930  GOSUB 4280
1940  FOR I=1 TO N
1950  F1=F1+((Y[I]-(A+B*X[I])) ** 2)
1960  F2=F2+((Y0-(A+B*X[I])) ** 2)
1970  NEXT I
1980  S=(F1/(N-2)) ** .5
1990  T=S*((1/D1) ** .5)
2000  U=T*((X2/N) ** .5)
2010  V=(P-N*X0*Y0)/(N-2)
2020  R0=(B/ABS(B))*(F2/D2) ** .5
2030  R1=R0
2040  F0=(N-2)*R0*R0/(1-R0*R0)
2050  IF ABS(F0)>A[N] THEN 2090
2060  IF ABS(F0)>B[N] THEN 2110
2070  P$="( Not significantly different from zero. P>0.05)"
2080  GOTO 2120
2090  P$="( Highly significant. P<0.01)"
2100  GOTO 2120
2110  P$="( Significantly different from zero. P<0.05)"
2120  IF D0 <> 1 THEN 2200
2130  PRINT " input a suitable y value to determine the corresponding x value"
2140  INPUT C[I]
2150  IF C[I]>0 THEN 2180
2160  PRINT "this will be logged and so must be positive and not zero. Try again."
2170  GOTO 2140
2180  G[I]=LOG(C[I])
```

```
2190 GOTO 2250
2200 PRINT " input suitable y values to determine corresponding x values"
2210 IF M$[1,2] <> "YE" THEN 2280
2220 PRINT " You may use up to 4 values, a zero will stop the data request"
2230 PRINT " Input one value per line"
2240 GOTO 2280
2250 V[1]=(Q[1]-A)/B
2260 V2=1
2270 GOTO 2410
2280 FOR I=1 TO 10
2290 INPUT Q[I]
2300 V2=I-1
2310 IF Q[I]=0 THEN 2330
2320 GOTO 2340
2330 I=10
2340 NEXT I
2350 IF SYS(4) <> 3 THEN 2410
2360 IF V2<2 THEN 2410
2370 PRINT " Your results will exceed the capacity of the VDU screen."
2380 PRINT " The output will pause at suitable points to allow you to copy"
2390 PRINT " your results. When you are ready to proceed type 1 and 'return'"
2400 INPUT O
2410 IF DO <> 1 THEN 2440
2420 GOTO 2440
2430 Q1=(Q1-A)/B
2440 FOR I=1 TO V2
2450 V[I]=(Q[I]-A)/B
2460 FOR M=1 TO 3
2470 K[I,M]=(S*S/B/B)*(1/M+1/N+(Q[I]-YO) ** 2/(B*B*D1))
2480 G[I,M]=H[N]*K[I,M] ** .5
2490 NEXT M
2500 NEXT I
2510 L1=X[N]
2520 L=X[N]
2530 FOR I=1 TO N
2540 IF X[I]>L THEN 2570
2550 IF X[I]<L1 THEN 2590
2560 GOTO 2610
2570 L=X[I]
2580 GOTO 2610
2590 L1=X[I]
2600 GOTO 2610
2610 NEXT I
2620 REM ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

```
2630  REM                              OUTPUT SEGMENT
2640  PRINT
2650  IF D0=1 THEN 2680
2660  IF D0=4 AND D0=1 THEN 2680
2670  GOTO 2710
2680  PRINT  USING 2690;A,B
2690  IMAGE "LOG(E) Y =",ddd.ddd,x,sddd.dddddd,x,"X"
2700  GOTO 2730
2710  PRINT  USING 2720;A,B
2720  IMAGE 10x,"Y=",3d.3d,x,s2d.3d,"x"
2730  PRINT
2740  PRINT " NUMBER OF POINTS = ";N
2750  PRINT " RANGE OF X = ";ABS(L-L1)
2760  PRINT
2770  PRINT "CORRELATION COEFFICIENT =";R0;" F RATIO =";F0
2780  PRINT "                    ";P$
2790  PRINT "standard error of estimate =",S
2800  PRINT "standard deviation of slope =",I
2810  R1=R0/(X2/N) ** .5
2820  B1=((2*(N-1)*(1-R1*R1)) ** .5)*I
2830  A1=((2*(N-1)*(1-R1*R1)) ** .5)*U
2840  B2=B1/N
2850  A2=A1/N
2860  PRINT "standard deviation of intercept =";U
2870  PRINT  USING 2880;U,S*100/X0
2880  IMAGE" covariance =",dddd.dd,"      coefficient of variation =",ddd.dd,"%"
2890  FOR I=1 TO V2
2900  PRINT
2910  IF D0=1 THEN 2950
2920  IF D0=4 AND D0=1 THEN 2950
2930  PRINT " FOR Y=";G[1];"  x=";V[1]
2940  GOTO 2970
2950  PRINT "FOR Y =";G[1];"  X=";V[1]
2960  IF M$ <> "DK" THEN 850
2970  PRINT
2980  PRINT " at 5% confidence level, limits for value of x are plus or minus"
2990  PRINT G[1,1],"(no replicates)"
3000  PRINT G[1,2],"(two replicates)"
3010  PRINT G[1,3],"(three replicates)"
3020  PRINT
3030  IF I=V2 THEN 3060
3040  IF SYS(4) <> 3 THEN 3060
3050  INPUT U
3060  NEXT I
```

```
3070  PRINT " CORRELATION OF SLOPE AND INTERCEPT = ";R1
3080  PRINT " independently-calculated limits (5%):"
3090  PRINT " slope +/-",B2,"     intercept +/- "A2
3100  PRINT
3110  PRINT " combined calculation of limits (5%):"
3120  PRINT " slope +/-",B1,"     intercept +/- "A1
3130  IF D0 <> 2 AND D0 <> 2 THEN 3190
3140  PRINT "Do you wish to see a graph of your data and plotted lines?"
3150  INPUT A$
3160  A$=UPS$(A$)
3170  IF A$[1,1] <> "Y" THEN 3190
3180  GOSUB 4680
3190  GOTO 850
3200  REM ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3210  REM           option 1 segment
3220  PRINT " HOW MANY DATA POINTS DO YOU WANT TO USE?"
3230  INPUT N1
3240  IF N1=0 THEN 850
3250  IF N1>2 THEN 3280
3260  PRINT "You need at least 3 points, try again"
3270  GOTO 3230
3280  PRINT " input pairs of x and y values, one pair to a line"
3290  FOR I=1 TO N1
3300  INPUT W[I],Z[I]
3310  IF W[I] <> 9999 THEN 3340
3320  I=0
3330  GOTO 3220
3340  IF Z[I]>0 THEN 3370
3350  PRINT "this point is not possible, y must be positive"
3360  GOTO 3220
3370  Z[I]=LOG(Z[I])
3380  NEXT I
3390  N=N1
3400  GOTO 1730
3410  REM ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3420  REM           option 2 segment
3430  PRINT " the followins values are unique to you"
3440  IF SYS(4) <> 3 THEN 3460
3450  PRINT " Please note them down."
3460  PRINT " Re-use these values should it be necessary to finish the "
3470  PRINT " experiment at a later date"
3480  IF SYS(4) <> 3 THEN 3500
3490  SYSTEM 20,"Pau-10"
3500  P0=BRK(0)
3510  N1=25
3520  PRINT
```

```
3530 PRINT "       code         x              y"
3540 PRINT "       ****       *****         *****"
3550 X=0
3560 FOR X=2 TO 50 STEP 2
3570 Z[X/2]=2+6*X
3580 Z[X/2]=Z[X/2]/5*(.5-RND(1)+5)
3590 W[X/2]=X
3600 Y[X/2]=X[X/2]=0
3610 PRINT  USING 3620;X/2,W[X/2],Z[X/2]
3620 IMAGE 6x,dd,6x,3d.d,8x,3d.d
3630 NEXT X
3640 GOTO 1200
3650 REM ------------------------------------------------------------
3660 REM           option 3 segment
3670 PRINT " HOW MANY DATA POINTS DO YOU HAVE ALL TOGETHER ?"
3680 INPUT N1
3690 IF N1=0 THEN 850
3700 IF N1>2 THEN 3730
3710 PRINT "You need at least 3 points, try again"
3720 GOTO 3680
3730 PRINT " input pairs of x and y values, one pair per line, separated by comma"
3740 FOR I=1 TO N1
3750 INPUT W[I],Z[I]
3760 IF W[I] <> 9999 THEN 3790
3770 I=0
3780 GOTO 3670
3790 NEXT I
3800 GOTO 1200
3810 REM ------------------------------------------------------------
3820 REM           option 7 segment
3830 PRINT "                    DATA SET"
3840 PRINT "                    ********"
3850 PRINT
3860 IF D0=1 OR D0=1 THEN 3950
3870 PRINT "             x              y"
3880 PRINT "          ******         ******"
3890 IF Y[1]=0 THEN 4010
3900 FOR I=1 TO N1
3910 PRINT  USING 3920;W[I],Z[I]
3920 IMAGE 6x,6d.2d,6x,6d.2d
3930 NEXT I
3940 GOTO 4060
3950 PRINT "          x          y          log(e) y"
3960 PRINT "        *****      *****       ********"
```

```
3970  FOR I=1 TO N1
3980  PRINT  USING 3990;W[I],EXP(Z[I]),Z[I]
3990  IMAGE 4x,6d.2d,5x,3d.2d,7x,3d.2d
4000  NEXT I
4010  IF Y[2]=0 THEN 4030
4020  GOTO 4060
4030  PRINT " x,y matrix set to zero i.e., there isn't a data set"
4040  IF O0 <> 0 THEN 4060
4050  O0=D0
4060  PRINT " The data was input via option";O0
4070  GOTO 850
4080  REM  T-value subroutine
4090  N7=1
4100  N8=N-2
4110  F8=F0
4120  IF F0>1 THEN 4160
4130  F8=1/F0
4140  N7=N8
4150  N8=1
4160  A7=2/9/N7
4170  A8=2/9/N8
4180  Z7=ABS(((1-A8)*F8 ** .333333-1+A7)/SQR(A8*F8 ** .666667+A7))
4190  IF N8>3 THEN 4210
4200  Z7=Z7*(1+.8*Z7 ** 4/N8 ** 3)
4210  F7=EXP(-Z7*Z7/2)*.398942
4220  W8=1/(1+Z7*.231642)
4230  P8=F7*W8*((((1.33027*W8-1.82126)*W8+1.78148)*W8-.356564)*W8+.319381)
4240  IF F0 >= 1 THEN 4260
4250  P8=1-P8
4260  P8=(1-P8)*100
4270  RETURN
4280  REM ^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^
4290  REM        monitor file segment (J002 only)
4300  FILES *
4310  F=0
4320  F$="FILE00.J001"
4330  ASSIGN F$,1,V1, NR
4340  GOTO V1 OF 4640,4640,4530,4640,4640,4640,4640,4640
4350  LOCK #1
4360  READ #1,I
4370  IF  END #1 THEN 4470
4380  GOTO TYP(1) OF 4390,4390,4410
4390  ADVANCE #1;1,I
4400  GOTO 4380
4410  S5=SYS(4)
```

```
4420  IF U0>0 THEN 4440
4430  PRINT #1;TIM(1),TIM(0),TIM(2)-273
4440  PRINT #1;N$,D0,A,B$, END
4450  UNLOCK #1
4460  RETURN
4470  UNLOCK #1
4480  F=F+1
4490  IF F<10 THEN 4520
4500  CONVERT F TO F$(5,6)
4510  GOTO 4530
4520  CONVERT F TO F$(6,6)
4530  CREATE V1,F$,9,256
4540  GOTO V1 OF 4330,4590,4460,4460
4550  S$="MWA "
4560  S$(5)=F$
4570  SYSTEM V1,S$
4580  GOTO V1+1 OF 4330,4610
4590  PRINT "File create error -";V1,F$
4600  GOTO 4640
4610  PRINT "System command error -";V1,S$
4620  REM Validation Label
4630  REM Validated by ^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^
4640  PRINT LIN(1);TAB(10)"If you have had any problems using this program, please"
4650  PRINT TAB(15)"contact Dave Scott(Pharmacy Dept.) room/220"
4660  RETURN
4670  REM*****************************************************************
4680  REM                          Graph Subroutine
4690  PRINT "Graph for ";N$
4700  PRINT "  * = data points  + = regression line  . = limits on regression line"
4710  PRINT "-------------------------------------------------------------------------------"
4720  FOR I=1 TO N1
4730  X[I]=INT(W[I])
4740  IF W[I]-INT(W[I])<.5 THEN 4760
4750  X[I]=X[I]+1
4760  Y[I]=INT(Z[I]/12)
4770  IF Z[I]-INT(Z[I]/12)<.5 THEN 4790
4780  NEXT Y[I]+1
4800  FOR I=2 TO 50 STEP 2
4810  S[I]=(A+B*I)/12
4820  R[I]=(A+B*(1+H[N]*T)*I)/12
4830  U[I]=(A-B*(1-H[N]*T)*I)/12
4840  S[I]=INT(S[I])
4850  R[I]=INT(R[I])
4860  U[I]=INT(U[I])
```

```
4870  IF S[I]-INT(S[I])<.5 THEN 4890
4880  S[I]=S[I]+1
4890  IF R[I]-INT(R[I])<.5 THEN 4910
4900  R[I]=R[I]+1
4910  IF U[I]-INT(U[I])<.5 THEN 4930
4920  U[I]=U[I]+1
4930  NEXT I
4940  FOR I=25 TO 3 STEP -1
4950  V$="     I                                                    "
4960  IF I/5 <> INT(I/5) THEN 4980
4970  CONVERT I*12 TO V$[1,4]
4980  FOR K=2 TO 50 STEP 2
4990  IF S[K] <> I-3 THEN 5010
5000  V$[K+7,K+7]="+"
5010  IF R[K] <> I-3 THEN 5030
5020  V$[K+7,K+7]="."
5030  IF U[K] <> I-3 THEN 5050
5040  V$[K+7,K+7]="."
5050  NEXT K
5060  FOR J=1 TO N1
5070  IF Y[J] <> I-3 THEN 5090
5080  V$[X[J]+7,X[J]+7]="*"
5090  NEXT J
5100  PRINT V$
5110  NEXT I
5120  PRINT "     ------------------------------------------------------------"
5130  PRINT "     0          10         20         30         40         50"
5140  RETURN
5150  REM                          replicate input segment
5160  PRINT "Input number of x values and number of y values for each."
5170  INPUT J,J0
5180  IF J*J0=0 THEN 850
5190  N1=J*J0
5200  IF N1 <= 30 AND J0 <= 6 AND J0>1 AND J>1 THEN 5250
5210  PRINT "You have selected";J;"x-values and";J0;"y-values for each x giving a total"
5220  PRINT "of";N1;"points. You should have at least 2 x-values, and 2 y-values for each."
5230  PRINT "You should also have a maximum of 6 y-values and 50 points. Please try again."
5240  GOTO 5170
5250  PRINT "Input data as x value followed by y values"
5260  FOR I=1 TO N1 STEP J0
5270  GOTO J0 OF 5280,5290,5320,5350,5380,5410
5280  GOTO 5210
5290  INPUT W[I],Z[I],Z[I+1]
5300  W[I+1]=W[I]
5310  GOTO 5430
5320  INPUT W[I],Z[I],Z[I+1],Z[I+2]
```

```
5330  W[I+1]=W[I+2]-W[I]
5340  GOTO 5430
5350  INPUT W[I],Z[I],Z[I+1],Z[I+2],Z[I+3]
5360  W[I+3]=W[I+2]-W[I+1]-W[I]
5370  GOTO 5430
5380  INPUT W[I],Z[I],Z[I+1],Z[I+2],Z[I+3],Z[I+4]
5390  W[I+4]=W[I+3]-W[I+2]-W[I+1]-W[I]
5400  GOTO 5430
5410  INPUT W[I],Z[I],Z[I+1],Z[I+2],Z[I+3],Z[I+4],Z[I+5]
5420  W[I+1]=W[I+2]-W[I+3]-W[I+4]-W[I+5]-W[I]
5430  IF W[I]=9999 THEN 5160
5440  NEXT I
5450  GOTO 1200
5460  END
```

FILER

(MONITOR FILE PROGRAM)

```
10  DIM N$[40]
20  FILES File00
30  FOR I=1 TO 300
40  GOTO TYP(1) OF 80,50,100
50  READ #1;N$,A,B,C
60  PRINT  USING 65;N$,A,B,C
65  IMAGE 9x,40a,2d,3d.3d,x,d
70  GOTO 40
80  READ #1;A,B,C,N$,D,E,F
90  PRINT  USING 93;A,B,C-31,N$,D,E,F
93  IMAGE 2d,2d,x,3d,x,40a,2d,x,3d.3d,x,d
95  NEXT I
100  GOTO 200
200  END
```

DOCUMENT MINIG1

PROGRAM TO CONVERT AUDIOLOGICAL DATA TO GRAPHICAL FORM

```
      TRACE 2
      MASTER OTOTEST
      REAL ZL(6),ZR(6),ZBR(4),ZBL(4)
      DIMENSION IL(6),IR(6),IBL(4),IBR(4)
      DIMENSION IL1(6),IR1(6),IBL1(4),IBR1(4),NAME(15)
      REAL X(6)/1.0,2.0,3.0,4.0,5.0,6.0/,X1(4)/2.0,3.0,4.0,5.0/
      CALL OPENGINOGP
      CALL UNITS(0.3)
      READ(1,9) NN
    9 FORMAT(I1)
      CALL DEVPAP(1000.0+NN*300.0,2020.0,0)
      CALL CHASIZ(0.,0.0)
      CALL AXIPOS(1,300.0,300.0,300.0,1)
      CALL AXISCA(2,0.0,6.0,1)
      DO 101 II=1,NN
      READ(1,10)(ITBL,ITEST,(NAME(J),J=1,15))
   10 FORMAT(I4,I6,15A4)
      WRITE(2,12)(ITBL,ITEST,(NAME(J),J=1,15))
   12 FORMAT(1H ,I5,I6,15A4)
      IF(II.GT.1)CALL SHIFT2(0.0,0.0)
      CALL AXIORA(1,0,1)
      CALL MOVTO2(120.0,0.0)
      CALL CHAHOL(20HAUDIO-FREQUENCY OF TEST*.)
      READ(1,20)((IL1(IB),IB=1,6),(IBL1(IB),IB=1,4),(IR1(IB),IB=1,6),
     1(IBR1(IB),IB=1,4))
   20 FORMAT(10I6,/,10I6)
      WRITE(2,13)(IL1(IB),IB=1,6)
      WRITE(2,13)(IR1(IB),IB=1,6)
   13 FORMAT(1H ,6I6)
      WRITE(2,14)(IBL1(IB),IB=1,4)
      WRITE(2,14)(IBR1(IB),IB=1,4)
   14 FORMAT(1H ,4I6)
      DO 40 II=2,ITEST
```

```
      I2=2
   50 CALL AXIPOS(1,30.0,30.0+(I1-2)*110,90.0,2)
      CALL AXISCA(2,9,-60.0,30.0,2)
      CALL AXIDRA(-1,-1,2)
      CALL DASHED(1,10.0,5.0,5.0)
      CALL MOVTO2(30.0,70.0+(I1-2)*120)
      CALL LINBY2(300.0,0.0)
      CALL MOVBY2(0.0,40.0)
      CALL LINBY2(-300.0,0.0)
      CALL MOVBY2(5.0,10.0)
      CALL CHAHOL(8H TEST *.)
      IF(I2.EQ.2)CALL CHAINT(I1+5,2)
       IF(I2.NE.2)CALL CHAINT(I1,2)
      CALL BROKEN(0)
      CALL PENSEL(1,0.0,0)
      CALL GRASYM(X,ZL,6,3,0)
      CALL GRAPOL(X,ZL,6)
      CALL PENSEL(2,0.0,0)
      CALL GRASYM(X,ZR,6,4,0)
      CALL GRAPOL(X,ZR,6)
      CALL PENSEL(5,0.0,0)
      CALL GRASYM(X1,ZBL,4,1,0)
      CALL GRAPOL(X1,ZBL,4)
      CALL PENSEL(7,0.0,0)
      CALL GRASYM(X1,ZBR,4,2,0)
      CALL GRAPOL(X1,ZBR,4)
      CALL PENSEL(1,0.0,0)
      IF(I2.EQ.2)I1=I1+6
   99 CONTINUE
      IF(ITEST.GT.7)I1=I1-6
      CALL MOVTO2(50.0,(I1-1)*120.0-40.0)
      CALL CHAARR(NAME,15,4)
      CALL MOVTO2(50.0,(I1-1)*120-60.0)
      CALL PENSEL(5,0.0,0)
      CALL SYMBOL(1)
      CALL CHAHOL(17HLEFT BONE       *.)
      CALL PENSEL(7,0.0,0)
      CALL SYMBOL(2)
      CALL CHAHOL(17HRIGHT BONE      *.)
      CALL PENSEL(1,0.0,0)
      CALL SYMBOL(3)
      CALL CHAHOL(17HLEFT AURAL      *.)
      CALL PENSEL(2,0.0,0)
      CALL SYMBOL(4)
      CALL CHAHOL(14H RIGHT AURAL*.)
      CALL PENSEL(1,0.0,0)
      I2=I
  101 CONTINUE
      CALL DEVEND
      STOP
      END
      FINISH
```

APPENDIX 3

PATIENT DATA

Containing:

(1) Some important parameters in tabular form.

(2) A key to the parameters (as in program "PARAMWRT" and stored in "PARAMFL".

(3) Case histories.

INPUT PARAMETERS

CODE		3	4	5	6	7	8	9	10	11	12	22	23	24	25
1	M	46.00	72.00	1.91	83.70	0.321	17.77	11.34	12.28	17.68	5.70	0.325	17.52	17.51	16.46
2	M	59.00	75.00	2.02	115.00	0.185	33.58	26.87	34.80	39.27	6.21	0.245	30.24	29.96	25.74
3	M	41.00	70.40	1.87	87.00	0.275	16.34	15.04	16.90	16.73	4.49	0.335	13.64	13.47	13.52
4	F	60.00	49.10	1.40	44.20	0.347	29.11	20.28	21.63	31.68	10.10	0.347	31.35	31.68	25.72
5	M	55.00	65.00	1.77	80.90	0.367	18.82	14.64	15.40	18.84	6.91	0.379	18.58	18.45	17.66
6	F	58.00	58.00	1.65	75.90	0.293	15.42	17.05	18.85	17.05	4.52	0.327	15.50	15.33	12.57
8	M	55.00	71.00	1.90	82.20	0.250	17.58	14.61	16.90	17.65	4.40	0.258	17.63	17.26	18.50
9	F	36.00	50.00	1.51	72.00	0.387	13.71	13.96	14.62	13.96	5.31	0.370	14.68	14.64	13.52
10	F	48.00	59.00	.00	66.70	0.459	13.73	12.65	12.98	13.53	6.30	0.482	12.31	12.35	12.42
11	M	24.00	80.00	2.10	79.20	0.277	24.87	24.87	27.91	26.30	6.89	0.308	23.41	23.56	23.29
12	F	69.00	70.00	1.97	85.80	0.222	22.29	20.01	24.07	23.86	4.95	0.237	22.62	22.47	20.25
13	M	29.00	80.00	2.08	87.60	0.232	18.88	16.21	19.21	20.69	4.38	0.221	21.70	21.47	18.60
14	M	70.00	59.20	1.67	87.20	0.149	21.20	14.30	20.50	21.50	3.16	0.152	21.58	21.38	20.25
15	M	53.00	71.00	1.85	81.10	0.309	20.88	18.16	19.82	21.34	6.45	0.341	19.38	19.23	18.82
16	M	27.00	75.00	2.00	98.00	0.223	17.63	15.31	18.39	19.00	3.93	0.000	.00	.00	.00
17	M	43.00	62.00	1.74	75.00	0.320	30.59	36.39	39.43	39.09	9.79	0.477	23.87	23.90	21.51
18	F	51.00	50.20	1.48	64.20	0.304	12.07	11.19	12.27	11.87	3.67	0.340	10.74	10.66	10.86
19	M	53.00	79.00	2.00	114.00	0.166	24.08	25.48	34.69	25.48	4.00	0.240	20.14	19.84	19.86
20	M	59.00	65.00	1.73	95.00	0.188	28.50	32.54	41.80	32.55	5.36	0.290	23.76	23.62	19.95
21	F	68.00	50.00	1.55	78.00	0.264	16.42	10.08	11.47	17.02	4.33	0.272	16.48	16.34	14.73
22	F	76.00	47.00	1.39	88.50	0.036	99.20	21.45	85.20	110.45	3.57	0.036	96.21	109.64	110.39
23	F	41.00	64.00	1.73	79.00	0.362	19.47	18.33	19.41	18.33	7.05	0.326	19.53	20.62	18.33
24	M	38.00	70.00	1.87	440.00	0.038	38.16	13.15	49.66	38.49	1.45	0.035	38.36	39.08	38.97
25	F	30.00	54.00	1.59	95.00	0.313	10.32	8.03	8.75	9.15	3.23	0.000	.00	.00	.00
26	M	65.00	73.00	1.88	117.00	0.136	19.32	14.66	22.12	19.75	2.63	0.131	18.77	20.41	20.27
27	F	52.00	50.00	.00	490.00	0.057	12.68	6.06	16.51	12.72	.72	0.084	10.89	10.83	10.76
28	M	61.00	77.00	1.97	175.60	0.203	18.30	13.42	16.72	17.20	3.71	0.209	18.69	16.83	16.59
29	M	65.00	73.00	1.88	80.00	0.136	19.32	14.66	22.12	19.75	2.63	0.131	18.77	20.41	20.27
30	M	67.00	77.60	1.90	137.50	0.250	10.02	14.67	20.32	16.96	2.51	0.250	20.02	20.59	20.32
31	M	34.00	58.00	1.65	89.00	0.263	45.54	29.08	30.94	46.07	11.98	0.328	51.09	52.22	54.57

INPUT PARAMETERS

CODE		54	55	56	57	58	59	60	61	70	71	72	73	74	75	76	77
1	M	7.00	84.50	.15	41.25	45.75	.18	34.19	37.44	.00	.00	.00	.00	.00	.00	.00	.00
2	M	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
3	M	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
4	F	17.00	47.50	.32	24.62	26.74	.32	23.03	25.45	.00	48.70	.00	.00	.00	.00	.00	.00
5	M	11.00	87.10	.29	22.25	28.65	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
6	F	32.00	76.80	.00	.00	.00	.23	19.95	21.93	.00	.00	.00	.00	.00	.00	.00	.00
8	M	21.00	99.10	.00	.00	.00	.19	.00	16.87	.00	.00	.00	.00	.00	.00	.00	.00
9	F	12.00	.00	.00	.00	.00	.42	11.36	11.28	.00	.00	.00	.00	.00	.00	.00	.00
10	F	9.00	.00	.00	.00	.00	.36	14.03	13.92	21.00	.00	.35	14.55	15.53	.34	15.52	15.60
11	M	24.00	.00	.00	.00	.00	.12	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
12	F	14.00	72.10	.00	.00	.00	.19	19.61	21.20	.00	.00	.00	.00	.00	.00	.00	.00
13	M	21.00	80.90	.30	13.59	13.44	.28	15.09	14.91	.00	.00	.00	.00	.00	.00	.00	.00
14	M	11.00	96.30	.15	18.25	18.15	.15	18.10	18.15	36.00	.00	.14	14.54	14.43	.14	14.39	14.90
15	M	16.00	78.90	.44	11.01	10.28	.44	10.95	10.28	.00	.00	.00	.00	.00	.00	.00	.00
16	M	2.00	102.00	.00	.00	.00	.21	16.92	17.19	14.00	101.30	.26	14.47	15.04	.26	15.24	15.04
17	M	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
18	F	15.00	70.30	.28	11.55	11.62	.33	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
19	M	17.00	.00	.21	22.15	22.61	.23	20.67	20.98	.00	.00	.00	.00	.00	.00	.00	.00
20	M	9.00	.00	.00	.00	.00	.25	18.77	18.62	15.00	.00	.00	.00	.00	.29	.00	15.34
21	F	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
22	F	31.00	93.80	.09	15.74	16.31	.08	15.58	16.34	.00	116.00	.00	.00	.00	.00	.00	.00
23	F	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
24	M	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
25	F	3.00	.00	.08	17.49	17.39	.08	17.34	17.39	6.00	.00	.00	.00	.00	.05	16.03	16.05
26	M	6.00	.00	.00	.00	.00	.09	17.40	17.26	.00	.00	.00	.00	.00	.00	.00	.00
27	F	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
28	M	6.00	.00	.00	.00	.00	.13	19.99	20.04	.00	.00	.00	.00	.00	.00	.00	.00
29	M	6.00	194.80	.00	.00	.00	.09	17.40	17.26	8.00	.00	.00	.00	.00	.07	20.22	20.13
30	M	10.00	89.00	.34	9.60	7.88	.34	9.22	9.07	.00	.00	.00	.00	.00	.00	.00	.00
31	M	9.00	.00	.24	35.39	41.83	.21	39.05	44.50	.00	.00	.00	.00	.00	.00	.00	.00

CALCULATED PARAMETERS

CODE		12	13	14	15	20	21	26	27	28	38	39	42	43	44
1	M	5.70	3.64	3.94	5.68	1.58	59.73	5.69	5.69	5.35	24.68	930.4	95.78	93.89	100.22
2	M	6.21	4.97	6.44	7.26	1.66	61.51	7.41	7.34	6.31	44.77	1662.4	62.47	61.92	69.05
3	M	4.49	4.14	4.65	4.60	1.28	48.06	4.57	4.51	4.53	23.21	873.8	91.99	93.91	98.16
4	F	10.10	7.04	7.51	10.99	4.11	144.30	10.88	10.99	8.92	59.29	2079.3	149.04	82.40	84.43
5	M	6.91	5.37	5.65	6.91	2.13	78.04	7.04	6.99	6.69	28.95	1063.3	91.84	74.36	82.13
6	F	4.52	5.00	5.52	5.00	1.56	54.76	5.07	5.01	4.11	26.59	934.5	80.77	62.44	64.31
8	M	4.40	3.65	4.22	4.41	1.24	46.26	4.55	4.45	4.77	24.76	925.3	99.52	86.06	95.06
9	F	5.31	5.40	5.66	5.40	2.12	70.28	5.43	5.42	5.00	27.42	907.9	86.54	71.96	81.75
10	F	6.30	5.81	5.96	6.21	2.14	.00	5.93	5.95	5.99	23.27	0.0	95.63	81.09	88.11
11	M	6.89	6.89	7.73	7.29	1.72	65.61	7.21	7.26	7.17	31.09	1184.3	104.79	137.35	141.95
12	F	4.95	4.44	5.34	5.30	1.41	50.24	5.36	5.33	4.80	31.84	1131.5	68.81	57.72	60.09
13	M	4.38	3.76	4.46	4.80	1.10	42.12	4.80	4.74	4.11	23.60	907.7	101.74	128.67	131.20
14	M	3.16	2.13	3.05	3.20	1.07	37.83	3.28	3.25	3.08	35.81	1269.5	79.90	50.44	56.10
15	M	6.45	5.61	6.12	6.59	1.82	69.75	6.61	6.56	6.42	29.41	1128.6	105.34	91.77	100.72
16	M	3.93	3.41	4.10	4.24	1.05	39.31	.00	.00	.00	23.51	881.5	74.48	98.07	100.31
17	M	9.79	11.64	12.62	12.51	3.16	112.51	11.39	11.40	10.26	49.34	1758.0	113.01	94.00	99.06
18	F	3.67	3.40	3.73	3.61	1.46	49.58	3.65	3.62	3.69	24.04	815.5	89.28	63.32	67.54
19	M	4.00	4.23	5.76	4.23	1.01	39.97	4.83	4.76	4.77	30.48	1204.0	63.23	70.67	77.56
20	M	5.36	6.12	7.86	6.12	1.65	61.94	6.89	6.85	5.79	43.85	1647.4	81.42	64.97	72.45
21	F	4.33	2.66	3.03	4.49	1.73	55.93	4.48	4.44	4.01	32.84	1059.4	77.94	45.99	47.63
22	F	3.57	.77	3.07	3.98	1.52	51.38	3.46	3.95	3.97	211.06	7136.7	61.25	31.95	35.18
23	F	7.05	6.64	7.03	6.64	2.20	81.48	6.37	6.72	5.98	30.42	1125.4	76.66	79.91	90.07
24	M	1.45	.50	1.89	1.46	.41	15.51	1.34	1.37	1.36	54.51	2040.6	9.71	19.02	19.66
25	F	3.23	2.51	2.74	2.86	1.20	40.63	.00	.00	.00	19.11	649.1	60.24	62.30	68.76
26	M	2.63	1.99	3.01	2.69	.72	27.95	2.46	2.67	2.66	26.47	1027.7	60.99	54.85	61.43
27	F	.72	.35	.94	.73	.29	.00	.91	.91	.90	25.36	0.0	7.06	8.95	9.49
28	M	3.71	2.72	3.39	3.49	.96	37.71	3.91	3.52	3.47	23.77	928.9	34.73	40.61	45.41
29	M	2.63	1.99	3.01	2.69	.72	27.95	2.46	2.67	2.66	26.47	1027.7	103.34	80.22	89.85
30	M	2.51	3.67	5.08	4.24	.65	26.37	5.01	5.15	5.08	12.91	527.4	89.13	74.61	83.43
31	M	11.98	7.65	8.14	12.12	4.13	145.18	16.76	17.13	17.90	78.52	2760.0	89.13	80.98	82.82

Input Parameto	Codes for doses 1st	2nd	3rd
Reference code	1		
Sex (1 = male, 2 = female)	2		
Age (years)	3		
Weight (Kg)	4		
Body surface Area (m^2)	5		
Dose intervals (8h) from 1st dose	-	54	70
Creatinine	6	55	71
K (full) (Kf)	7	56	72
V (AUC) (Vf)	8	57	73
V (intercept) (V_I)	9	-	-
V (intercept-calc. trough) (V_I-ctr)	10	-	-
V (intercept-trough) (V_{I-tr})	11	58	74
K (3 points) (K_3)	22	59	75
V (5 points AUC) (V_5)	23	60	76
V (4 points AUC) (V_4)	24	-	-
V (intercept-trough, 3 points) ($V_{I\,3}$)	25	61	77
Dose given (mg)	171	-	-
Mean error in 2-point K_s	172	-	-

PARAMETERS CALCULATED BY "PARAMWRT"

SD refers to Standardised differences of the form:(A-B)/B

D = dose (mg), Cr = creatinine Cl = clearance

Description				Parameter codes for measurements 1st	2nd	3rd
Clearance using appropriate K and Volumes:						
	8	57	73	12	66	82
	9	-	-	13	-	-
	10	-	-	14	-	-
	11	58	74	15	67	83
	23	60	76	26	68	84
	24	-	-	27	-	-
	25	61	77	28	69	85
Reciprocal volumes for:						
	8	57	73	16	62	78
	9	-	-	17	-	-
	10	-	-	18	-	-
	11	58	74	19	63	79
	23	60	76	29	64	80
	24	-	-	30	-	-
	25	61	77	31	65	81
Volume as percentage of						
	Weight			38		
	Body surface Area			39		
	(Body Surface Area)$^{3/2}$			40		
Dose to give mean steady-state level of 2.5mg/L (from AUC)						
	mg/Kg			20		
	mg/m^2			21		

SD K_3 and Kf			41	92	93
SD V_I and Vf			48	-	-
SD V_{I-Ctr} and Vf			49	-	-
SD V_I-tr and Vf			50	86	89
SD V_5 and Vf			51	87	90
SD V_{4AUC} and Vf			52	-	-
SD $V_{I\,3}$ and Vf			53	88	91
SD Doses from clearances					
	12	66	82		
by means of					
	13	-	-	32	-	-
	14	-	-	33	-	-
	15	67	83	34	117	120
	26	68	84	35	118	121
	27	-	-	36	-	-
	28	69	85	37	119	122
Dose in mg/Kg				173		
Total Dose (mg/Kg) between 1st and 2nd doses				174		

	1st	2nd	3rd
Creatinine clearance by methods of:			
Jadrný	42	135	141
Cockcroft	43	136	142
Siersback-Nielsen	44	137	143
K from Jeliffe formulae using:			
Jadrný	129	147	150
Cockcroft	130	148	151
Siersback-Nielsen	131	149	152
SD between CrCl and Cl			
Jadrný	45	138	144
Cockcroft	46	139	145
Siersback-Nielsen	47	140	146
SD between dose calculated from K by Cr and V = 0.3 Weight and dose calculated from AUC (Dose from clearances in parantheses)			
Jadrný	132(124)	153	156
Cockcroft	133(125)	154	157
Siersback-Nielsen	134(126)	155	158
SD serum creatinine compared to first dose	-	159	163
SD CrCl compared to 1st dose			
Jadrný	-	160	164
Cockcroft	-	161	165
Siersback-Nielsen	-	162	166

Standardised differences between 2nd/3rd and initial measurements

		2nd	3rd
K		102	104
K_3		103	105
Vf		106	107
V_{I-tr}		167	168
V_{I-tr} cf Vf (1st)		169	170
V_5		115	116
V_5 cf Vf (1st)		127	128
Clearances from:			
66	82	108	110
68	84	109	123
Doses calculated from			
66	82	94	98
67	83	95	99
68	84	96	100
69	85	97	101

PATIENT HISTORIES

Code	Initials	Diagnosis, clinical course, other drugs (Dose and mean level (μg/ml) are given)
1	PB	neutropenic, febrile. Lincomycin previously. 80mg tds (Mean 1.75)
2	TD	perforated colon, pyrexia 120 mg tds (Mean 2.41)
3	AlDu	faecal fistula, surgery performed. Post-operative pyrexia and erythema. Clindamycin with gentamicin 120 mg tds (Mean 3.33)
4	NF	Inoperable cancer, bronchus. 1/7 history of rigors and malaise. Clindamycin with gentamicin. Inappropriate ADH secretion lead to deafness and dizziness after 7/7. Antibiotics replaced with cefoxitin. VP16 trial finished 5/7 previously 80 mg tds (Mean 0.99)
5	WF	urethral bleeding following straddle injury. Became pyrexial, had tachycardia and rigors. Blood culture -ve, urine grew Proteus mirabilis, later sterile. 100 mg tds (Mean 1.81)
6	MG	PUO (2/12) unresponsive to ampicillin or co-trimoxazole. ? endocarditis. Did not respond to gentamicin and penicillin. Surgery found carcinoma of gall bladder. Also given Spironolactone 2.5 mg bd, Penicillin 2.4 g qds iv 111.25 mg tds (Mean 3.08)
7		Blank for program audit
8	HJ	Severe peripheral artery disease. Surgery for bilateral common iliac occlusion. Sudden fever, with rigors, after 2/7. No obvious source or organism found. Good initial progress but still pryexial until penicillin (1.2 g qds) added after 4/7 103 mg tds (Mean 2.93)
9	JK	Cystic fibrosis. Pseudomonas infection. Dose reduced (160 to 120 mg) after 3/7 due to high-tone hearing loss with good drug levels. 140 mg tds (Mean 3.30)
10	AL	hydronephritis with renal stone. Loin pain, fever, rigors, did not respond to co-trimoxazole. E. coli found in urine but not blood. Responded rapidly to gentamicin. 100 mg tds (Mean 1.98)

11	JP	Aspiration pneumonia with bacterial infection. E. coli found in blood and sputum. Responded well. Signs of nephrotoxicity after 1/52. Drug and creatinine levels rose. Drug stopped and creatinine returned to normal in 1/52. Erythromycin 500 mg iv qds. Chloramphenicol 500 mg qds 160 mg tds (Mean 2.90)
12	LP	Prophyllaxis following colectomy. Carcinoma of the cervix. No pyrexia. Frusemide 40 mg od. Slow K 600 mg bd 128 mg tds (Mean 3.03)
13	SP	UTI with pyelonephritis and clinical bacteraemia. Quadriplegic following accident. Good response but rising levels lead to dose reduction. Later recurrence of pyrexia due to gentamicin-resistant Pseudomonas species. Treated with amikacin, levels also rose during treatment. 94.5 mg tds (Mean 3.65)
14	LS	Small bowel resection, poor nutritrion investigated. 1/12 later sudden pyrexia, tachycardia, low BP. Pseudomonas UTI, E. coli septicaemia. 14/7 therapy with Penicillin (2/7), Carbenicillin (2/7). Recurrence of septicaemia 6/7, deterioration leading to death 5/52. 105 mg tds (Mean 3.74)
15	GS	Abdomino-perineal resection. (diffuse adenocarcinoma of rectum). Post-operative prophyallaxis. 80 mg tds (Mean 2.03)
16	MT	Haemophilus parainfluenzae endocarditis 80 mg tds (Mean 2.54)
17	RT	Jaundice and ulcerative colitis. Fever and rigors following percutaneous cholangiogram. Presumptive gram negative septicaemia. Patient improved but fever remained until transfer to Queen Elizabeth Hospital for ERCP. Metronidazole and cephazolin at Queen Elizabeth Hospital. Azathioprine (2/12) Prednisolone (1/12) Sulphasalazine (16 years) 120 mg tds (Mean 1.53)
18	AuWh	Pyrexia with rigors following T tube cholangiogram. Gram negative rods cultured from T tube. Resolved satisfactorily. Navidrex K o.m. Prednisolone 0.5/0.75 mg alt.diem. 100 mg tds (Mean 3.41)

19	AlWh	Diarrhoea, vomiting, abdominal pain, hypotensive. Oliguric for 24 hours but responded well to treatment. All investigations normal, negative cultures. 137.8 mg tds (Mean 4.31)
20	AlWi	Perforated appendix, post-op pyrexia. Successful prophyllactic therapy. 200 mg bd (Mean 3.10)
21	LW	Colonic perforation. Post-op prophyllaxis. Refused venepuncture for third profile. 100 mg tds (Mean 2.88)
22	VB	Pyloric oedema, secondary to benign gastric ulcer. Signs of bilateral bronchopneumonia, pyrexia, rigors 80 mg tds (Mean 2.80)
23	SW	Febrile, rigors, vaginal discharge. Fractured pelvis and clavicle. Alcoholic, pregnant, multiple suicide attempts. No organisms cultured. Good response. 100 mg tds (Mean 1.77)
24	CH	Severely ill, clinical diagnosis of septicaemia. Oliguric on admission going to acute renal failure. Haemodialysis necessary. Renal function and fever resolved. 120 mg tds (Mean 10.34)
25	MW	Renal surgery with complications. Post-op bleeding, 'Shock lung', pneumothorax, endocarditis. Became pyrexial, ampicillin resistant. Progressive oliguria leading to renal failure and dialysis. Deteriorated to death. Severe renal damage found post-mortem. 80 mg tds (Mean 3.10)
26	BW	Stenosing carcinoma. Faecal contamination of peritoneum. Transfusion on day 3. 120 mg tds (Mean 5.71)
27	JN	Perforated gastric ulcer, peritonitis, broncho-pneumonia, renal failure, shock. Initial resolution of pyrenia and pneumonia. Unable to come off ventilator, developed recurrent Pseudomonas infection. Died within 1/12. 80 mg tds (Mean 13.83)
28	WC	Aplastic anaemia. Febrile. Klebsiella blood culture. Lincomycin given with gentamicin. Fever reduced in 3/7 but patient died in 6/7. PM refused. 80 mg tds (Mean 2.69)

29	BC	Obstructive jaundice, fever. Streptococeal culture treated with gentamicin then ampicillin after sensitivity report. Poor hepatic function, cholangsistectomy on day 4, no improvement. Renal function deteriorated steadily. Died 4/7 after treatment. PM showed intrahepatic bile duct carcinoma with metastases. 80 mg tds (Mean 3.80)
30	AM	Case notes missing. 52 year old male, 67Kg. 80 mg tds (Mean 3.99)
31	ArDu	Oat cell bronchial carcinoma (VP16 trial). Pyrexia with leukopenia. Clindamycin, frusemide, spironolactone. 80 mg tds (Mean 0.83)

N.B. Mean level is that predicted to arise from unchanged dosing, not that actually attained.

APPENDIX 4

SUPPORTING PUBLICATIONS

Quarterly Journal of Medicine, New Series XLVIII, No. 191, pp. 473–491, July 1979

Human Botulism Caused by Clostridium Botulinum Type E: The Birmingham Outbreak

A. P. BALL[1], R. B. HOPKINSON[2], I. D. FARRELL[6], J. G. P. HUTCHISON[6], R. PAUL[7], R. D. S. WATSON[3], A. J. F. PAGE[3], R. G. F. PARKER[4], C. W. EDWARDS[4], M. SNOW[1], D. K. SCOTT[5], A. LEONE-GANADO[2], A. HASTINGS[1], A. C. GHOSH[8] AND R. J. GILBERT[8]

From the Departments of Communicable and Tropical Diseases[1], Anaesthesia[2], Cardiology[3], Pathology[4] and Pharmacy[5], and the Regional Public Health Laboratory[6], East Birmingham Hospital, Birmingham; Department of Neurophysiology[7], Walsgrave Hospital, Coventry, and Central Public Health Laboratory, Colindale, London[8]

Journal of Clinical and Hospital Pharmacy (1980) 5, 175–195

REVIEW OF RECENT STUDIES ON CONTROL OF AMINOGLYCOSIDE ANTIBIOTIC THERAPY

P. G. Davey, I. Gonda*, E. S. Harpur* and D. K. Scott*

*Department of Communicable and Tropical Diseases, East Birmingham Hospital, and *Department of Pharmacy, University of Aston, Birmingham*

Rapid Development of a High-performance Liquid Chromatographic Assay for 4-Aminopyridine in Body Fluids

D. K. Scott

Pharmacy Department, East Birmingham Hospital, and University of Aston in Birmingham, Gosta Green, Birmingham, B4 7ET

Journal of Clinical Pharmacy (1979) 4, 25–32.

THE STABILITY OF COMPOUND TABLETS CONTAINING ASPIRIN AND PHENACETIN: THE EFFECT OF LEGISLATION

W. J. Irwin, A. Li Wan Po and D. K. Scott

The Department of Pharmacy, The University of Aston in Birmingham, Gosta Green, Birmingham, U.K.

SCIENCE
AND TECHNOLOGY

Hospital quality control of white uncoded tablets using HPLC

By A. T. CHAMBERLAIN, BSc, PhD, CChem, MRIC* , and D. K. SCOTT, BSc MPS†

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ABBREVIATIONS AND THEIR DEFINITIONS

Abbreviations are all defined at their first usage, but the following may occur in several chapters.

Cl	Plasma clearance (volume per unit time)
Cr	Serum creatinine concentration (μM/L) or Creatinine
im	intramuscular
iv	intravenous
K	unless otherwise defined locally, see below
$K, K_o, Ka, K_{10}, K_{12}, K_{21}$	Defined at page 68
OPA	o-phthalaldehyde
SDS	Sodium dodecyl sulphate
SPS	Sodium pentane sulphonate
SMZ	Sulphamethoxazole
TMP	Trimethoprim
V	Volume of distribution (litres) of 1-compartment model