THE CLINICAL PHARMACOKINETICS OF CHLORAMPHENICOL AND CHLORAMPHENICOL SUCCINATE

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MASTER OF PHILOSOPHY

THE UNIVERSITY OF ASTON IN BIRMINGHAM

NOVEMBER 1986

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A H.P.L.C. method of analysis of serum and urine for chloramphenicol and chloramphenicol succinate was developed which demonstrated no interference from drugs likely to be co-prescribed with chloramphenicol. A method of analysing serum and urine for the major metabolic product of chloramphenicol was described.

A study was conducted in 21 paediatric patients (age 20 days - 6 1/2 years) measuring peak and trough serum samples and urine samples collected over a dosage interval to calculate pharmacokinetic parameters of chloramphenicol and chloramphenicol succinate in paediatric patients. The B.N.F. recommended dosage was employed for all patients and a wide range of peak, steady state serum concentrations of chloramphenicol was found. Only 31% of patients were within the therapeutic range (15-25 mg/L). The half-life of chloramphenicol was found to be 3.65 hours (range 1.4 - 24 hours) at steady state and the volume of distribution 0.9L/kg (range 0.3 - 1.7L/kg). There was a decrease in the half-life of chloramphenicol as the course progressed. The half-life of chloramphenicol succinate was found to be 0.9 hours (range 0.3 - 2.2 hours) and volume of distribution 0.7L/kg (range 0.2 - 1.6L/kg).

It was found that phenobarbitone increased the serum concentration of chloramphenicol in some patients, but paracetamol did not appear to interact with chloramphenicol. It was recommended that blood samples should be drawn 2 hours post intravenous dose for measurement of peak serum chloramphenicol concentrations.

Key words

Chloramphenicol
Chloramphenicol Succinate
Paediatrics
Pharmacokinetics

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INTRODUCTION

1.1 BACKGROUND

Chloramphenicol was isolated as a secretion from the bacterium Streptomyces Venezuela found in mulched soil near Caracas, Venezuela in 1947 (1). When it was determined that it contained two molecules of chlorine it was christened "Chloromycetin". The compound was chemically synthesised in 1948 and was marketed in 1949 as the first broad spectrum antibiotic. It was initially considered to be free from toxic effects, but shortly after it's introduction, serious blood dyscrasias associated with it's use were reported (2) and later a complication descriptively entitled "Grey-Baby Syndrome" (3).

A combination of these adverse effects and the introduction of safer alternative antibiotics, particularly ampicillin in 1961, lead to a decline in the usage of chloramphenicol. In 1974 the first report of ampicillin-resistant Haemophilus influenzae appeared in the United States (4). In 1977 a British multicentre study found that 11.8% of H. influenzae type b strains were ampicillin resistant. The study was repeated in 1981, when 14% of type b strains were found to be resistant (5). It is due to this significant and increasing resistance pattern that dual chloramphenicol/ampicillin therapy is now considered to be mandatory for the initial treatment of meningitis, before the sensitivity of the infecting organism is known (6).

Resistance to chloramphenicol is not unknown. Two strains of H. influenzae resistant to chloramphenicol have been reported from the USA (7) and a case of meningitis due to chloramphenicol-resistant H. influenzae type b has been described in the UK (8). This organism was not β-lactamase producing and thus proved susceptible to treatment with ampicillin. Bacterial resistance to chloramphenicol in organisms likely to cause meningitis is not, at present, a significant clinical consideration if dual therapy is used.

Nevertheless there has been a search for a new, effective antibiotic treatment for bacterial meningitis. Cefuroxime has attracted the most attention since it has more activity against H. influenzae and is also more resistant to β-lactamase than other cephalosporins (9). In a random controlled study in Sweden it was found that cefuroxime was as effective as dual chloramphenicol/ ampicillin in the treatment of bacterial meningitis. However, there was a higher incidence of adverse reactions in the cefuroxime group (n = 21) than in the dual therapy group (n = 19) (10). Until a cephalosporin can demonstrate either greater efficacy, or fewer adverse effects, than the inexpensive chloramphenicol/penicillin alternative then the British National Formulary is likely to continue to recommend dual treatment with chloramphenicol/benzylpenicillin as first line therapy for the treatment of bacterial meningitis (9).

1.2 STRUCTURE/ACTIVITY RELATIONSHIP

The structure of chloramphenicol is shown in Figure 1.1

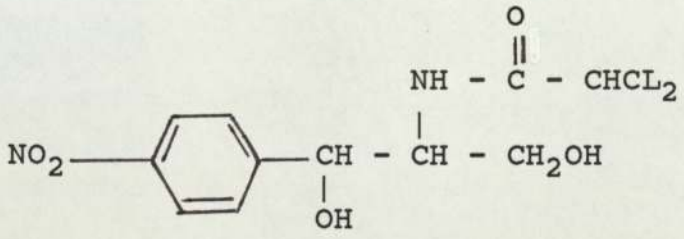


Figure 1.1 Chloramphenicol

The antibacterial activity of chloramphenicol is dependant on an intact propanol moiety. Activity dramatically decreases if there is any alteration of this group (11). The dichloroacetic acid group is also required for maximal antibiotic activity, substitution of this group decreases, but may not eliminate antibacterial activity (12).

Attempts have been made to manipulate the molecule in order to augment the antibacterial activity and eradicate toxicity. Thiamphenicol is the only analogue to have been marketed (in Europe only) in which the nitro group is replaced with methylsulphonyl group (Figure 1.2). There have been no published reports of aplastic anaemia associated with thiamphenicol. However the incidence of reversible bone marrow suppression is greater, and the antibiotic activity is less than chloramphenicol (13).

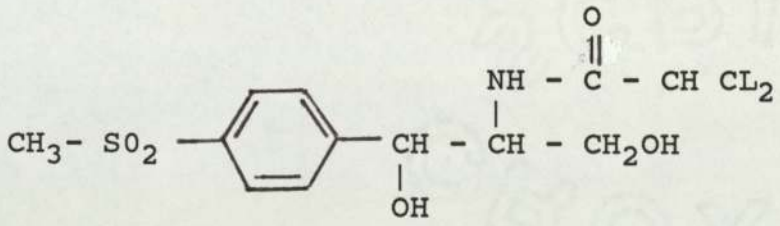


Figure 1.2 Thiamphenicol

The antibacterial activity of chloramphenicol is effected by the binding of the molecule to the 50S subunit of bacterial ribosomes. This reversibly inhibits the peptidyl transferase reaction at the ribosome, thus preventing peptide bond formation (14). The inhibition of protein synthesis is maximal when one molecule of chloramphenicol is bound per ribosome (15). When resistance develops this is usually as a result of enzymatic acetylation of the molecule, and is R-factor transmitted (16).

1.3 SPECTRUM OF ACTIVITY

Chloramphenicol is active against aerobic and anaerobic, gram positive and gram negative bacteria. It is generally bacteristatic but has bactericidal activity against H. influenzae, Streptococcus pneumoniae and Neisseria meningitidis (the latter only at concentrations of 50 mg/L which would be potentially toxic in vivo). Bactericidal

TABLE 1.1

SUSCEPTIBILTY OF BACTERIA TO CHLORAMPHENICOL

VERY SUSCEPTIBLE (MIC < 4 mg/L)	SUSCEPTIBLE (MIC 4.0 - 12.5 mg/L)	RELATIVELY RESISTANT (MIC 12.5 - 25.0 mg/L)	RESISTANT (MIC > 25mg/L)
Groups A and B Streptococci*	S. Aureus		
Clostridium Spp	Streptococci	Group D Streptococci	Pseudomonas Spp
N. Meningitidis*	L. Monocytogenes	Serratia Marcescens	Proteus (Indole Positive) Spp
N. Gonorrhoeae	E. Coli	Enterobacteriaceae	
H. Influenzae*	Klebsiella Spp		
Bordetella Pertussis	Proteus Spp		
Shigella Spp	Salmonella Spp		
Peptococci and Peptostreptococci	Vibrio Cholerae		
Fusobacterium Fusiforme	Clostridium Spp		
	Bacteroides Fragilis		

* Common Meningitis Causative Organisms

From "Drug Therapy in Infants" - R.J. Roberts

W.B. Saunders 1984

concentrations against H. influenzae and Streptococcus pneumoniae are 0.78 mg/L and 12.5 mg/L respectively, and these may be attained in the cerebrospinal fluid at therapeutic doses (17). The susceptibilty of various bacteria to chloramphenicol is shown in Table 1.1.

1.4 INDICATIONS AND DOSAGE

In the UK chloramphenicol is licensed for use in typhoid fever, H. influenzae meningitis, serious chest infections and in situations where clinical assessment, usually supplemented by laboratory studies, indicates that no other antibiotic would suffice (18).

Locally chloramphenicol therapy is used in cases of bacterial meningitis or acute epiglottitis, where the causative organism is found to be H. influenzae. In cases of suspected bacterial meningitis therapy is commenced immediately and a lumber puncture specimen of C.S.F. is obtained and sent to microbiology. Antibiotic therapy may then be terminated if the cultured C.S.F. does not show growth of a sensitive organism or the patient spontaneously improves.

The dosage of chloramphenicol in neonates (less than 14 days old) is 25 mg/kg daily in divided doses. In older infants and children 50 mg/kg daily in divided doses every six hours is recommended, except for the treatment of pyogenic meningitis when 50-100 mg/kg in divided doses every six hours should be used (19).

The dose may be administered by the oral, intravenous or intramuscular route. The intramuscular route has been associated with unreliable absorption and, as a result, inadequate serum concentrations of chloramphenicol (20). Consequently this route of administration is not used at this centre.

1.5 ADVERSE REACTIONS

A summary of the adverse effect of chloramphenicol is contained in Table 1.2. However, the most serious may be divided into three categories; bone marrow suppression, idiopathic aplastic anaemia and grey baby syndrome.

Bone marrow suppression is dose related, readily reversible and is manifested by anaemia with a normocellular marrow. Thrombocytopenia or leucopenia may also be present (21). Marrow suppression is thought to be mediated by the inhibition of mitochondrial protein synthesis in bone marrow (22). Clinical evidence of bone marow suppression has been associated with sustained peak serum concentrations in excess of 25m/L and trough concentrations of 10mg/L (23). Resolution of the dyscrasias occurs within 12 days of discontinuation of treatment.

Aplastic anaemia is well accepted as an adverse reaction to chloramphenicol therapy, although the relationship is difficult to demonstrate. The reaction is

TABLE 1.2

ADVERSE REACTIONS ASSOCIATED WITH CHLORAMPHENICOL

REACTION	IMPORTANCE	DETECTION
Dose Related		
Marrow Suppression	Reversible, primarily erythrocytic series	Decrease in reticulocyte count, increase in serum iron and total iron binding capacity. Follow serum chloramphenicol levels
Grey Baby Syndrome	Potentially fatal	Monitor serum concentrations
Retinal, Peripheral Neuropathy	Usually reversible	Monitor serum concentrations
Diarrhoea, Vomiting glossitis	Due to overgrowth of non-susceptible organisms	-
Inhibition of Immune Function	Unknown	Only shown in experimental studies
Unrelated to Drug Concentration		
Idiopathic Marrow Aplasia	Often fatal	Unknown
Allergic reactions	Fever, Maculovesicular rash	Occurs in about 0.5 - 1% of patients
Superinfections	Superinfections bacteria are often resistant to multiple antibiotics	

From "The Current Status of Chloramphenicol" - Meissner, H.C., Smith, A.L. - Paediatrics 64 3. 1979.

not related to dose, duration of therapy or route of administration. Furthermore it usually develops weeks to months after termination of therapy (24). The haemotological effect is usually pancytopenia, less commonly leucopenia or thrombocytopenia may be seen. The incidence of aplastic anaemia after exposure to chloramphenicol has been reported as between 1 in 24,500 to 1 in 40,800 therapeutic courses (25) and it has been speculated that it may involve a genetic predisposition (26) which is, at present, unpredictable.

The most notable toxic effect of chloramphenicol is the grey baby syndrome. This condition is manifested by vomiting, abdominal distension, decreased bowel sounds, respiratory depression, cardiovascular collapse and a characteristic ashen grey appearance of the skin. In 40% of infants who develop grey baby syndrome death results within 24-48 hours. Serum concentrations of 40-200mg/L of chloramphenicol have been reported in association with grey baby syndrome and although it is classically associated with premature infants (28) and neonates (29) it has been reported in both children (30) and adults (31).

1.6 THERAPEUTIC RANGE

The therapeutic range is the range between the minimum serum concentration required to achieve a therapeutic effect and the maximum serum concentration which can be maintained without significant toxicity.

The toxic reactions to chloramphenicol and their relation to serum concentration has already been discussed (Section 1.5). Authors have mainly related toxic effects to peak serum concentrations, but it has been suggested that trough serum concentrations greater than 15 mg/L should be avoided (32).

The therapeutic effect of an antibiotic at a given serum concentration is more difficult to quantify than, for example, that of theophylline when serum concentration may be correlated with a measurable physiological parameter viz. FEV (forced expiratory volume) in individual patients. The therapeutic effect of an antibiotic depends upon the M.I.C. of the antibiotic for the infecting organism and the penetration of the antibiotic to the site of the infection. In the treatment of meningitis patients the unbound chloramphenicol concentration in the C.S.F. is, together with the M.I.C. of the antibiotic for the pathogen, the most important consideration. Table 1.1 details the susceptibility of various organisms to chloramphenicol and those most likely to cause meningitis are indicated. M.I.C.'s for most organisms susceptible to chloramphenicol are below 12.5 mg/L. However the activity of chloramphenicol in the C.S.F. is greater due to the reduced concentration of albumin and therefore the greater fraction of unbound chloramphenicol. Inflammation of the meninges produces a dramatic rise in the concentration of albumin in the C.S.F., but this is not sufficient to produce the same extent of chloramphenicol protein binding that is present in the serum.

Penetration of chloramphenicol into the C.S.F. from serum will be discussed later (Section 1.7.2) but it has been found to be approximately 50% of the simultaneous serum concentration, with little decline over a dosing interval.

Peak concentrations of chloramphenicol in serum for the treatment of meningitis should not be below 15 mg/L or penetration of the C.S.F. becomes unreliable.

It has also been established that adverse reactions to chloramphenicol (other than idiopathic aplastic anaemia) are unlikely if the peak serum concentration does not exceed 25 mg/L.

Therefore the therapeutic range for chloramphenicol may be defined as peak serum concentrations between 15 - 25 mg/L although other authors have suggested ranges of 10 - 20 mg/L (33) and 10 - 25 mg/L (34). The alternative lower maximum recommended concentration of 20 mg/L may be seen as a conservative suggestion erring on the side of caution the lower minimum peak concentration of 10 mg/L is more difficult to justify since it may well produce subtherapeutic levels in the C.S.F. and needlessly expose the patient to the risk of developing aplastic anaemia.

The most recent report on the use of chloramphenicol in the treatment of meningitis in the UK recommended that peak serum concentrations should fall within the range 15 - 25 mg/L and trough concentration should be less than 15 mg/L (35).

1.7 PHARMACOKINETICS

1.7.1 ABSORPTION

Chloramphenicol is available commercially as three distinct preparations. An oral capsule containing 250 mg chloramphenicol base, a suspension of chloramphenicol palmitate containing the equivalent of 125 mg of chloramphenicol base in 5 mls, and a parenteral preparation of chloramphenicol sodium succinate containing the equivalent of 300 mg, 1g or 1.2g chloramphenicol base per vial.

Since chloramphenicol base has an extremely bitter taste the suspension is formulated using chloramphenicol palmitate, which has no antibacterial activity. This palmitate ester is hydrolysed in the small intestine by pancreatic esterases, yielding free chloramphenicol which is subsequently absorbed (36). The rate of hydrolysis of the ester is an inverse function of particle size (37). The bioavailability of chloramphenicol, when administered in the form of chloramphenicol palmitate suspension, is approximately 80% as measured by recovery of total nitro compounds in the urine (37).

The succinate ester of chloramphenicol is used for the parenteral formulation since it is very water soluble (13). Clinically chloramphenicol succinate is similar to the palmitate in that it has no intrinsic antibacterial activity. Hydrolysis is required to yield active chloramphenicol (38). Unlike the palmitate this occurs in the liver, lungs and kidney (39).

Early research workers assumed that this hydrolysis occurred rapidly and to completion but more recent work has demonstrated that a variable proportion of chloramphenicol succinate is excreted renally without being hydrolysed (40). This discovery invalidates much of the pharmacokinetic research in chloramphenicol before 1977 and accounts for the wide variations in pharmacokinetic parameters described by authors before this date.

1.7.2 DISTRIBUTION

Chloramphenicol penetrates well into most tissues and tissue fluids. Detectable concentrations have been found in the brain (41) and in the heart, lung, kidney, liver and spleen (42). It also diffuses in ascitic fluid (43), bile (44), breast milk (45), saliva (46) and crosses the placenta (47).

The most significant aspect of chloramphenicol distribution when treating bacterial meningitis is it's penetration into the cerebrospinal fluid. In adults concentrations of chloramphenicol in the C.S.F. are approximately 50% of the levels in serum samples obtained simultaneously (48). In newborn and young infants penetration is reported to be even better, concentrations in C.S.F. are up to 99% of the simultaneous serum levels (49). However, it should be noted that wide variations exist in these figures and C.S.F. concentrations as low as 20% of the simultaneous serum concentrations have also been recorded.

It has been shown that the fluctuation in C.S.F. concentration throughout a dose interval is much less than the corresponding serum concentration profile (50).

Neither the pharmacokinetic importance nor the quantity of chloramphenicol present in the bile has been asessed. Following secretion of the bile into the gastro-intestinal tract the drug would be reabsorbed, but the total quantity involved is likely to be small and of dubious clinical significance. However, of potentially more importance is the concentration of chloramphenicol glucuronide in the bile (44). Microflora in the gastro-intestinal tract contains β-glucuronidase which may hydrolyse chloramphenicol glucuronide, liberating chloramphenicol and allowing reabsorption of the active drug (51). Specifically patients with impaired renal function may accumulate chloramphenicol glucuronide which may then be hydrolysed by lysosomal β-glucuronidase present in the liver, kidney, spleen, endocrine and reproductive organs producing high, sustained chloramphenicol serum concentrations (52).

The degree of plasma protein binding of chloramphenicol in healthy adults has been variably reported as 53% (53), 66% (54) and 60% (55) depending on the technique used. Binding is primarily with albumin at a single binding site, and of a hydrophobic nature (55). Serum protein binding of chloramphenicol is not altered by the addition of extrinsic bilurubin to the sera of normal adults or premature infants (53).

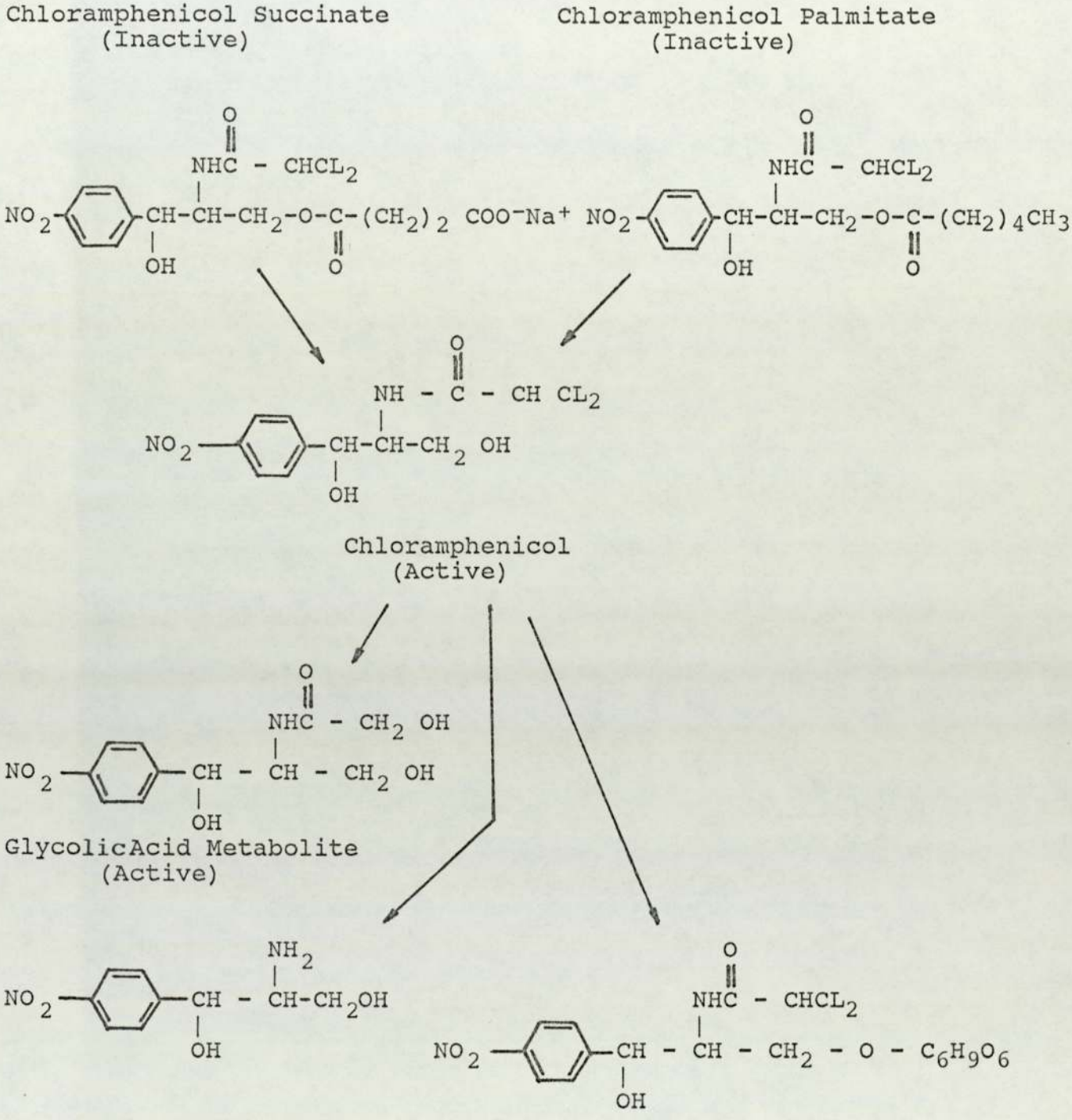
The unbound chloramphenicol is the active entity and only unbound chloramphenicol is able to cross the blood brain barrier into the C.S.F. The percentage of unbound chloramphenicol increases as serum albumin concentrations decrease (55). In patients with advanced cirrhosis the half-life of chloramphenicol increases fourfold, this may be correlated with the increased level of bilirubin and decreased level of albumin in serum (53). Furthermore, body fluids which have a lower protein content than serum exhibit higher ratios of unbound drug and therefore higher antibacterial activity. One study found that a total serum concentration of 18mg/L of chloramphenicol was required to produce the same antibacterial titre as that achieved by a total concentration of 8.8mg/L chloramphenicol in the C.S.F. (55).

1.7.3 METABOLISM AND ELIMINATION

Chloramphenicol is eliminated both renally and by metabolism in the liver. Approximately 5-15% of the dose is excreted unchanged in the urine by glomerular filtration (56) and this range has been demonstrated in both paediatric (57) and adult populations (58). Chloramphenicol renal clearance shows a direct relationship to creatinine clearance in adults (59), but since this is only the minor route of elimination, dosage adjustment in renal failure is not considered necessary even in patients with a glomerular filtration rate less than 10 mls/min (60).

Figure 1.3

CHLORAMPHENICOL; ITS ESTERS AND METABOLITES



Adapted from "The Current Status of Chloramphenicol"
Meissner H.C., Smith A.L. Paediatrics 64 3 1979

The major elimination route is the liver where chloramphenicol is metabolised to mostly inactive products (61). Chloramphenicol glucuronide is the principal metabolite produced by hepatic glucuronyl transferase present in the encloplasmic reticulum of the hepatocyte (62). Chloramphenicol glucuronide is then excreted renally by tubular secretion (44). Other metabolites are a deacetylated amine, which has no antibacterial activity and a glycolic acid metabolite which has limited activity (11). The hydrolysis of chloramphenicol's esters and metabolism of the active drug are illustrated in Figure 1.3.

In view of the toxicity of chloramphenicol it has been recommended that dosage should be adjusted in the event of liver dysfunction (16). However, the author makes no attempt to define liver dysfunction in terms of measurable parameters. It has been suggested that the extent of hepatocellular damage and thus reduction in the liver's ability to conjugate chloramphenicol might be quantified by the degree of hyperbilirubinaemia and hypoalbuminaemia (63). This is supported by another study which demonstrated that in adult patients with elevated total serum bilirubin concentrations (> 1.5mg/100 mls) the mean apparent total body clearance of chloramphenicol was only 56% of that in patients with normal liver function (53). Currently there are no recognised guidelines for establishing a chloramphenicol dose in the presence of liver disease.

1.8 DRUG INTERACTIONS WITH CHLORAMPHENICOL

1.8.1 PENICILLINS/CHLORAMPHENICOL

The bactericidal effect of penicillins is mediated by inhibition of a specific step in cell wall synthesis, and is greatest during the early phase of bacterial growth. Chloramphenicol inhibits new protein synthesis and is (for most organisms) a bacteristatic agent, preventing growth and thus blunting the effect of the penicillin (64).

This theoretical interaction has been supported by a limited number of in vitro and in vivo studies, but its clinical significance has not been sufficiently evaluated. Two studies have compared the efficacy of dual chloramphenicol/ampicillin therapy versus chloramphenicol only in the treatment of Salmonella typhi; one study found no advantage in dual therapy (65) whereas the second found dual therapy to be superior to chloramphenicol alone (66).

Two clinical studies have reported possible antagonism between a penicillin and chloramphenicol for the treatment of meningitis. The first compared ampicillin/chloramphenicol/ streptomycin (streptomycin for the first day only) with ampicillin only and found the mortality rate to be greater (11.4%; n = 123) in the triple therapy group compared with (4.1%; n = 145) in the ampicillin only group (67). The second study found no difference in the mortality rate between single and dual therapy group, but found that

children with Haemophilus influenzae meningitis had more eighth nerve sequelae when treated with ampicillin/ chloramphenicol than when treated with ampicillin alone (68). However, this second study was neither randomised nor controlled and the number of infants studied was too small to draw meaningful conclusions. Both studies were performed before the emergence of ampicillin resistant H. influenzae was reported, and an ampicillin-only treatment group would now be considered ethically unacceptable.

Although no study has been published comparing ampicillin/chloramphenicol and chloramphenicol alone in the treatment of H. influenzae meningitis the theorisation for a penicillin/chloramphenicol interaction lacks clinical credibility in this type of meningitis since chloramphenicol is claimed to be bacteriacidal against H. influenzae and S. Pneumoniae (17).

1.8.2 PARACETAMOL/CHLORAMPHENICOL

The prolongation of chloramphenicol half life by concurrent administration of paracetamol is considered sufficiently significant to be included in the B.N.F. drug interactions section. This interaction is based on a paper published in the B.M.J. in 1979 (69) where the authors, while studying hepatic microsomal glucuronidation in children with kwashiorkor, noted that the half life of chloramphenicol was prolonged from the normal 2 - 3 hours to 18 - 24 hours in

patients administered paracetamol simultaneously. A follow up investigation was performed on six adult intensive care patients receiving intravenous chloramphenicol where the effect of single doses of paracetamol on chloramphenicol kinetics were studied.

The authors reported that the half life of chloramphenicol was increased from 3.25 hours to 15 hours as a result of paracetamol administration.

The authors concluded that the inhibition of hepatic metabolism by paracetamol reduces chloramphenicol clearance thus prolonging the half life. The clinical implications are that chloramphenicol accumulation would occur within the body producing greatly raised serum concentrations as a result of concurrent paracetamol administration. The paper as published gives no information on methods of analysis, nor the range of results obtained in such a small study group. A subsequent study in 26 children did not find this interaction (70).

Although paracetamol is often prescribed for its antipyretic action in the treatment of meningitis the potential interaction reported has not been supported by any other research.

1.8.3 PHENOBARBITONE/CHLORAMPHENICOL

Phenobarbitone is an anticonvulsant frequently used as part of the treatment of patients with central nervous system infections. Studies have indicated that each drug interferes with the metabolism of the other. Phenobarbitone is a potent hepatic enzyme inducer and it has been demonstrated in rats that phenobarbitone administered with chloramphenicol increases the rate of glucuronidation of the latter (71). One study investigating this interaction in children (age 1 month to 12 years) employing a 100 mg/kg/day chloramphenicol intravenous dose found a peak, steady state chloramphenicol concentration of 25.3 mg/L in patients not receiving phenobarbitone (n = 17) and 16.6 mg/L in patients also receiving phenobarbitone (n = 6; dose not specified). The half life of chloramphenicol and area under the curve of serum concentration of chloramphenicol versus time were also reduced, 3.6 hours and 93.9 mg hours/L respectively in the chloramphenicol only group and 3.3 hours and 53.3 mg hours/L in the chloramphenicol and phenobarbitone patients (72). The reduction in area under the curve reflecting either a decrease in bioavailable fraction of chloramphenicol, or increased clearance. The results of this study are summarised in Table 1.3. Another report cites the case of two paediatric patients receiving the maximum dose of intravenous chloramphenicol (100mg/kg/day) and phenobarbitone (10mg/kg/day) who both only attained markedly subtherapeutic peak chloramphenicol levels (73).

TABLE 1.3

COMPARISON OF PHARMACOKINETIC PARAMETERS OF CHLORAMPHENICOL MEASURED IN PATIENTS RECEIVING ANTICONVULSANT THERAPY

	Serum Concentration (mg/L)		Serum Half-Life (Hours)	AUC (mg hrs/L) AUC (mg/kg/L)
	Peak	Trough		
CHLORAMPHENICOL ONLY (n = 17)				
Mean	25.3	13.4	3.6	93.9
Range	10.4-50	< 2-18	0.5-12.8	102-178
Standard Deviation	8.7	6.0	2.8	41.1
Standard Error	2.11	1.47	0.7	11.3
CHLORAMPHENICOL AND PHENOBARBITONE (n = 6)				
Mean	16.6	7.5	3.3	53.3
Range	10.2-22	2.3-9.4	2.2-6.4	30-109
Standard Deviation	5.2	3.4	1.5	30.3
Standard Error	2.3	1.38	0.64	12.4
CHLORAMPHENICOL AND PHENYTOIN (n = 6)				
Mean	41.7	26.5	4.1	108.3
Range	28-57	8.5-36.5	2.1-5.5	50-167
Standard Deviation	10.15	9.0	1.50	42.9
Standard Error	4.14	3.7	0.60	17.5

From "Pharmacologic Interactions Among Chloramphenicol, Phenytoin and Phenobarbital." Krasinski K, Kusmiesz H, Nelson J. Paediatric Infectious Diseases Vol.1 No. 4 p.232-235 (1982)

A further study in premature infants did not find any consistent effect of phenobarbitone on chloramphenicol kinetics, although this may simply reflect saturation of the immature conjugating mechanism in neonates (74). The bulk of evidence supports the view that chloramphenicol clearance is increased when given simultaneously with phenobarbitone.

Chloramphenicol is known to inhibit phenobarbitone metabolism and elevation of serum phenobarbitone concentrations have been reported when chloramphenicol is also administered.

1.8.4 PHENYTOIN/CHLORAMPHENICOL

Phenytoin is used as an alternative anticonvulsant in patients with infections of the central nervous system. Phenytoin is believed to inhibit the metabolism of chloramphenicol by mechanisms which have not been determined. The study quoted above which examined the change in pharmacokinetic parameters of chloramphenicol when administered with phenobarbitone also included a chloramphenicol/phenytoin group (n = 6). This found peak steady state chloramphenicol levels of 41.7mg/L when administered with phenytoin (control group concentration = 25.7 mg/L). The half life was extended from 3.6 hours in the control group to 4.1 hours in the chloramphenicol/ phenytoin group and area under the serum chloramphenicol concentration versus time curve increased from 93.9 mg hr/L to 108/3 mg hr/L. See also Table 1.3 (72).

A short report on a 7 year old child found chloramphenicol peak and trough serum concentrations decreased by 46% and 74% respectively after two days of phenytoin administration (75).

Clearly there is good evidence to support an interaction between phenytoin and chloramphenicol, but the result of this interaction is not conclusively established.

Chloramphenicol also inhibits the metabolism of phenytoin.

1.8.5 OTHER DRUGS/CHLORAMPHENICOL

Chloramphenicol is reported to interact with other drugs. Drugs affected include tolbutamide, chlorpropamide, nicoumalone and dicoumarol (not commercially available in the U.K.). The mechanism of this interaction appears to be chloramphenicol induced inhibition of the liver microsomal enzymes leading to reduced metabolism of the drug affected and thus enhanced serum concentration and activity (76).

Similarly the breakdown of cyclophosphamide to its active metabolite is inhibited by chloramphenicol, thus reducing its therapeutic effect (77).

These interactions are with drugs only likely to be co-prescribed with chloramphenicol in exceptional

circumstances and therefore do not need to be discussed in detail.

1.9 BACKGROUND OF CURRENT PROJECT

The recommended dosage for chloramphenicol is 25 mg/kg/day for infants less than 14 days old and 50 mg/kg/day for older children and adults. For severe infections in older children the dose may be doubled.

In paediatric patients, following an acute admission, therapy is invariably commenced via the intravenous route. Chloramphenicol succinate is subject to metabolism to active chloramphenicol and also to renal excretion. It has been found that the renal clearance of chloramphenicol succinate is four times the creatinine clearance, suggesting that it is actively secreted at the renal tubule (33). The proportion of unhydrolysed chloramphenicol succinate that is excreted in the urine, expressed as a percentage of the total dose has been found to vary between 6-80% (33) and 8-45% (75) in paediatric patients.

The mechanism for the hydrolysis of chloramphenicol succinate in vivo is unclear. In vitro it has been demonstrated that breakdown occurs non-enzymatically, the rate being pH dependant (33). In vivo it has been suggested

that hydrolysis occurs in the liver, lungs and kidney, and enzyme involvement has not been demonstrated at these sites (58). Whatever the mechanism of the hydrolysis, it occurs at a variable and unpredictable rate.

In the healthy kidney excretion of chloramphenicol succinate by glomerular filtration and tubular secretion is proportional to serum concentration (although the secretion mechanism is saturable, at an undefined concentration). The slower the rate of metabolic clearance of chloramphenicol succinate from serum (i.e. conversion to chloramphenicol) the larger the proportion which will be excreted renally. This effectively reduces the dose of active chloramphenicol which is administered. A dose of chloramphenicol succinate equivalent to 100 mg chloramphenicol may produce a bioavailable dose of 20-94 mg of chloramphenicol (see proportion of chloramphenicol succinate excreted unchanged above). With such a wide inter-patient variability in conversion it is difficult to make dosage recommendation which will produce serum concentrations within the optimum range.

Several studies have demonstrated that the theoretical problems raised above are reflected in clinical results. Kauffman et al (33) found that only 51% of 45 patients aged 3 days to 16 years had peak serum chloramphenicol concentrations within the desired range (10-25 mg/L for this study). 16% of the remaining patients exhibited

subtherapeutic levels and 33% had peak serum concentrations in excess of 25 mg/L. The doses used in this study ranged from 15 - 200 mg/kg/day and the author makes no comment on the proportion of the patients who were administered doses within the recommended dosage guidelines.

Mulhall et al (35) published a similar study which was retrospective and performed in the U.K. (n = 64). Patients enrolled in the study were all neonates less than 28 days old. Of 45 patients who did not show signs of toxicity, 11 received the "recommended" dose. 4 of these patients had peak serum chloramphenicol concentrations within the therapeutic range of 15-25 mg/L, 4 were subtherapeutic and 3 had either peak concentrations greater than 25 mg/L or trough concentrations in excess of 15 mg/L. This division of a group of 11 patients demonstrates the effect of the variable excretion of chloramphenicol succinate on peak serum chloramphenicol concentrations. The "recommended" dose in this study was 25 mg/kg/day for patients under 7 days and 37.5-50mg/kg/day for older patients, which is lower than that usually accepted. Neither Kauffman nor Mulhall stated whether patients enrolled in the study were receiving any other medication.

Both studies may be criticised on the basis that they failed to control the dosage. Safety and efficacy of treatment are of paramount importance and are related to serum concentration of chloramphenicol rather than dosage.

Therefore the guidelines on dosage compiled within the current state of knowledge of the pharmacokinetics of chloramphenicol and chloramphenicol succinate should be followed at the initiation of therapy. However, provided that careful monitoring of peak and trough serum concentrations of chloramphenicol is performed, deviations in dosage above or below that recommended may be justified if they are required to maintain an individual patient's serum concentration within the therapeutic range. The results of these studies would be more valid if they had succeeded in attaining this, but Kauffman found that 49% of his patients fell outside the therapeutic range, while Mulhall found that of 54 patients who received the prescribed dose, only 8 (15%) were within the therapeutic range. Furthermore Mulhall noted that 9 (17%) of her patients demonstrated clinical signs or symptoms of toxicity.

Dosage and peak serum chloramphenicol concentration are two variables which should be investigated by holding one constant and noting the changes in the other. There is a need for a study to examine whether doses within the recommended guidelines will reliably produce steady state serum chloramphenicol concentrations within the therapeutic range. If the suspicion that this is not the case proves to be justified then there is a need for a further study to examine whether pharmacokinetic parameters calculated for an individual patient at the beginning of the course of therapy

remain constant during the course in order that they can be used in predictive equations to establish steady state serum concentrations and to alter the dosage in order to achieve appropriate serum concentrations.

1.10 OBJECTIVES

1. To develop a method of measuring chloramphenicol, chloramphenicol sodium succinate and chloramphenicol glucuronide concentrations in serum and urine.

2. To examine the pharmacokinetics and disposition of chloramphenicol, chloramphenicol sodium succinate and chloramphenicol glucuronide in the body, and to investigate any changes which may occur in these during the course of treatment.

3. To establish a method, based on data known about the patient, and pharmacokinetic parameters, of individualising chloramphenicol succinate doses to produce predictable, effective, non-toxic serum concentrations of chloramphenicol.

4. To assess the influence of other concurrent medication on the pharmacokinetics of chloramphenicol, chloramphenicol succinate and chloramphenicol glucuronide.

METHODS AND MATERIALS

2.1 Design of Study

Samples were requested on the first day of therapy (preferably after the first dose), the third day of therapy (steady-state) and the last day of the course in order that any change in pharmacokinetic parameters could be monitored. Serum and urine samples were collected as described under sampling protocol (section 2.3.4).

A full blood count was taken in order to detect toxic reactions. Where possible blood samples were drawn for measurement of creatinine levels, albumin and bilirubin concentrations and routine liver function tests.

2.2 Ethical Review

The study protocol was submitted to the Ethical Committee of the Medical Advisory Committee at the North Staffordshire Hospital Centre and was accepted by this committee as suitable and ethical.

2.3 Clinical Protocol

2.3.1 Patient Selection

All patients presenting to the paediatric wards between

1st April 1985 - 30th April 1986 with suspected bacterial meningitis, acute epiglottitis with H. influenzae as the suspected pathogen, or other infection for which chloramphenicol was considered to be the antibiotic of choice by the admitting clinician, were considered for inclusion in the study.

Before admission into the study, informed consent was required from the parents or legal guardians of the patients. During the study period 8 patients were not admitted to the study due to either refusal of consent or inability to contact the parents to obtain consent.

2.3.2 Clinical Investigations

Upon admission to the study, and during the course of therapy, clinicians were asked to monitor the plasma creatinine concentration, serum albumin concentration and conjugated and unconjugated bilirubin plasma concentrations and perform liver function tests. These were only performed when the medical staff felt that they were clinically indicated - otherwise renal and hepatic function were assumed to be normal. In practice bilirubin levels and liver function tests were only performed if there was clinical evidence of jaundice.

Full blood counts were requested at initiation of therapy, at regular intervals during the course of therapy,

and at the next out-patient appointment following discharge in order to monitor any toxic reactions.

2.3.3 Administration Protocol

On the day of admission to the study all the patients were over 14 days old. The B.N.F. recommended dosage is 50-100 mg/kg/day in divided doses every six hours. Clinicians accepted this guideline, although the dosage was adjusted in order that the volume of intravenous chloramphenicol succinate solution to be injected was convenient for accurate measurement and administration. The weight of the patient was taken to be the weight on admission.

All except one patient had the total dose equally divided into four parts, administered at six hourly intervals (6, 12, 18, 24 hours). The one exception had doses initially administered at four hourly intervals, the total daily dose being 100 mg/kg/day. Once the decision to initiate chloramphenicol therapy had been taken, the first dose was administered as soon as practical. In severely ill children the second dose was administered at the next regular dosing time, regardless of how short a period this was after the first dose, in order to achieve a loading effect, and doses thereafter were administered every six hours.

Preparation of the intravenous chloramphenicol succinate solution and drawing up into the syringe of the correct dose

was performed on the ward by a staff nurse and checked by another nurse, following normal ward routine. Administration of the dose was performed by the doctor on duty and was given as an intravenous bolus dose. The time of administration was recorded since it was not always possible for the dose to be given at a precise time.

Oral doses were measured and administered by a nurse and checked by another nurse. The volume of oral suspension to be administered was measured using an Medisco oral syringe medicine dispenser. This ensures accurate measurement of volume and transfer of the total dose into the oral cavity.

When practical, preparation and administration of doses was supervised by the research pharmacist.

2.3.4 Sampling Protocol

2.3.4.1 Serum

Blood samples were required during the first 24 hours of therapy (preferably the first dosage interval), the 9th and last dosage interval of the course. Both peak and trough samples were requested. Peak samples were defined as being 2 hours after an intravenous dose and 4 hours after an oral dose. Trough samples were taken immediately before the next dose, or at the time the next dose was due in cases where the course of therapy had been terminated.

Samples were usually taken by the research pharmacist by heel prick. Approximately 250 µl was expressed into a plastic blood tube. When other clinical investigations were required an aliquot of a venepuncture specimen obtained by a clinician was taken, on these occasions the research pharmacist was present to ensure that the sample was taken at the correct time.

The blood was allowed to clot and then centrifuged at 2,000 r.p.m. for 5 minutes. 50 µl of serum was removed using a SMI Digitron micropippette and placed in a plain glass blood/gas tube. The sample was either analysed immediately or frozen at - 20°C and analysed the following day for chloramphenicol and chloramphenicol succinate.

When the volume of blood was sufficient, a second 50 µl of serum was taken and placed in a separate blood gas tube and stored at -20°C for analysis for chloramphenicol glucuronide at a later date.

2.3.4.2 Urine

Urine samples were collected during the same dosage intervals as blood samples, and were taken on the first, third and last day of chloramphenicol therapy. Patients that were not catheterised had urine bags fitted immediately before the dose was administered. One patient was sufficiently old to void into a urine bottle during the dosage interval. Urine and catheter bags were emptied every

hour during the dosage interval. The total volume of urine produced at each interval was measured, and placed into separate urine bottles marked with the time the bag was emptied and volume obtained. The specimens were frozen at -20°C and analysed within 5 days for chloramphenicol and chloramphenicol succinate.

2.4 Preparation of Samples

Urine samples were thawed, shaken to ensure thorough mixing and then centrifuged at 3000 r.p.m. for 5 minutes. 1 ml of the supernatant was measured with a SMI digital adjust micropippette and transferred to a 10 ml volumetric flask. The urine was diluted to 10 mls with distilled water. The dilution was shaken and 50 µl measured using a SMI digitron micropippette and transferred to a 10 ml glass blood/gas tube.

The 50µl serum sample in the blood gas tube was thawed, if appropriate. Urine and serum samples were then treated similarly as described below.

2.5 Extraction of Samples

To the serum or diluted urine samples was added 950 µl of 0.05M sodium acetate buffer adjusted to pH=5 with glacial acetic acid (Analar grade, B.D.H.). 1 ml of ethyl

acetate (H.P.L.C. grade, Aldrich Chemical Company) containing benzocaine (Analar grade, B.D.H.) 750 µg/L as an internal standard was added and vortex mixed for 60 seconds, care being taken to avoid emulsification. The two phases were separated by centrifuging at 3,000 r.p.m. for five minutes. As much of the organic phase as possible was removed by glass pippette and transferred to a glass 10 ml sample tube. A further 1 ml of ethyl acetate was added to the aqueous component and vortex mixed and separated as described above, and the organic phase added to the first extract.

The ethyl acetate was evaporated under a steady stream of nitrogen on a sample concentrator (Jencons Dri-Block DB-3) at a temperature not exceeding 20°C. The dry residue was reconstituted with 50 µl methanol (H.P.L.C. grade, Koch-Light Ltd).

2.6 Chromatography

The equipment consisted of a pump (Pye - Unicam 4011), an injector (Rheodyne 7125) and a variable wavelength ultra-violet detector (Pye - Unicam 4020) operating at a wavelength of 277 nm with a detector range of 0.16 aufs. The column was a reverse phase Partisil 10 ODS-2 of length 25 cms and internal diameter 4.6mm. The mobile phase was 23% acetonitrile (H.P.L.C grade, Koch-Light Ltd) 77% sodium acetate 0.05M solution adjusted to pH=5 with glacial acetic acid. The final pH of the mobile phase was 5.5 and the flow rate 1.75 mls/min.

The analysis was performed at ambient temperature and samples were injected via a 20 µl injection loop. Retention times were of the order of 5 minutes for chloramphenicol-1-sodium succinate, 7 minutes for chloramphenicol-3-sodium succinate, 9 minutes for chloramphenicol and 19 minutes for benzocaine. A copy of a typical trace is shown in Figure 2.1. The areas under the peaks were calculated by a computing integrator (Pye-Unicam 4810) in arbitrary units.

The chromatography method is an adaptation of that used by Burke, Wargin and Blum (Journal of Pharmaceutical Sciences p.909- 912, Vol. 69, No.8, 1980).

2.7 Preparation of Standards

A solution of chloramphenicol 250 mg/L and chloramphenicol succinate 250 mg/L in distilled water was prepared using chloramphenicol and chloramphenicol succinate supplied by Parke-Davis Ltd. 40 µl and 100 µl of this solution was added to 960 µl and 900 µl respectively of blank serum to produce serum spiked with 10 mg/L chloramphenicol and chloramphenicol succinate; and 25 mg/L chloramphenicol and chloramphenicol succinate respectively. Serum for these standard solutions was obtained from blood samples obtained from the investigator by venepuncture.

The standard serum solutions were stored at -20°C and were discarded 48 hours after preparation.

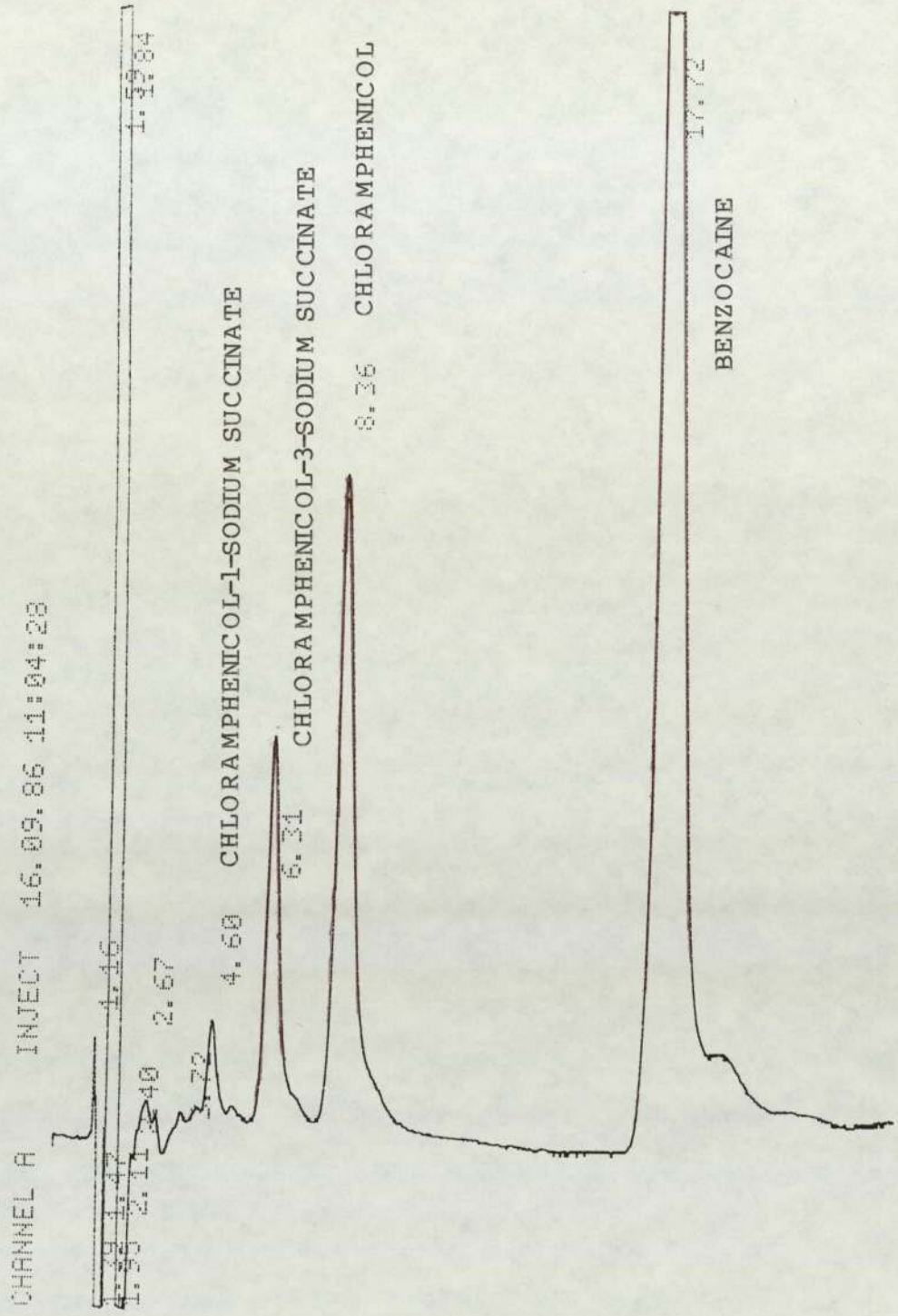


Figure 2.1 H.P.L.C. Trace of Serum Spiked with 15mg/l chloramphenicol and chloramphenicol sodium succinate, extracted and analysed as described in text.

2.8 Validation of Analytical Methods

2.8.1 Calibration

In order to establish that the calibration plot would be linear serum was spiked with the following amounts of chloramphenicol and chloramphenicol succinate; 2.5, 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100 mg/L. The spiked serum was produced by mixing blank serum with the appropriate quantity of an aqueous solution. For serum concentrations in the range 2.5 - 25 mg/L an aqueous solution of 250 mg/L chloramphenicol and chloramphenicol succinate was used, for serum concentrations 30-100 mg/L an aqueous solution of 1000 mg/L chloramphenicol and chloramphenicol succinate was used. The serum was then subjected to the extraction and chromatography techniques described above. The peak areas measured by the computing integrator for chloramphenicol-3-sodium succinate and chloramphenicol were divided by the peak area obtained for the internal standard (benzocaine). This ratio was then plotted against the serum concentration. The results are set out in Table 2.1 and Figure 2.2.

On each day of analysis the standard serum samples were extracted and analysed as described above. A daily calibration plot was prepared of concentration of drug in serum against ratio of peak areas using the linear regression facility of a Casio fx 180p scientific calculator. Concentrations for the urine and serum samples were found by interpolation/extrapolation of this plot by the calculator.

Table 2.1

Calibration Curve for Chloramphenicol and Chloramphenicol Sodium Succinate

Serum Conc (mg/L)	RATIOS OF PEAK AREAS	
	Chloramphenicol-3-Sodium Succinate	Chloramphenicol
100	0.936173	2.130448
80	0.776849	1.835691
60	0.641144	1.255256
50	0.486088	1.039266
40	0.415235	0.865405
30	0.331274	0.615534
25	0.279985	0.554306
20	0.220265	0.442503
15	0.161218	0.346423
10	0.104636	0.256015
5	0.060964	0.111605
2.5	-	0.055023
Correlation Coefficient	0.997	0.998
Gradient	9.344×10^{-3}	2.155×10^{-2}
Intercept	0.031730	0.006571

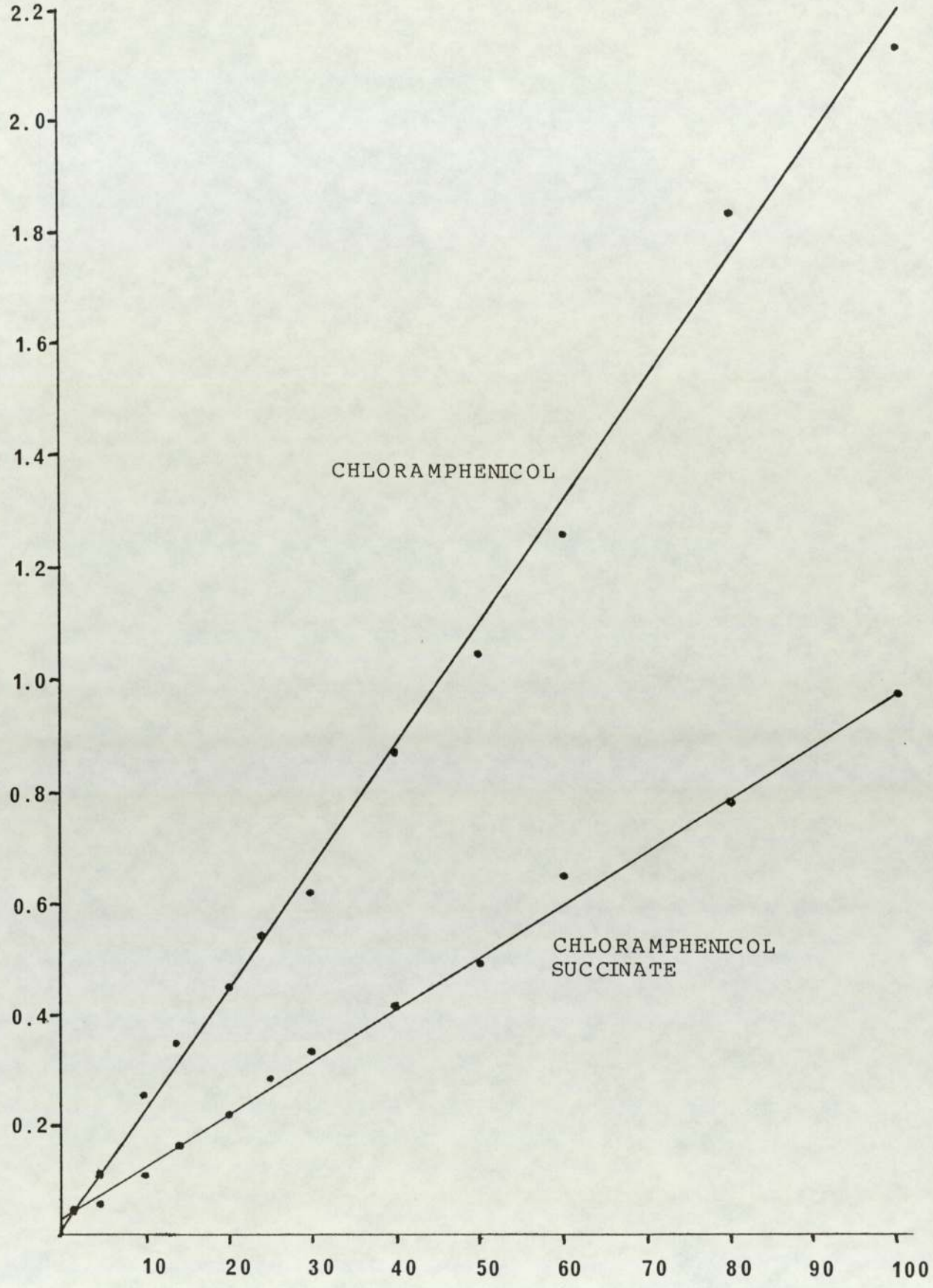


Figure 2.2 Plot of calibration data for chloramphenicol and chloramphenicol succinate.

2.8.2 RECOVERY

To determine the recovery from serum and urine, 50 µl of serum spiked with 15 mg/L chloramphenicol and 15 mg/L chloramphenicol succinate (prepared as described in section 2.4) was subjected to the full extraction and chromatography techniques described above (sections 2.5,2.6). This was repeated six times. The peak areas obtained were compared with those obtained with a reference solution of 15 mg/L chloramphenicol and 15 mg/L chloramphenicol succinate in distilled water injected directly onto the column. The reference solution was also injected six times and for both samples the injection volume was 20 µl.

The results are shown in Table 2.2. The peak areas are shown in arbitrary units:

Average Recovery

Chloramphenicol-3-sodium succinate = 61%

Chloramphenicol = 89%

2.8.3 Intrasample Reproducibility

In order to determine the intrasample reproducibility (within day variation) solutions of 15 mg/L chloramphenicol and 15 mg/L chloramphenicol succinate were prepared in

Table 2.2

Absolute Peak Areas Obtained for Chloramphenicol and Chloramphenicol-3-Sodium Succinate

Unextracted

	Chloramphenicol	Chloramphenicol-3-Sodium Succinate
	40862	22956
	41000	23389
	40129	23074
	40812	22822
	40265	23501
	40018	22634
Mean	40514	23062
Standard Deviation	424.8	332.2
Coefficient of Variance	1.0%	1.44%

Extracted

	Chloramphenicol	Chloramphenicol-3-Sodium Succinate
	34464	13727
	37360	14261
	36461	15044
	34931	14353
	39028	14501
	34180	12890
Mean	36070.7	14129.3
Standard Deviation	1898.2	740.3
Coefficient of Variance	5.3%	5.2%

distilled water, phosphate buffer pH=7.4 and serum. Six 50µl aliquots of each were taken and extracted and analysed as described above (section 2.5, 2.6). The results are shown in Table 2.3.

2.8.4 Intersample Reproducibility

In order to determine the intersample reproducibility (day to day variation) solutions of 15 mg/L chloramphenicol and 15 mg/L chloramphenicol succinate were prepared in distilled water, phosphate buffer pH=7.4 and serum. One 50 µl aliquot of each was analysed daily on six days over a two week period. The results are shown in Table 2.4.

2.8.5 Assessment of Specificity

Serum spiked with drugs which are likely to be administered concurrently to patients receiving chloramphenicol was extracted and analysed as described above (section 2.5, 2.6). Any peaks produced on the H.P.L.C trace were noted. The serum concentrations of drugs used are those that may typically be produced following therapeutic dosage.

Drugs Which Did Not Produce A Peak

Phenytoin (20 mg/L)
Phenobarbitone (30 mg/L)
Ampicillin (10 mg/L)
Benzylpenicillin (15 mg/L)

Drugs Which Produced A Peak Which Did Not Interfere With Analysis

	Retention Time
Gentamicin (10 mg/L)	10.28 minutes
Cefotaxime (25 mg/L)	2.99 minutes
Paracetamol (60 mg/L)	2.79 minutes

Drugs Which Produced A Peak Which Did Interfere With Analysis

	Retention Time
Sulphadiazine (150 mg/L)	3.56 minutes
Sulphadimidine (100 mg/L)	4.99 minutes
Co-Trimoxazole (48 mg/L)	6.45 minutes
	7.91 minutes

2.9 Analytical Method For Chloramphenicol Glucuronide

50 µl of serum or diluted urine sample was obtained as described above. 450 µl sodium acetate solution (pH=5) 0.05m was added to the sample and 500 µl of glucurase (Sigma Chemical Co.) was also added. Glucurase is a prepared solution of bovine liver β-glucuronidase which contains 5000 Sigma units per ml. One Sigma unit will hydrolyse 1.0 µg of phenolpthalein from phenolpthalein glucuronide per hour at pH=5 at 37°C.

The mixture was gently shaken, the cap was tightly screwed on each tube and they were placed in a sample block, with the temperature regulated at 37°C for fifteen hours (overnight).

The following day the samples were extracted and analysed as described in sections 2.5, 2.6.

Table 2.3. Intrasample (Within Day) Variability of Analysis of 15mg/L Samples of Chloramphenicol and Chloramphenicol Succinate in Water, Buffer (pH = 7.4) and Serum

RATIO OF PEAK AREAS

Water	Chloramphenicol-3-Succinate	Chloramphenicol
	0.14752	0.32263
	0.14730	0.35352
	0.15200	0.31822
	0.16105	0.32066
	0.16134	0.31942
	0.25567	0.34012
Standard Deviation	0.00628	0.01444
Mean	0 15414	0.32909
Coefficient of Variance	4.1%	4.4%
Buffer (pH = 7.4)		
	0.16751	0.32753
	0.16378	0.31375
	0.15397	0.30706
	0.16958	0.31251
	0.16012	0.33414
	0.16792	0.31924
Standard Deviation	0.00590	0.01014
Mean	0.16381	0.31903
Coefficient of Variance	3.6%	3.13%

Table 2.3 (continued)

Serum

	0.14381	0.32917
	0.13527	0.34779
	0.15006	0.35064
	0.14016	0.33203
	0.13682	0.33751
	0.14394	0.34697
Standard Deviation	0.00542	0.00902
Mean	0.14167	0.34068
Coefficient of Variance	3.8%	2.6%

Table 2.4. Intersample (Day-to-Day) Variability of Analysis of 15mg/L of Chloramphenicol and Chloramphenicol Succinate in Water, Buffer (pH = 7.4) and Serum

RATIO OF PEAK AREAS

Water	Chloramphenicol-3-Succinate	Chloramphenicol
	0.12878	0.32483
	0.15488	0.34141
	0.15539	0.33684
	0.17705	0.39075
	0 14245	0.32406
	0.14272	0.33261
Standard Deviation	0.01640	0.02493
Mean	0.15021	0.34175
Coefficient of Variation	10.9%	7.3%
Buffer (pH = 7.4)		
	0.13681	0.31128
	0.15770	0.37297
	0.18506	0.32098
	0.14889	0.33036
	0.16387	0.29434
	0.15585	0.35077
Standard Deviation	0.01614	0.02821
Mean	0.15803	0.33012
Coefficient of Variation	10.2%	8.5%

Table 2.4. (continued)

Serum

	0.13598	0.36503
	0.14974	0.35422
	0.15748	0.34403
	0.12773	0.32681
	0.12212	0.40726
	0.14538	0.32665
Standard Deviation	0.01350	0.03015
Mean	0.13974	0.35400
Coefficient of Variation	10.3%	8.5%

2.10 Validation of Chloramphenicol Glucuronide Analytical Method

5 mls of a 1 in 10 dilution of a patients urine sample was added to 45 mls of sodium acetate 0.05m solution (pH=5) and placed in a 250 ml glass beaker. The beaker was placed in a water bath at 37°C and the solution stirred. 50 mls of Glucarase, which had previously been warmed to 37°C was added to this solution.

Immediately, and at ten minute intervals thereafter a 1 ml aliquot of the mixture was withdrawn. To this aliquot was added 250 µl of a solution containing saccharo 1,4 lactone (10 mg/250 µl, Alldrich Chemical Company). This is a specific β-glucuronidase inhibitor.

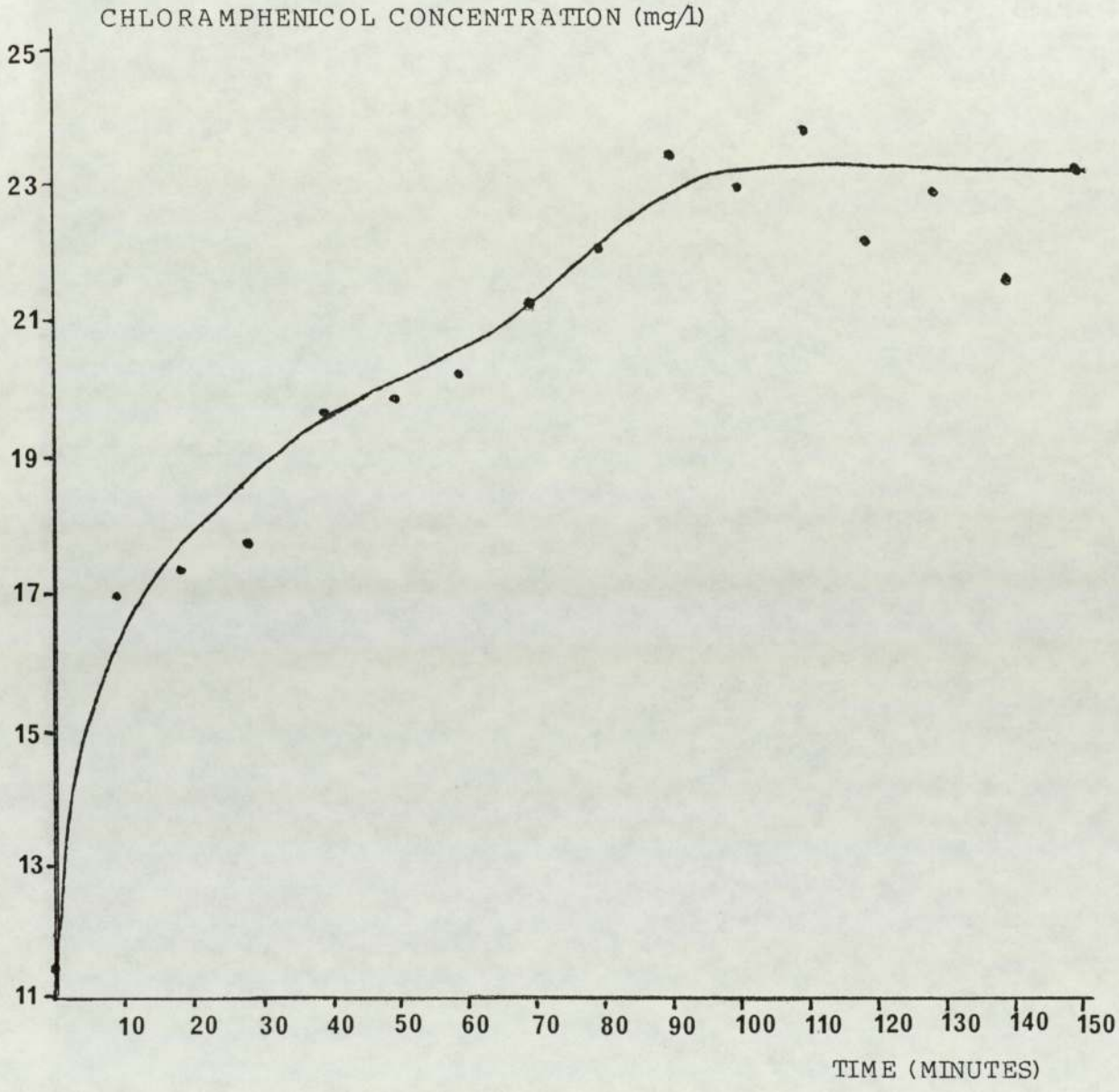
The aliquots were then extracted and analysed as described in Section 2.5 and 2.6.

The results are shown in Table 2.5 and Figure 2.3.

Table 2.5 Increase in Concentration of Chloramphenicol With Time in Urine Incubated With β-Glucuronidase

Time (minutes)	Concentration of Chloramphenicol (mg/L)
0	11.3
10	16.9
20	17.2
30	17.5
40	19.5
50	19.9
60	20.5
70	21.1
80	21.9
90	23.4
100	22.9
110	23.8
120	22.1
130	22.7
140	21.4
150	23.2

Figure 2.3 Increase of chloramphenicol concentration with time in urine sample incubated with β-Glucuronidase.



PHARMACOKINETIC ANALYSIS APPLIED TO CHLORAMPHENICOL AND CHLORAMPHENICOL SUCCINATE

2.11 Intravenous Administration

2.11.1 Pharmacokinetics of Chloramphenicol Succinate

Chloramphenicol is administered intravenously as its succinic acid ester. This is far more water soluble than chloramphenicol base and is more convenient for administration via this route.

In the USA intravenous drugs are usually administered by short infusion, i.e. gradual introduction of the total dose of drug into the blood stream over a period of time usually between 10 and 60 minutes. This avoids very high initial blood concentrations which may produce extreme pharmacological effects and increase the risk of phlebitis at the injection site. In the UK, unless such risks have been particularly identified with the specific drug being administered, intravenous preparations tend to be given by "fast-push" bolus. Chloramphenicol succinate is non-toxic and thus in the UK is administered over a period of a few seconds. The two methods of administration do not produce identical pharmacokinetic situations and the following discussion is applicable only to bolus administration.

The change in serum concentration of chloramphenicol succinate with time after an intravenous bolus dose follows a first order process and a semilogarithmic plot of serum

concentration versus time is illustrated in Figure 2.4.

There are two distinct phases in the decline of the serum concentration, a rapid phase (AB) and then a slower decline (BC).

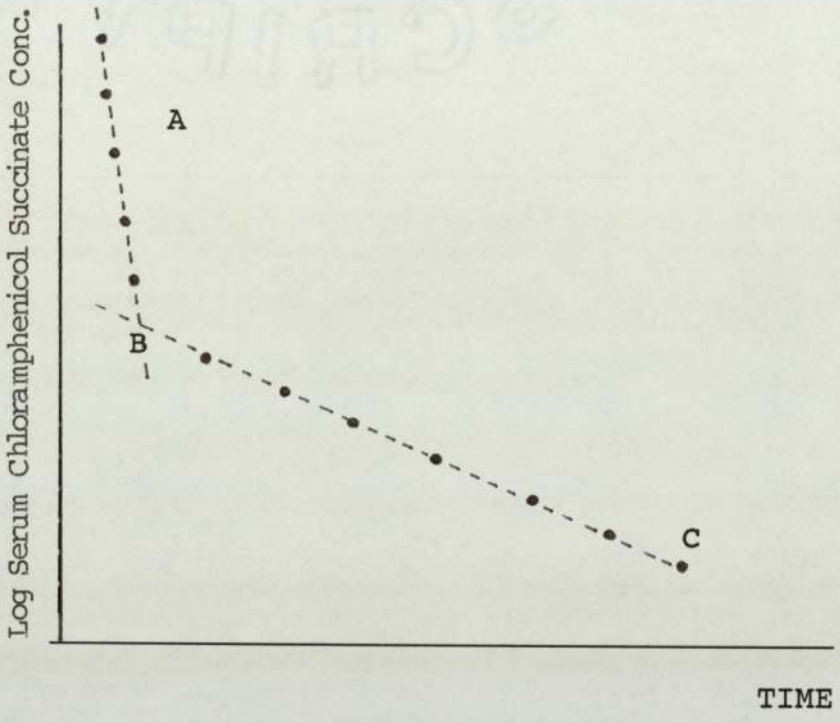


Figure 2.4 Semilogarithmic plot of chloramphenicol succinate serum concentration against time following intravenous bolus administration.

The rapid decline (AB) is referred to as the distribution phase and represents the drug diffusing from the serum into other tissues. Factors which influence the duration of this phase include the degree of binding to plasma and tissue proteins (not evaluated for chloramphenicol succinate), the perfusion of tissues into which it diffuses, and the diffusion rate into the tissue. Chloramphenicol succinate is a weak acid (pKa = 5.5) and considering the Henderson-Hasselbalch equation:

$$\text{Log}\left(\frac{\text{IONISED CONCENTRATION}}{\text{UNIONISED CONCENTRATION}}\right) = \text{pH} - \text{pKa for acids}$$

at physiological pH=7.4, 99% exists in the ionised form. Only the unionised form is free to pass through the capillary wall into tissue. Once the unionised chloramphenicol succinate has diffused into the tissue, 99% reverts to the ionised form, maintaining the concentration gradient down which the chloramphenicol succinate continues to pass until the concentration of unionised form is equal on both sides of the capillary wall. The distribution phase is a state of physiological equilibrium and is reversible.

The gradual decline (BC) in the serum concentration of chloramphenicol succinate is termed the elimination phase and reflects the proportional change in concentration (and total amount) of the drug throughout the body, since all tissues are now in equilibrium with serum. During this phase the body acts as a single compartment and changes that occur are not reversible. The situation is illustrated in Figure 2.5.

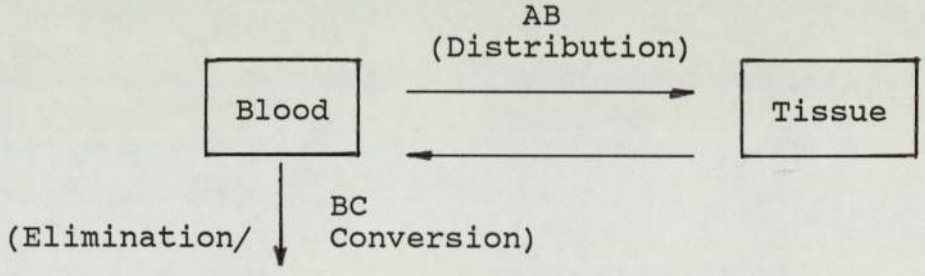


Figure 2.5 Distribution and Elimination of Chloramphenicol Succinate in the body

The two phases are not distinct, both commence with the administration of chloramphenicol succinate into the body and only terminate with complete elimination of chloramphenicol succinate from the body. However, each phase overshadows the importance of the other at different stages of the dosing interval.

Elimination of chloramphenicol succinate is known to occur in two ways, conversion to active chloramphenicol and renal excretion.

The mechanism for the hydrolosis to active drug has not been elucidated, although it has been suggested that is is pH rather than enzyme dependant. It is not possible to define directly a rate at which chloramphenicol succinate is eliminated by this route.

Excretion by the kidney is a product of three separate processes, glomerular filtration, active secretion and reabsorption. Approximately 10% of plasma presented to the kidney is filtered at the glomerulus. Unbound chloramphenicol succinate dissolved in plasma is also filtered, but the proportion bound to plasma protein is not. The concentration of chloramphenicol succinate in the filtrate is the same as the unbound plasma concentration.

The second process contributing to renal excretion is active secretion, and this is inferred when the rate of excretion of a drug exceeds the rate of glomerular filtration. It has been found that the renal clearance of chloramphenicol succinate in adults is four times that of creatinine (a compound which is completely filtered at the glomerulus, not secreted or reabsorbed) implying that it must undergo active secretion. Separate secretory mechanisms exist for acids and bases, but other than this they lack a

high degree of specificity and compounds secreted into the proximal tubule by the same mechanism compete with each other. The active secretion mechanism is saturable, but whether saturation or competition have any clinical effect has not been evaluated.

Passive reabsorption of exogenous compounds e.g. drugs may occur all along the nephron. The degree of reabsorption depends upon the physical properties of the compound e.g. its non polar/polar nature, degree of ionisation (25%-75% of chloramphenicol succinate in infant urine in the pH range 5-6) and molecular weight. Although the proportion of chloramphenicol succinate which is theoretically available to be reabsorbed is highly variable it has been shown that urinary pH and flow rate do not affect excretion of chloramphenicol succinate.

The situation is clearly subject to many potential variations but most variables are under physiological control and stay within narrow limits. Plasma protein concentration, glomerular filtration rate, active secretion rate (provided that the mechanism is not saturated by another compound) will only fluctuate in healthy individuals to a small extent, in disease states variations will be more marked and less predictable. The importance of reabsorption should not be overestimated. Since total renal clearance of chloramphenicol succinate is far greater in adult patients than creatinine clearance, active secretion must be of much greater significance than reabsorption.

2.11.2 Determination of Elimination Rate Constant for Chloramphenicol Succinate from Urine Data

From standard pharmacokinetic equations it is possible to define the elimination rate and clearance by filtration at the glomerulus of chloramphenicol succinate. Without a method of assessing the extent of active secretion clinically in individual patients there is little advantage in these calculations

The most direct approach to quantifying total renal excretion of chloramphenicol succinate is to measure the quantity excreted in the urine over a dosage interval. If all the chloramphenicol succinate administered is cleared from the body within this interval then the proportion which is cleared renally can be calculated.

A more useful pharmacokinetic parameter would be the renal clearance, if this could be derived from urine data. The rate of renal excretion of chloramphenicol succinate is porportional to the amount of drug in the body.

$$\frac{dAe}{dt} \propto Ab$$

Ae = cumulative amount excreted unchanged in the urine

Ab = total amount of drug in body

t = time

The constant which links these two variables is the renal elimination rate constant KeCAPSS.

i.e. $\frac{dAe}{dt}$ = KeCAPSS Ab

The total amount excreted unchanged at the end of a dosing interval is obtained by integration.

$$Ae_t = KeCAPSS \int_o^t Ab.dt \qquad (1)$$

At any given time the amount of chloramphenicol succinate in the body may be derived from

$$Ab = Dose.e^{-KCAPSS.t}$$

KCAPSS = elimination rate constant for chloramphenicol succinate

Substituting into equation (1)

$$Ae_t = KeCAPSS \int_o^t . \ Dose\ e^{-KCAPSSt}.dt$$

$$Ae_t = KeCAPSS\ Dose \left[\frac{e^{-KCAPSSt.}}{-KCAPPS} \right]_o^t$$

$$Ae_t = KeCAPSS\ Dose \left[\frac{e^{-KCAPSS.t}}{-KCAPSS} - \frac{1}{-KCAPSS} \right]$$

or $$Ae_t = \frac{KeCAPSS.}{KCAPSS}\ Dose\ [\ 1 - e^{-KCAPSS.t}] \qquad (2)$$

If Dose. $e^{-KCAPPSt}$ represents the proportion of a dose remaining in the body after time t, then Dose $(1-e^{-KCAPPS.t})$ represents the proportion eliminated in time t. As the time increases then $e^{-KCAPSSt} \longrightarrow 0$ and the cumulative amount excreted equals the product of the dose and the ratio of the renal and total elimination rate constants.

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$$Ae_{\infty} = \frac{KeCAPSS}{KCAPSS} \text{ Dose} \quad (3)$$

or $$\frac{Ae_{\infty}}{Dose} = \frac{KeCAPSS}{KCAPSS}$$

Ae_{∞} = Total amount of chloramphenicol succinate excreted unchanged in the urine.

Thus it has been demonstrated mathematically that the proportion of chloramphenicol succinate excreted unchanged in the urine is the same as the ratio of renal elimination constant to whole body elimination constant.

Substituting the value for dose obtained from equation (3) into equation (2).

$$Ae_t = \frac{KeCAPSS}{KCAPSS} \cdot \frac{KCAPSS\ Ae_{\infty}}{KeCAPSS} \left(1 - e^{-KCAPSS.t}\right)$$

or $$Ae_t = Ae_{\infty} - Ae_{\infty} .e^{-KCAPSSt}.$$

Rearranging and taking logs

$$\log (Ae_{\infty} - Ae_t) = \log Ae_{\infty} - \frac{KCAPSSt}{2.3} \quad (4)$$

Since Ae_{∞} and KCAPSS are constant this is of the form $y = mx + c$ and plotting $\log (Ae_{\infty} - Ae_t)$ versus time should produce a straight line of gradient $\frac{-KCAPSS}{2.3}$. If urine samples passed at hourly intervals are collected and analysed individually for the concentration of chloramphenicol succinate, knowing the volume that is excreted the amount excreted can be found. The total amount of chloramphenicol succinate excreted unchanged (Ae_{∞}) is determined by addition and then the cumulative hourly totals (Ae_t) are

subtracted from this resulting in the amount remaining to be excreted (ARE). This may then be plotted on semilogarithmic graph paper against time of urine sample as in Figure 2.6.

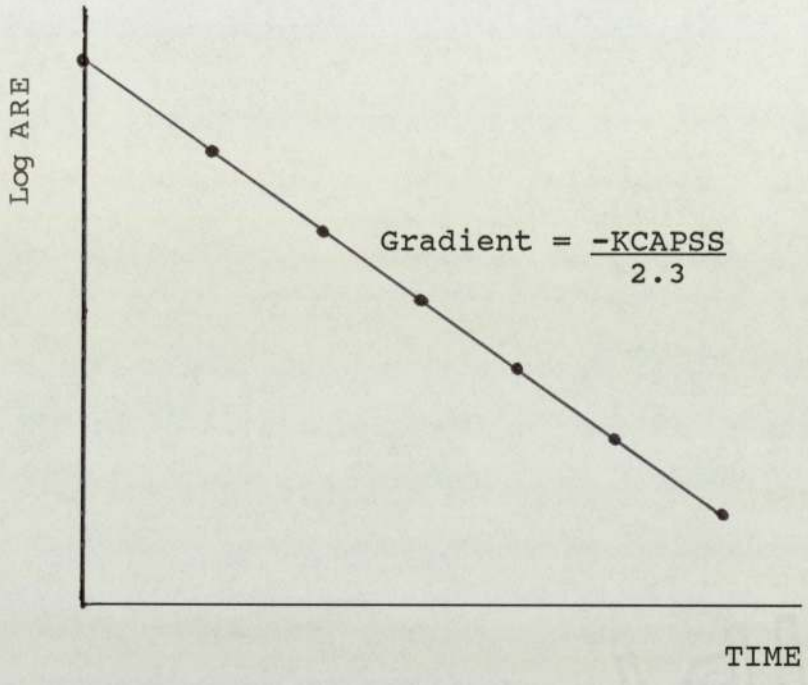


Figure 2.6. The exponential relationship between the amount remaining to be excreted (ARE) and time using urinary analysis after intravenous bolus dose of chloramphenicol succinate.

KCAPSS may thus be found either by calculations based on the graph or by regression analysis to find the best fit line from the data. It should be stressed that KCAPSS found by this method is the total elimination rate constant, not the renal elimination rate constant.

2.11.3 Determination of Renal and Metabolic Elimination Rate Constants for Chloramphenicol Succinate

Returning to equation (3) it has been derived that the

fraction of chloramphenicol succinate excreted unchanged (Fe) is equal to the ratio between the elimination rate constants.

$$Fe = \frac{Ae\infty}{Dose} = \frac{KeCAPPPS}{KCAPPS}$$

or $Fe.KCAPSS = KeCAPSS$

Furthermore, elimination rate constants are additive i.e. the whole body elimination rate constant is equivalent to the sum of the renal elimination rate constant and the metabolic elimination rate constant in a drug which is eliminated by both routes.

$KCAPSS = KeCAPSS + KmCAPSS$

KmCAPSS = metabolic elimination rate constant for chloramphenicol succinate

$$KmCAPSS = KCAPSS\ (1 - Fe) \qquad (5)$$

From knowledge of the total elimination rate constant and fraction excreted unchanged the metabolic elimination rate constant may be calculated.

The importance of this constant is that it defines the rate at which chloramphenicol succinate is converted to active chloramphenicol. The rate of release of chloramphenicol is:

$$\frac{dAbCAP}{dt} = S.Ab\ KmCAPSS$$

AbCAP = Amount of chloramphenicol in body

Ab = Amount of chloramphenicol succinate in body

S = Salt Factor

or $\frac{dAbCAP}{dt} = S.\ Dose\ e^{-KCAPSSt}.KmCAPSS$

N.B. Dose refers to the amount of chloramphenicol succinate administered.

Chloramphenicol is liberated over a period of time and the rate at which this occurs decays exponentially, administration of active drug should therefore be viewed as an oral dose of absorption rate S. Dose.e-KCAPSSt. KmCAPSS. As the value of t increases so the administration rate tends to zero, reflecting the decline in the quantity of chloramphenicol succinate available for conversion to chloramphenicol.

2.11.4 Determination of the Elimination Rate for Chloramphenicol

Immediately that the active drug begins to be liberated, it also begins to be eliminated from the body. The principle route of elimination of chloramphenicol is by metabolism in the liver, although a small proportion is renally excreted. Renal excretion is believed to be by filtration and neither secretion or reabsorption have been implicated. The rate of elimination of chloramphenicol is the product of the amount of drug in the body and the elimination rate constant for chloramphenicol (KCAP).

Rate of Elimination = AbCAP.KCAP KCAP = Elimination rate constant for chloramphenicol

The rate of change of the amount of chloramphenicol in the body is the difference between the administration rate and the elimination rate:

$$\frac{dAbCAP}{dt} = S.\ Dose.e^{-KCAPSSt}.\ Km - AbCAP\ .\ KCAP$$

It has been demonstrated above that the administration rate term is initially large at small values of t, and the amount of chloramphenicol in the body is small, producing a small elimination rate. Accummulation of chloramphenicol occurs until the stage where the value of AbCAP and t are sufficiently large (the value of the term $e^{-KCAPPSt}$ decreases with increasing value of t) that there is no change in the amount of chloramphenicol in the body.

$$\frac{dAbCAP}{dt} = 0$$

$$\therefore S.Dose\ e^{-KCAPSSt}\ \ KmCAPSS = AbCAP.\ KCAP$$

At the point where the rate of administration of chloramphenicol (i.e. the rate of hydrolysis of chloramphenicol succinate) is equal to the rate of elimination, the concentration of chloramphenicol present in the serum is termed the "Peak Concentration".

After this point the value of the rate of elimination of chloramphenicol exceeds the rate of administration, and the rate of change of amount of chloramphenicol in the body becomes negative, indicating the decreasing amount of drug in the body. As the value of $t \rightarrow \infty$, the rate of administation $\rightarrow 0$ and the rate of change of the amount of chloramphenicol in the body becomes equal to the rate of elimination of chloramphenicol (assuming that all of the chloramphenicol succinate is cleared in a dosage interval).

The situation is illustrated in Figure 2.7.

2.11.5 Basis for Predictive Pharmacokinetic Methods Applied to Chloramphenicol

Clinically it is more convenient to relate efficacy and toxicity to serum concentrations rather than to the amount of drug in the body, which cannot be measured directly. Clearly there is a direct relationship between serum concentration and amount of drug in the body, and the constant which links the two is termed the "volume of distribution." The volume of distribution is that volume of serum containing a known concentration of drug which would be required to account for the total amount of drug in the body, i.e.:

$$Vd \times Cp = Ab$$

Vd = Volume of Distribution (L^{-1})

Cp = Serum concentration (mgL^{-1})

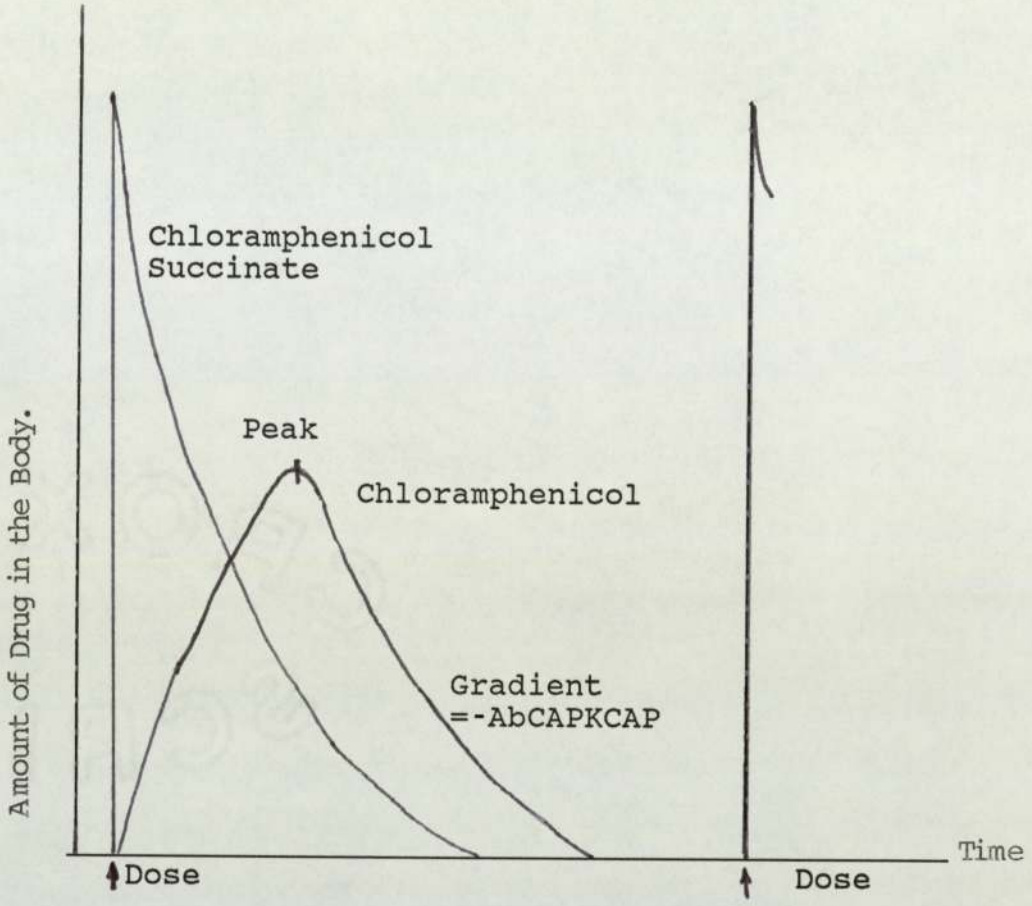


Figure 2.7. The change with time of the amount of chloramphenicol succinate and chloramphenicol in the body following an intravenous bolus dose of chloramphenicol succinate.

For most drugs the volume of distribution is larger than the plasma volume since drugs distribute throughout the body. The volume of distribution is a theoretical concept which does not usually equate with any particular body compartment.

The value for the volume of distribution of a drug depends upon the properties of the drug molecule, degree of tissue and plasma protein binding and is characteristic for a particular drug. It may be modified by disease states which produce symptoms such as low plasma protein levels, oedema, etc. Given a target serum concentration of a drug, and knowledge of the population value of the volume of distribution of that drug the dose required may be estimated.

Alternatively for individualisation of drug therapy the peak serum concentration achieved for a given dosage may be measured and thus the volume of distribution for that patient may be calculated.

In that situation depicted in Figure 2.7, the same dose administered at the interval shown will produce the same peak plasma concentration, provided that there is no change in the volume of distribution and that the fraction of chloramphenicol succinate converted to chloramphenicol does not alter. In a more clinically realistic situation the second dose would be administered before the chloramphenicol serum concentration had declined to zero. If there is no change in the variables described above then the INCREASE in the serum concentration obtained after the second dose is equal to the serum concentration obtained after the first dose. At the end of the second dosage interval the trough serum concentration is higher than at the end of the first and thus the peak concentration obtained with the third dose is higher still than with previous doses, thus the chloramphenicol accummulates in the body.

With the increase in the amount of chloramphenicol in the body the elimination rate increases until the amount that is eliminated over a dosage interval is equal to the amount which is administered. This is termed "steady-state" and the maximum and minimum serum chloramphenicol concentrations at this time are the steady-state peak and trough concentrations respectively.

The amount of chloramphenicol which is eliminated in a dosing interval is the difference between the peak and trough serum concentrations, multiplied by the volume of distribution.

Amount of chloramphenicol administered = Amount of chloramphenicol eliminated.

$= (Cp_{Peak} - Cp_{Trough})\ Vd$

Cp_{Peak} = Peak serum concentration (mg/L)

Cp_{Trough} = Trough serum concentration (mg/L)

or $F.S.\ Dose = (Cp_{Peak} - Cp_{Trough})\ Vd$ (6)

F = Bioavailable fraction = (1-Fe)

It has been demonstrated above that the fraction of a dose remaining after time t is e^{-KCAPt}, and by extension the fraction of serum concentration after time t is also e^{-KCAPt} and therefore:

$Cp_{Trough} = Cp_{Peak}\ e^{-KCAPt_{pt}}$ t_{pt} = time between peak and trough samples (7)

Substituting into Equation (6)

$$F.S.\ Dose = (Cp_{Peak} - Cp_{Peak} \cdot e^{-KCAPt_{pt}}) \cdot Vd$$

Rearranging $F.S.\ Dose = Cp_{Peak} \cdot Vd\ (1 - .e^{-KCAPt_{pt}})$

$$\text{or } Cp_{Peak} = \frac{F.S.\ Dose}{Vd(1 - e^{-KCAPt_{pt}})} \quad (8)$$

==========

Alternatively substituting equation (7) into equation (8)

$$Cp_{Trough} = \frac{F.S.\ Dose.e^{-KCAPt_{pt}}}{Vd(1 - e^{-KCAPt_{pt}})} \qquad (9)$$

These formulae are adaptions of standard pharmacokinetic expressions. It is proposed that they may form the basis of a predictive method of assessing steady state peak and trough chloramphenicol concentrations from values of F, Vd and KCAP which are calculated from samples drawn after the first intravenous dose. The assumption is made that these values do not alter significantly during a course of therapy, although samples drawn later in the course of therapy will allow recalculation of these values, and it will be possible to either support or challenge this assumption.

RESULTS

3.1 Study Population

21 patients were enrolled into the study during the period 1st April 1985 - 30th April 1986. 8 patients who were prescribed chloramphenicol were not enrolled due to either refusal of consent or inability to contact the parents or legal guardians in order to obtain consent.

All the patients enrolled were white and of European origin. Eight of the subjects were female; thirteen were male. The ages ranged from 3 weeks to 6 1/2 years, the mean age being 15 months. The weight of the patients varied from 2.1 kg to 22.6 kg, with a mean weight of 8.8 kg.

Twenty of the subjects were diagnosed as meningitis patients, in nine cases no organism was cultured from the C.S.F. and the causative organism was assumed to be viral. Of the eleven bacterial cases, 7 were caused by infection with N. meningitidis, 2 by S. pneumoniae and 2 by H. influenzae. The non-meningitis case was the oldest subject (6 1/2 years) and was diagnosed as suffering from H. influenzae tonsillitis

The breakdown of patients is shown in Table 3.1.

Table 3.1

Study Population

Patient No.	Age (months)	Sex M/F	Weight (kg)	Diagnosis
1	24.0	M	11.55	H. Influenzae Meningitis
2	24.0	M	12.50	Meningococcal Meningitis
3	23.0	F	14.70	Meningococcal Meningitis
4	78.0	M	22.60	H. Influenzae Tonsillitis
5	9.5	M	9.35	Meningococcal Meningitis
6	19.0	M	11.50	Meningococcal Meningitis
7	25.0	F	8.50	Viral Meningitis
8	6.5	M	6.80	Viral Meningitis
9	4.0	F	6.90	Viral Meningitis
10	26.0	F	11.90	Meningococcal Meningitis
11	7.0	F	7.50	H. Influenzae Meningitis
12	6.5	F	6.10	Strep. Pneumoniae Meningitis
13	5.0	M	7.00	Strep. Pneumoniae Meningitis
14	10.5	M	9.20	Viral Meningitis
15	10.5	M	9.30	Meningococcal Meningitis
16	22.5	M	11.50	Meningococcal Meningitis
17	0.67	M	3.17	Viral Meningitis
18	1.0	M	2.10	Viral Meningitis
19	2.25	F	4.40	Viral Meningitis
20	2.5	M	4.50	Viral Meningitis
21	2.25	F	4.40	Viral Meningitis

3.2 Medication

The mean dose of chloramphenicol during the course of therapy was 19.72 mg/kg q.d.s. (78.9 mg/kg daily). The minimum dose was 12.3 mg/kg q.d.s. (49.2 mg/kg daily) and the maximum regular dose was 26.7 mg/kg q.d.s. (106.8 mg/kg). One patient was given a stat. loading dose of 50 mg/kg. Most of the patients were also prescribed additional antibiotics (usually benzylpenicillin) and an antipyretic (paracetamol either regularly or as required), some patients were also prescribed aspirin suppositories if required. Full details of other medication and chloramphenicol dosage is included in the raw data in Appendix 1.

3.3 Treatment of Results

According to the length of time after commencement of therapy that the samples were obtained, results from the analysis of samples were split into three sections. The first section consists of samples obtained within the first 24 hours of therapy, the second section is from the middle of a full therapeutic course (range 36-132 hours) when steady state has been reached, and the third section is the end of a course of therapy (range 183-270 hours).

Not all the samples were obtained for each patient.

3.4 Excretion of Chloramphenicol Succinate and Chloramphenicol

The percentage of the dose administered which is excreted as chloramphenicol and chloramphenicol succinate in the urine during a six hour dosing period is shown in Table 3.2.

3.5 Serum Concentrations of Chloramphenicol and Chloramphenicol Succinate

The serum peak and trough steady state concentrations of chloramphenicol are shown in Table 3.3. Peak concentrations were all obtained two hours post intravenous dose or four hours post oral dose. Trough samples were obtained immediately before the next dose.

Table 3.3 also quotes the dose administered and bioavailable dose, calculated from the amount of chloramphenicol succinate excreted unchanged in the urine.

Bioavailable Dose = Total Dose x (1-Fe)

Fe = Fraction of Chloramphenicol Succinate Excreted Unchanged

Table 3.2

Percentage of Chloramphenicol And Chloramphenicol Succinate Excreted Unchanged in the Urine

Patient	Beginning of Course		Middle of Course	
	C'Phenicol %	C'Phenicol Succinate %	C'Phenicol %	C'Phenicol Succinate %
1	-	-	22.8	49.8
2	-	-	1.2	3.1
3	0.8	3.8	-	-
4	7.0	25.6	-	-
5	-	-	4.8	5.7
6	1.6	1.9	-	-
7	0.5	1.8	-	-
8	4.9	6.5	-	-
9	-	-	4.9	6.5
10	-	-	2.2	6.6
11	3.9	26.5	8.8	9.9
12	-	-	-	-
13	3.7	1.3	-	-
14	-	-	1.4	0.9
15	5.7	7.1	22.3	42.1
16	-	-	1.9	3.3
17	7.4	12.9	-	-
18	-	-	5.2	4.8
19	10.9	26.0	3.7	0.0
20	2.9	4.8	-	-
21	10.9	27.0	-	-
Mean	5.0	12.1	7.2	12.1
Standard Deviation	3.5	10.9	7.9	17.1

Table 3.3

Steady State Peak and Trough Serum Chloramphenicol Concentrations

Patient No.	C'Phenicol Conc (mg/L)		Dose/kg (mg/kg)	Bioavailable Dose/kg (mg/kg)
	Peak	Trough		
1	57.0	33.0	25.65	13.11
2	14.1	2.0	23.97	23.26
5	7.4	2.7	13.32	12.58
8	3.2	2.1	21.37	-
9	38.2	28.3	25.21	23.68
10	51.6	24.3	20.64	19.61
11	13.5	12.7	13.36	12.02
12	7.0	0.4	12.32	-
13	-	7.6	-	-
14	22.9	-	13.51	13.51
15	17.3	8.3	24.74	14.36
16	39.7	20.8	26.80	25.20
17	7.6	5.1	12.62	-
18	19.8	16.2	14.25	13.66
19	18.0	20.3	22.68	22.68
20	13.8	3.6	22.22	-
21	24.6	8.3	22.63	-
Mean	22.2	12.2	-	-
Standard Deviation	16.2	10.3	-	-

3.6 Half-Life and Elimination Rate Constant of Chloramphenicol

The calculated values for the half-life and elimination rate constant of chloramphenicol are shown in Table 3.4 The elimination rate constant was calculated from the relationship:

$$\frac{\ln \text{Peak Conc} - \ln \text{Trough Conc}}{\text{Time Between Samples}} = \text{KCAP}$$

and the half-life from the relationship

$$T\ 1/2 = \frac{0.693}{\text{KCAP}}$$

Values marked with an asterisk* have been excluded when calculating the mean since they lie more than 3 standard deviations away from the mean.

3.7 Half Life and Elimination Rate Constant of Chloramphenicol Succinate

This was calculated by plotting log amount remaining to be excreted against time from urine data. The total amount of chloramphenicol succinate excreted in the dosage interval was calculated, then the amount excreted in each sample subtracted sequentially from the total. The gradient of the plot of log amount remaining to be excreted versus time was multiplied by -2.303 to give the elimination rate constant. The explanation for this method is contained in Section 2.11. The results are shown in Table 3.5.

Table 3.4 Half Life and Elimination Rate Constant for Chloramphenicol

Patient No.	Beginning of Course		Middle of Course		End of Course	
	T 1/2 (Hrs)	KCAP (Hrs^{-1})	T 1/2 (Hrs)	KCAP (Hrs^{-1})	T 1/2 (Hrs)	KCAP (Hrs^{-1})
1	-	-	4.44	0.16	-	-
2	-	-	1.42	0.49	-	-
3	1.99	0.35	-	-	-	-
4	-	-	-	-	-	-
5	1.75	0.39	2.4	0.29	-	-
6	2.09	0.33	-	-	-	-
7	6.10	0.11	-	-	-	-
8	-	-	33.51*	0.02*	-	-
9	-	-	9.24	0.07	3.98	0.17
10	-	-	3.22	0.21	-	-
11	75.79*	0.01*	34.03*	0.02*	-	-
12	-	-	0.97	0.71	1.36	0.51
13	4.53	0.15	-	-	-	-
14	-	-	-	-	-	-
15	4.27	0.16	3.77	0.18	-	-
16	-	-	4.29	0.16	2.36	0.29
17	11.44	0.06	3.47	0.20	-	-
18	14.00	0.05	6.90	0.10	-	-
19	3.60	0.19	-	-	-	-
20	-	-	2.19	0.32	-	-
21	5.60	0.12	1.53	0.45	-	-
Mean	5.53	0.19	3.65	0.28	2.56	0.32
Standard Deviation	4.35	0.12	2.40	0.19	1.32	0.17

* These figures have not been used to calculate the mean since they are more than three Standard Deviations from the mean result

In order to give some estimation of the accuracy of the method the correlation coefficient (or how closely the plot corresponds to a straight line) is also stated. Plots with a correlation coefficient of -1.0000 are those where only two points were available to plot the line, but the other values show plots which generally correspond closely to a straight line.

The half-life of chloramphenicol succinate was calculated from the relationship above linking elimination rate constant and half-life.

3.8 Metabolic Elimination Rate Constant of Chloramphenicol Succinate

The calculated values for the metabolic elimination rate constant of chloramphenicol succinate are shown in Table 3.6. It was shown in Section 2.11 that the elimination rate constant of chloramphenicol succinate was equal to the sum of the excretion rate constant and the metabolic rate constant of chloramphenicol succinate. Furthermore, the ratio of the excretion rate constant of chloramphenicol succinate to the total elimination rate constant is equivalent to the fraction of chloramphenicol succinate excreted unchanged in the urine. Therefore, the metabolic elimination rate has been calculated from the elimination rate constant x (1-Fe). The elimination

Table 3.5 Half Life and Elimination Rate Constant for Chloramphenicol Succinate

Patient No.	Beginning of Course			Middle of Course		
	KCAPSS (Hrs^{-1})	T 1/2 (Hrs)	C.C.	KCAPSS (Hrs^{-1})	T 1/2 (Hrs)	C.C.
1				0.31	2.21	-0.822
4	2.23	0.31	-1.000	-		
5	-	-	-	0.96	0.72	-0.971
6	0.95	0.73	-0.908	-	-	-
7	0.40	1.73	-1.000	-	-	-
8	0.94	1.36	-1.000	-	-	-
9	-	-	-	0.73	0.95	-1.000
10	-	-	-	1.34	0.52	-0.984
11	1.35	0.52	-0.856	0.84	0.83	-0.920
13	0.76	0.92	-1.000	-		
14	-			0.65	1.07	-1.000
15	0.82	0.85	-0.957	1.67	0.41	-1.000
17	0.61	1.15	-1.000	-	-	-
18	-			1.36	0.51	-1.000
19	0.84	0.83	-1.000	-	-	-
20	0.54	1.28	-0.950	-	-	-
21	0.91	0.76	-0.975	-	-	-
Mean	0.94	0.95	-	0.98	0.90	-
Standard Deviation	0.49	0.40	-	0.44	0.58	-

Table 3.6 Metabolic Elimination Rate Constant of Chloramphenicol Succinate

Patient No.	Beginning of Course			Middle of Course		
	KCAPSS Hrs^{-1}	Fe	KmCAPSS Hrs^{-1}	KCAPSS Hrs^{-1}	Fe	KmCAPSS Hrs^{-1}
1	-	-	-	0.31	0.23	0.24
4	2.23	0.26	1.66	-		
5	-	-	-	0.96	0.05	0.91
6	0.95	0.02	0.94	-	-	-
7	0.40	0.05	0.38	-	-	-
8	-	-	-	-	-	-
9	-	-	-	0.73	0.05	0.69
10	-	-	-	1.34	0.02	1.31
11	1.35	0.04	1.29	0.84	0.09	0.76
13	0.76	0.04	0.73	-	-	-
14	-	-	-	0.65	0.01	0.64
15	0.82	0.06	0.77	1.67	0.22	1.30
17	0.61	0.07	0.56	-	-	-
18	-	-	-	1.36	0.05	1.29
19	0.84	0.11	0.74	-	-	-
20	0.54	0.05	0.51	-	-	-
21	0.91	0.11	0.81	-	-	-
Mean	-	-	0.84	-	-	0.89
Standard Deviation	-	-	0.36	-	-	0.39

rate constants are shown in Table 3.5 and the fraction of chloramphenicol succinate excreted unchanged is detailed in Table 3.2.

3.9 Volume of Distribution of Chloramphenicol

The volume of distribution of a drug is the apparent volume that is required to contain the total amount of the drug in the body at the serum concentration of the drug.

The volume of distribution of chloramphenicol has been calculated by dividing the bioavailable dose of chloramphenicol (in mg/kg body weight) by the increase in serum concentration obtained by this dose. In order to calculate the increase in serum concentration it was necessary to extrapolate the peak serum concentration to that which would theoretically have been obtained at time zero. This was calculated using the formula:

$$\text{Serum Conc at time zero} = \frac{\text{Peak Serum Concentration}}{e^{-2KCAP}}$$

When the peak concentration sample is taken two hours after the dose.

The calculated values for the volume of distribution of chloramphenicol are shown in Table 3.7.

Table 3.7 Volume of Distribution of Chloramphenicol

Patient No.	Volume of Distribution L/kg	
1	0.29	(2)
2	0.65	(2)
3	0.50	(1)
5	1.20	(2)
7	1.37	(1)
9	1.47	(2)
10	0.36	(2)
15	0.86	(2)
16	0.74	(2)
18	1.70	(2)
Mean	0.91	
Standard Deviation	0.49	

(1) Calculated from data obtained in the first 24 hours of therapy

(2) Calculated from data obtained in the middle of the course

Table 3.8 Volume of Distribution of Chloramphenicol Succinate

Patient No.	Volume of Distribution L/kg	
4	0.19	(1)
5	0.33	(2)
6	1.51	(1)
7	0.97	(1)
10	0.31	(2)
11	0.20	(1)
13	0.61	(1)
15	0.99	(1)
16	0.27	(2)
17	0.22	(1)
18	0.26	(2)
19	1.63	(1)
21	1.42	(1)
Mean	0.68	
Standard Deviation	0.55	

(1) Calculated from data obtained in the first 24 hours of therapy

(2) Calculated from data obtained in the middle of the course

3.10 Chloramphenicol Glucuronide

The serum concentrations of chloramphenicol glucuronide measured are reported in Table 3.9 and the percentage of the dose recovered in the urine as chloramphenicol glucuronide is reported in Table 3.10.

Table 3.9 Serum Concentrations of Chloramphenicol Glucuronide

CHLORAMPHENICOL GLUCURONIDE SERUM CONCENTRATION (mg/L)

	Beginning of Course		Middle of Course	
Patient	Peak	Trough	Peak	Trough
3	1.8	1.0	-	-
5	-	1.9	2.9	1.1
8	0.6	0	-	1.2
9	-	-	1.1	1.2
11	3.4	2.0	-	-
15	2.4	0.1	4.1	0
16	4.5	4.8	2.2	2.1
17	5.0	1.9	4.6	2.5
18	1.7	-	-	2.9
19	2.0	2.2	2.0	1.7
20	1.4	-	4.0	3.5
21	3.2	1.9	4.7	3.0

Table 3.10 Percentage of Dose Excreted as Chloramphenicol Glucuronide

Patient	Beginning of Course	Middle of Course
1	-	22.0%
2	-	4.5%
3	3.9%	-
4	18.5%	-
5	-	5.6%
6	7.6%	-
7	6.5%	-
8	4.8%	-
9	-	14.2%
10	-	12.4%
11	31.0%	54.0%
13	18.0%	-
14	-	5.2%
15	16.0%	20.0%
16	-	8.4%
17	35.0%	-
18	-	15.7%
20	8.3%	-
21	23.0%	-
Mean	15.7%	16.2%
Standard Deviation	10.6%	14.6%

DISCUSSION

4.1 Study Population

All the patients admitted to to the paediatric wards at the City General Hospital, Stoke-on-Trent who were prescribed chloramphenicol were considered for inclusion in the study. The only reason for exclusion was lack of parental consent and other than this there was no discernable pattern to patients excluded.

Stoke-on-Trent does not have a large African or West Indian community. Therefore the racial origins of the study population are representative of this area and the patients excluded from the study were also white Europeans.

Of the 20 patients admitted into the study with suspected meningitis 13 (65%) were less than 12 months old, 7 were 12-26 months. Epidemiological studies have demonstrated that Haemphilus influenzae meningitis occurs predominantly in the age range 2 months to 3 years; and two thirds of meningococcal infections occur in the first five years, over one-half of these in the first 12 months of life. The incidence of meningococcal infections is higher in males than in females, and this is reflected in the study.

The distribution of bacterial causative organisms in the study population is probably not typical. Analysis of cases of bacterial meningitis reported to the Public Health Laboratory Service between 1967-1970 showed that 42% were meningococcal infections; 31% were H. influenzae infections and 27% penumococcal meningitis. The distribution between the various causative organisms will have changed since these figures were reported, and there is also a regional variation in the proportion of cases due to each organism. Nevertheless the incidence of Haemophilus and pneumococcal meningitis appears to be low in this study. This may be ascribed to the failure to successfully culture the causative bacterium in some of the cases which were assigned to the viral meningitis group.

In other respects, given the limitations of such a small population, the study group appears to conform to a normal paediatric group of meningitis patients as described by epidemiological studies.

4.2 Method of Analysis

The H.P.L.C. method utilised is an adaptation of a method which has been described by several authors. The only major changes were the proportions of sodium acetate and acetonitrile in the mobile phase. This was necessitated by the administration of sulphadimidine to some of the patients. Sulphonamides strongly absorb ultra-violet light and upon

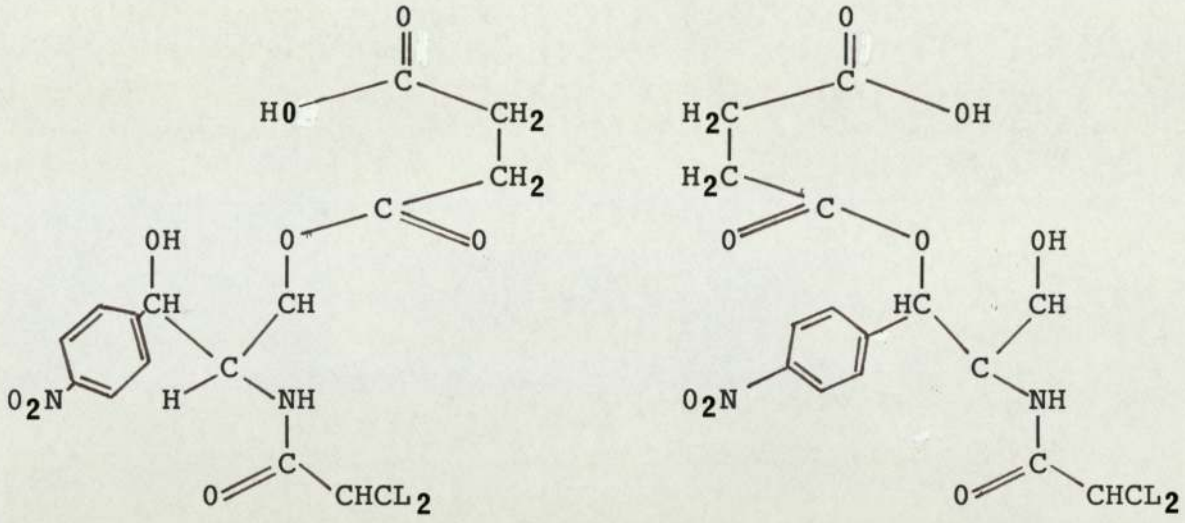
analysis of the serum a peak was obtained which obscured the chloramphenicol succinate peaks. Adaptation of the mobile phase to the proportions described, although increasing the time required to perform a run, eradicates interference by either sulphadimidine or sulphadiazine (the sulphonamide considered to be more effective in the treatment of meningitis) with the chloramphenicol succinate peaks.

In order to produce a "cleaner" sample and to prolong column life the chloramphenicol and chloramphenicol succinate were extracted into ethyl acetate. Due to the differing partition coefficients into this phase recovery was inevitably better for chloramphenicol (89%) than for chloramphenicol succinate (62%). Direct injection of serum (+ internal standard) onto the column would have avoided any loss of the compounds, but it would have been necessary to monitor the column effluent at a u.v. wavelength below 255 n.m. in order to minimise interference from serum and this is significantly removed from the optimum absorbance wavelength for chloramphenicol (278 n.m) and chloramphenicol succinate (276 n.m) that sensitivity, as well as column life, would have been reduced. Burke (78) utilised trichloroacetic acid to precipitate plasma proteins and then injected the supernatant directly onto the column, due to plasma protein binding he only obtained recovery of 63% for chloramphenicol and 40% for chloramphenicol succinate. Aravind (79) claims recovery of 100% for both chloramphenicol and chloramphenicol succinate using a method also based on extraction into ethyl acetate,

but he compared unknown samples extracted into ethyl acetate with a calibration curve produced after extraction of spiked serum samples into ethyl acetate, which does not reflect total recovery.

The calibration plot showed good correlation to a straight line for both chloramphenicol (correlation coefficient = 0.998) and chloramphenicol succinate (cc = 0.997). The chloramphenicol succinate plot deviated from the origin leading to possible errors where extrapolating the calibration plot to low concentrations. Consequently concentrations of chloramphenicol succinate reported below 5mg/L were not utilised in calculations.

Chloramphenicol succinate is supplied as a crystalline powder of chloramphenicol-3-succinate. In solutions at pH near neutrality it partially rearranges to chloramphenicol-1-succinate (Figure 4.1). The assay utilises the preparation of a standard plot of total chloramphenicol succinate concentration versus ratios of peak areas for chloramphenicol -3-succinate. This is justified only if the proportion of chloramphenicol-3-succinate which rearranges to chloramphenicol-1-succinate is constant. Burke (78) found that the proportion of chloramphenicol-1-succinate formed declines as the pH lowers and Aravind (79) found the ratio of peak areas of chloramphenicol-1-succinate to chloramphenicol-3-succinate to be 1:4 at pH = 6.4 over a range of concentrations of chloramphenicol succinate. This ratio was also found in the analysis performed for this project.



CHLORAMPHENICOL-3-SUCCINATE CHLORAMPEHNICOL-1-SUCCINATE

Figure 4.1 Configuration of Chloramphenicol-1-Succinate and Chloramphenicol-3-Succinate

It is believed that only chloramphenicol-3-succinate is converted to chloramphenicol in vivo. Burke has demonstrated that the conversion between chloramphenicol-3-succinate and chloramphenicol-1-succinate occurs rapidly at physiological pH and temperature, so the formation of a second isomer should have no effect on the pharmacokinetics or therapeutic effect of chloramphenicol.

Within day variations in serum samples were found to be 3.8% for chloramphenicol-3-succinate and 2.6% for chloramphenicol. Day to day variations were 10.3% and 8.5% respectively. These results demonstrate that the method is highly reproducible. Since calibration was performed before each day's analysis the within day variation gives a more accurate representation of errors which may be present in the results reported.

4.3 Sampling

Samples were taken as described (Section 2.3.4). Due to the compromise necessitated between the care of the patient and the demands of the study only two blood samples could be taken per dosage interval. The timing of the peak sample was, therefore, critical. If the sample was drawn before the peak had been attained, a misleading impression of the risk of toxicity would be produced, furthermore any calculations which assumed first order decay would produce erroneous results. The time to peak concentration is largely dependant on the rate of hydrolysis of chloramphenicol succinate, and this is certainly subject to individual variation. The actual sampling time was chosen to ensure that samples were either at or past peak levels, rather than pre-peak. There is little guidance in the literature as to when peak chloramphenicol samples should be taken. The Alder Hey Book of Children's Doses suggests 30 minutes after an intravenous dose and makes no recommendation on oral doses. This sampling time appears to be early, gentamicin is sampled at 60 minutes and is administered as an active drug, the 60 minute period representing the time for the drug to distribute throughout the body compartments (the volume of distribution of gentamicin is 0.25mg/kg, considerably less than that reported for chloramphenicol). Although hydrolysis of chloramphenicol succinate has been reported to be rapid it is not instantaneous and samples taken 30 minutes after intravenous administration would seem to represent a pre-peak level. Mulhall in her study took samples 15-60

minutes after intravenous administration and 1-4 hours after oral dosing. The significance of results from samples drawn over such a wide time interval, given the relatively short half-life of chloramphenicol, and the possibility that samples drawn after intravenous administration may well be pre-peak, is questionable.

Several American studies have been able to take samples at more regular intervals. Although many of them do not quote the time at which peak concentration was reached after dosing the findings of those that do are quoted in Table 4.1. Care must be taken in the interpretation of the times stated, since administration is invariably by intravenous infusion and this does not produce a situation which can be directly applied to the timing after an intravenous bolus injection.

Study	Age of Patient	Method of Administration	Time to Peak (After End of Dose)
Pickering et al (80)	3 months - 12 years	60 min i.v. infusion	60 minutes
Pickering et al (80)	3 months - 12 years	Oral	2-3 hours
Yogev et al (81)	5-23 months	30 min. i.v. infusion	45 minutes
Kauffman et al (57)	3 days - 16 years	10 min. i.v. infusion	90 minutes - 3 hours (no intervening samples
Burke et al (58)	Adult	5 min. i.v. infusion	<20 minutes
Slaughter et al (59)	43-69 years	15 min i.v. infusion	1.7 hours

Table 4.1 Time to Peak Concentration found in other studies

The time at which the peak sample should be drawn is clearly subject to interpatient variability. Since it was only possible to draw two samples it was decided that two hours post intravenous administration was sufficiently close to peak concentration and provided a consistent time interval for a sample to be taken. The central reason for monitoring serum concentrations is to avoid toxicity which has been related to peak serum chloramphenicol concentrations. The clinical relevance of the timing at which samples are drawn is only apparent when compared to the time at which samples were taken by the investigators who correlated toxic effects to serum chloramphenicol concentration. However, this comparison is difficult to draw since correlation of bone marrow suppression to serum chloramphenicol concentration has been performed on adult patients receiving oral chloramphenicol base (Scott et al). The timing of samples drawn by workers investigating Grey Baby Syndrome are not applicable since the therapeutic range which has been defined is based on the risk of toxicity from bone marrow suppresssion. In summary, it is not possible to confidently state a time interval at which a peak sample may be drawn, but two hours after intravenous dosing represents a good compromise. This was confirmed by the clinical results obtained (see Section 4.4.5). Investigators who have linked toxic effects to serum chloramphenicol concentrations have no evidence that the concentrations that they have measured were the maximum (peak) concentrations.

Urine samples were taken as described. Few of the patients were catheterised so urine was collected in a urine bag. This method of collection is subject to leakage and collection of the total volume is difficult to guarantee. Inspection of the bedding and nappies was carried out by nursing staff and if there was any sign of urine leakage the urine samples from that dosage interval were discarded.

Calculating elimination rate constant of a drug by the method described in section 2.11 for chloramphenicol succinate is well established. However, for total accuracy the bladder must be completely emptied at the time of administration of the dose and immediately before the next dose and there should be several samples passed in one dosage interval. These conditions are difficult to fulfil in paediatric patients. The method requires that complete elimination of chloramphenicol succinate occurs within a dosage interval (i.e. a dosage interval is greater than 4 half-lives for chloramphenicol succinate). If total elimination of chloramphenicol succinate occurs within a significantly shorter period of time, emptying of the bladder at the beginning and end of the dosage interval is of less importance. Trough serum concentrations of chloramphenicol succinate and urine concentrations at the end of the dosage interval support the view that that chloramphenicol succinate has a sufficiently short half-life for it to be completely eliminated in six hours.

However the disadvantage to this method is that a drug which is completely eliminated in a short period of time is only likely to produce a small number of points on the log ARE versus time plot and the accuracy of determinations from a straight plot which consists of only two or three points may be questioned.

Particular difficulty was experienced in obtaining samples at both the beginning and the end of the therapeutic course. There were several contributing factors for the missing data at the beginning of the course:

(1) ignorance of the staff of the requirements of the study which was overcome eventually;

(2) many parents were reluctant to give immediate consent on presentation of the patient;

(3) the inconvenient time lapse between administration of the first dose and peak sample, particularly since many patients presented between the hours of 10 p.m. and 2 a.m. (although the researcher made himself available to take samples at all times);

(4) and two patients who presented had shutdown peripheral circulation resulting in difficulty obtaining heel prick samples and no urine samples.

From the point of view of the study these obstacles were disappointing, but they represent difficulties which will always be encountered when attempting to gather first dose data for chloramphenicol in order to calculate individual pharmacokinetic parameters;

Two problems were commonly experienced in gaining samples towards the end of the course of therapy:

(1) Patients were frequently discharged without samples being taken once they had been transferred to oral medication;

(2) discontinuation of therapy once viral meningitis had been diagnosed.

4.4 Clinical Results

Because of the deficit of data at some stages due to the above limitations it was sometimes only possible to draw empirical observations and conclusions from the data rather than statistically demonstrate significance.

4.4.1 Excretion of Chloramphenicol Succinate

Table 3.2 records the percentages of chloramphenicol succinate excreted unchanged in the urine. In the first 24

hours of the course a mean of 12.1% of the administered dose of chloramphenicol succinate (n = 12, range 1.3-27.0%) was excreted unchanged in the urine. At steady state the mean was also 12.1% (n = 11, range 0 - 49.8%) and there was no significant difference between the two groups. However, close examination of the data shows that where results can be "paired" for individual patients there are large and conflicting differences in the results. Therefore, it is not possible to draw the conclusion that there is no change in the proportion of unhydrolysed chloramphenicol succinate which is excreted in the urine during a dosage interval.

The proportions of chloramphenicol succinate excreted in the urine which other studies have reported are shown in Table 4.2. The values are generally higher than those found in this study, but most of the study populations were adult. Significantly when Nahata's data is recalculated to exclude patients over 24 months the mean proportion excreted unchanged in his study falls from 31% (n = 12) in the original study to 23% (n = 9) in the recalculated data. It is known that in adult patients renal excretion is principally achieved by active secretion, these results suggest that this process may be deficient in patients less than two years old.

Elimination by active tubular secretion is important for excretion of penicillins by adult patients. In neonates a prolonged half-life has been found for benzylpenicillin and this has been attributed to immature renal active secretion.

With increasing post natal age and maturing renal function urinary excretion rate increases and serum half-life decreases. At 14 days the half-life is three times that obtained in an adult population, by age 3-4 years the rate of renal excretion benzylpenicillin is more rapid than in adults. The age at which full maturation of the tubular secretion mechanism is achieved has not been defined and is presumably subject to interpatient variability. However the results of this study suggest that renal elimination of chloramphenicol succinate is still deficient at 26 months (see Table 3.2). It was not possible to demonstrate a relationship between the age of the patients and proportion of chloramphenicol succinate excreted unchanged.

Study	Number of Patients	Age of Patients	Mean % Excreted Unchanged	Range
Burke et al (58)	8	19-64 years	26%	13-36%
Kauffman et al (57)	45	3 days - 16 years	33%	6-80%
Nahata et al (34)	12	2 1/2 months - 20 years	31%	7.6-42%
Slaughter et al (59)	6	50-72 years	20%	6.5-43.5%
Kramer et al (82)	12	16-67	27%	10-44%

Table 4.2 Previously reported percentages of chloramphenicol succinate eliminated unchanged in the urine.

4.4.2 Excretion of Chloramphenicol

At steady state it was found that 7.2% of the dose of chloramphenicol administered was excreted in the urine. Burke found 11% of the bioavailable chloramphenicol was excreted unchanged in the urine (range 5-19%).

There was a strong direct relationship between the percentage of chloramphenicol and chloramphenicol succinate excreted unchanged in the urine ($r_{11} = 0.98$, $P < 0.001$) at steady state. Since renal elimination of chloramphenicol is by glomerular filtration this supports the hypothesis that tubular secretion is not as significant in the renal excretion of chlormaphenicol succinate in patients of this age as in more mature patients. This relationship was also demonstrated in urine samples obtained during the first 24 hours of therapy but it was less statistically significant ($r_{10} = 0.77$, $P < 0.01$), probably due to the fact that chloramphenicol had not reached steady state.

4.4.3 Serum Concentrations of Chloramphenicol

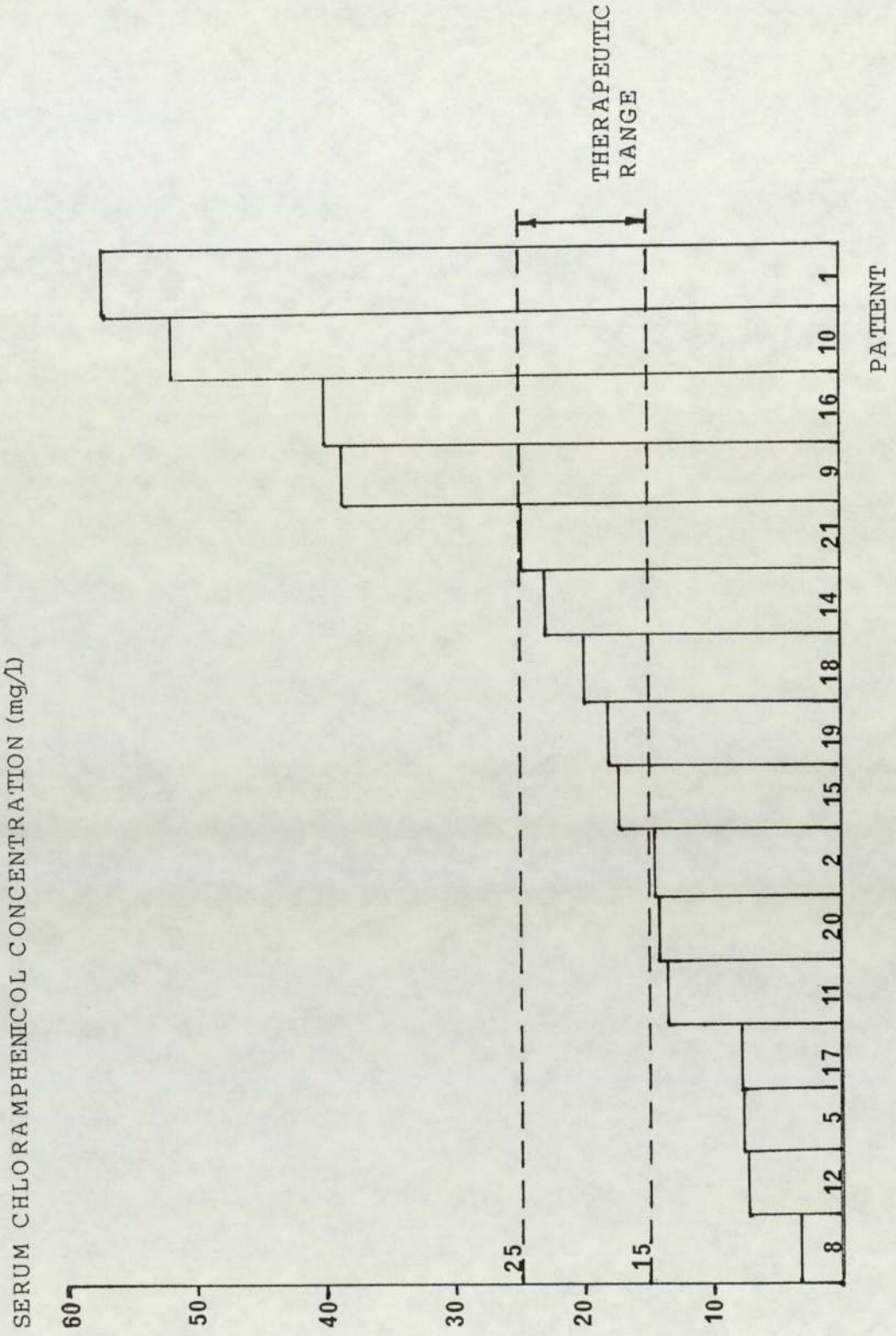
Table 3.3 details the steady state peak and trough serum concentrations of chloramphenicol measured. 7 patients (44%) demonstrated peak concentrations which were subtherapeutic, 4 patients (25%) displayed levels which were potentially toxic and only 5 patients (31%) were within the therapeutic range.

The results are demonstrated in the form of a histogram in Figure 4.1. Kauffman and Mulhall both demonstrated wide variations in peak steady state chloramphenicol concentration, (although they also used wide variations in dose) the results of this study confirm that even using recommended dosages only a minority of patients will be within the therapeutic range, the majority may be either sub or supratherapeutic. The need to monitor chloramphenicol serum concentrations in all patients is thus confirmed.

Most of the patients who were subtherapeutic were receiving a total daily dose 60 mg/kg/day chloramphenicol. Other pharmacokinetic parameters (half-life, volume of distribution, proportion of chloramphenicol succinate excreted unchanged) in these patients are not significantly different to those found in the other patients. The only consistent explanation for the low levels in these patients is that a dose of 60 mg/kg/day is inadequate. However, it should also be noted that patient 14 who was receiving a dose of 54 mg/kg/day had a peak chloramphenicol concentration of 22.9 mg/L.

Examining patients who exhibited peak chloramphenicol serum concentrations above the therapeutic range also does not highlight any pharmacokinetic parameters which may identify patients who were likely to be at risk. All of the patients with serum concentrations>25 mg/L were receiving doses 100mg/kg/day, but patient 2 who was also receiving this dose

Figure 4.2 Peak Chloramphenicol Steady State Serum Concentrations



showed subtherapeutic levels. There were no consistent differences in the pharmacokinetic parameters between the group that were supratherapeutic and the group as a whole.

4.4.4 Half-Life and Elimination Rate Constant of Chloramphenicol

Table 3.4 details the half-life and elimination rate constant found for chloramphenicol. The mean results and standard deviations follow closely results found in numerous other studies in patients of this age group.

The results for patients 8 and 11 were omitted from the calculations for the mean since they deviate so much from the norm. Failure of chloramphenicol serum concentrations to decline during a dosage interval in paediatric patients has been previously noted (57) but not explained. Patient 8 demonstrated a long half-life (33.5 hours) at steady state. Significantly he was the only patient in the study to be receiving chloramphenicol orally at this stage. Yogev (81) has noted that patients receiving oral therapy have a longer apparent half-life than those receiving parental therapy, this may be attributed to delayed absorption of chloramphenicol. Patient 11 also had a prolonged half-life, both initially and at steady state. Interpretation of this result is complex. Examination of the raw data reveals that levels at steady state were acceptable and did not show evidence of gross

accumulation of chloramphenicol, which would be expected from such a long half-life. This patient was hydrocephalic and it is possible that this formed a second compartment into which the chloramphenicol succinate preferentially diffused, and from which it was slowly released into the serum to be hydrolysed to chloramphenicol. Within the raw data there is some evidence which supports this view. At the beginning of the course chloramphenicol succinate also had a long half-life in this patient and (unusually) was detected in the serum when the trough sample was taken. Serum samples drawn in the middle of the course did not show the presence of chloramphenicol succinate, but the chloramphenicol succinate was excreted in the urine throughout the dosage interval, possibly indicating that diffusion from the second compartment was at a rate which was not sufficient to accumulate and produce detectable serum concentrations (the dose was almost halved between the first and second set of samples).

The half-life of chloramphenicol appears to decline as the period of treatment progresses. Statistically at a 95% confidence interval the probability of a correlation between the decline in half-life and duration of course of chloramphenicol for 7 paired results is $P = 0.08$, which is approaching significance. Further research may confirm that the half-life of chloramphenicol decreases during a therapeutic course.

The decrease in half-life of chloramphenicol implies that clearance has increased. Tuomanen has also noted that the A.U.C. of chloramphenicol decreases as therapy progresses and Nahata reported that the A.U.C. of both chloramphenicol and chloramphenicol succinate decreased during a therapeutic course. The results of this study confirm that clearance of chloramphenicol increases as therapy progresses.

4.4.5 Half-Life and Elimination Rate Constant of Chloramphenicol Succinate

The half-life and elimination rate constant found for chloramphenicol succinate are shown in Table 3.5. The mean half-life was found to be 0.9 hours, which suggests that at least 75% will have been eliminated after 2 hours, confirming that this is a reasonable time take a peak sample. Interestingly Kauffman and Nahata in separate papers reported significantly longer half-lives (2.2 and 2.7 hours respectively) in mixed paediatric/adult populations but Slaughter and Burke independantly reported half-lives in adult populations (0.6 and 1.2 hours respectively) similar to those found in this study.

The half-life of chloramphenicol succinate did not show any significant change between initiation of therapy and the middle of the course.

4.4.6 Volume of Distribution of Chloramphenicol and Chloramphenicol Succinate

Tables 3.7 and 3.8 details the volume of distribution of chloramphenicol and chloramphenicol succinate. The results correlate well with other published results for chloramphenicol, the range of mean values reported previously being 0.7 - 1.0L/kg. The volume of distribution found for chloramphenicol succinate is higher than that found in adult studies - but lower than Nahata reported (2.1L/kg). Weber and Smith (83) have suggested that the results obtained in this study are not supported by the serum concentrations measured and have recalculated his data to produce a volume of distribution of 0.9L/kg. It should be noted that the volume of distribution found in patient 11 does not support the existence of a second compartment for chloramphenicol succinate.

The results from this study demonstrate that chloramphenicol succinate is rapidly eliminated from the body and this is confirmed by the small volume of distribution found coupled with low serum concentrations measured after two hours.

4.5 Pharmacokinetic Predictive Method

At the end of Section 2 it was suggested that it may be possible to predict steady state chloramphenicol serum

concentrations. Any method attempting to do so relies on pharmacokinetic parameters remaining the same throughout a dosage interval. It has been shown that excretion of chloramphenicol succinate varies during a course of therapy and that the elimination rate of chloramphenicol increases as the course progresses.

The predictive equations 8 and 9 overestimate the peak and trough concentrations which were obtained for individual patients. This is because the equations make no allowances for the quantity of chloramphenicol which is eliminated between administration of the dose and drawing the peak sample. It is possible to build a correction factor for this into the equations, but given the change in pharmacokinetic parameters of chloramphenicol during a course of therapy the use of predictive methods rather than routine monitoring of serum concentrations should be discouraged.

4.6 Drug Interactions With Chloramphenicol

Many patients were also being administered other drugs which have been reported to interact with chloramphenicol. Most of the patients were prescribed paracetamol and there was no significant increase in the half life of chloramphenicol in these patients.

Several patients were also receiving anticonvulsant therapy. Some of those prescribed phenobarbitone demonstrated high peak chloramphenicol serum concentrations, but this did not correlate with a long half-life and therefore it is difficult to draw any conclusion regarding the effect of the phenobarbitone on the pharmacokinetics of chloramphenicol.

Several studies have reported interactions between chloramphenicol and anticonvulsant drugs, the results of this study imply that there is considerable clinical significance in this interaction.

4.7 Toxicity

Several patients demonstrated very high steady state serum concentrations of chloramphenicol. Since these patients immediately had the dosage adjusted no patient maintained in high serum concentration for longer than 24 hours after the steady state sample was drawn.

Two patients (9 and 10) with steady state serum concentrations of 38 mg/L and 52 mg/L respectively did not show raised white blood cell counts in films taken several days after steady state samples despite the infection this may have been a result of chloramphenicol toxicity. Both patients blood counts were normal on discharge when the chloramphenicol had either been discontinued or the dosage adjusted.

No other evidence of toxicity was found in any of the patients and all were treated successfully.

4.8 Chloramphenicol Glucuronide

Tables 3.9 and 3.10 detail the serum concentrations of chloramphenicol glucuronide measured and percentage of the dose administered which is excreted as the glucuronic acid conjugate.

The proportion of the dose excreted as chloramphenicol glucuronide appears to be low. Table 4.3 collates the proportions of the dose excreted as chloramphenicol, chloramphenicol succinate and chloramphenicol glucuronide. It can be seen that in most patients it does not approach 100%. This implies either that chloramphenicol glucuronide is not the major metabolite or that the enzymatic hydrolysis does not go to completion. Figure 2.3 appears to demonstrate that hydrolysis is complete after 2 hours, and therefore more than sufficient time was allowed in the analysis of clinical samples.

Aravind (84) has used a similar method and claimed complete hydrolysis - supported by the identification on his H.P.L.C. trace of a chloramphenicol glucuronide peak which disappeared after incubation, and also 100% recovery of the dose in the urine.

Table 4.3

Percentage of Chloramphenicol, Chloramphenicol Succinate, And Chloramphenicol Glucuronide Excreted in the Urine

Patient	Beginning of Course			Middle of Course		
	CAP %	CAPSS %	CAPG %	CAP %	CAPSS %	CAPG %
1	-	-	-	22.8	49.8	22.0
2	-	-	-	1.2	3.1	4.5
3	0.8	3.8	3.9	-	-	-
4	7.0	25.6	18.5	-	-	-
5	-	-	-	4.8	5.7	5.6
6	1.6	1.9	7.6	-	-	-
7	0.5	1.8	6.5	-	-	-
8	4.9	6.5	4.8	-	-	-
9	-	-	-	4.9	6.5	14.2
10	-	-	-	2.2	6.6	12.4
11	3.9	26.5	31.0	8.8	9.9	54.0
12	-	-	-	-	-	-
13	3.7	1.3	18.0	-		
14	-	-	-	1.4	0.9	5.2
15	5.7	7.1	16.0	22.3	42.1	20.0
16	-	-	-	1.9	3.3	8.4
17	7.4	12.9	35.0	-	-	
18	-	-		5.2	4.8	15.7
19	10.9	26.0		3.7	0.0	
20	2.9	4.8	8.3	-	-	
21	10.9	27.0	23.0	-	-	
Mean	5.0	12.1	15.7	7.2	12.1	16.2
Standard Deviation	3.5	10.9	10.6	7.9	17.1	14.6

CAP = Chloramphenicol

CAPSS = Chloramphenicol Succinate

CAPG = Chloramphenicol Glucuronide

Kauffman (33) could only recover 18% of the dose as chloramphenicol glucuronide (range 3-60%), and a total of 55% of the dose as the base and esters, using a B-glucuronidase hydrolysis method.

Glazko (85) has stated that it is only possible to hydrolyse a maximum of 70% of chloramphenicol glucuronide by this method.

It is possible that some of the patients were deficient in glucuronidating capacity due to immature liver function. However there was no statistical correlation between age and percentage of dose excreted as chloramphenicol glucuronide.

Since there is some considerable doubt about the accuracy of the method of analysis for chloramphenicol glucuronide it is not possible to draw any conclusions from the serum concentrations of chloramphenicol glucuronide.

CONCLUSIONS

1. There is considerable variation in the peak, steady state serum chloramphenicol concentrations obtained when using B.N.F. recommended doses. Since the levels obtained have been shown to be either subtherapeutic or potentially toxic, monitoring of chloramphenicol serum concentrations is mandatory.

2. The B.N.F. dosage range (50-100 mg/kg/day) may be employed when supported by therapeutic drug monitoring. However an initial dose of 75 mg/kg/day is most likely to produce steady state serum concentrations within the therapeutic range.

3. Blood samples should be drawn two hours after intravenous bolus administration of chloramphenicol succinate. Current recommendations that samples should be drawn after 30 minutes produce pre-peak levels.

4. Pharmacokinetic parameters of chloramphenicol change during the therapeutic course and predictive pharmacokinetic equations should not be used as a substitute for regular monitoring of serum chloramphenicol concentrations.

5. Phenobarbitone increases serum chloramphenicol concentrations in some patients, emphasising the need for therapeutic monitoring in patients receiving anticonvulsant therapy. There was no evidence of an interaction between chloramphenicol and paracetamol.

APPENDIX I

RAW DATA

A = Concentration of Chloramphenicol (mg/l)
B = Concentration of Chloramphenicol Succinate (mg/l)
C = Concentration of Chloramphenicol Glucuronide (expressed as mg/l Chloramphenicol base).

Administration of p.r.n drugs has only been recorded if it occured on the day of sampling.

PATIENT 1.

Weight = 11.55 kg Age = 15 months Diagnosis H. Influenzae Meningitis

Commenced 20-30 hours 11.12.1985 Dose 300 mg i.v. q.d.s.

Serum Samples

13.12.85	12.45	Dose 14.45	Sample	A = 57;	B = 0;	
13.12.85	12.45	Dose 18.15	Sample	A = 33;	B = 0;	C = 6

Urine Samples

13.12.85	12.45 - 15.00 hrs	7 ml	A = 1453;	B = 4673;	C = 1840.
13.12.85	15.00 - 16.00 hrs	28 ml	A = 1236;	B = 3928;	C = 1270.
13.12.85	16.00 - 18.00 hrs	18 ml	A = 1320;	B = 3553;	C = 1133.

Concurrent Medication

Ampicillin	1g i.v. q.d.s
Sulphadimidine	600 mg i.v. q.d.s
Aspirin	150 mg p.r. p.r.n

PATIENT 2

Weight = 12.5 kg Age = 2 years Diagnosis Meningococcal Meningitis

Commenced 18.30 09.03.1986 Dose 300 mg i.v. q.d.s.

Serum Samples

12.03.86	12.00	Dose 14.00	Sample	A = 14.1;	B = 6.9;	
12.03.86	12.00	Dose 18.00	Sample	A = 2.0;	B = 0.5;	

Urine Samples

12.03.86	12.00 - 17.00 hrs	22 ml	A = 160;	B = 592;	C = 616

Concurrent Medication

Benzylpenicillin 600 mg i.v. 4 hourly

PATIENT 3

Weight = 14.7 kg Age = 23 months Diagnosis Meningococcal Meningitis

Commenced 12.00 16.04.1986 Dose 350 mg i.v. q.d.s.

Serum Samples

16.04.86	12.00	Dose 14.00	Sample	A = 23.0;	B = 1.5;	C = 1.8
16.04.86	12.00	Dose 18.00	Sample	A = 5.7;	B = 0;	C = 1.0

Urine Samples

16.04.86	12.00 - 18.00	15 ml	A = 197	B = 1220;	C = 910

Concurrent Medication

Benzylpenicillin	750 mg i.v.	4 hourly	
Paracetamol	180 mg p.o.	p.r.n.	(12.30, 16.04.86)
Aspirin	150 mg p.r.	p.r.n.	(15.00, 16.04.86)

PATIENT 4

Weight = 22.6 kg Age = 6.5 years Diagnosis H. Influenzae Tonsillitis

Commenced 20.55 14.01.1986 Dose 400 mg i.v. q.d.s.

Serum Samples

14.01.86	21.00 Dose 23.00	Sample	A = 10.2;	B = 1.5	

Urine Samples

14.01.86	21.00 - 22.00 hrs	115 ml	A = 113;	B = 1100;	C = 388
14.01.86	22.00 - 24.00 hrs	40 ml	A = 380	B = 382;	C = 737

Concurrent Medication

Benzylpenicillin	1.5g i.v.	4 hourly			
Paracetamol	480mg p.o. p.r.n.		(09.00,	18,15,	14.01.86)

PATIENT 5

Weight = 9.35 kg Age = 9.5 months Diagnosis Meningococcal Meningitis

Commenced	14.00 08.04.1986	Dose 250mg i.v. q.d.s.
	18.00 09.04.86	Dose 125mg i.v. q.d.s.

Serum Samples

08.04.86	14.00 Dose 16.00	Sample	A = 12.8;	B = 4.8;	
08.04.86	14.00 Dose 18.00	Sample	A = 5.8;	B = 0;	C = 1.9
11.04.86	12.30 Dose 14.30	Sample	A = 7.4;	B = 8.2;	C = 2.9
11.04.86	12.30 Dose 18.00	Sample	A = 2.7;	B = 4.5;	C = 1.1

Urine Samples

11.04.86	12.30 - 13.00 hrs	11 ml	A = 32;	B = 116;	C = 333
11.04.86	13.00 - 14.00 hrs	22 ml	A = 93;	B = 274;	C = 124
11.04.86	14.00 - 15.00 hrs	25 ml	A = 67;	B = 100;	C = 115
11.04.86	15.00 - 16.00 hrs	10 ml	A = 66;	B = 0;	C = 449
11.04.86	16.00 - 17.00 hrs	10 ml	A = 25;	B = 0;	C = 463
11.04.86	17.00 - 18.00 hrs	25 ml	A = 43;	B = 0;	C = 53

Concurrent Medication

Benzylpenicillin	300 mg i.v. 4 hourly	
Paracetamol	120 mg p.o. p.r.n	(13.30, 21.55, 08.04.86; 02.00, 07.00 11.45, 22.00, 11.04.86)
Aspirin	150 mg p.r. p.r.n.	(16.45, 08.04.86)

PATIENT 6

Weight = 11.5 kg Age = 19 months Diagnosis Meningococcal Meningitis

Commenced 18.00 10.03.1986 Dose 150mg i.v. q.d.s.

Serum Samples

11.03.86	12.00 Dose	14.00 Sample	A = 10.5;	B = 1.7
11.03.86	12.00 Dose	18.00 Sample	A = 2.8;	B = 0

Urine Samples

11.03.86	12.00 - 15.00 hrs	8 ml	A = 78;	B = 386;	C = 632
11.03.86	15.00 - 16.00 hrs	20 ml	A = 56;	B = 37;	C = 145
11.03.86	16.00 - 18.00 hrs	19 ml	A = 37;	B = 3;	C = 182

Concurrent Medication

Paracetamol	240mg p.o.	4 hourly
Benzylpenicillin	300mg i.v.	q.d.s.
Chloramphenicol Eye Drops	2 drops q.d.s.	Both eyes

Additional Medical problems

Eye infection

PATIENT 7

Weight = 8.5 kg Age = 25 months Diagnosis Viral Meningitis

Commenced 12.40 20.06.1985 Dose 425mg i.v. Stat.

Serum Samples

20.06.85	12.40	Dose 14.40	Sample	A = 28.6; B = 32.0
20.06.85	12.40	Dose 18.15	Sample	A = 19.0; B = 0;

Urine Samples

20.06.85	12.40 - 14.00 hrs	13 ml	A = 105;	B = 330;	C = 1979
20.06.85	14.00 - 16.45 hrs	14 ml	A = 54;	B = 437;	C = 130

Concurrent Medication

Paracetamol	120mg p.o.	4 hourly
Benzylpenicillin	360mg i.v.	4 hourly
Sulphadimidine	400mg i.v.	q.d.s

PATIENT 8

Weight = 6.8 kg Age = 6.5 months Diagnosis Viral Meningitis

Commenced 24.00 18.08.1985 Dose 90mg i.v. q.d.s.
20.08.1985 100mg p.o. q.d.s.

Serum Samples

19.08.85	12.00	Dose 14.00	Sample	A = 3.2; B = 0;	C = 0.6
19.08.85	12.00	Dose 18.00	Sample	A = 0; B = 0;	C = 0
22.08.85	18.00	Dose 20.45	Sample	A = 2.2; B = 0	
22.08.85	18.00	Dose 24.00	Sample	A = 2.1; B = 0;	C = 1.2

Urine Samples

19.08.85	12.00 - 15.00 hrs	35 ml	A = 50;	B = 135;	C = 91
19.08.85	15.00 - 17.00 hrs	25 ml	A = 10;	B = 12;	C = 47

Concurrent Medication

Ampicillin	90mg i.v. q.d.s	125mg p.o. q.d.s.
Paracetamol	120mg p.o. p.r.n.	(07.10, 22.00, 19.08.85; 06.00, 22.10.85)

PATIENT 9

Weight = 6.9 kg Age = 4 months Diagnosis Viral Meningitis

Commenced 18.00 04.11.1985 Dose 175mg i.v. q.d.s.
07.11.85. 130mg i.v. q.d.s

Serum Samples

06.11.85	12.00	Dose 14.00	Sample	A = 38.6;	B = 0.4	
06.11.85	12.00	Dose 18.00	Sample	A = 28.3;	B = 0	
13.11.85	07.00	Dose 10.00	Sample	A = 3.4	B = 0;	C = 1.1
13.11.85	07.00	Dose 12.30	Sample	A = 2.2;	B = 0;	C = 1.2

Urine Samples

06.11.85	12.00 - 15.10 hrs	21 ml	A = 189;	B = 680;	C = 634
06.11.85	15.10 - 17.35 hrs	20 ml	A = 228	B = 78;	C = 582

Concurrent Medication

Phenobarbitone	30mg i.v. b.d.	
Cefotaxime	300mg i.v. q.d.s.	
Acyclovir	70mg i.v. q.d.s.	
Paracetamol	120mg p.o. p.r.n.	(06.00, 12.30, 06.11.85)

PATIENT 10

Weight = 11.93 kg Age = 26 months Diagnosis Meningococcal Meningitis

Commenced 01.30 24.03.1986 Dose 250mg i.v. g.d.s.

Serum Samples

25.03.86	12.45	Dose 14.45	Sample	A = 51.6;	B = 6.3.
25.03.86	12.45	Dose 18.15	Sample	A = 24.3;	B = 2.5.

Urine Samples

25.03.86	12.00 - 13.35 hrs	4 ml	A = 61;	B = 59;	C = 1018
25.03.86	13.35 - 14.45 hrs	26 ml	A = 139;	B = 777;	C = 215
25.03.86	14.45 - 16.15 hrs	8 ml	A = 160;	B = 247;	C = 1369
25.03.86	16.15 - 17.25 hrs	4 ml	A = 70;	B = 66;	C = 2589

Concurrent Medication

Benzylpenicillin	600mg i.v. q.d.s.
Phenobarbitone	30mg i.v. q.d.s.

PATIENT 11

Weight = 7.5 kg	Age = 7 months	Diagnosis	H. Influnzae Meningitis
Commenced	13.00 01.12.1985	Dose 125mg i.v.	4 hourly
		02.12.85	100mg i.v. q.d.s.
		06.12.85	100mg p.o. q.d.s.

Serum Samples

02.12.85	14.00	Dose 16.00	Sample	A = 44.2;	B = 7.9;	C = 3.4
02.12.85	14.00	Dose 18.15	Sample	A = 43.3;	B = 3.3;	C = 2
05.12.85	13.00	Dose 15.00	Sample	A = 13.5;	B = 0	
05.12.85	13.00	Dose 18.00	Sample	A = 12.7;	B = 0	
12.12.85	06.00	Dose 10.15	Sample	A = 7.4;		
12.12.85	06.00	Dose 12.15	Sample	A =0		C = 1.8

Urine Samples

02.12.85	14.00 - 15.00 hrs	10 ml	A = 85;	B = 1414;	C = 710
02.12.85	15.00 - 16.00 hrs	3 ml	A = 393;	B = 3028;	C = 2541
02.12.85	16.00 - 17.00 hrs	15 ml	A = 70;	B = 1446;	C = 1072
02.12.85	17.00 - 18.00 hrs	6 ml	A = 300;	B = 162;	C = 1338
05.12.85	12.00 - 13.00 hrs	2.5 ml	A = 226;	B = 40;	C = 879
05.12.85	13.00 - 15.00 hrs	11 ml	A = 183;	B = 650;	C = 1353
05.12.85	15.00 - 16.00 hrs	16 ml	A = 203;	B = 353;	C = 1293
05.12.85	16.00 - 17.00 hrs	12 ml	A = 153;	B = 52;	C = 964
05.12.85	17.00 - 18.00 hrs	15 ml	A = 78;	B = 14;	C = 330

Concurrent Medication

Benzylpenicillin	300mg i.v. q.d.s.	
Phenobarbitone	40mg i.v. b.d.	
Dexamethasone	1mg i.v. t.d.s.	
Phenytoin	35mg p.o. b.d.	
Aspirin	150mg p.r. p.r.n.	(06.30, 05.12.85; 02.15, 12.12.85)

PATIENT 12

Weight = 6.1 kg Age = 6.5 months Diagnosis Strep. Pneumoniae Meningitis

Commenced	22.30.27, 02.1986	Dose 110mg i.v. q.d.s.	
		01.03.86	75mg i.v. q.d.s.
		10.03.86	125mg p.o. q.d.s.

Serum Samples

05.03.86	12.30 Dose 14.20	Sample	A = 7.0;	B = 3.9
05.03.86	12.30 Dose 18.30	Sample	A = 0.4;	B = 0.9
11.03.86	12.00 Dose 16.00	Sample	A = 10.1	
11.03.86	12.00 Dose 18.00	Sample	A = 3.65	

Concurrent Medication

Benzylpenicillin	300mg i.v. q.d.s./penicillin V	250mg q.d.s.
Paracetamol	120mg p.o. q.d.s.	

PATIENT 13

Weight = 7 kg Age = 5 months Diagnosis Strep. Pneumoniae Meningitis

Commenced 02.30 18.11.1985 Dose 130mg i.v. q.d.s.

Serum Samples

18.11.85	14.00	Dose 16.00	Sample	A = 9.6;	B = 9.2	
18.11.85	14.00	Dose 18.25	Sample	A = 4.9;	B = 0	
21.11.85	12.00	Dose 18.30	Sample	A = 7.6;	B = 0;	C = 1.3

Urine Samples

18.11.85	14.00 - 15.00 hrs	12 ml	A = 160;	B = 0;	C = 910
18.11.85	15.00 - 16.40 hrs	35 ml	A = 46;	B = 56;	C = 224
18.11.85	16.40 - 18.30 hrs	33 ml	A = 40	B = 9;	C = 140

Concurrent Medication

Benzylpenicillin 300mg i.v. 4 hourly
Chlorpheniramin 1mg p.o.b.d.

PATIENT 14

Weight = 9.2 kg Age 10.5 months Diagnosis Viral Meningitis

Commenced 18.00 11.02.1986 Dose 230mg i.v. q.d.s.

Serum Samples

13.02.86	12.30	Dose 14-20	Sample	A = 22.9;	B = 0

Urine Samples

13.02.86	12.30 - 15.30 hrs	15 ml	A = 59;	B = 56;	C = 218
13.02.86	15.30 - 16.30 hrs	30 ml	A = 25;	B = 25;	C = 146
13.02.86	16.30 - 18.00 hrs	110 ml	A = 1;	B = 0;	C = 40

Concurrent Medication

Benzylpenicillin 450mg i.v. 4 hourly

PATIENT 15

Weight = 9.3 kg	Age = 10.5 months	Diagnosis	Meningococcal Meningitis
Commenced	16.30 22.04.1986	Dose 125mg i.v. Stat. Then 230mg i.v. q.d.s.	

Serum Samples

22.04.86	16.30	Dose 18.30	Sample	A = 10.5; B = 3.65;	C = 2.4
22.04.86	16.30	Dose 24.00	Sample	A = 4.3; B = 0;	C = 0.1
24.04.86	12.00	Dose 14.00	Sample	A = 17.3; B = 0;	C = 4.1
24.04.86	12.00	Dose 18.00	Sample	A = 8.3; B = 0;	C = 0

Urine Samples

22.04.86	16.30 - 18.00 hrs	23 ml	A = 63;	B = 235;	C = 48
22.04.86	18.00 - 19.00 hrs	22 ml	A = 58;	B = 188;	C = 203
22.04.86	19.00 - 20.00 hrs	25 ml	A = 60;	B = 85;	C = 427
22.04.86	20.00 - 21.00 hrs	25 ml	A = 62;	B = 26;	C = 63
22.04.86	21.00 - 24.00 hrs	21 ml	A = 69;	B = 0;	C = 107
24.04.86	12.00 - 13.30 hrs	58 ml	A = 763;	B = 2130;	C = 600
24.04.86	13.30 - 17.00 hrs	25 ml	A = 282;	B = 400;	C = 462

Concurrent Medication

Benzylpenicillin	1g i.v.	4 hourly
Aspirin	150mg p.r. p.r.n.	(15.40, 22.04.86)
Paracetamol	120mg p.o. p.r.n.	(06.00, 24.04.86)

PATIENT 16

Weight = 11.5 kg	Age = 22.5 months	Diagnosis	Meningococcal Meningitis
Commenced	21.00 222.04.86	Dose 300mg i.v. q.d.s.	

Serum Samples

24.04.86	12.00	Dose 14.00	Sample	A = 39.7;	B = 3.9;	C = 4.5
24.04.86	12.00	Dose 18.00	Sample	A = 20.8;	B = 0;	C = 4.8
30.04.86	12.30	Dose 14.30	Sample	A = 12.0;	B = 8.7;	C = 2.2
30.04.86	12.30	Dose 18.15	Sample	A = 4.0;	B = 0;	C = 2.1

Urine Samples

24.04.86	12.00 - 14.15 hrs	10 ml	A = 580;	B = 1372;	C = 2515
30.04.86	12.30 - 15.30 hrs	14 ml	A = 128;	B = 47;	C = 1360
30.04.86	15.30 - 17.30 hrs	50 ml	A = 40;	B = 30;	C = 91

Concurrent Medication

Benzylpenicillin	800mg i.v. 4 hourly	
Paracetamol	120mg p.o. p.r.n.	(14.00, 24.04.86; 12.30, 30.04.86)

PATIENT 17

Weight = 3.2 kg	Age = 20 days	Diagnosis Viral Meningitis
Commenced	24.00 12.08.1985	Dose 50mg i.v. q.d.s
		12.15, 13.08.85 40mg i.v. q.d.s.

Serum Samples

13.08.85	12.15	Dose 14.15	Sample	A = 22.4;	B = 30.0;	C = 5.0
13.08.85	12.15	Dose 19.00	Sample	A = 16.8;	B = 3.0;	C = 1.9
15.08.85	14.00	Dose 16.00	Sample	A = 7.6;	B = 9.0;	C = 4.6
15.08.85	14.00	Dose 18.00	Sample	A = 5.1;	B = 0;	C = 2.5

Urine Samples

13.08.85	12.15 - 15.45 hrs	18 ml	A = 96;	B = 350;	C = 593
13.08.85	15.45 - 18.00 hrs	16.5 ml	A = 75;	B = 52;	C = 202

Concurrent Medication

Benzylpenicillin	150mg i.v. q.d.s.	
Gentamicin	10mg i.v. t.d.s.	
Phenobarbitone	15mg i.v. p.r.n.	(12.10, 13.08.85)

PATIENT 18

Weight = 2.1 kg	Age = 28 days	Diagnosis Viral Meningitis
Commenced	11.45 18.08.1985	Dose 30mg i.v. q.d.s.

Serum Samples

18.08.85	11.45	Dose 13.45	Sample	A = 11.6; B = 15.1;	C = 1.7
18.08.85	11.45	Dose 18.00	Sample	A = 9.4; B = 0.8;	
22.08.85	14.00	Dose 16.00	Sample	A = 19.8; B =5.0	
22.08.85	14.00	Dose 18.00	Sample	A = 16.2; B = 3.1;	C = 2.9

Urine Samples

22.08.85	14.00 - 15.30 hrs	13 ml	A = 43;	B = 134;	C = 234
22.08.85	15.30 - 17.30 hrs	40 ml	A = 25;	B = 6;	C = 42

Concurrent Medication

Benzylpenicillin	125mg i.v. b.d.
Gentamicin	5.6mg i.v. b.d.
Phenobarbitone	12mg i.v. b.d.

PATIENT 19

Weight = 4.4 kg	Age = 9 weeks	Diagnosis Viral Meningitis
Commenced	07.00, 11.11.1985	Dose 100mg i.v. q.d.s.

Serum Samples

11.11.85	12.30	Dose 14.30	Sample	A = 23.1; B = 3.6;	C = 2.0
11.11.85	12.30	Dose 18.00	Sample	A = 11.8; B = 0;	C = 2.2
13.11.85	07.00	Dose 09.30	Sample	A = 18.0; B = 0;	C = 2.0
13.11.85	07.00	Dose 12.25	Sample	A = 20.3; B = 0;	C = 1.7

Urine Samples

11.11.85	12.30 - 14.00 hrs	11 ml	A = 768;	B = 2339
11.11.85	14.00 - 15.30 hrs	16 ml	A = 152;	B = 643
13.11.85	09.50 - 10.30 hrs	17 ml	A = 42;	B = 0
13.11.85	10.30 - 11.15 hrs	17 ml	A = 69;	B = 0
13.11.85	11.15 - 11.45 hrs	18 ml	A = 103;	B = 0

Concurrent Medication

Cefotaxime 200mg i.v. q.d.s.

PATIENT 20

Weight = 4.5 kg Age = 2.5 months Diagnosis Viral Meningitis

Commenced 24.00, 28.11.1985 Dose 100mg i.v. q.d.s.

Serum Samples

29.11.85	14.15 Dose 17.15	Sample	A = 4.3;	B = 0;	C = 1.4
03.12.85	12.00 Dose 14.00	Sample	A = 13.8;	B = 7.5;	C = 4.0
03.12.85	12.00 Dose 18.15	Sample	A = 3.6;	B = 7.5;	C = 3.5
13.12.85	12.00 Dose 14.00	Sample	A = 10.9;	B = 0;	
13.12.85	12.00 Dose 18.00	Sample	A = 0;	B = 0	

Urine Samples

29.11.85	14.15 - 15.30 hrs	4 ml	A = 85;	B = 526;	C = 425
29.11.85	15.30 - 16.45 hrs	40 ml	A = 50;	B = 102;	C = 82
29.11.85	16.45 - 17.30 hrs	50 ml	A = 11;	B = 43;	C = 63
13.12.85	12.00 - 13.00 hrs	11 ml	A = 115;	B = 1192;	C = 912
13.12.85	13.00 - 15.00 hrs	5 ml	A = 229;	B = 618;	C = 518
13.12.85	15.00 - 17.00 hrs	30 ml	A = 166;	B = 466;	C = 337
13.12.85	17.00 - 18.00 hrs	25 ml	A = 62;	B = 170;	C = 968

Concurrent Medication

Benzylpenicillin	200mg i.v. q.d.s.	
Gentamicin	12mg i.v. t.d.s.	
Phenobarbitone	15mg p.o. b.d.	(from 24.00, 29.11.5)
Phenytoin	12mg p.o. b.d.	(from 24.00, 29.11.85)

Additional Medical Problems

Spina Bifida

PATIENT 21

Weight = 4.4 kg Age = 9 weeks Diagnosis Viral Meningitis

Commenced 03.30 11.11.1985 Dose 100mg i.v. q.d.s.

Serum Samples

11.11.85	12.30 Dose	14.30 Sample	A = 24.6;	B = 3.6;	C = 3.2
11.11.85	12.30 Dose	18.30 Sample	A = 15.0;	B = 0;	C = 1.9
13.11.85	07.00 Dose	09.50 Sample	A = 24.6;	B = 0;	C = 4.7
13.11.85	07.00 Dose	12.15 Sample	A = 8.3.;	B = 0;	C = 3.0

Urine Samples

11.11.85	12.30 - 13.30 hrs	32 ml	A = 50;	B = 472;	C = 290
11.11.85	13.30 - 14.00 hrs	4 ml	A = 80;	B = 380;	C = 330
11.11.85	14.00 - 15.00 hrs	19 ml	A = 124;	B = 880;	C = 215
11.11.85	15.00 - 15.30 hrs	7 ml	A = 344;	B = 291;	C = 175
11.11.85	15.30 - 16.00 hrs	9 ml	A = 58;	B = 20;	C = 23
11.11.85	16.00 - 17.00 hrs	7 ml	A = 48;	B = 168;	C = 75
11.11.85	17.00 - 18.00 hrs	34 ml	A = 103;	B = 12;	C = 195
13.11.85	09.50 - 11.15 hrs	35 ml	A = 52;	B = 0;	C = 166
13.11.85	11.15 - 12.00 hrs	30 ml	A = 64;	B = 0;	C = 264

Concurrent Medication

Cefotaxime 200mg i.v. q.d.s

APPENDIX 2

PHARMACOKINETIC ABBREVIATIONS USED IN THE TEXT

Ab	=	Total amount of chloramphenicol succinate in the body
AbCAP	=	Total amount of chloramphenicol in the body
Ae_t	=	Cumulative amount of chloramphenicol succinate excreted unchanged in the urine
Ae_∞	=	Total amount of chloramphenicol succinate excreted unchanged in the urine
ARE	=	Amount remaining to be excreted (chloramphenicol succinate)
Cp_{PEAK}	=	Peak chloramphenicol serum concentration
Cp_{TROUGH}	=	Trough chloramphenicol serum concentration
F	=	Bioavailable fraction
Fe	=	Fraction of chloramphenicol succinate excreted unchanged
KCAP	=	Elimination rate constant for chloramphenicol
KCAPSS	=	Elimination rate constant for chloramphenicol succinate
KeCAPSS	=	Renal elimination rate constant for chloramphenicol succinate
KmCAPSS	=	Km = Metabolic elimination rate constant for chloramphenicol succinate
S	=	Salt factor (0.723)
t	=	Time elapsed after dose
T_{PT}	=	Time between peak and trough samples
$T_{1/2}$	=	Half life of chloramphenicol

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