THE PHARMACOKINETICS OF SULFADIMIDINE IN THE RAT.

A Thesis Presented to the Faculty of
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the Degree of Master of Science.

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Abstract.

The metabolism of sulfadimidine was studied in the rat. The main excretory products were found to be the free drug and the N-4 acetyl derivative. The N-4 glucuronide and the N-4 sulfamate were assumed to be minor metabolic products.

The rates of excretion of the total drug were found to follow a similar pattern after oral, I.V. and I.P. administrations. The rate plots after all three routes showed an initial rise followed by a secondary linear phase. After I.V. injection an initial phase similar to an absorption phase is most unusual and several experiments were conducted to determine the cause. It was found that there was:

1) No apparent saturation of binding sites or excretion processes.
2) No crystallisation of drug after injection.
3) No effect due to anesthetic.
4) No explanation indicated by blood levels of the drug.

The blood level data was fitted to a two compartment open model using the "NONLIN" program and a 360/65 IBM digital computer.

Acknowledgement

The author wishes to express his gratitude to Dr. W.J. O'Reilly for his supervision and to Dr. Kerry Ann McMahon for her assistance during the course of this work, at The School of Pharmacy.

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Chapter 1.

Symbols used in text.

All k values are apparent first order rate constants with units of reciprocal hours.

Two compartment model.

k_{12} - from central compartment to peripheral compartment.

k_{21} - from peripheral compartment to central compartment.

k_{el} - elimination from central compartment into urine

k_m - elimination of metabolite from central compartment into urine.

k_u - elimination of free drug from central compartment into urine.

D_T - amount of drug in peripheral compartment.

D_B - amount of drug in central compartment.

D_U - amount of drug in urine.

D_o - amount of drug administered.

Two compartment model with absorption.

Symbols are the same as above with;

D_G - amount of drug in the gastro-intestinal tract.

k_a - absorption of the administered drug into the central compartment.

k_e - elimination from central compartment into urine.

$$\text{factor, } f = \frac{D_U^{\infty}}{M_U^{\infty} + D_U^{\infty}} = \frac{k_u}{k_e}$$

D_U - amount of free drug excreted at infinite time.

M_U - amount of metabolite excreted at infinite time.

One compartment model.

Symbols are the same as above with;

M_B - amount of metabolite in the blood.

M_U - amount of metabolite in the urine.

k_1 - rate constant for metabolism of the drug.

k_2 - rate constant for excretion of the metabolite.

k_3 - rate constant for excretion of the free drug.

$$\text{factor, } f = \frac{k_3}{k_1+k_3} = \frac{k_3}{K} = \frac{D_U^{\infty}}{M_U^{\infty} + D_U^{\infty}}$$

and $K = k_1 + k_3$

k - rate constant for excretion of the metabolite after injection of the metabolite.

Chapter 2.

Introduction.

2-A) Metabolism and excretion of sulfonamides.

When sulfonamides are administered to animals, a number of metabolites may be detected in the urine and tissues (Bridges and Williams, 1963, 1962, 1966). The metabolites include N-1 and N-4 glucuronides, N-4 acetyl and N-4 sulfate derivatives.(Fig. 2.1). The free drug is also found in the tissues (including blood) and urine.

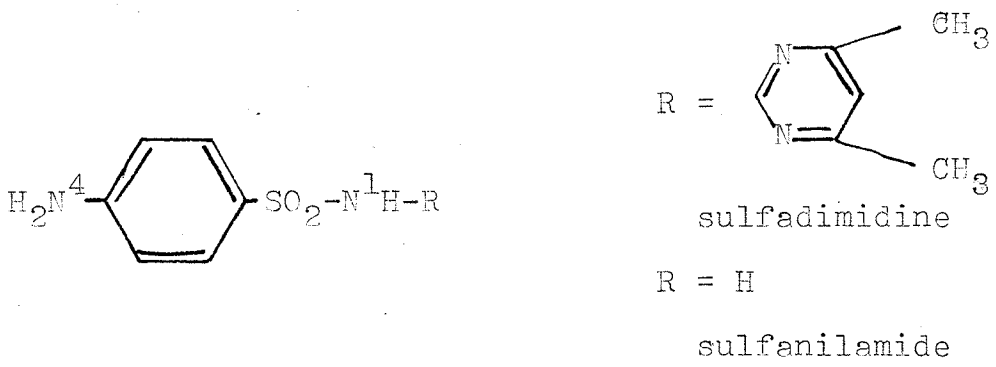


Fig. 2.1. Generalised Sulfonamide.

The amounts of free drug and each metabolite excreted depend on many factors including species, dose and the drug administered. Bray et al (1951) stated, " There are several pathways available for metabolism and while it is to be expected that all possible reactions will occur simultaneously, the proportions of the dose which actually follow the various routes differ greatly." Such factors as the efficiency of the necessary enzyme systems, the availability of the conjugating molecules

(e.g. glucuronic acid) and the availability of free energy for synthetic reactions, can contribute to these differences. Taken together, the above determine the velocity of each individual reaction in the intact animal, which in turn determines the amount of metabolite.

1) Metabolism of sulfonamides.

a) Site of metabolism.

The site of metabolism of sulfonamides may vary from species to species.

Table 2.1 Acetylation of sulfanilamide by various tissues in the rat (Hartiala and Terho, 1965)

Tissue.	Percentage of sulfanilamide acetylated.
Control	0.0
Liver	22.8
Stomach	10.0
Duodenum	26.0
Ileum	14.9
Kidney	31.6
Skeletal Muscle	0.0

Hartiala and Terho (1965) investigated the ability of isolated organs to detoxicate sulfanilamide. Small pieces of isolated organs were incubated in a modified Krebs-Ringer solution with the sulfanilamide. Table 2.1 indicates the percentage of the added

sulfanilamide converted to the acetyl derivative by the tissues of the rat.

Although conducted *in vitro* , the results indicate that the various tissues in the rat have the ability to acetylate sulfanilamide.

Sykes (1944) studied the ability of various tissues of different species to carry out the metabolism of sulfadimidine. On addition of the compound to liver slices from rats and guinea pigs, the acetylation never exceeded 10 % , whereas 60 % of the added sulfadimidine was acetylated in sliced or minced pigeon liver.

Glucuronides of sulfonamides are often assumed to be formed non - enzymatically to a small extent in the kidney and urine (Uno *et al* , 1967 ; Bridges and Williams , 1963.) When glucuronides are the major metabolites , a synthesising system is involved . This system is present in the kidney , the skin , the liver , and the gastro - intestinal tract of the dog , the rabbit , and the rat. (Boyland and Booth , 1962.)

Benzi *et al*,(1968) studied the metabolic ability

of isolated perfused kidneys in situ of dogs and monkeys. The kidneys of both animals were capable of demethylating and acetylating aminopyrine and also of conjugating oxazepam with glucuronide. The compounds and their metabolites were present in the excreted urine and in the renal tissues.

Govier (1965) found that the main site of acetylation of sulfanilamide and para amino benzoic acid (PABA) in the rabbit, was the liver. The acetylation occurred in the reticulo-endothelial cells. The lung and spleen also contain a high percentage of reticulo-endothelial cells and are capable of acetylating PABA and sulfanilamide.

(b) Amounts of Metabolites.

The percentages of the various metabolites and free drug also vary with different species and drug. Sykes (1944) noted that on administration of sulfadimidine to man, about 90% of the dose was excreted in the conjugated form. On the other hand, only 50% of the dose was excreted in the conjugated form when the drug was administered to pigeons.

Gilligan and Beck (1945) noted the variability in the extent to which sulfonamide drugs are metabolised in man. Sulfadiazine and sulfathiazole are

acetylated to a lesser extent than sulfadimidine. Glucuronidated sulfonamides were formed in some instances *in vivo*, varying from 5% with sulfathiazole to 68% with sulfadimidine.

Salvi and Plancher (1964) compared the metabolic fate of such long acting drugs as sulfaphenazole and sulfadimethoxine with sulfadimidine. Both showed high percentages of glucuronide compared with sulfadimidine. After administration, at least four excretory products were recognised i.e. the free drug, and the acetyl, sulfate, and glucuronide derivatives.

Bohni *et al* (1969) studied the metabolism of sulformethoxine in both animals and man. In man, four days after the administration of 1.0 Gm. of sulformethoxine, the total sulfonamide in the urine consisted of approximately two-thirds inactive acetylated substance and approximately one-third non-acetylated substance. 37% of the latter (or 13.5% of the total sulfonamide) consisted of inactive glucuronide.

Various workers have listed metabolites of many sulfonamide drugs. Uno *et al* (1967) administered sulfadimethoxine to rabbit and man and obtained the following results (Table 2.2.)

Table 2.2. Percentages of metabolites found in urine after administration of sulfadimethoxine to man and rabbit. (Uno et al 1967).

Metabolite	Man %	Rabbit %
Free drug	4.9	22.9
N-4 acetyl	17.1	55.1
N-1 glucuronide	77.1	8.9
N-4 glucuronide	0.9	12.8

The existence of the N-4 glucuronide in humans was doubted because the figure quoted lies within the range of error, and the compound is very unstable.

Bridges and Williams (1963) studied the metabolism of sulfasomizole (Table 2.3).

Table 2.3. Percentages of metabolites found in urine after administration of sulfasomizole to various animals. (Bridges and Williams, 1963).

Metabolite	Rabbit %	Rat%	Dog %	Man %
Free drug	26.9	68.1	90.2	61.8
N-4 acetyl	68.3	29.0	0.0	36.8
N-4 glucuronide	1.1	1.5	1.4	0.7
N-4 sulfate	0.0	1.4	3.9	0.1
Oxidation product	2.2	0.0	4.2	0.0

The results suggested that N-glucuronides may be formed *in vivo* non-enzymatically and if they occurred in urine, they could be artifacts.

Bridges and Williams (1966) after administration of sulfadimethoxine to rats, found the major metabolite to be the N-4 acetyl derivative with only small amounts of N-1 glucuronide.

The free drug, N-4 acetyl and the N-1 glucuronide were found in human urine after administration of sulfamethomidine (Ueda et al, 1968). The N-4 acetyl and free sulfamethomidine were found in the urine of rabbits and rats (DiCarlo et al, 1962)

2) Acetylation and deacetylation.

Deacetylation also occurs to varying extents in different species. With man after administration of the acetyl derivative only 1% appeared in the urine as free sulfadimidine whereas with pigeon 40% of the free drug was recovered after acetyl derivative was administered (Sykes, 1944)

Krebs et al(1947) found;

a) Rat liver extracts were able to hydrolyse N-4 acetyl and other acyl derivatives.

b) acetanilide was hydrolysed by rat liver and kidney extracts.

c) acetanilide and phenacetin were deacetylated by the intact rat.

d) in man and rabbit, conjugation appeared to proceed as long as free sulfadimidine was present and

the acetyl derivative was not significantly hydrolysed. It was concluded that the relative amounts of free and acetylated sulfonamides were dependent on the relative activities of the acetylating and deacetylating systems. The results of Smith and Williams (1949) confirmed Krebs' work.

3) Sulfonamide excretion and correlation of in vivo and in vitro properties.

Sulfonamides are excreted by the kidney by a combination of three processes, glomerular filtration, secretion and tubular reabsorption. A wide variety of organic acids are secreted by the para-amino hippurate mechanism which can be blocked by iodopyracet. Arita et al (1969) used this infomation to investigate the excretion of sulfanilamide (pK_a = 10.5) and sulfisoxazole (pK_a = 5.1) from rabbits. Sulfisoxazole was actively secreted whereas sulfanilamide was not. More sulfanilamide than sulfisoxazole was filtered through the glomerulus. Reabsorption occurred with both compounds but slightly more sulfisoxazole was reabsorbed when the active secretion mechanism was blocked. When the urine was made alkaline, sulfanilamide was un-ionised and the reabsorption was the same. Sulfisoxazole, at an alkaline pH, is ionised and the tubular reabsorption

decreases below the normal level. This may suggest a non-ionic diffusion process for the reabsorption of sulfanilamide but does not elucidate the reabsorption process for sulfisoxazole.

Despopoulos and Callahan (1962) assumed the secretion to require a physicochemical interaction between the substrate and a postulated intracellular receptor molecule. The substrate specificity in this transport system depended on the presence of the intramolecular sequence, SO_2N^-, in the sulfonamide. The reactivity of this group required ionisation at the nitrogen, localization of the net charge at the nitrogen, and electro-negativity at each oxygen sufficient for the formation of hydrogen bonds.

Poulsen (1959) studied the renal clearance of sulfadimidine and sulfathiazole in cows. The former was cleared by a combination of filtration, secretion and reabsorption, whereas the latter was cleared only by filtration and secretion.

Rieder (1963) worked with five sulfonamides in man and rabbits. The renal excretion rate was related to the diffusibility of the sulfonamides from aqueous to lipid solvent. There was no correlation between the extent of protein binding and elimination rate. These results were confirmed in rats (Yamazaki

et al, 1968).

Yamazaki et al (1968) administered a series of sulfonamides to rats. In alkaline urine those sulfonamides with a low pK_a were more ionised and exhibited fast excretion rates. These findings were in agreement with those of Arita et al (1969) in that the reabsorption is a non-ionic process.

Reiss (1963) stated that colorimetric determinations of blood levels did not differentiate between free sulfonamide and that bound to proteins or between ionised and un-ionised forms. There could also be binding of the ionised forms to proteins. Since it appears that only free anions of sulfonamides are active against bacteria (Bell and Roblin, 1942), Reiss attempted to correlate physical properties such as pK_a, formation of complexes with bovine serum albumin and distribution coefficient between buffer and chloroform, with known antibacterial properties. A three dimensional diagram was plotted and the coordinates (x,y and z respectively) were solubility in chloroform, solubility in pH 7.4 buffer, and concentrations of the sulfonamides which yield 10^{-4} moles/litre concentrations of free anions in 3% bovine serum albumin solution. With this diagram the pharmacological properties of the sulfonamides could be predicted

with some accuracy, e.g. (a) elimination is slower the nearer the sulfonamide approaches the xz plane, (b) it is increased as the sulfonamide approaches the yz plane, (c) the pharmacological activity increases as the position of the sulfonamide approaches the xy plane.

Austin (1967) concluded that the distribution phase was primarily a passive factor which depended on the pK_a value of a given sulfonamide, and the pH value of the fluids involved. The un-ionised lipid soluble fraction is the fraction transported across cell membranes or from one fluid to another.

4) Sulfonamides and protein binding.

Many protein binding experiments with sulfonamides have been complicated by the fact that workers considered binding capacity in one species and attempted to extrapolate these results to other species. Anton and Boyle (1964) considered the alteration of acetylation of sulfonamides due to protein binding by using a 3% bovine serum albumin to evaluate the percentage acetylation of pigeon liver extracts. Anton (1960, 1961) found that the bound fraction of sulfonamide in rat's blood was devoid of antibacterial activity. The bound fraction could be displaced and the net effect of the displacement was a drop in plasma

concentration of total drug and an increase in the unbound fraction. This resulted in an increased concentration of sulfonamide (unbound) in tissue, mainly muscle.

5) Sulfadimidine - metabolism and excretion for this work.

Sulfadimidine could undergo several metabolic steps but acetylation was assumed to be the main one. Reports of other sulfonamides in different species indicate that sulfadimidine is protein bound in the rat (Witzgall and Boyens, 1964; Toth et al, 1961; Scholtan, 1964; and Toth et al, 1964). Yamazaki et al, (1968) in a series of sulfonamides found there was no correlation between the excretion rate and protein binding in rats. In this work, therefore, the protein binding was not taken into consideration. Sulfadimidine has a pK_a value of 7.37 and is assumed to be excreted by glomerular filtration and active secretion, with a minimum of tubular reabsorption (Arita et al, 1969)

2-B) Pharmacokinetics and Modelling.

1) Theory of Modelling.

The kinetics of biological systems involving enzymatic reactions, diffusion and flow across membranes are complex. To simplify biological systems, models are derived which may or may not be appropriate but simplify the problem, (Rescigno and Segre, 1966). Pharmacokinetics is the study of the kinetic processes involved in the absorption, distribution, and elimination of drugs.

Nooney (1966) stated that the model was derived indirectly from the biological system by way of an intermediate abstraction from the real situation. Basically the abstraction involves the recognition of the governing features of the system and the formulation of their essential interrelationships. The model is, therefore, limited by the abstraction and can only deal with the terms derived. There are three main reasons for converting to a formal mathematical model;-

1) Mathematics offers a concise notation for quantitative matters. This is suited to precise expression.

2) Inclusiveness and generality - the brevity of mathematics permits simultaneous consideration of a broad array of variables and their relations.

3) There is a large body of existing mathematical formalism i.e. there is a vast collection of theorems and methods comprising mathematics which can be applied.

Pharmacokinetic models are virtually all composed of five basic types of unit processes (Wagner, 1968).

a) the conversion of a chemical species to another chemical species by an enzymatic or hydrolytic reaction catalysed by a chemical constituent of the body.

b) transfer of a species across a barrier or membrane where the volume of a compartment on one side of the barrier may be ignored.

c) transfer of a species across a barrier or membrane where the volumes of the compartments on both sides of the membrane must be considered.

d) transfer of a species from inside the body to the urine, sweat or expired air, all of which are considered to be outside the body.

e) a system composed of free, or non-protein bound species and the corresponding protein bound species.

Riegelman et al (1968) went on to describe the body in more detail with respect to pharmacokinetic modelling. Compounds or species are distributed through the body by the blood and vascular system.

(Kety et al, 1951). A model of this system represents drug distribution and elimination and consists of a central compartment with interchanging connections with one or more peripheral compartments, that is, a mamillary model (Shepherd, 1962).

Blood is the major constituent of the central compartment and the rate of uptake by the tissues is controlled by various forces i.e.

1) rate of blood flow through the tissues,

2) mass of the tissues,

3) partition characteristics between the plasma and tissues. The interrelationships of these forces is described by Fick's Law of Diffusion, (Kety, 1951). Blood flows can vary from 500ml. to 2ml. per 100ml. of tissue and the partition characteristics are dependent on pH, lipid solubity and complexation with proteins and nucleic acids. These factors all render very difficult the assignment of physiological meaning to a compartment. Price (1960), working with anesthetics, defined four groups of tissues according to the degree of perfusion of the blood and partition coefficients between lipid and water;

1) a highly perfused, lean tissue group e.g. heart, lung, liver, kidney etc.

2) poorly perfused, lean tissue group e.g. muscle

and skin tissue.

3) fat group e.g. adipose tissue, including bone marrow.

4) negligible perfused tissue group, e.g. bone, teeth, ligament, - can be ignored except for compounds which concentrate therein, e.g. calcium in bone. Group one is considered to be part of the central compartment.

The experimental data is fitted to the simplest model that is relevant to the study. Then, the model must be increased in complexity until the correct "fit" is obtained. (When the difference between the theoretical and experimental curves is a minimum, the "fit" is considered correct.) Nelson (1961a), however, stated that with increasing complexity in the chosen model, increasingly more complex mathematical expressions result from integration of the differential equations describing the rate of transfer or modifications of the drug. The constants in the mathematical expressions must be evaluated from the experimental data. Since the mathematical expressions have no power of discrimination in themselves, the fact that the expression, with constants evaluated, does fit the experimental results may mislead one into believing the model chosen is correct. Nooney (1966)

insisted that the interpreted results must agree with already established biological knowledge before the model can be assumed valid. Wagner (1968) stated that the closeness of fit (of a model) as measured by residuals, is a strong point; but cautioned that one must look for trends in areas of poor fit to test the adequacy of the model.

There are three possibilities after the model is re-examined, in relation to the fit obtained (Berman et al, 1962).

1) The data is adequate to define the model and final fit yields a random scatter of the data about the theoretical calculated values. Calculated correction terms tend towards zero as the solution converges to a least squares fit and the standard deviations of the variable parameters are relatively small compared to their values,ie. the model is considered compatible with the data.

2) Data is inadequate to permit the definition of the model.

3) The data is adequate to define the model but does not have the random scatter about the calculated values, i.e. the presence of systematic deviations suggests that the model is inadequate to fit the data and it requires additional degrees of freedom (i.e.

the release of constraints or the introduction of more parameters.)

2) Models Used.

a) One compartment model.

A one compartment model of the type, Fig. 2.2.

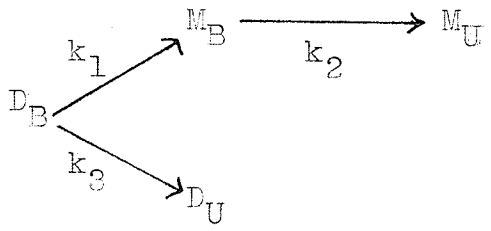


Fig.2.2. One compartment model.

was commonly used to describe the time course of a drug in the body. That is, plotting blood levels at various times on semi-logarithmic graph paper led to a straight line which could be described by a mono-exponential term. Nelson and O'Reilly (1961) studied the kinetics of sulfisoxazole acetylation and excretion in man. The single compartment model of the above type was written to describe the post-absorptive and equilibrative phases of drug excretion. A time of four hours after drug ingestion was arbitrarily chosen as zero time. This allowed interpretation of excretion data in terms of the equation developed. The equations excluded the absorptive and equilibrative phases of drug distribution. The kinetics of sulfaethylthiadiazole, sulfamethylthiadiazole, sulf-

isomidine and sulfamethoxine were also considered by the same approach (Nelson, 1964; Kushima, 1967).

b) Two compartment model.

Nelson (1964) recognised that after rapid I.V. injection, the kinetics of sulfaethylthiadiazole could be described by the two compartment model, Fig.2.3.

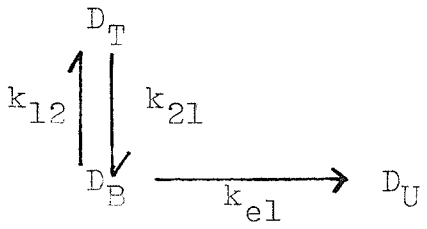


Fig.2.3. Two compartment model.

This model had the minor disadvantage of more complex mathematical expressions.

After rapid I.V. injection, provided blood samples are taken sufficiently quickly, at least a bi-exponential curve is obtained when plotting blood levels at various times on semi-logarithmic graph paper. The two compartment model will fit this type of experimental data and is also physiologically compatible with the distribution of the drug through the body under perfusion and diffusion forces already described. (Riegelman et al, 1968a; Teorell, 1937; Dominguez, 1935; Riggs, 1963) The time courses of thiopentone, acetylsalicylic acid and griseofulvin in man fitted the two

compartment model.(Riegelman et al, 1968a). Wagner et al (1968) used a two compartment model to describe the pharmacological effects of L.S.D. in man.

c) Complex models.

Models of greater complexity have been described, e.g.

1) Gibaldi and Feldman (1969) proposed a three compartment model to explain the influence of route of administration; oral versus I.V.. In the oral case a three compartment model accounted for the hepato-portal system.

2) Gibaldi et al (1969) proposed a three compartment model to describe the pharmacological effects of L.S.D.

3) Garrett et al (1963) proposed a five compartment model to explain the time course of radioactive calcium in the dog.

It seems then that the simplest model which will accurately describe the time course of a drug in the body requires two compartments. However, in simplifying the model one can focus on the process of interest i.e. metabolism and excretion.

3) Model Parameters.

According to Doluisio and Dittert (1968), There are two fundamental types of parameters describing

any model;

1) Volume terms which describe the volumes or capacities of any compartments into which the drug is distributed.

2) Rate terms which describe the rates at which the reactions depicted in the model, occur.

There are three types of volume terms used in pharmacokinetics, (Benet and Ronfield, 1969).

a) A volume term with some physiological meaning in that it describes the actual size of a body region e.g. adipose tissue in thiopental pharmacokinetics (Bischoff and Dedrick, 1968).

b) Dominguez (1934) described the volume of distribution or "the hypothetical volume of body fluid dissolving the substance at the same concentration as the plasma." This term has no real physiological meaning.

c) The hypothetical volume employed with reference to the volume of an unsampled compartment, e.g. the volume of the tissue compartment in the two compartment model.

Riegelman et al (1968b) stated that the volume of distribution is a parameter of a model, used to describe the distribution of a drug in the body. Its physiological meaning is limited by the model.

As such volume terms can be misleading and at the most are of limited use.

Rate constants have not yet been assigned any real physiological meaning in that they represent a series of reactions rather than one particular reaction. They give an indication of the length of time a drug will stay in the body.

4) The Parameters, k_1 and k_3.

k_1 and k_3 are related by the following expressions;

$$k_3 = fK$$

$$\text{and } k_1 = K - k_3$$

$$\text{where } f = \frac{Du^{\infty}}{Du^{\infty} + Mu^{\infty}}$$

Du^{∞} is the amount of free drug excreted in infinite time.

Mu^{∞} is the amount of metabolite excreted in infinite time. (infinite time can be defined as the time it takes for all of the drug to be excreted from the body. This is usually taken as about ten half lives of the drug. In the case of sulfadimidine, the half life in rats is about 4 to 5 hours and infinite time was taken as 48 hours.)

That is, the amounts of free drug and metabolite in the urine depend on the relative sizes of k_1 and k_3.

k_1 - the rate constant for metabolism.

The value of k_1 represents the conversion of free drug to the metabolite in the central compartment and its excretion into the urine. The chemical reaction for transformation is, Fig.2.4

$$RNH_2 \quad + \quad AcCoA \longrightarrow RNHAc \quad + \quad CoA$$

Sulfonamide acetyl CoA

Fig.2.4. Conversion of sulfonamide to acetyl-sulfonamide.

The rate constant, therefore, represents transport to and from the metabolic site, the actual metabolic transformations and then excretion by the kidneys.

k_3 - the rate constant for excretion of the free drug.

The value of k_3 represents a complex series of reactions leading to the excretion of the free drug by the kidney, into the urine.

5) Pharmacokinetic Studies of Sulfadimidine.

Turco et al (1966) studied the kinetics of intestinal absorption of sulfadimidine in man. A model of the type, Fig.2.5 was proposed to explain the time course of sulfadimidine.

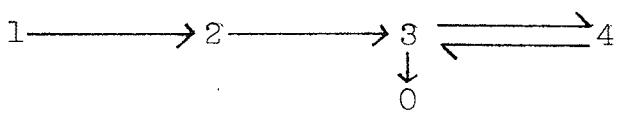


Fig.2.5 Model to explain the time course of sulfadimidine in man

where, 1 = gut.

2 = intermediary state between blood and gut.

3 = blood.

4 = tissue.

0 = urine.

Using a suitable digital computer program the data was found to fit the theoretical line generated by the computer, after both oral and I.V. administration. From the program the authors obtained relatively large standard deviations of the generated parameters. The large errors were explained as due to poor experimental data, rather than any definite areas of deviation which would indicate a poor fit of the model.

Koizumi et al (1964c) studied the kinetics of renal excretion of sulfonamides, including sulfadimidine, from man after oral administration of the drug. To obtain the various excretion rates, several assumptions were made;

1) glomerular filtration rate was constant during the experiment.

2) rate of water removal at the proximal tubule was constant and large enough compared with that of the sulfonamide.

3) volume of fluid in tubule where the drug was passively absorbed, was constant.

4) reabsorption of drug was a first order process.

5) fraction of protein bound drug was constant during the duration of the experiment.

Koizumi et al (1964a and b) studied the absorption of sulfonamides, including sulfadimidine, through the stomach and intestinal walls of rats. The walls were assumed to be of a lipid nature. The experimental data supported a relationship between the absorption rate and the partition coefficient between chloroform and buffer, pH = 7.4. Because of the lipoid nature of the barrier, absorption of the dissociated and undissociated forms was assumed to be different. The absorption rates increased to a maximum at the optimum pH, when the greatest amount is in the undissociated form. This pH differed by about one pH unit from the theoretical pH at which there will be the minimum amount of dissociation. It was concluded that stomach and intestine conditions i.e. acidity and surface conditions, played a part in the absorption rate. Kakemi et al (1969) found that the degree of binding to the mucosa was correlated to absorption characteristics, in vitro. The uptake of the barbituric acid used, was classified into two processes, absorption

of the drug onto the mucosal surfaces and the accumulation of the drug in the tissues; with the former as the more important step.

In the following work with sulfadimidine in the rat, both one and two compartment models are used.

2-C) Computing and Pharmacokinetics.

With the formulation of complex models, more sophisticated methods of processing the experimental data have been developed. The analog and digital computers are capable not only of processing the data but also of simulating curves from hypothetical models.

1) Analog Computer.

The analog computer deals with continuously varying voltages. The input of the analog computer is a voltage which varies with time, determined by setting the potentiometers in the circuit. The output is also in the form of a voltage which changes with time. It is visualised either as a repetitive sweep on an oscilloscope or as a graph on an X-Y plotter. It is primarily a device for solving differential equations using time as the independent variable (Wagner, 1967).

2) Comparison of Analog and Digital Computers.

Many authors (Garrett et al, 1963; Taylor and Wiegand, 1962; Yeisley and Pollard, 1964; Garfinkel, 1966) preferred the analog computer over the digital computer. Garfinkel (1966) stated "If the problem can be solved using the analog computer, use it; if not, use a digital computer."

Garrett et al (1963) and Taylor and Wiegand (1962) consider the analog computer better because of its

simplicity and more flexible programming, having the advantage that an understanding of the dynamic aspects of the problem variables is readily achieved.

Garfinkel (1966) prefers the analog computer because of the ease with which the user can control it and the rapidity of the solution process. However, three major disadvantages, as compared with the digital computer, were also pointed out;

1) The analog computer can handle a limited number of chemical reactions whereas the digital can handle up to 10,000 or more.

2) There is a limitation on the range of numerical problems that can be represented, and on their accuracy.

3) The capabilities of the analog computer are increasing less rapidly than the digital computer. Despite these disadvantages, the analog computer has been used on comparatively complex problems.

3) Digital Computers.

As already mentioned, digital computers deal with discrete observations expressed as integers or binary numbers in the memory of the computer. They are capable of addition, multiplication, exponentiation etc. and hence, are very useful for rapid numerical analysis of data. Most programmes are of the iterative

type and deal mainly with the statistical analysis of experimental data. Many, however, contain a proviso which can be used for simulation purposes.

4) Examples of Use of Digital Computers.

Berman et al (1962) developed a program that can simultaneously treat 25 separate functions that may arise in response to a system having up to 55 parameters of which a maximum of 25 may be simultaneously variable. With an input of experimental data, the mathematical expressions describing the model, the initial estimates of the rate constants of the experiment, the program automatically obtained a least squares fit of the data by an iterative procedure involving the model parameters. The program was divided into various subroutines which can determine solutions of the systematic equations, e.g.;

1) linear differential equations - solved by using a fourth order Runge-Kutta method.

2) sum of exponentials - solved analytically.

The iterative procedure was employed because the functions are in general, non-linear with respect to the variables and a first approximation is usually inadequate to obtain a least squares solution in one step. The values of the parameters were incremented or decremented to give functions which in turn gave a

minimum least squares solution. The newly calculated values for one iteration were used as initial estimates for the following one and convergence to the best fit was obtained. Because of this, it was found necessary to put constraints on the initial values of the parameters so that the computer would not decrease one parameter at the expense of the others.

Ackerman et al (1966) developed a program which included a technique described as "iterative guessing". Their program mainly used the equation;

$$E = \sum_{i=1}^{i=n} (yi - C_1 e^{-b_1 t_i} - C_2 e^{-b_2 t_i})^2$$

knowing the values of b_1 and b_2, the unique values of C_1 and C_2 were determined to give a minimum sum, E, of the squared deviations between the simulated curve $(C_1 e^{-b_1 t} - C_2 e^{b_2 t})$ and the data points, yi and t_i. The procedure was to guess values of b_1 and b_2 until one found a set of results that gave a minimum deviation. Holding one and varying the other often fails to converge to the best answer or takes too long to do so. The technique, "iterative guessing " was, therefore, developed, Fig.2.6.

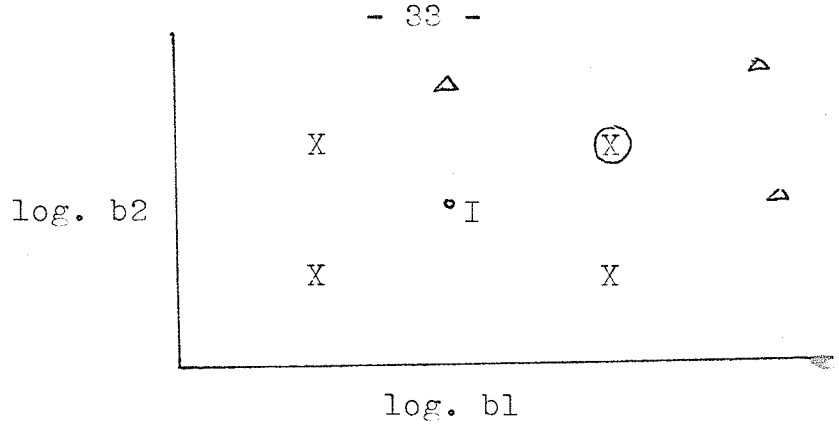


Fig. 2.6. "Iterative Guessing " Technique.

(Ackerman et al, (1966)

The X's represent values of b_1 and b_2 separated by about 30% from the original, I. The squared deviations of each are found, compared and the lowest one becomes the centre of the next square. The process is repeated, except points previously used are not recalculated, and continued until the centre is the best point. The step size is then reduced to 10% and so on until convergence is obtained. The parameters can be guessed from the technique or can be originally estimated using an analog computer. The program was used to generate curves for various models, e.g.

1) Blood glucose regulation - their comparatively simple model for the passage of glucose through the body, was found to generate curves which "Although there can be no doubt that the fit is not within the limits of equipment error, it is well within the limits of repeatability." No systematic trends for

errors were found in a series of thirty curves. Thus, the agreement of data and simulated curves supported the validity of the simplified model as emphasising the most important features of blood glucose regulation.

2) Calcium in bone - using a four compartment model including two in the bone, curves were generated that corresponded with the passage of radioactive calcium through the body.

Lowenthal and Vitsky (1967) wrote a program that calculated the minimum variance of the input values k_a and k_d/k_a, the apparent first order rate constants for absorption and elimination in the model

$$D_G \xrightarrow{k_a} D_B \xrightarrow{k_d} D_U$$

concentration in blood, $$C = \frac{a_o k_a}{V_d(k_a - k_d)} (e^{-k_d t} - e^{-k_a t})$$

$$C = \frac{\gamma}{\delta} k_a (e^{-k_d t} - e^{k_a t})$$

$$C = \frac{\gamma}{\delta} k_a e^{-k_a t} (e^{-\delta t} - 1)$$

$$\ln.C - \ln.(e^{-\delta t} - 1) = \ln.(\gamma/\delta k_a) - k_a t$$

where $\gamma = a_o/V_d$ and $\delta = k_a - k_d$

where a_o is the dose and V_d is the volume of distribution. As with Ackerman et al (1966), a grid search routine was used, Fig.2.7.

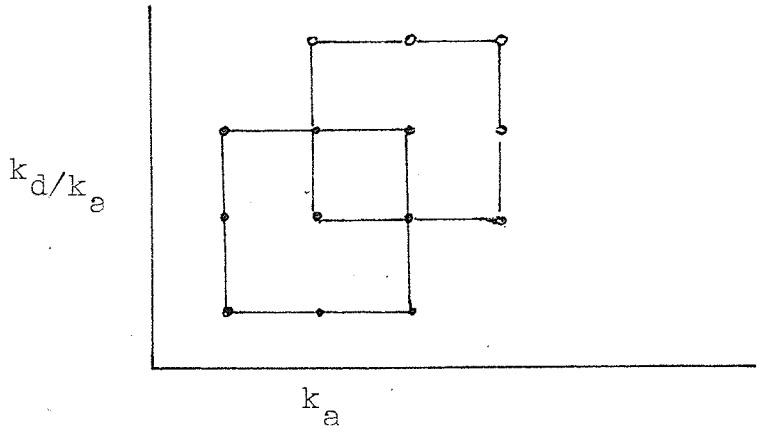


Fig. 2.7 Grid Search Routine. (Lowenthal and Vitsky, 1967.)

The initial estimates were incremented or decremented by 0.1, 0.05, 0.005, etc. and eight points were calculated about the initial.

Various blood concentration versus time data from the literature were fitted. Some data could not be fitted by the equation because of;

1) poor spacing of time intervals.

2) insufficient data points.

3) data not collected over a sufficiently long period of time.

With this program, separate tests must be made to determine the goodness of fit of the calculated curve. The χ^2 test was suggested.

$$\chi^2 = \frac{(C_{obs} - C_{calc})^2}{C_{calc}}$$

The χ^2 values are compared with tables. It was found

that the computer calculated variance gave just as good an indication of the goodness of fit as the test.

Garfinkel (1966) considered the glycolytic pathway for the metabolism of glucose as a comparatively simple system. With a digital computer and an input of rate constants, concentrations and a series of differential equations, curves were simulated which corresponded with actual experimental results from the glycolytic pathway in ascites cells. Using an isolated, beef heart glycolytic system, which overcame the problems of compartmentalisation, limiting rates of entry into cells, etc., the number of differential equations that directly referred to the enzyme system was increased. This again led to a good correlation between the simulated and experimental data.

Wagner (1967) used a digital computer to simulate curves for three antibiotics, lincomycin, novobiocin and tetracycline. In each case, good correlation was found between the simulated curves and the experimental data of the blood levels. The program employed an iterative procedure to generate a set of parameters which provided a least squares fit of estimated to observed serum levels. The analog computer was used to give initial estimates of the parameters.

5) "NONLIN" - the digital computer program used in this work.

The program used to process the data in this work, was "NONLIN" (Metzler, 1969) which consists of the main program and eleven subroutines. The basic idea behind the program is similar to those already described, i.e. the calculated data is compared with the experimental data and the weighted sum of squares found e.g.

weighted sum of squares for parameter values, $\underline{\Theta}$,

$$SS(\underline{\Theta}) = \sum_{i=1}^{NC} \sum_{j=1}^{Ni} (Yij\ (Xij) - fi(\underline{\Theta}, Xij))^2 Wij$$

where NC is the number of functions

Yij(Xij) is the j-th. observation on the i-th. function and the i-th. function having Ni observations.

fi($\underline{\Theta}$,Xij) is the calculated data from the parameter values, Θ, and times, Xij.

Wij is the weighting factor of the j-th. observation on the i-th. function.

Briefly, the subroutines are as follows;

1) INPUT - inputs all the data from punched cards.

2) SUMARY - causes the parameter values and weighted sum of squares to be printed after the iterations converge. It also causes other calculated data to be printed.

3) SUMSQ - computes weighted sum of squares.

4) MELS - solves a system of simultaneous equations by Hartley's modification of the Gauss-Newton method.

5) EIGEN - computes eigenvalues and eigenvectors of the correlation matrix of the estimates.

6) COMPUT - accepts values of parameters and time and then either calls DEFUNC to algebraically calculate values or controls the integration subroutine in NUMINT which again calculates values.

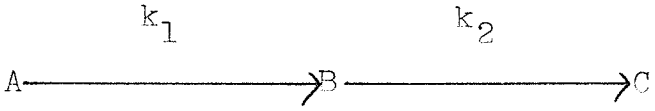
7) NUMINT - uses a fourth order, variable step size, Runge-Kutta method to integrate a set of differential equations which are defined in DEFUNC.

8) PLOTN - if desired will plot theoretical and observed values.

9) COR - computes correlation between observed and calculated values.

10) DLIMIT - adjusts the correction factor of the parameters to make sure they lie within the limits.

11) DFUNC - this subroutine must be written for each problem run on NONLIN i.e. for the model;



the differential equations are;

$$\frac{DA}{dt} = -Ak_1$$

$$\frac{DB}{dt} = Ak_1 - Bk_2$$

if these are input, the theoretical values are calculated through NUMINT.

Integrating the above;

$$A = Ao\ e^{-k_1 t}$$

$$B = \frac{Aok_1}{k_1 - k_2} (e^{-k_2 t} - e^{-k_1 t})$$

if these forms are placed in DFUNC, the theoretical values are calculated directly.

The equations input into DFUNC have been summarised in Chapter 4.

Chapter 3

Experimental methods and materials.

3-1) Materials and animals.

a) Sulfadimidine.

The sulfadimidine used was a commercial sample purified by recrystallisation from 95% ethanol. Fig.3.1.

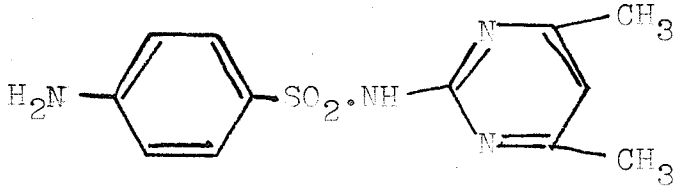


Fig.3.1. Sulfadimidine.

M.Pt. sample 197°-198°C. (uncorrected)

M.Pt. literature 198°-199°C. (Merck Index, 1968)

b) Synthesis of N-4 acetyl sulfadimidine. Fig.3.2.

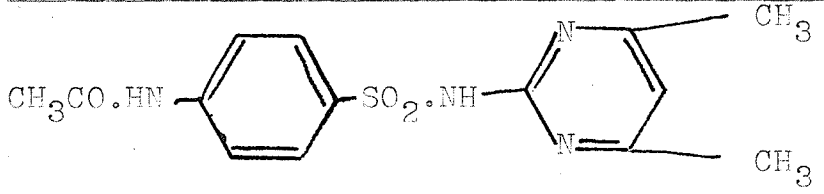


Fig.3.2. N-4 acetyl sulfadimidine.

The acetyl compound was prepared by dissolving sulfadimidine in weak sodium hydroxide solution and then adding the requisite amount of acetic anhydride, (Noller, 1965, P.192.) The acetyl compound thus obtained was recrystallised twice from 95% ethanol. Its purity was determined using the method of Bratton and Marshall (1939) and by comparison of melting points.

M.Pt. sample = 247°-248°C. (uncorrected)

M.Pt. literature = 249°-250°C. (Dictionary of Organic Compounds, 1965)

By Bratton and Marshall (1939) assay, the sample contained 0.16% free sulfonamide.

c) Animals.

Male Sprague-Dawley rats(The Colony, Madison,Wisc.) between 150 and 400 grams were used in all experiments. Before experiments, they were starved for 24 hours but allowed water ad libitum.

3- 2) Animal Techniques.

a) Metabolic Studies.

To determine the main metabolites, the rats were anesthetised with ether and dosed with one ml. of the drug solution (25mg./ml. - the drug was dissolved with with the aid of a minimum amount of sodium hydroxide), by stomach tube. The urine was collected for 24 hours and the volume noted.

b) Pharmacokinetic Studies.

To maintain urine flow, the rats were given 2 mls. of water by stomach tube, every hour for two hours before administration of the drug. Water administration was continued every half hour after the drug had been given. The drug was administered (oral and I.P.) while the rat was restrained but conscious. (Doses of 15mg./ml. and 15mg./½ml., respectively.)

To give the drug I.V., the rats were mildly anesthetised with ether, and the injections given into a femoral vein. (Dose of 15mg./½ml.)

c) Urine Collection.

After administration by the appropriate route, the rat was placed in an improvised metabolic cage and urine samples were taken at various times, the time being accurately noted. Any urine excreted between times was added to the next sample. The rats were made to urinate by causing them to inhale sulfuric ether. (The ether causes spontaneous contraction of the bladder wall, resulting, in most cases, in micturition)(Nelson et al, 1966.) Each sample was thoroughly washed into a beaker.

The total urine output was collected for at least 48 hours in order to estimate the total excretion of free drug and metabolite.

d) Blood Collection.

Blood samples were obtained when the rats were sedated with pentobarbitone sodium. A mild to heavy sedation was obtained for the duration of the experiment using 50mg./kg., then the rats were allowed to recover and transferred to metabolism cages for at least 48 hours. Two methods were used to obtain the blood levels, namely catheterisation of a jugular vein

(Van Petten et al, 1970) or retro-orbital puncture (Nagashima et al, 1968).

In the former method, the rats were anesthetised with pentobarbitone sodium, 50mg./kg., and then a catheter, 0.023 inches internal diameter and 0.045 inches outer diameter, was placed into the jugular vein to within about a quarter of an inch of the heart; it was secured using three cotton threads along the length of the vein. The other end of the catheter was led in front of the right foreleg underneath the skin and out at the back of the head. To secure the catheter it was led through a polythene saddle stitched beneath the skin.(Van Petten et al, 1970) Within 24 hours the injection was given through the catheter and blood samples taken at the required times. After each sample was taken, the catheter was washed out with heparinised saline. (It was hoped to undertake more than one experiment on each rat, but it was found that even though only half an inch of catheter protruded from the skin, the rat was still able to dislodge it from the vein.) The rats were then left and urine samples collected after 48 hours.

In the latter method, the rats were sedated with pentobarbitone sodium and an injection of the drug given into one femoral vein and one of 50 units of

heparin into the other. A heparinised glass capillary, of approximately the same dimensions as the above catheter, was inserted through the membranes at the corner of the rat's eye. The blood flowed freely through this, and was collected in a small plastic cup, previously greased with silicone grease. As soon as possible, the blood was taken up in a syringe to obtain a known volume. Presumably because of clotting, about five samples were obtained from each eye.

3-3) Preparation of Samples.

a) Urine.

All urine samples were filtered and individually made up to volume. To assay for the free drug, 2mls. of the diluted urine were made up to 5mls. with water and N.HCl and assayed. For the total drug in the urine, 10mls. of the diluted urine were heated under reflux with N.HCl, 2.5mls., cooled, made up to 25mls. and assayed.

b) Blood.

Each blood sample was immediately placed into 4mls. of potassium oxalate solution, 0.5mg./ml.. The blood proteins were then precipitated using 1.0ml. of a 15% solution of trichloracetic acid. This was then centrifuged, filtered and the supernatant assayed.

(No significant amounts of sulfadimidine were precipitated with the proteins (McMahon, 1970))

3- 4) Analytical Methods.

a) Assay Methods.

A calibration curve was prepared using 0.15% sulfadimidine in the range 6-44 mg./ml.. To 5ml. of solution, either standard solution, suitably diluted urine sample, or blood sample, was added 0.2ml. of 0.25% w./v. sodium nitrite. Three minutes later 0.2ml. of 1.25% w/v. ammonium sulfamate was added and in a further two minutes 0.2ml. of 0.25% w./v. N-1-naphthylethylene diamine dihydrochloride. (Fischer Scientific Co.)(Bratton and Marshall, 1939.) An Oxford Sampler (Oxford Laboratories. California.) was used to pipette 0.2ml. volumes of reagents. The amount of metabolite was determined by the difference in free compound before and after acid hydrolysis.

b)Metabolic Studies.

The urine was mixed with an equal volume of 95% ethanol, centrifuged, and the supernatant used for T.L.C.. Silica gel plates, 0.25mm. thick, were used to achieve a separation. (All types of cellulose plate were unable to stand the high temperatures required.) (Stahl, 1965) The solvent systems found to give the best separation were :-

1-butanol saturated with water : pyridine 8 : 1.

1-butanol : concentrated NH_4OH : pyridine : water

40 : 10 : 5 : 50 (pH = 9.0)

The volume of ammonium hydroxide was adjusted to give the above ratio which gave the best results.

The spots associated with the free drug were detected by spraying with Bratton-Marshall reagents, those of the glucuronide by spraying with phosphoric acid/naphthoresorcinol reagent (Bridges et al, 1965), and those of the metabolites by spraying with N.HCl, heating at 110°C. for 10 minutes, and then spraying with Bratton-Marshall reagents. To achieve complete separation of the acetyl and free sulfadimidine, the plates were run for about five hours which included about two hours after the solvent had reached a pre-marked solvent front. Both the acetyl and free drug were recognised by comparison with standards.

To determine the relative amounts of free drug and each metabolite present, the plates were run, sprayed along the edge, and then scraped at the corresponding areas. The separate scrapings were boiled with N.HCl for one hour to hydrolyse off the glucuronide, acetyl, etc.. The solution was cooled, filtered, made up to volume and assayed.

The urine was also assayed for total and free

drug.

3-5) Digital Computer Methods.

NONLIN, a computer program for parameter estimation in non-linear situations, kindly supplied by Dr. C.M. Metzler of the Upjohn Company, was used to obtain the best values of the model parameters. The computer used was the IBM 360/65 model at the University of Manitoba Computer Centre. For the required modifications of the program, see Chapter 4.4.

3-6) Statistical Methods.

All straight lines were fitted using the regression formula:-

$$\text{slope, } b = \frac{\sum xy - \frac{\sum x \sum y}{N}}{\sum x^2 - \frac{(\sum x)^2}{N}}$$

according to Saunders and Fleming (1957)

Sets of results with unequal numbers were compared using a student t-test (Saunders and Fleming, 1957).

Sets of results with equal numbers were compared using analysis of variance (Goldstein,1967).

Chapter 4.

Theory of Results.

The experimental data was fitted to one and two compartment models. The models are representations of the absorption, distribution, metabolism, and excretion of the drug in the body and as such they focus on the more important transfers and reactions. The one compartment model has the minor advantage of more simple mathematics.

4-1) One compartment model.

Examples of one compartment models are shown in Fig.4.1.

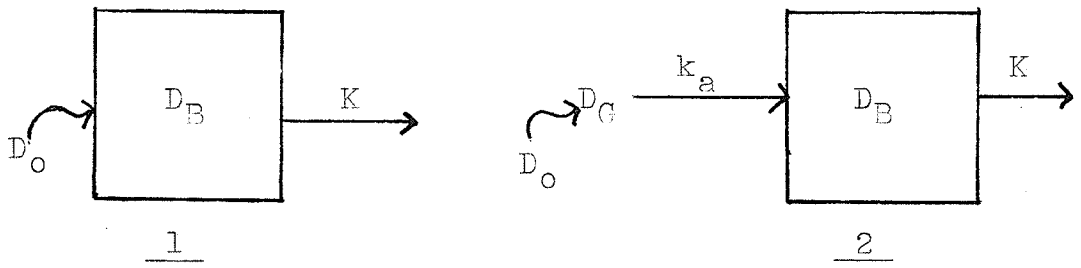


Fig.4.1. One compartment models.

The drug and its metabolites are assumed to be in equilibrium with these materials in other fluids of distribution. The k's are first order rate constants in reciprocal hours for the respective processes and are assumed to proceed at a rate directly proportional to the amounts of the materials in the indicated fluids. The volume of distribution of the drug and

its metabolites is assumed to remain constant through out the experiment.

The differential equations describing model 1 are;

$$\dot{D}_B = -K\ D_B \qquad (1)$$

$$\dot{D}_U = K\ D_B \qquad (2)$$

giving solutions;

$$D_B = D_o\ e^{-Kt} \qquad (3)$$

$$D_U = D_o(1 - e^{-Kt}) \qquad (4)$$

The differential equations describing model 2 are;

$$\dot{D}_G = -k_a\ D_G \qquad (5)$$

$$\dot{D}_B = k_a\ D_G - K\ D_B \qquad (6)$$

$$\dot{D}_U = K\ D_B \qquad (7)$$

giving solutions;

$$D_G = D_o\ e^{-k_a t} \qquad (8)$$

$$D_B = \frac{k_a\ D_o}{(k_a - K)}\ (e^{-Kt} - e^{-k_a t}) \qquad (9)$$

$$D_U = \frac{k_a\ D_o}{K(k_a - K)}\ (k_a(1 - e^{-Kt} - K(1 - e^{-k_a t})) \qquad (10)$$

In the above solutions;

fraction of free drug, $$f = \frac{D_U^{\infty}}{M_U^{\infty} + D_U^{\infty}} = \frac{k_3}{k_1 + k_3} = \frac{k_3}{K} \qquad (11)$$

$$k_3 = Kf \qquad (12)$$

and $$k_1 = K - k_3 \qquad (13)$$

a) Methods used to obtain constants.

1) Blood level data.

a) Without absorption from the gut.

A plot of logarithm of blood levels versus times would yield a straight line with a slope of -K/2.303 i.e. taking logarithms of equation (3)

$$\log. D_B = \text{Log. } D_o - \frac{K\ t}{2.303} \qquad (14)$$

Constants k_3 and k_1 can be obtained from equations (12) and (13)

b) With absorption from the gut.

A plot of logarithms of blood levels versus times would yield a biexponential curve of the type, Fig.4.2.

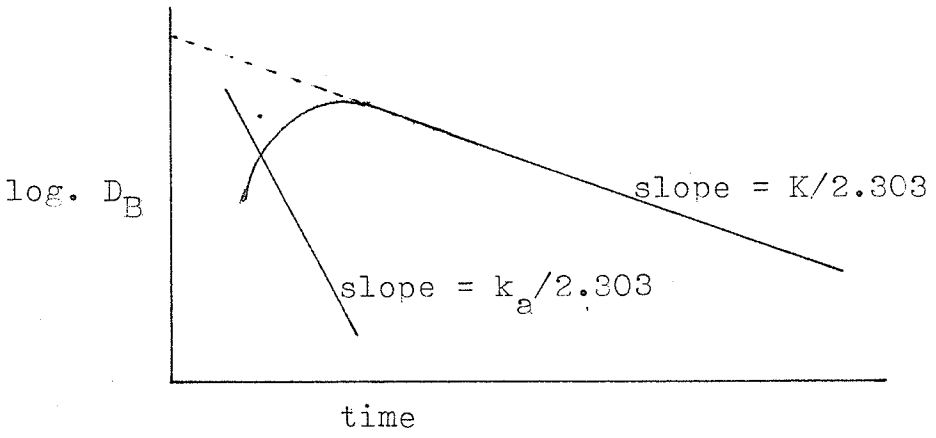


Fig.4.2. Plot of D_B versus time on semi-logarithmic graphy paper. Oral dosage.

In Fig. 4.2., the line for k_a is found by the method of residuals. Fig 4.2. is the normal case where the rate of absorption is much faster than the rate of excretion. If the situation were reversed, the later, linear slope would be equal to k_a/2.303.

2) Urine level data.

Parameters are obtained from the urinary excretion data by plotting the logarithm of the rate of excretion versus time. From equation (3), the curve obtained from a rate plot can be characterised by equation (15). (Swintosky, 1957)

$$\log.\frac{dD_U}{dt} = \log. K C_o - \frac{Kt}{2.303} \qquad (15)$$

where C_o is the apparent maximum amount of excretable substance. Therefore, the secondary, linear portion of the curve will have a slope equal to K/2.303.

With model 2, if the rate of absorption is very slow compared to the rate of excretion, the slope of the secondary linear portion will be equal to $k_a/2.303$.

4-2) Two Compartment Model.

An example of a two compartment model is shown in Fig.4.3.

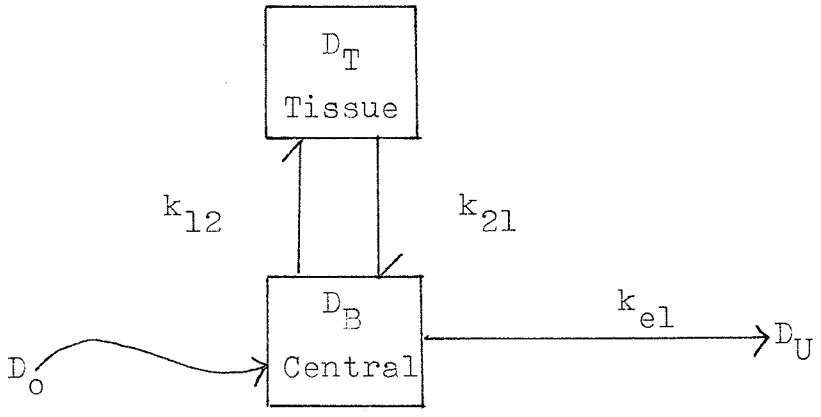


Fig. 4.3. A Two Compartment Model.

As already mentioned (Chapter 2, Section 2-B), a two compartment model is physiologically compatible with the distribution of a drug through the body under perfusion and diffusion forces. The volumes of the tissue and central compartments are assumed to be equal through the experiment. Metabolism and excretion occurs in the central compartment and the k's represent first order rate constants with units of reciprocal hours. The differential equations describing the model are;

$$\dot{D}_B = k_{21}D_T - GD_B \tag{16}$$

$$\dot{D}_T = k_{12}D_B - k_{21}D_T \tag{17}$$

$$\dot{D}_U = k_{el}D_B \tag{18}$$

where $G = k_{12} + k_{el}$

giving solutions;

$$D_B = \frac{D_o(k_{12} - r_1)e^{-r_1 t}}{r_2 - r_1} + \frac{D_o(k_{12} - r_2)e^{-r_2 t}}{r_1 - r_2} \tag{19}$$

$$D_U = \frac{k_{el}D_o(k_{12}-r_1)(1-e^{-r_1 t})}{r_1(r_2 - r_1)} + \frac{k_{el}D_o(k_{12}-r_2)(1-e^{-r_2 t})}{r_2(r_1 - r_2)} \tag{20}$$

$$D_T = \frac{D_o k_{12}(e^{-r_1 t} - e^{-r_2 t})}{r_2 - r_1} \tag{21}$$

where;

$$r_1 = \tfrac{1}{2}(-(k_{21}+G) + \sqrt{(G+k_{21})^2 - 4 \cdot k_{el} \cdot k_{21}} \tag{22}$$

and;

$$r_2 = \tfrac{1}{2}(-(k_{21}+G) - \sqrt{(G+k_{21})^2 - 4.k_{el}.k_{21}}) \quad (23)$$

In the above solutions;

fraction of free drug, $$f = \frac{D_U^{\infty}}{M_U^{\infty} + D_U^{\infty}} = \frac{k_u}{k_m + k_u} = \frac{k_u}{k_{el}} \quad (24)$$

$$k_u = k_{el}.f \quad (25)$$

$$k_m = k_{el} - k_u \quad (26)$$

It is noted that the constants describing a two compartment model are hybrid constants, that is, they are complex products of the actual rate constants.

b) Methods used to obtain rate constants.

1) Blood level data.

A plot of the logarithm of the blood level versus time gave a biphasic plot of the type, Fig.4.4.

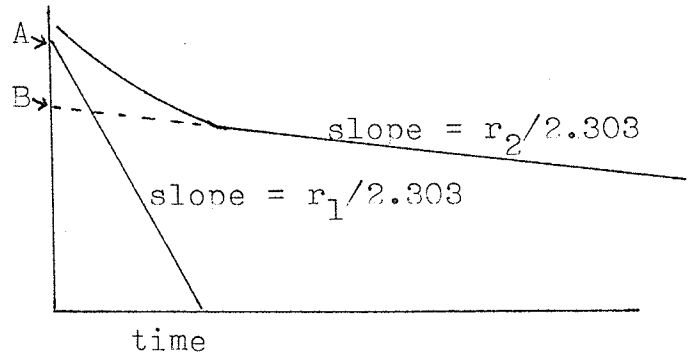


Fig. 4.4 Plot of D_B versus time on semi-logarithmic paper - rapid I.V. injection.

r_1 is obtained by the method of residuals. A and B are found as shown in Fig 4.4.

The values of the microconstants may be obtained

from the above parameters by the method of Riegelman et al, (1968a).

$$C_p^o = A + B \tag{27}$$

$$1 = \frac{A}{C_p^o} + \frac{B}{C_p^o} = A' + B' \tag{28}$$

$$k_{21} = A'r_2 + B'r_1 \tag{29}$$

$$k_{12} = \frac{A'B'(r_2 - r_1)^2}{k_{21}} \tag{30}$$

$$k_{el} = \frac{1}{A'/r_1 + B'/r_2} \tag{31}$$

4-3) Simulation of two compartment model with absorption.

For ease of administration, most drugs are given orally. Simulations of the two compartment model with absorption, Fig.4.5., were carried out to determine whether the constants obtained graphically were in agreement with those used as input.

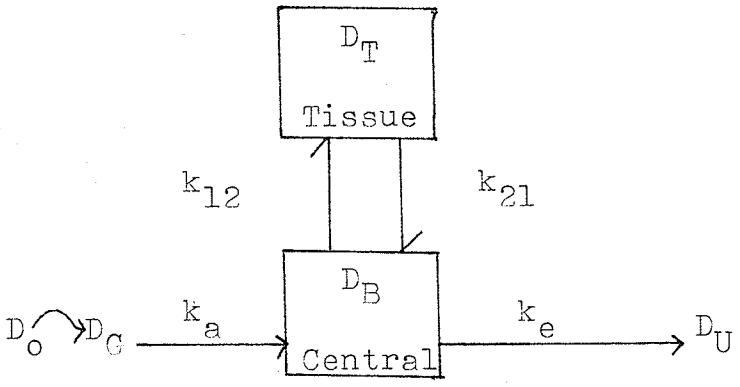


Fig. 4.5. Two compartment model with absorption.

The differential equations describing the model are,

$$\dot{D}_G = -k_a D_G \tag{32}$$

$$\dot{D}_B = k_{21} D_T + k_a D_G - K' D_B \tag{33}$$

$$\dot{D}_U = k_e D_B \tag{34}$$

$$\dot{D}_T = k_{12} D_B - k_{21} D_T \tag{35}$$

where $K' = k_{12} + k_e$

Taking Laplace Transforms of equations (32),(33) & (35)

$$s\tilde{D}_G - \tilde{D}_o = -k_a \tilde{D}_G \tag{36}$$

$$s\tilde{D}_B = k_a \tilde{D}_G + k_{21} \tilde{D}_T - K' \tilde{D}_B \tag{37}$$

$$s\tilde{D}_T = k_{12} \tilde{D}_B - k_{21} \tilde{D}_T \tag{38}$$

Rearranging equations (36),(37) and (38) and transferring to matrix form;

$$\begin{vmatrix} s + k_a & 0 & 0 \\ -k_a & s + K' & -k_{21} \\ 0 & -k_{12} & s + k_{21} \end{vmatrix} = \begin{vmatrix} D_o \\ 0 \\ 0 \end{vmatrix} \tag{39}$$

$$\Delta_1 = s + k_a((s + K')(s + k_{21}) - k_{21}k_{12})) \tag{40}$$

which is equivalent to;

$$\Delta_1 = (s + k_a)(s + r_1)(s + r_2) \tag{41}$$

where;

$$r_1 = \tfrac{1}{2}(-(k_{21}+K') + \sqrt{(K'+k_{21})^2 - 4 \cdot k_e \cdot k_{21}})$$

and;

$$r_2 = \tfrac{1}{2}(-(k_{21}+K') - \sqrt{(K'+k_{21})^2 - 4 \cdot k_e \cdot k_{21}})$$

transferring matrix (39) to determinant form and

solving for D_C

$$D_C = \frac{\begin{vmatrix} D_o & 0 & 0 \\ 0 & s + K' & -k_{21} \\ 0 & -k_{12} & s + k_{21} \end{vmatrix}}{\Delta_1} = \frac{D_o}{s + k_a}$$

therefore, taking antitransforms;

$$\underline{D_C = D_o e^{-k_a t}} \qquad (42)$$

transferring matrix (39) to determinant form and solving for D_B;

$$D_B = \frac{\begin{vmatrix} s + k_a & D_o & 0 \\ -k_a & 0 & -k_{21} \\ 0 & 0 & s + k_{21} \end{vmatrix}}{\Delta_1} = \frac{D_o(-k_a)(S + k_{21})}{(s+k_a)(s+r_1)(s+r_2)} \qquad (43)$$

By partial fractions and taking antitransforms of equation (43);

$$D_B = \frac{k_a D_o (k_{21} - k_a) e^{-k_a t}}{(r_1 - k_a)(r_2 - k_a)} + \frac{k_a D_o (k_{21} - r_1) e^{-r_1 t}}{(r_2 - r_1)(k_a - r_1)} + \frac{k_a D_o (k_{21} - r_2) e^{-r_2 t}}{(r_1 - r_2)(k_a - r_2)} \qquad (44)$$

From equation (34);

$$D_U = k_e \int D_B \cdot dt$$

Therefore;

$$D_U = \frac{k_a k_e D_o (k_{21} - k_a)(1 - e^{-k_a t})}{k_a (r_1 - k_a)(r_2 - k_a)}$$

$$+ \frac{k_a k_e D_o (k_{21} - r_1)(1 - e^{-r_1 t})}{r_1 (k_a - r_1)(r_2 - r_1)} + \frac{k_a k_e D_o (k_{21} - r_2)(1 - e^{-r_2 t})}{r_2 (k_a - r_2)(r_1 - r_2)} \qquad (45)$$

Values of D_U and D_B were then calculated at arbitrary time units of 1,2,3 to the required number and D_o equal to 100 units. The values of the rate constants were varied in an attempt to simulate most types of absorption, distribution, and excretion.

Rates of excretion and blood levels were plotted against time on semi-logarithmic graph paper. The slopes and any residual slopes were found and attempts made to recalculate the rate constants.

4-4) Digital Computation Methods.

The computer program, NONLIN, is capable of solving systems of differential equations. If the integrated solutions of the differential equations are used as input, the program provides the numerical solutions. The former method required more time but was slightly more accurate. The latter method was more convenient and was used in this study.

As input data, the program required initial estimates of the parameters governing the model, with high and low limits. It also required the blood levels and the times of sampling. The computer then

took the initial estimates of the parameters and calculated, using the equation input into the subroutine DFUNC, a theoretical drug level at that time. It then compared the theoretical with the experimental to find the weighted sum of squares, SS, i.e. $SS = \sum (\text{theoret.} - \text{expt.al})^2 W$, where W is the weighting factor. The program then changed the values of the parameters until a minimum weighted sum of squares was found.

The main points of interest in the output were;

1) The final estimates of the parameters with their standard deviations.

2) The weighted sum of squares of the final estimates of the parameters and the percentage deviation of the theoretical curve from the experimental points.

3) The theoretical curve.

The standard deviation is taken as the square root of the weighted sum of squares divided by the degrees of freedom. The weights are taken as the reciprocals of the diagonal elements of the inverse matrix generated by the subroutine MELS (Deming,1964)

Two separate programs were used to fit the blood level data. The first incorporated equation (46), with the initial estimates obtained as in Fig.4.4.

$$D_B = Ae^{-r_1 t} + Be^{-r_2 t} \qquad (46)$$

From the output of this program values of k_{12}, k_{21} and k_{el} were found from equations (27) to (31). These values were then used as input for a program that incorporated equation (19). This procedure was found to be better than the one in which rate constants were calculated directly from the graph, and only the latter program used.

Chapter 5.

Results.

5-A) Metabolic studies.

1) Thin layer chromatography of urine samples.

After oral dosage of sulfadimidine, the metabolites in the urine were separated using T.L.C., Fig.5.1.

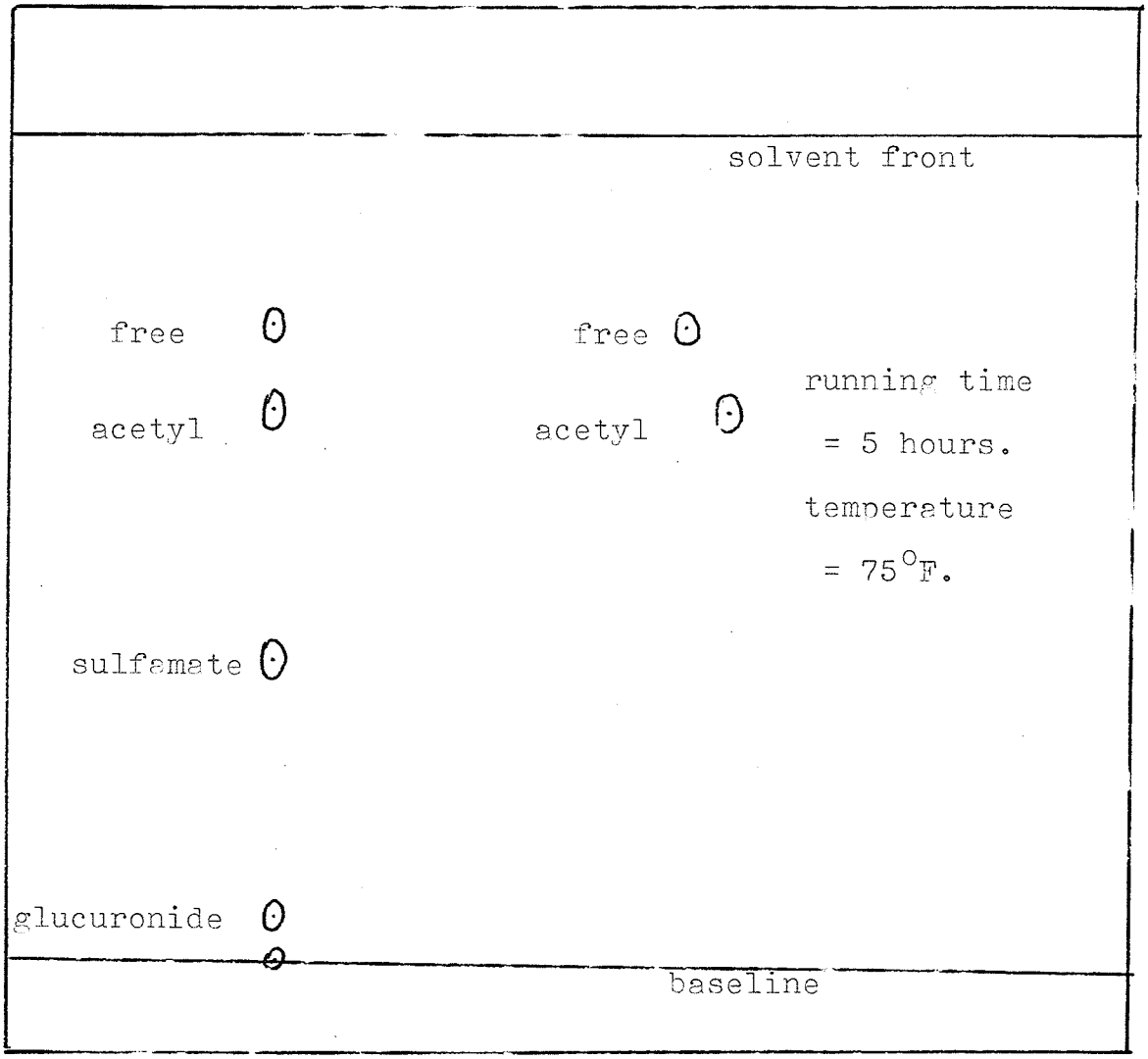


Fig.5.1. Reproduction of T.L.C. of urine sample run on silica gel plate in butanol-water/pyridine system 1.

All urine samples gave identical results with the above system and with the silica gel in butanol-water/pyridine/NH_3,system,2. Initial spraying of the plates with Bratton-Marshall reagent detected one spot corresponding to the free drug. After hydrolysis on the plate with normal acid, (Chapter 3, Section 3-4) four spots were detected with Bratton-Marshall reagent. When acid treated plates were sprayed with naphthoresorcinol/phosphoric acid a light blue spot was detected, corresponding to a positive area on the plate. This was assumed to be sulfadimidine N-4 glucuronide. The relative R_f values obtained from T.L.C. are shown in Table 5.1.

Metabolite	R_f
free drug	0.80
N-4 acetyl	0.72
N-4 sulfamate	0.39
N-4 glucuronide	0.043

Table 5.1. Relative R_f values of free drug and metabolites.

For a complete separation of the N-4 acetyl and free sulfadimidine spots, the thin layer systems had to be run for at least five hours. These two products were verified by standard free drug and N-4 acetyl derivative on the same plate. Because of the long

running time, the solvent front ran well over a preset mark and, therefore, only relative R_f values were obtained. The relative positions and R_f values were compatible with the results obtained by Bridges and Williams, (1963), with sulfasomizole in the rat. The N-4 glucuronide and N-4 sulfamate of sulfasomizole were confirmed by comparison with standards. The relative positions and R_f values corresponded with two spots obtained with sulfadimidine in the rat. As the slower running spot was assumed to be the N-4 glucuronide (naphthoresorcinol/phosphoric acid spray.) the other was assumed to be the N-4 sulfamate.

2) Percentage of each metabolite from T.L.C.

The percentage of each metabolite was calculated directly from spectrophotometric determinations, Table 5.2.(Chapter 3, Section 3-4)

Metabolite.	System 1.%.	System 2.%.
free drug	20.81	21.65
N-4 acetyl	61.49	53.57
N-4 sulfamate	13.44	17.85
N-4 glucuronide	4.36	6.93

Table 5.2. Percentages of each metabolite from T.L.C. oral dosages.

From the above figures, Table 5.2, it was assumed that the free and acetyl compounds constitute the

major excretory products of the drug, sulfadimidine. Henceforth, the excretory products of the drug will be referred to as the free and metabolised compounds. The metabolite was taken as the difference between readings before and after hydrolysis of the urine samples.

3) Analysis of urine samples.

The total amounts of drug and metabolites recovered after oral ingestion of 25mg. and 100mg. of sulfadimidine are shown in Tables 5.3 and 5.4.

Rat Number	Weight, Gm.	Free Drug, mg.	Total Drug, mg.	Free / Total %	mg./kg.
1	-	4.38	10.94	40.05	-
2	-	5.88	14.69	42.64	-
3	210	5.60	13.01	43.05	119.04
4	221	5.13	15.38	33.33	113.12
5	264	1.91	3.28	58.15	94.69
6	194	3.88	7.64	50.77	128.86
			Mean*±	44.66 ± 3.54	

Table 5.3. Amounts of drug and metabolite after administration of 25mg. orally.

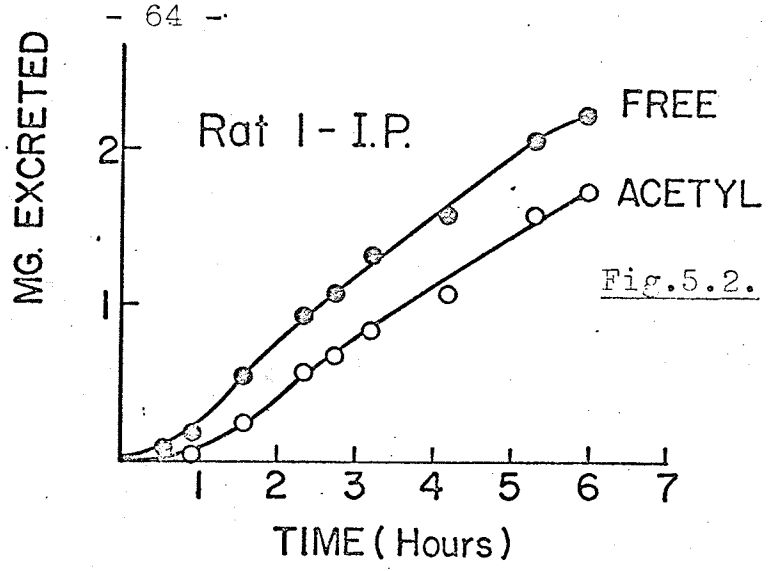


Fig.5.2.

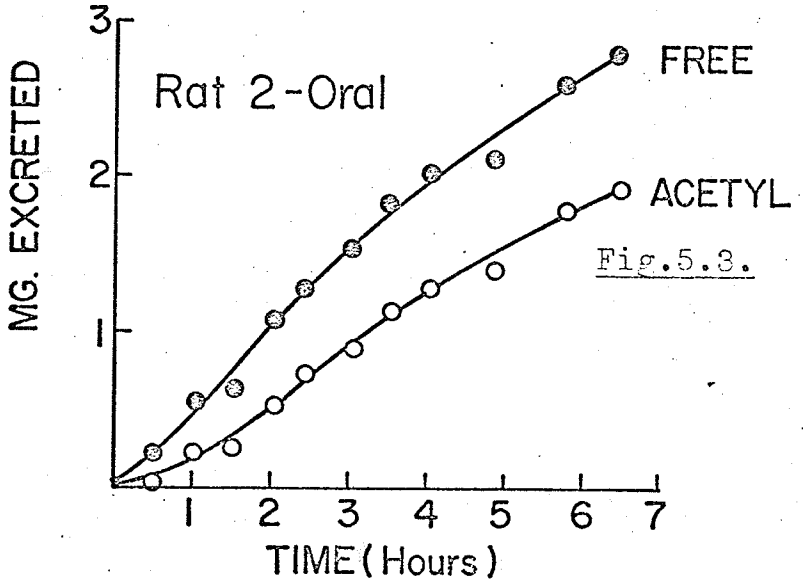


Fig.5.3.

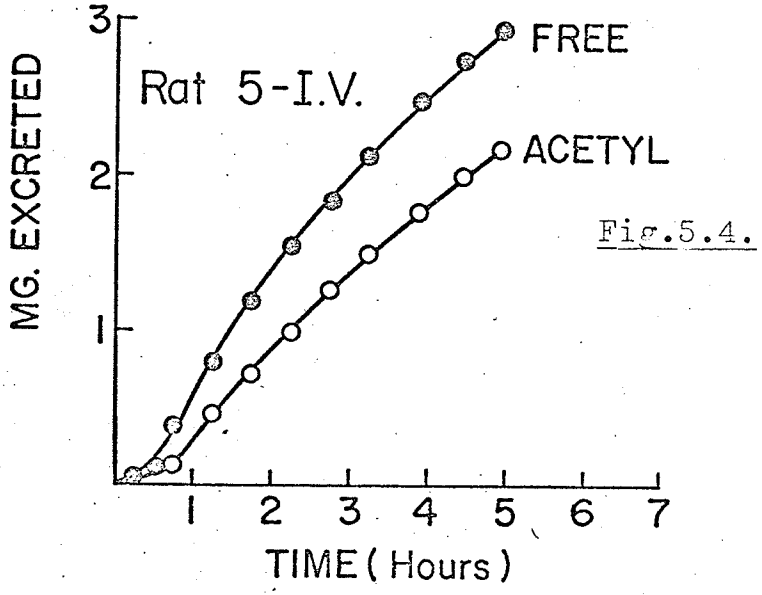


Fig.5.4.

Cumulative excretions of free and acetyl sulfadimidine in the urine after different routes of administration

Rat Number.	Weight Gm.	Free Drug, mg.	Total Drug, mg.	Free / Total %	mg./kg.
1	325	8.13	17.34	48.86	307.69
2	325	14.45	25.50	56.66	307.69
3	340	18.21	37.19	48.96	294.11
4	340	9.15	28.75	31.83	294.11
5	330	13.05	37.69	34.63	303.03
6	295	14.03	37.19	37.71	338.98
7	365	21.85	54.63	40.00	273.97
8	305	13.05	34.31	38.03	327.86
9	350	22.17	42.90	51.67	285.71

Mean* = 43.15 ± 2.86

*Mean ± standard error of the mean.

Table 5.4. Effect of increasing the dose to 100mg. administered orally.

In both cases about 55% of the dose was recovered in the urine after 24 hours.

The two dose levels were compared using a "t-test" on the ratios free/total %

$$t = 0.3353$$

from tables t = 2.15, at probability level of 0.01. Therefore, the difference between the two mean values of the ratios free/total % was attributed to chance, and there was no significant difference between the ratios obtained at the two dose levels.

5-B) Pharmacokinetic Studies

1) Comparison of routes of administration, i.e. oral vs. I.P. vs. I.V.

The drug was administered to groups of animals orally, I.P. and I.V., urine samples collected, and the cumulative totals of free drug and metabolite in the urine were plotted against time, Figs.5.2,5.3 and 5.4. All three methods of dosage showed what seems to be an initial, slow period of excretion.

From the cumulative urinary data, logarithm of rate of excretion versus time plots were constructed (Swintosky, 1957).

The plots for all three routes showed an initial rise followed by a secondary linear phase, (Figs.5.5, 5.6 and 5.7). Such behaviour is expected after oral administration and is usually interpreted as an initial absorption phase followed by the elimination of the drug, (Cummings et al, 1967). After I.V. an initial phase similar to an absorption phase is most unusual and cannot be explained readily, (Chiou and Riegelman, 1969; Jusko and Levy, 1970). Very little work has been done on pharmacokinetics of drugs after I.P. injection but generally the kinetics are similar to I.V. administration, (McMahon, 1970).

To determine if the elimination phase in each

Table 5.5. First order rate constants obtained from urinary excretion rate data.

Rat Number.	K $hrs.^{-1}$	f	k_3 $hrs.^{-1}$	k_1 $hrs.^{-1}$	$t_{\frac{1}{2}}$ hrs.
I.P.					
1	0.257	0.563	0.144	0.122	2.700
2	0.158	0.541	0.086	0.073	4.381
3	0.129	0.531	0.069	0.061	5.358
4	0.161	0.489	0.079	0.083	4.293
5	0.164	0.487	0.080	0.084	4.225
Mean #	0.174±.02	0.522±.06	0.092±.02	0.082±.01	4.197±.426
Oral					
1	0.152	0.572	0.087	0.065	4.545
2	0.193	0.545	0.105	0.088	3.587
3	0.203	0.712	0.144	0.058	3.422
4	0.227	0.556	0.127	0.101	3.047
5	0.121	0.523	0.063	0.058	5.712
Mean #	0.179±.02	0.582±.03	0.105±.02	0.074±.01	4.063±.481
I.V.					
1	0.218	0.584	0.127	0.090	3.185
2	0.132	0.530	0.070	0.063	5.241
3	0.176	0.514	0.091	0.086	3.928
4	0.156	0.563	0.088	0.068	4.446
5	0.168	0.574	0.096	0.071	4.136
Mean #	0.170±.02	0.553±.02	0.094±.01	0.076±.01	4.187±.336

Mean ± standard error of the mean.

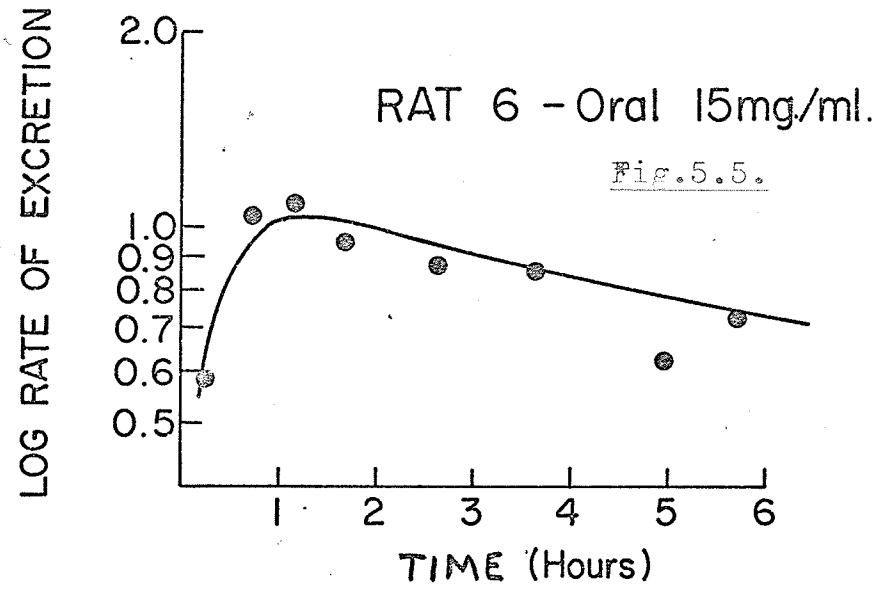


Fig.5.5.

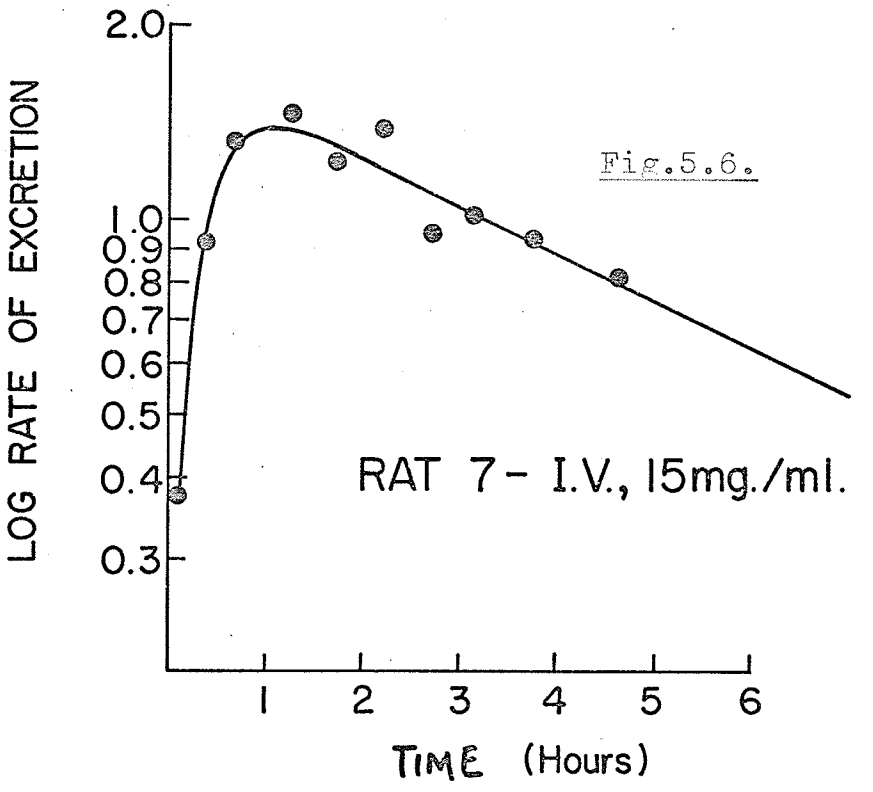


Fig.5.6.

Plots of logarithm of the rate of excretion of total drug versus midpoint of time of collection of urine.

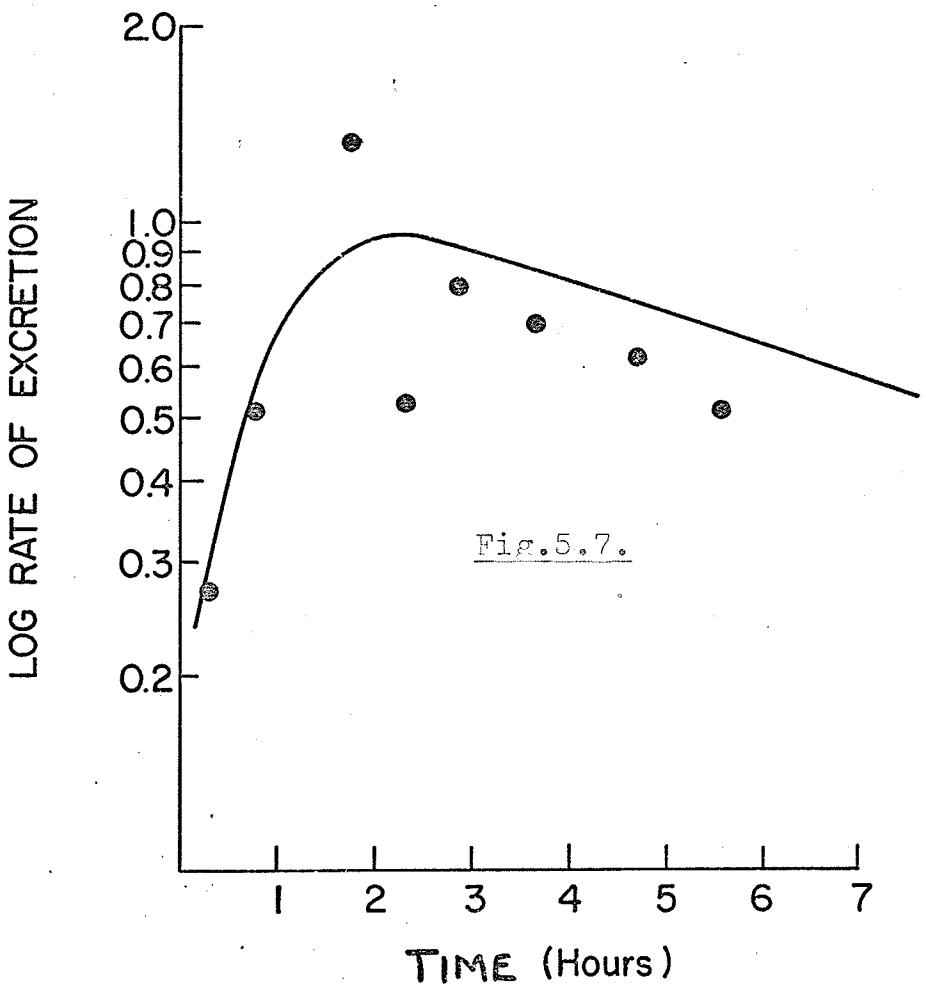


Fig.5.7.

Plot of logarithm of the rate of excretion of total drug versus midpoint of time of collection of urine Rat 7 - dosed I.P., 15mg./1/2ml.

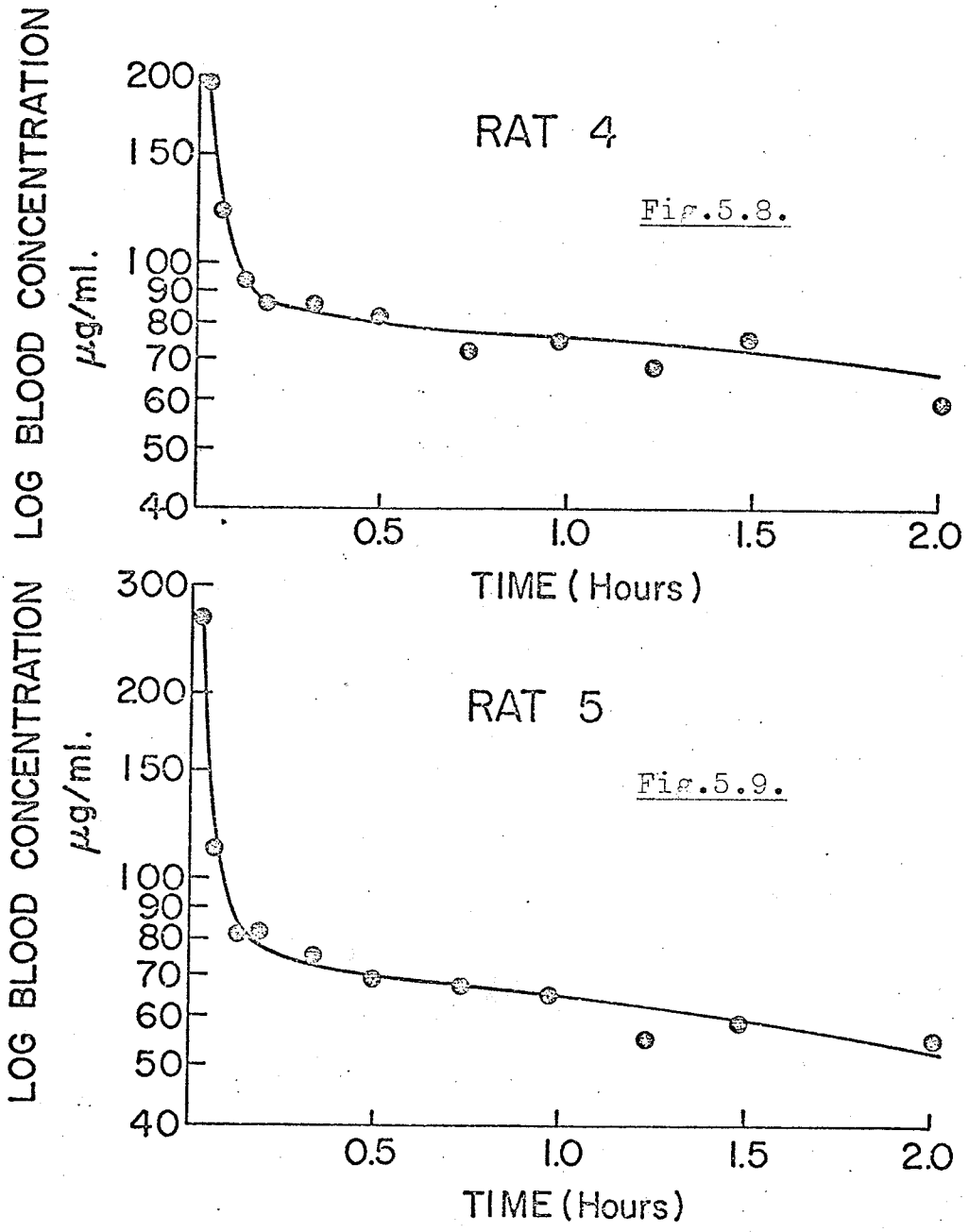


Fig.5.8.

Fig.5.9.

Plots of logarithm of free sulfadimidine concentrations in the blood versus time after I.V. injections, 15mg./1/2ml

case (oral, I.V., I.P.) are similar, the slopes of the later parts of the curves were calculated and values for the one compartment rate constants obtained, (Chapter 4, Section 4-1, Table 5.5.).

The one and two compartment models both imply that renal excretion of drug is a first order process. This requires that rate of renal excretion be proportional to the blood concentration and the maximal urinary excretion rate should occur at the point of maximal blood level i.e. at a time immediately after I.V. injection. Such urinary excretion curves have been obtained with griseofulvin (Chiou and Riegelman, 1969) and riboflavin (Jusko and Levy, 1970). The urinary excretion rate plot obtained here (Fig.5.7) does not seem to have been obtained in any other case and experiments were conducted to determine the possible causes of this unusual result. Possible causes may have been the anesthetic, saturation of any binding sites, and crystallisation of the sulfadimidine.

2) Blood Level Work.

The graphs obtained from the semi-logarithmic plots of the blood levels of drug versus time indicated that the drug showed two compartment behaviour, Figs.5.8 and 5.9.

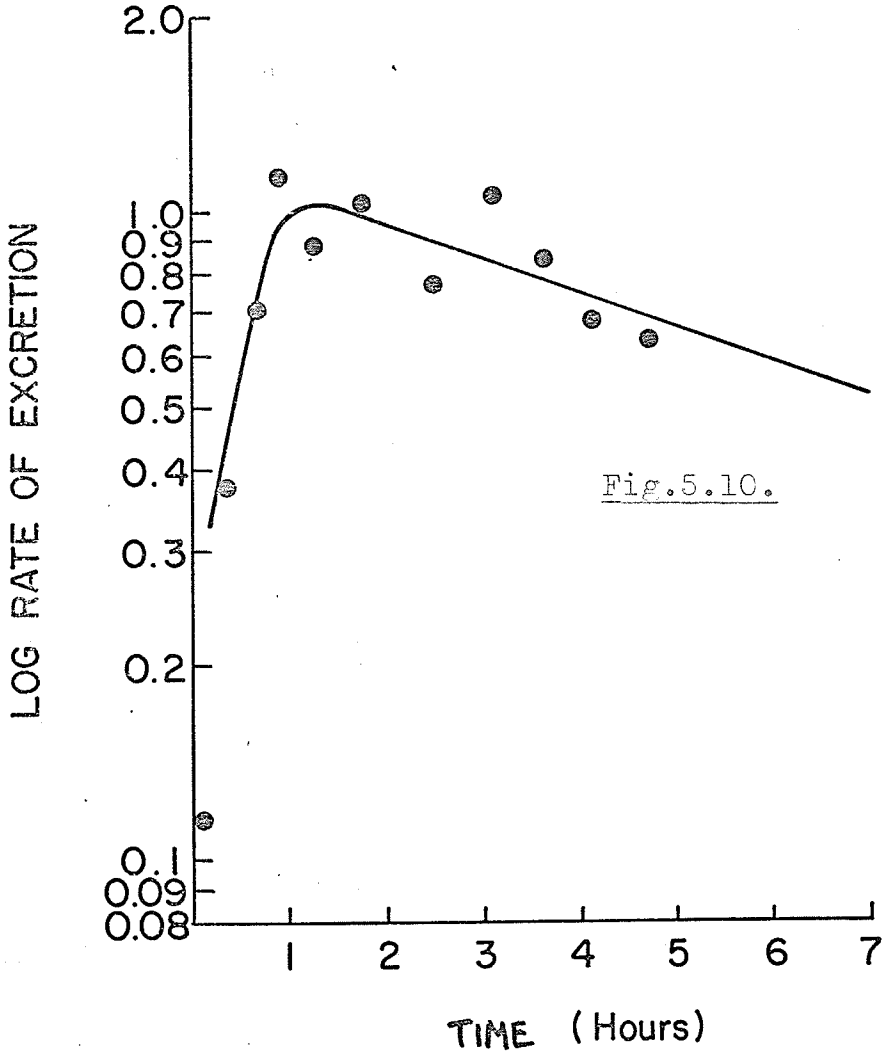


Fig.5.10.

Plot of logarithm of the rate of excretion of total drug versus midpoint of time of collection of urine dose of 15mg./1/2ml., administered I.V. without anesthesia Rat 1

There was nothing in the blood level plots to explain the initial rise in the urinary excretion rate data after I.V. injection. This indicated that the unusual effect may be due to the excretory processes for the drug and its metabolites.

From the biexponential curves, estimates of the constants r_1 and r_2 can be made. Table 5.6 shows the two compartment hybrid constant, r_2 and the one compartment elimination constant, K.

	K hrs.$^{-1}$ from urinary data	r_2 from blood levels
	0.218	0.054
	0.132	0.213
	0.176	0.0605
	0.156	0.134
	0.168	0.159
Mean*	0.170±0.011	0.124±.0.030

Mean*± standard error of the mean

t = 1.38

from tables, at P = 0.05, t = 2.31

There is no significant difference between r_2 and K.

Table 5.6. Comparison of r_2 from blood levels and K from urinary excretion rate data, both after I.V. injection.

This result confirms that the post-equilibrium rate of

drug decline in the blood is proportional to the drug concentration in the blood.

3) Effect of anesthesia.

To test whether the unusual effect observed with the urinary excretion rate data after I.V. injection was due to the anesthetic, the dose was administered I.V. after the rat had recovered from the anesthesia. In all rats, the rate plots were of a similar shape to those obtained with anesthetic, and the K values did not differ significantly, Fig. 5.10; Table 5.7.

	K hrs.$^{-1}$ with anesthetic	K hrs.$^{-1}$ without anesthetic
	0.218	0.118
	0.132	0.214
	0.176	0.141
	0.156	
	0.168	
Mean*	0.170±0.014	0.157±0.029

*Mean± standard error of the mean.

$t = 0.445$

from tables at $P = 0.05$, $t = 2.45$

The values are not significantly different.

Table 5.7. Values of K after I.V. injection with and without anesthesia.

It was assumed, therefore, that the anesthetic was

probably not the cause of the unusual effect after I.V. administration.

4) Effect of increasing and decreasing the dose.

Sulfonamides are bound to plasma proteins (Anton, 1960,1961) and have been known to crystallise out in the kidney (Nelson, 1961b). To determine whether the initial slow excretion rate observed after I.V. administration was due to these effects, the dose was increased and decreased to 30mg. and 7.5mg. in 0.5ml. and administered under anesthetic via a femoral vein. The rate plots were of a similar shape to those obtained with a 15mg. dose and the K values did not differ significantly, (Figs.5.11 and 5.12, Table 5.8).

K hrs.$^{-1}$	K hrs.$^{-1}$	K hrs.$^{-1}$
7.5mg. dose	15mg. dose	30mg. dose
0.175	0.218	0.155
0.139	0.132	0.274
0.183	0.176	0.173
0.197	0.156	0.226
Mean*0.173±0.013	0.171±0.018	0.207±0.027

*Mean± standard error of the mean

Analysis of variance was carried out on the three sets of values.

Table 5.8. K values for the 7.5mg., 15mg. and 30mg. doses administered I.V.

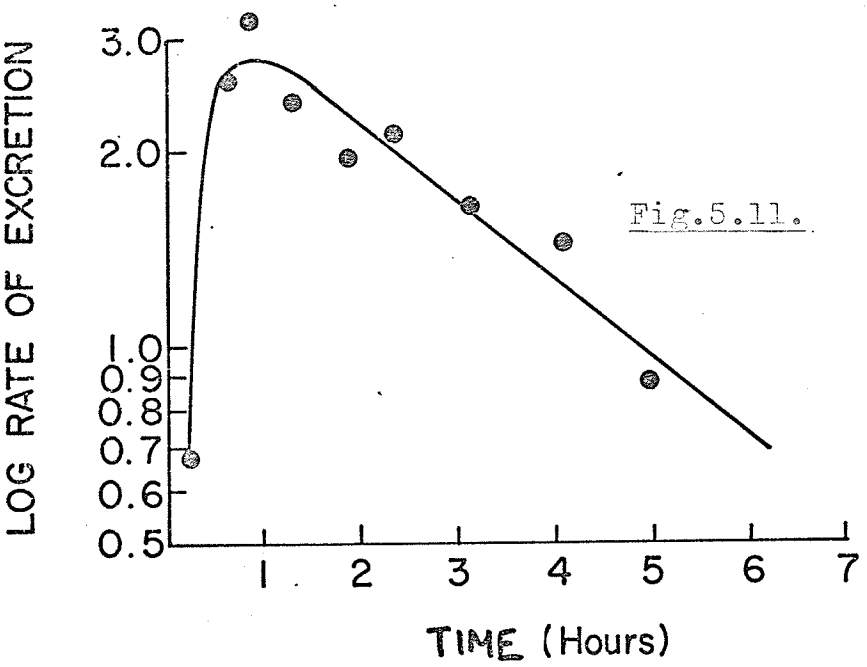


Fig.5.11.

RAT 2 - 30mg./1/2ml. administered I.V.

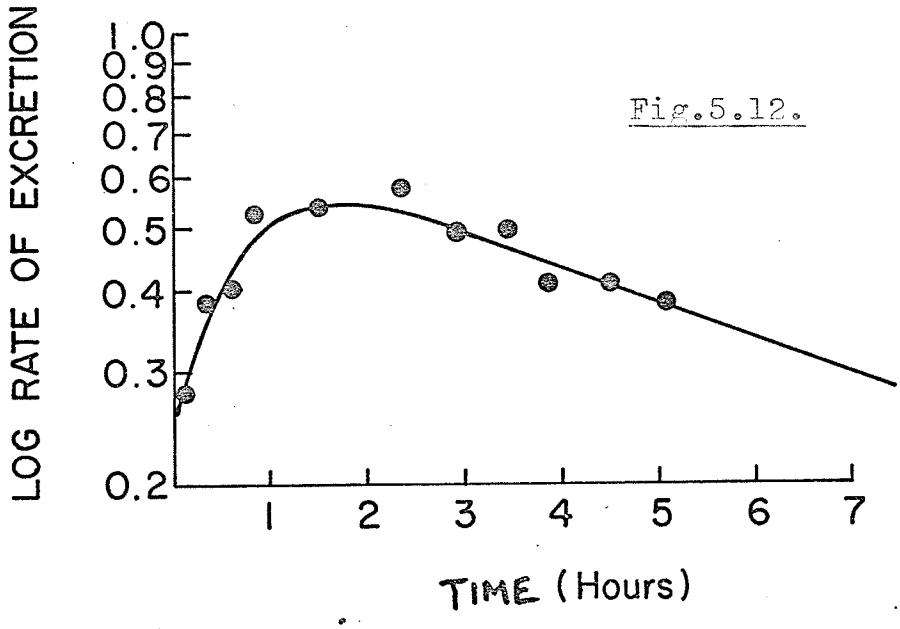


Fig.5.12.

RAT 2- 7.5mg./1/2ml. administered I.V.

Plots of logarithm of the rate of excretion of total drug versus midpoint of collection of urine.

After administration of the 7.5mg., 15mg. and 30mg. doses I.V., the excretion rates reached their maxima after about 90, 90 and 60 minutes respectively. It could be assumed, therefore, that the unusual effect was probably not due to saturation of binding sites in the plasma or closely associated tissues, nor was it due to the saturation of excretory processes in the kidney.

5) Effect of increasing the dose volume.

Because the solubility of sulfadimidine is low at physiological pH, there was the possibility the drug was crystallising out after injection to give a depot effect. However, on examining a mixture of blood and a solution of the drug under the microscope, no recognisable crystals of sulfadimidine could be seen. To confirm this the dose volume was increased threefold to 1.5ml. and administered I.V. under anesthetic, via a femoral vein. There was no change in the shape of the rate plots, and the K values obtained did not differ significantly from those obtained when the volume was only 0.5ml. (Fig.5.13, Table 5.9.)

That is, the shape of the curve is probably not due to the drug crystallising out in the blood.

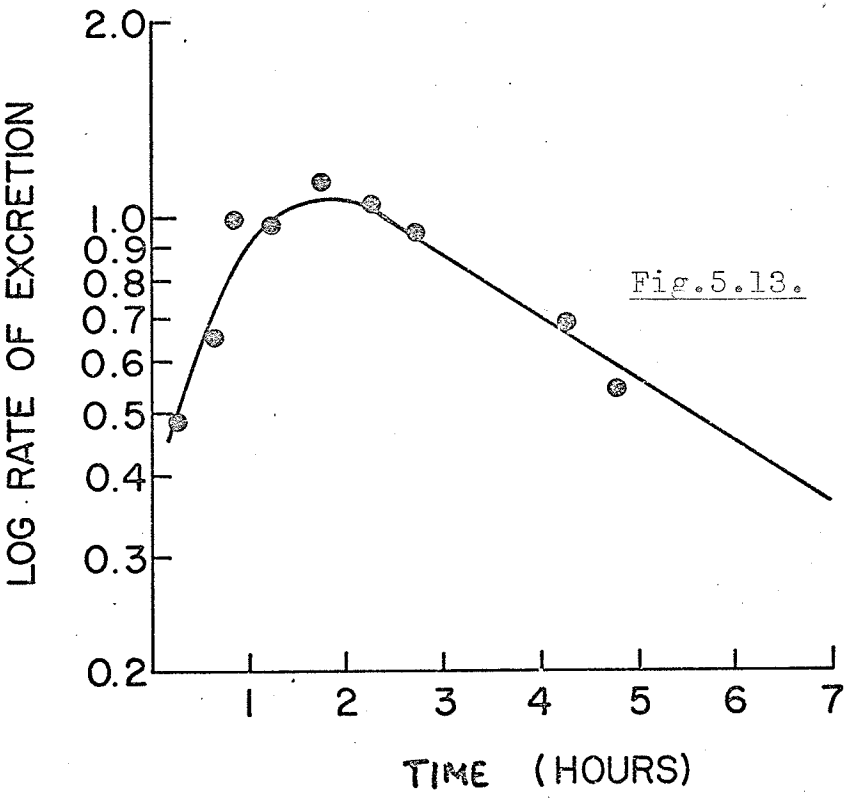


Fig.5.13.

Plot of logarithm of the rate of excretion of total drug versus midpoint of time of collection of urine Rat 8-15mg/1.5ml. administered I.V.

K hrs.$^{-1}$ 0.5ml. dose volume.	K hrs.$^{-1}$ 1.5ml.dose volume.
0.218	0.183
0.132	0.231
0.176	0.085
0.156	0.216
0.168	
Mean* 0.170 ± 0.014	0.179 ± 0.033

*Mean ± standard error of the mean.

$t = 0.276$

From tables, $t = 2.37$, at $P = 0.05$

Table 5.9. K values for dose volumes of 0.5ml. and 1.5ml. after I.V. injection.

6) Two compartment analysis of blood levels.

As can be seen from all semi-logarithmic plots of blood levels versus time, the drug showed a two compartment type behaviour exemplified by the bi-exponential curves, Figs. 5.14, 5.15, 5.16. In all cases the initial distribution phase was extremely rapid with the plots becoming linear after only about thirty minutes. For this reason the blood sampling was continued for about two hours. In each case there was little or no metabolite in the blood, indicating one of two actions;

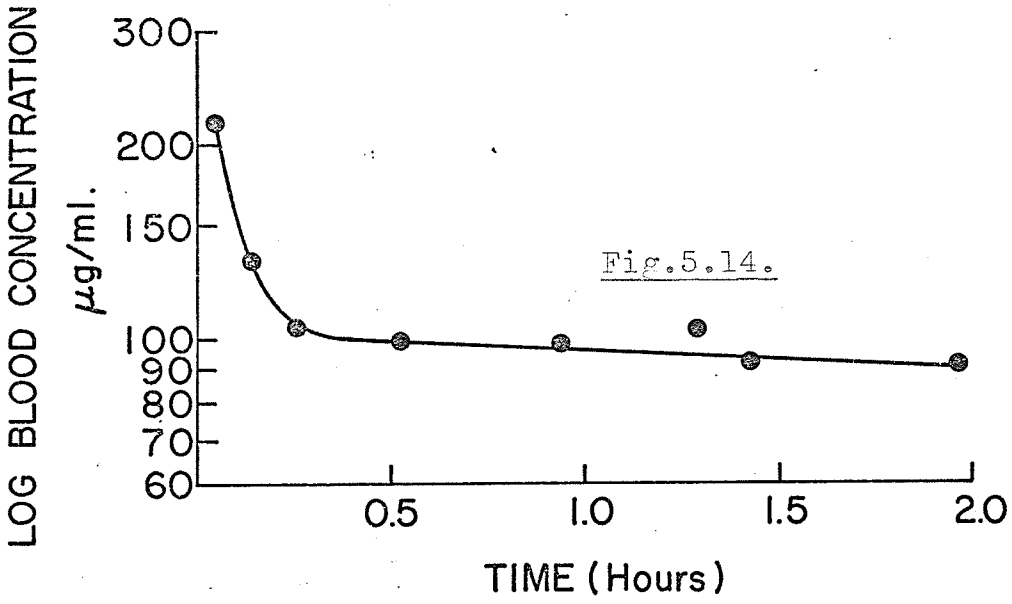


Fig.5.14.

RAT 1

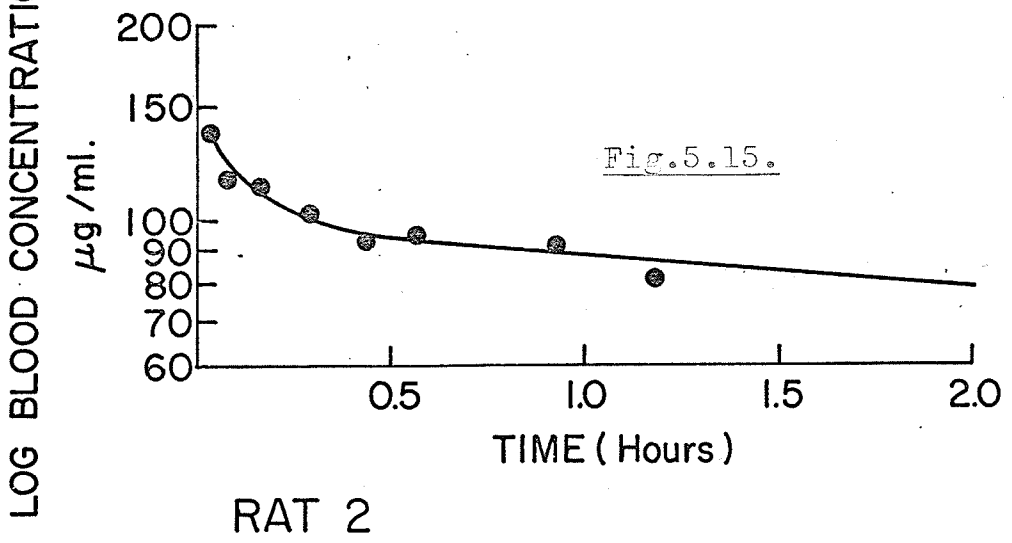


Fig.5.15.

RAT 2

Plots of logarithm of free sulfadimidine concentrations in the blood versus time after I.V. injection 15mg./1/2ml.

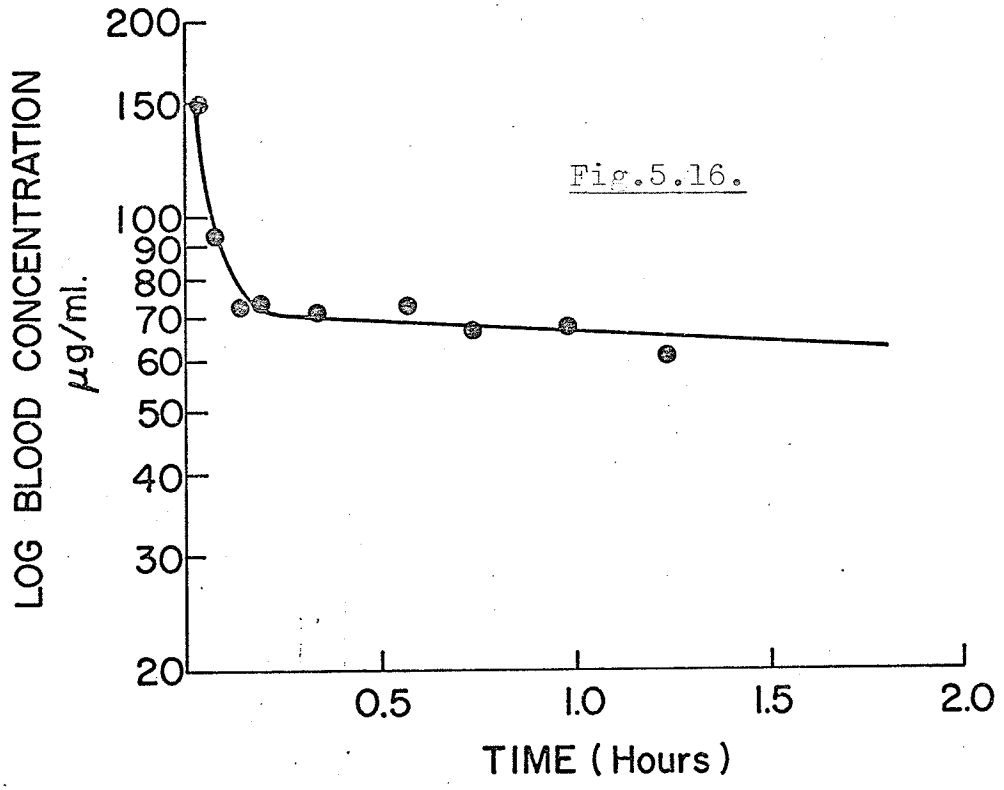


Fig.5.16.

Plot of logarithm of free sulfadimidine concentrations in the blood versus time after I.V. injection, 15mg./ml. Rat 3

1) Metabolism may occur in the kidney.

2) The elimination and/or diffusion constants of the metabolite are much larger than those of the free drug and the metabolite is removed as rapidly as it is formed.

The experimental data was processed as mentioned in Chapter 4, Section 4-2, and the values are shown in Table 5.10.

In all but rat (2), k_{12}, the diffusion constant into the tissue compartment, was about twice as large as k_{21}, the diffusion constant out of the tissue compartment. There seemed to be a trend toward the rate constant for elimination increasing as the initial plasma concentration increased. r_1 was large compared with r_2, accounting for the rapid distribution phase.

The standard deviations of the generated parameters are large, but this is more probably due to the scatter of the experimental points than to a deviation from the model. As Turco et al (1966) point out, experimental error is exhibited by scatter in data, such as obtained here, while deviations from the model give rise to consistent areas of poor fit in certain parts of the experimental curve.

Table 5.10. Computed values of k_{12}, k_{21}, r_1, r_2, k_{el} and C_p^o, the initial plasma concentration in μg./ml.

Rat No.	k_{el} # $hrs.^{-1}$	k_{12} # $hrs.^{-1}$	k_{21} # $hrs.^{-1}$	C_p^o # μg./ml.	r_1	r_2
1 f=0.458	0.188±0.113	21.97±3.7	8.885±0.57	247.8±24.27	30.989	0.054
2 f=0.476	0.298±0.083	3.192±1.384	8.56±2.429	148.2±9.84	11.387	0.213
3 f=0.428	0.372±0.282	12.38±8.93	6.083±2.05	357.8±141.2	18.714	0.0605
4 f=0.523	0.421±0.121	14.39±2.91	6.813±0.78	265.0±26.87	21.491	0.134
5 f=0.429	0.531±0.147	14.93±2.40	6.539±0.77	258.1±17.78	21.841	0.159
Mean $	0.362±0.058	13.372±3.017	7.376±0.56	255.4±33.3	20.88±3.2	0.124±0.03

Values ± the standard deviation, generated as in Theory Section 4.

$ Mean ± standard error of the mean.

7) Acetylation and Deacetylation

A dose of 15mg./½ml. of the N-4 acetyl sulfadimidine was administered I.V. to the rats while under ether anesthesia.

On plotting the logarithm of the rate of excretion versus time, a straight line was obtained (Fig.5.17), showing that the level of the metabolite in the blood falls exponentially. This behaviour of the metabolite, therefore, could be explained in one of two ways;

1) The passage of the metabolite obeys a one compartment model.

2) The passage of the metabolite obeys a two compartment model, but the initial distributive phase is so rapid that it does not show on the graphs.

a) Calculation of k_2 from Urinary Excretion Data after I.V. Injection of the Free Drug.

There are three accepted methods available for the calculation of k_2;

1) Iterative method of Nelson (1961b)

2) "Rate versus amount" method of Cummings et al (1967)

3) The "Terminal Ratio" method of Cummings et al (1967)

The second method is based on the equation;

$$\frac{dM_U}{dt} = k_2 M_B$$

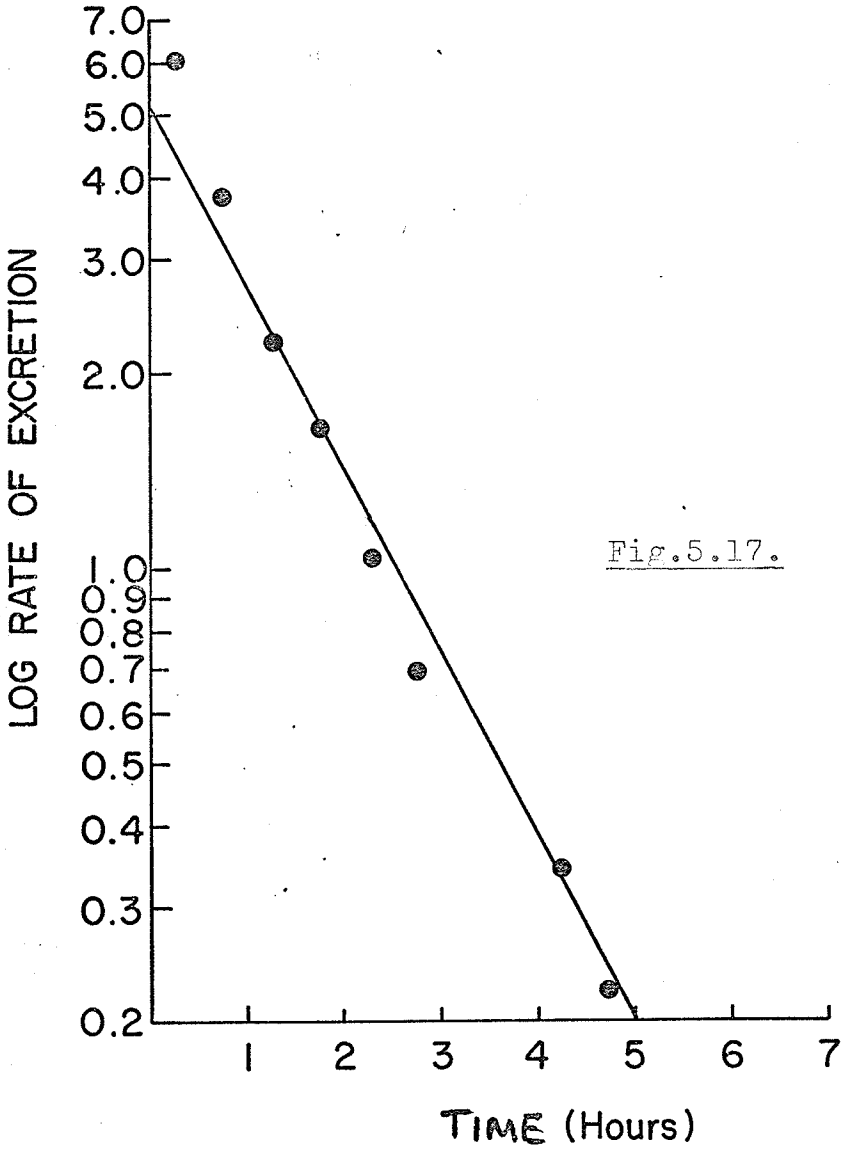


Fig.5.17.

Plot of logarithm of the rate of excretion of total drug versus the midpoint of time of collection of urine Rat-2 Injection of N-4 acetylsulfadimidine I.V. 15mg./1/2ml.

that is, the rate of excretion of the metabolite is proportional to the metabolite level;

where $M_B = \frac{M_U^\infty}{D_U^\infty}(D_U - M_U)$

As already mentioned, there was little or no metabolite present in the blood. In using this method it was assumed that the metabolite was removed as rapidly as it was formed. Hence, this indirect method of calculating M_B had to be used.

b) Values of k, k_1 and k_2.

These values are listed in Table 5.13.

k $hrs.^{-1}$, after injection of metabolite	k_1 $hrs.^{-1}$, after injection of free drug	k_2 $hrs.^{-1}$, after injection of free drug
0.719	0.090	2.048
0.710	0.062	5.774
0.629	0.086	
0.697	0.068	
0.715	0.071	
Mean*		
0.694±0.014	0.076±0.005	3.911±1.863

*Mean ± standard error of the mean.

Table 5.13. Comparison of values of k, k_1 and k_2 from urinary excretion data.

The values of k, k_1 and k_2 were significantly different from each other.

c) Deacetylation.

There was a significant amount of deacetylation in all the rats studied. The longer the drug remained in the body, the greater the percentage of deacetylation (Table 5.14.)

Rat 2		Rat 4	
Time in hrs.	Free drug % Total drug. after injection of metabolite	Time in hrs.	Free drug % Total drug. after injection of metabolite.
0.48	0.554	0.22	0.824
0.96	1.171	1.01	1.651
1.48	1.860	1.87	2.219
1.98	2.103	2.82	4.370
2.48	3.427	3.86	7.398
3.00	4.011	4.33	23.484
3.50	8.695	5.37	30.208
4.00	7.361		
4.47	9.395		
5.03	11.607		

Table 5.14. Percentages of deacetylation at various times after injection of the N-4 acetyl sulfadimidine assuming 100% metabolite.

Chapter 6.

Discussion.

6-A) Metabolic studies.

1) Thin layer chromatography of urine samples.

The main excretory products of sulfadimidine in the rat were found to be the free drug and its N-4 acetyl derivative. Two spots were tentatively identified as the N-4 glucuronide and N-4 sulfamate, but were minor metabolites. Acetylation of the aromatic amino group is a common metabolic transformation of sulfonamides and the percentage of acetylation obtained is governed by the drug and species used. (Bridges and Williams, 1963; Bohni et al, 1969; Bridges et al, 1969.)

2) Percentages of excreted products.

The dose was increased from 25mg. to 100mg. and the 'f' values found for each level. (Tables 5.3 and 5.4.) From the t-test performed, the difference between the two 'f' values was attributed to chance and there was no significant difference between the ratios obtained at the two dose levels. Table 6.1 shows the mean values of the free drug, and total drug excreted into the urine after 24 hours and the 'f' values obtained at the two dose levels.

Dose	Free Drug, mg.	Total Drug, mg.	'f'
25mg.(6)*	4.462	10.822	0.467
Range	1.908-5.88	3.218-15.375	0.333-0.582
100mg.(9)*	14.898	35.277	0.432
Range	8.13-22.165	17.34-54.625	0.318-0.567

* The number in brackets is the number of animals used in each experiment.

Table 6.1. Mean values of free drug, total drug and 'f' obtained after oral dosage of 25mg. and 100mg.

Drucker et al (1964) noted that the extent of acetylation of para amino benzoic acid (P.A.B.A.) decreased as the dose increased. Levy (1965) noted that the rate of conjugation of salicylic acid with glycine reached a maximum as the dose of drug was increased. In each case the relative decline in metabolic efficiency was correlated with a saturation of the drug metabolic system at high drug concentrations. The kinetics of the metabolic reaction changed from apparent first order to zero order behaviour with increasing dose, analogous to the enzyme saturation in classical Michaelis-Menten kinetics. If such a saturation occurs then f, the fraction of drug excreted free, should increase with increasing dose, (McMahon, 1970).

After a four fold increase of dose, saturation of the metabolic and excretory processes involving the drug is unlikely.

6-B) Pharmacokinetic Studies.

1) Comparison of the routes of administration.

a) After all three forms of dosage, the cumulative plots were similar. An apparent slow initial excretion phase (Fig.5.6) after I.V. dosage was surprising since immediate fast excretion from the more concentrated 'I.V. pool' would be expected. The rate plots appeared to be the same shape after each method of administration, confirming the initial slow excretion rate in the I.V. case. (Figs. 5.5,5.6 and 5.7)

b) The initial slow excretion rate may be due to renal inhibition, a direct result of the injection technique. It was noted that when rabbits were submitted to stress, there was a significant decrease in blood flow to the kidneys, resulting in a decrease in urine formation and flow, (Brod and Sirota, 1949). It is conceivable that rats under a similar type of stress, either anesthetic or restraint, will experience a similar decrease in blood flow, resulting in the slow excretion rate.

The chief problem with the "rate method" is that it requires somewhat more points than the "sigma-

minus method" (Bray et al, 1951) because urinary flow fluctuations cause greater deviations in a log. rate than in a log. sigma minus plot.

The rate method gave consistent results from rat to rat. Therefore, it was decided to use this method rather than the "sigma-minus method" or a combination of the two.

c) One compartment rate constants from urinary excretion data.

Calculated rate constants are listed in Table 5.5. The K values from each form of dosage did not differ significantly. In most cases the values of k_1 and k_3 are about the same, that is, the sulfadimidine is about 50% acetylated. The K values are obtained from the secondary linear portions of the rate plots, when sufficient time should have passed for any absorption and equilibration phases to be completed.

Various experiments were attempted to find an explanation for the unusual initial, slow excretion rate observed after I.V. injection.

2) Blood level work.

The plots of the logarithm of drug level in the blood versus time indicated that the drug followed two compartment behaviour. The biexponential curves obtained from blood level data were similar to those

expected from a two compartment model.(Riegelman et al 1968a). There was no rise or other discrepancy in the blood level plots to account for the initial, slow excretion rate into urine after I.V. injection. The unusual effect could be due to the excretory processes of the free drug and its metabolites from the kidney.

a) Comparison of K from urinary excretion data and r_2 from blood levels.

In the theory section it was shown that a value of K could be obtained from the slope of the blood level plot. If the data were fitted to a two compartment model, r_2 was obtained. The values of K and r_2 were not significantly different even though the former was obtained from urinary excretion data.(Table 5.6)

As can be seen from the rate plots (Figs. 5.5, 5.6 and 5.7) and blood level plots (Figs. 5.8 and 5.9), there was less variability in the blood level work than in the urinary excretion data. With the techniques employed constant volumes of blood could be taken, whereas with urinary excretion work the rats were not sedated, and as such were subjected to a certain amount of stress, provoked by the method of urine sampling. The stress led to urinary flow variation, and could lead to the same urinary

retention experienced by rabbits.(Brod and Sirota, 1949). However, sufficient numbers of urine samples were taken to enable the slopes of the linear portion to be found by regression analysis.

3) Urinary excretion data was studied in various experiments in an attempt to explain the initial slow excretion rate after I.V. injection. In all cases the shapes of the rate plots were the same and the K values were not significantly different. Table 6.2 lists the experiments and conclusions.

Experiment	Conclusion
a) Dose administered with anesthetic	Initial slow excretion rate not due to anesthetic*
b) Dose increased and decreased	Initial slow excretion rate not due to saturation
c) Dose administered in large dose volume	Initial slow excretion rate not due to crystallisation of sulfadimidine

Table 6.2. List of experiments to explain initial slow excretion rate, and conclusions.

*This is in agreement with Anton (1961), who found that brief exposure to ether did not alter the distribution of sulfonamides in rats.

4) Two compartment analysis of blood levels.

The absence of metabolite in the blood may be

explained in several ways. Metabolism may occur in the kidney or in some other compartment not directly connected to the central compartment, but directly connected to the excretory compartment. Benzi et al, (1968) and Hartiala and Terho (1965) have noted that the kidney is capable of metabolising drugs.

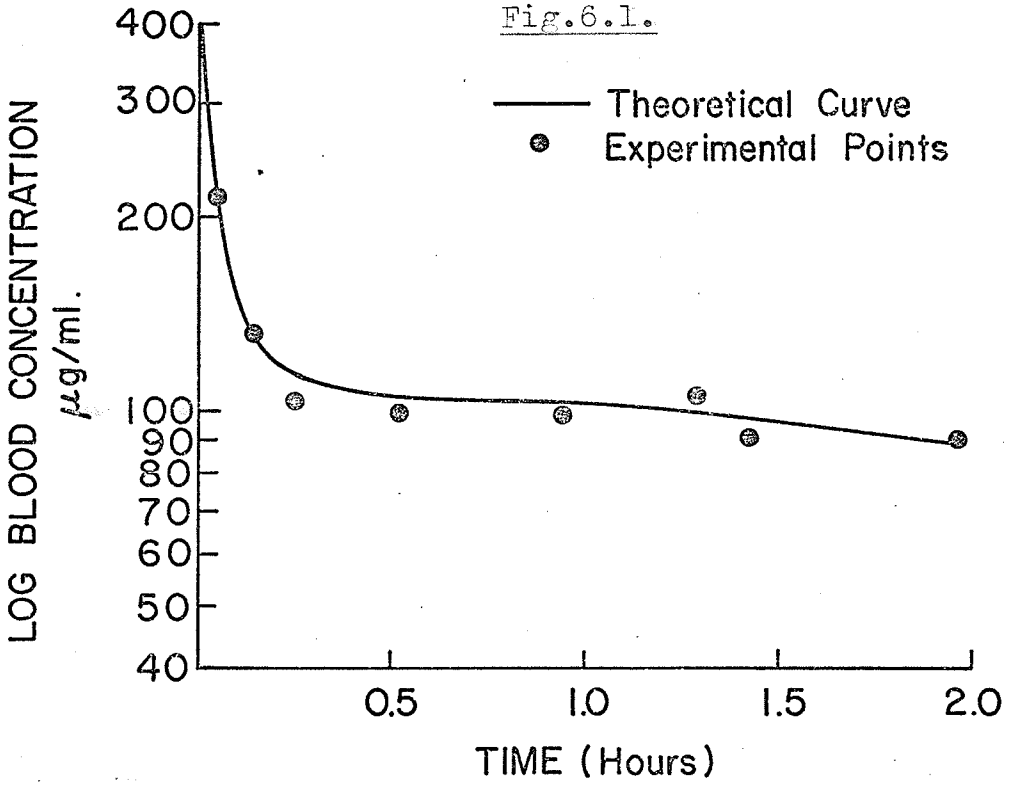
Another explanation may be that the metabolite was removed as rapidly as it was formed. Calculated values of k_2 were very large compared with k_3. Despopoulos and Sonnenberg (1967) stated that in vitro acetyl sulfadimidine was actively transported by the kidney whereas sulfadimidine was not. If this is the case in vivo, a comparatively larger amount of metabolite would be removed and the near absence of the metabolite in the blood would be explained. To confirm such a theory would necessitate the simultaneous sampling of blood and urine and with the present techniques this was not practical.

The computed values of the two compartment parameters are shown in Table 5.10. The standard deviations are large but as can be seen in Fig. 6.1, there are no definite areas of poor fit.

5) Comparison of one and two compartment parameters.

From one and two compartment models, the

Fig.6.1.



Comparison of theoretical curve with experimental points after I.V. injection , 15mg./1/2ml. Rat 1

parameters k_1 and k_m can be compared to determine whether there is any relation between the two, Table 6.3.

From blood level data;

$$k_1 + k_3 = r_2 \qquad (1)$$

and $$r_2 f = k_3 \qquad (2)$$

and $$k_u + k_m = k_{el} \qquad (3)$$

and $$k_{el} f = k_u \qquad (4)$$

In no case did the ratio k_1/k_m approach unity. Riegelman *et al* (1968a) have criticised the one compartment model as not being compatible with physiological facts. If this is the case, the value of k_1 is misleading and k_m should be used as a measure of the metabolic capacity.

From equations (1) and (3), k_1 and k_m are related to r_2 and k_{el} respectively. k_{el} is the elimination rate constant for the two compartment model. r_2 includes both distribution and elimination and is referred to as the "disposition rate constant". (Riegelman *et al*, 1968a). k_{el} and r_2 may be related by the equation;

$$k_{el} = (C_p^o/B)\ r_2$$

The ratio C_p^o/B calculated from r_2 and k_{el} generally ranges from 1.5-2.5. In this case, the values are higher, >3.0, indicating that sulfadimidine distributes into the peripheral compartment to a large degree

Table 6.3. Comparison of one and two compartment parameters from blood levels.

Rat No.	k_3 hrs.$^{-1}$	k_1 hrs.$^{-1}$	k_u hrs.$^{-1}$	k_m hrs.$^{-1}$	k_1/k_m
1	0.025	0.029	0.086	0.102	0.284
2	0.101	0.112	0.142	0.156	0.718
3	0.026	0.035	0.159	0.213	0.163
4	0.070	0.064	0.220	0.201	0.318
5	0.068	0.091	0.228	0.303	0.300
Mean #	0.058±0.014	0.066±0.014	0.167±0.024	0.195±0.033	0.357±0.094

Mean ± standard error of the mean.

Table 6.4. Clearances of free drug and metabolite calculated from blood levels.

Rat No.	Vp mls.	Vp/Body Weight,%	C_f mls./min.	C_m mls./min.	Total plasma clearance $C_f + C_m$ mls./min.
1	60.53	13.5	0.103	0.0868	0.1898
2	101.23	22.5	0.263	0.239	0.502
3	41.92	10.5	0.149	0.111	0.260
4	56.60	14.2	0.190	0.208	0.398
5	58.12	13.7	0.294	0.221	0.515
Mean #	63.68±9.93		0.200±0.035	0.173±0.030	0.373±0.064

Mean ± standard error of the mean.

(Riegelman et al, 1968a.)

6) Volume of distribution and clearance of drug.

The volume of distribution, Vp, was found from the formula, $Vp = Dose/C_p^o$. The clearances of the drug and its metabolites were calculated from the formulae, (McMahon and O'Reilly, 1971).

$$\text{Free drug clearance } C_f = \frac{k_u \cdot Vp}{60}$$

$$\text{Metabolic clearance, } C_m = \frac{k_m \cdot Vp}{60}$$

The values are shown in Table 6.4.

Riegelman et al (1968b) stated that the volume of distribution was a parameter of the model used and, therefore, changed with it. The clearance is also an indirect model parameter and is not, therefore, too reliable a measure of the ability of the animal to remove the drug from the plasma. However, Riggs (1963) pointed out that " the magnitude of the rate constant (of removal of the drug from the compartment) depends as much upon the volume of the compartment as it does on the effectiveness of the process of removal." The clearance, therefore, probably provides a better basis on which to compare the rates of metabolism of related drugs in the same species. (McMahon and O'Reilly, 1971)

7) Acetylation and deacetylation.

After I.V. injection of the metabolite, N-4 acetyl sulfadimidine, a plot of logarithm of the rate of excretion versus time gave a straight line. That is, the passage of the metabolite obeys a one compartment model or it obeys a two compartment model, but the initial absorption and distribution phases are so rapid that they do not appear on the graphs.

The values of k, k_1 and k_2 are significantly different, indicating that they may represent three different steps leading to the excretion of the metabolite into the urine.(Table 5.13.)

Krebs (1947) and Smith and Williams (1948) hypothesised that the amount of acetyl metabolite excreted depended on the relative activities of the acetylating and deacetylating systems; generally the former is more efficient. It was shown that deacetylation does occur to a significant extent in rats when N-4 acetyl sulfadimidine was administered.(Table 5.14.)

Urinary excretion data has been used to study the pharmacokinetics of sulfadimidine in the rat. After I.V. administration, the initial excretion rate was surprisingly low and various experiments were carried out to determine the cause. Water was administered to a group of rats every hour for four

hours before injection to maintain a more constant urine flow, and other experiments indicated that the unusual effect was not due to saturation, anesthesia or crystallisation. The experiments did not discount the possibility of the effect being due to a combination of the above.

Blood level data indicated a two compartment model but did not give any explanation of the unusual slow initial excretion rate given by urinary excretion data after I.V. injection. The latter method was used more often because of the ease of the techniques employed.

6-C) Simulations.

As shown in Chapter 4, Section 4.4, a two compartment model with absorption gave a mathematical solution with three exponential terms for the amount of drug excreted into the urine. The simulations were undertaken to determine the accuracy of parameters estimated graphically from triexponential curves.

Artificial data were generated according to equation 45, Chapter 4, Section 4.4, using selected values of k_a, which were assumed to have sufficient scope to include noted values of absorption rates of sulfonamides. (Koizumi et al, 1964a; Turco et al, 1966.) Values of k_{12}, k_{21} and k_e were random selections. (Table 6.5a, Assigned Values.) The artificial data were analysed graphically using rate plots.(Fig.6.5.)

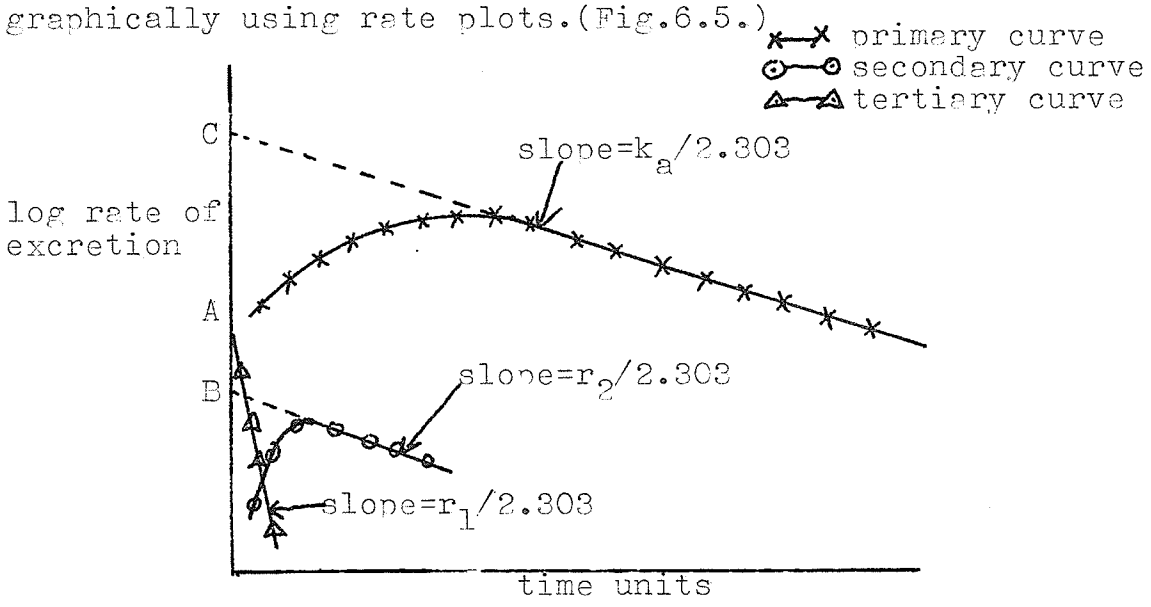


Fig.6.5. Theoretical log. rate of excretion versus mid-point of the time of collection. In this case, $r_1 \rangle r_2 \rangle k_a$.

Tables 6.5a, b, and c. Assigned, graphical and computed values of parameters used in simulations.

Simulation Set.	k_a units^{-1}	k_{12} units^{-1}	k_{21} units^{-1}	k_e units^{-1}
1	0.10	0.50	1.50	0.50
2	0.25	1.50	0.50	0.50
3	1.00	1.50	0.50	0.50
4	0.10	0.50	1.50	0.80
5	0.60	0.50	1.50	0.80
6	1.00	1.50	0.50	0.80
7	0.25	1.50	0.50	1.30
8	1.00	1.50	0.50	2.00

Table 6.5a Assigned values of parameters.

Simulation Set.	k_a units^{-1}	k_{12} units^{-1}	k_{21} units^{-1}	k_e units^{-1}
1	0.13	0.66	0.31	0.045
2	0.24	0.42	0.20	0.46
3	0.61	0.23	0.38	0.20
4	0.10	0.065	0.40	0.30
5	0.75	0.77	0.42	1.22
6	1.16	5.96	1.02	3.80
7	0.47	0.0079	0.17	0.15
8	1.00	4.97	1.22	3.60

Table 6.5b Graphical values of parameters.

Simulation Set.	D_o Units.	k_a units^{-1}	k_{12} units^{-1}	k_{21} units^{-1}	k_e units^{-1}
1	99.9±.06	0.34±.005	0.34±.012	1.08±.05	0.14±.003
2	99.3±.4	0.12±.007	0.55±.03	0.32±.02	0.82±.06
3	100.6±.2	3.20±.19	0.22±.02	0.41±.01	0.17±.004
4	99.3±.2	0.13±.0005	0.14±.0009	0.17±.0001	0.52±.002
5	100.0±.02	0.54±.016	0.41±.01	1.38±.025	0.87±.026
6	97.2±95.7	1.26±113.3	11.5±478.6	1.40±125.3	1.74±227.4
7	100.7±.86	0.31±.088	1.27±.23	0.49±.094	0.92±.35
8	100.0±.003	1.00±.0019	1.50±.002	0.50±.008	2.00±.006

Table 6.5c Computed values of parameters ± standard deviation.

A straight line was drawn through the last few points and extended to the co-ordinate axis. The last few points were assumed to represent the "slowest term," in this case the absorption of the drug, and its coefficent and rate constant found from the intercept and slope of the straight line.(Riggs,1963) Values of the last term for each of the earlier lying above the observed line can be read from the straight line. These values minus the observed rate values gave a series of differences which gave the secondary curve when plotted on semilog. paper. By repeating this procedure the tertiary curve was obtained.(Riggs,1963)

The cumulative data from which the rate plot in Fig.6.5. was derived, can be represented by the equation;

$$D_U = A(1 - e^{-r_1 t}) + B(1 - e^{-r_2 t}) + C(1 - e^{-k_a t}) \quad (6.1)$$

where

$$A = \frac{k_a \cdot k_e \cdot D_o (k_{21} - r_1)}{r_1 (k_a - r_1)(r_2 - r_1)} \quad (6.2)$$

$$B = \frac{k_a \cdot k_e \cdot D_o (k_{21} - r_2)}{r_2 (k_a - r_2)(r_1 - r_2)} \quad (6.3)$$

$$C = \frac{k_a \cdot k_e \cdot D_o (k_{21} - k_a)}{k_a (r_1 - k_a)(r_2 - k_a)} \quad (6.4)$$

In the case of Fig.6.5., k_a was the smallest constant input and was found from the primary curve. By the method of residuals, the secondary curve was generated. The"primary" slope of this curve gave r_2, the second smallest constant. Again by the method of

residuals on the secondary curve, the tertiary curve was generated. The slope of the tertiary curve gave r_1, the largest constant. As shown in Fig.6.5., the intercepts of the straight lines gave A,B and C.

Assigned and graphically estimated values of r_1, r_2 and k_a are shown in Tables 6.6a and 6.6b. As can be seen from Tables 6.6a and 6.6b, the estimates of r_1 were subject to great error. Reasonable estimates of k_a and r_2 were obtained. It was hoped to submit this data to computational analysis using a DFUNC subroutine incorporating equation (6.1). However, such a subroutine would not accurately fit the data.

Values of k_e and k_{21} were calculated from equations (6.5) and (6.6) which were derived from equations (6.2) and (6.4).

$$k_e = \left(\frac{C(r_1 - k_a)(r_2 - k_a)}{D_o} + \frac{Ar_1(k_a - r_1)(r_2 - r_1)}{k_a D_o}\right)\frac{1}{(r_1 - k_a)} \qquad (6.5)$$

$$k_{21} = \frac{\dfrac{C(r_1 - k_a)(r_2 - k_a)}{D_o} + k_a k_e}{k_e} \qquad (6.6)$$

The constant k_{12} was found by substituting values of k_{21}, k_e and r_2 into equation (6.7).

$$r_2 = \tfrac{1}{2}(k_{12}+k_{21}+k_e - \sqrt{(k_{12}+k_{21}+k_e)^2 - 4.k_e.k_{21}} \qquad (6.7)$$

Equation (6.7) was derived from the equation for r_2 generated in Chapter 4, Section 4.4.

Table 6.6. Assigned and graphically estimated values of r_1, r_2 and k_a.

Table 6.6a. Assigned values of parameters.

Simulation Set	r_1	r_2	k_a units^{-1}
1	2.15	0.35	0.10
2	2.15	0.35	0.60
3	2.27	0.53	0.60
4	2.40	0.11	0.25
5	2.65	0.15	0.25
6	2.65	0.15	1.00
7	2.72	0.89	0.60
8	3.73	0.27	1.00

Table 6.6b. Graphical estimates of parameters.

Simulation Set	r_1	r_2	k_a units^{-1}
1	0.48	0.29	0.13
2	1.50	0.34	0.70
3	1.01	0.75	0.48
4	0.82	0.10	0.24
5	0.39	0.16	0.26
6	3.73	0.12	1.16
7	12.76	1.46	0.61
8	2.14	0.27	1.20

Plots of cumulative amounts of drug excreted into the urine versus time.

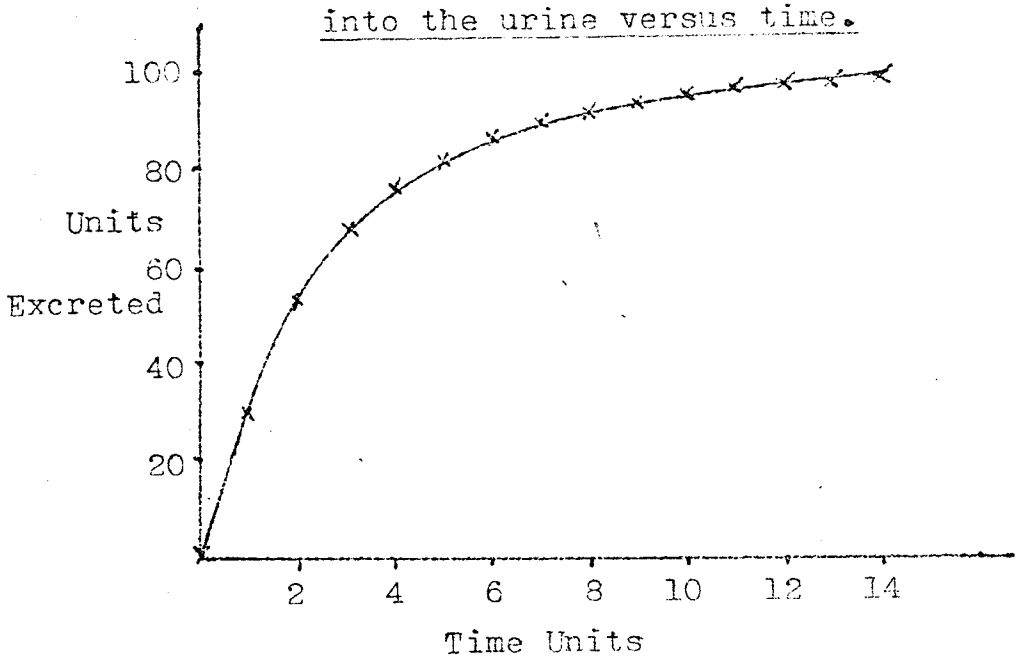


Fig.6.2. Simulation Set 8.

X - indicate original points.

Line indicates computer generated curve.

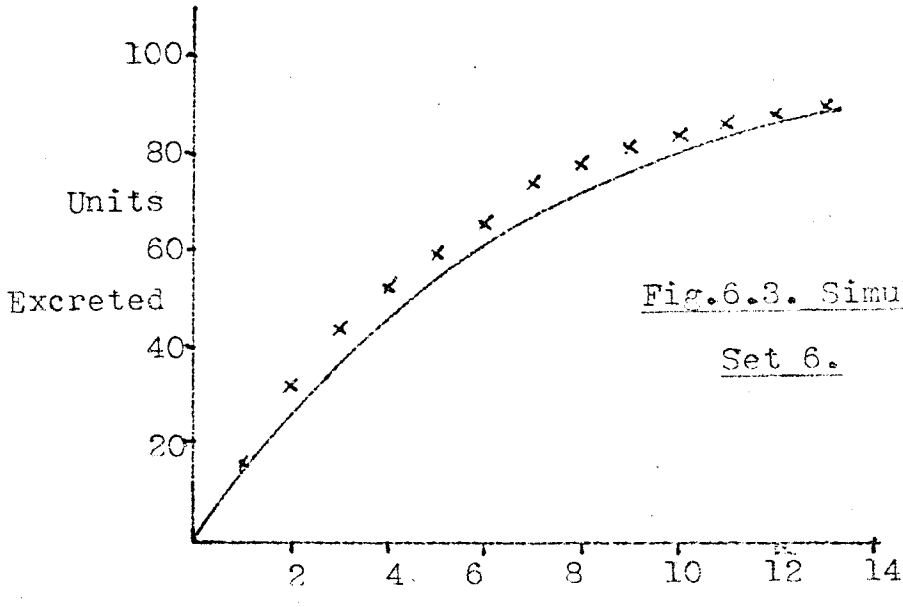


Fig.6.3. Simulation Set 6.

X - indicates original points.

Line indicates computer generated curve.

Graphical estimates of k_a, k_{12}, k_{21} and k_e are listed in Table 6.5b. The errors in k_{12}, k_{21} and k_e reflect the errors in the graphical estimates of r_1.

The graphical estimates of the parameters were used as input for a computer program which incorporated equation 45, Chapter 4, Section 4.4., in the subroutine DFUNC (D_o was made equal to 100 units for this program). By using this procedure it was hoped to generate computed values equal to the original values of the parameters. (Computed values $\pm$ the standard deviations are shown in Table 6.5c.)

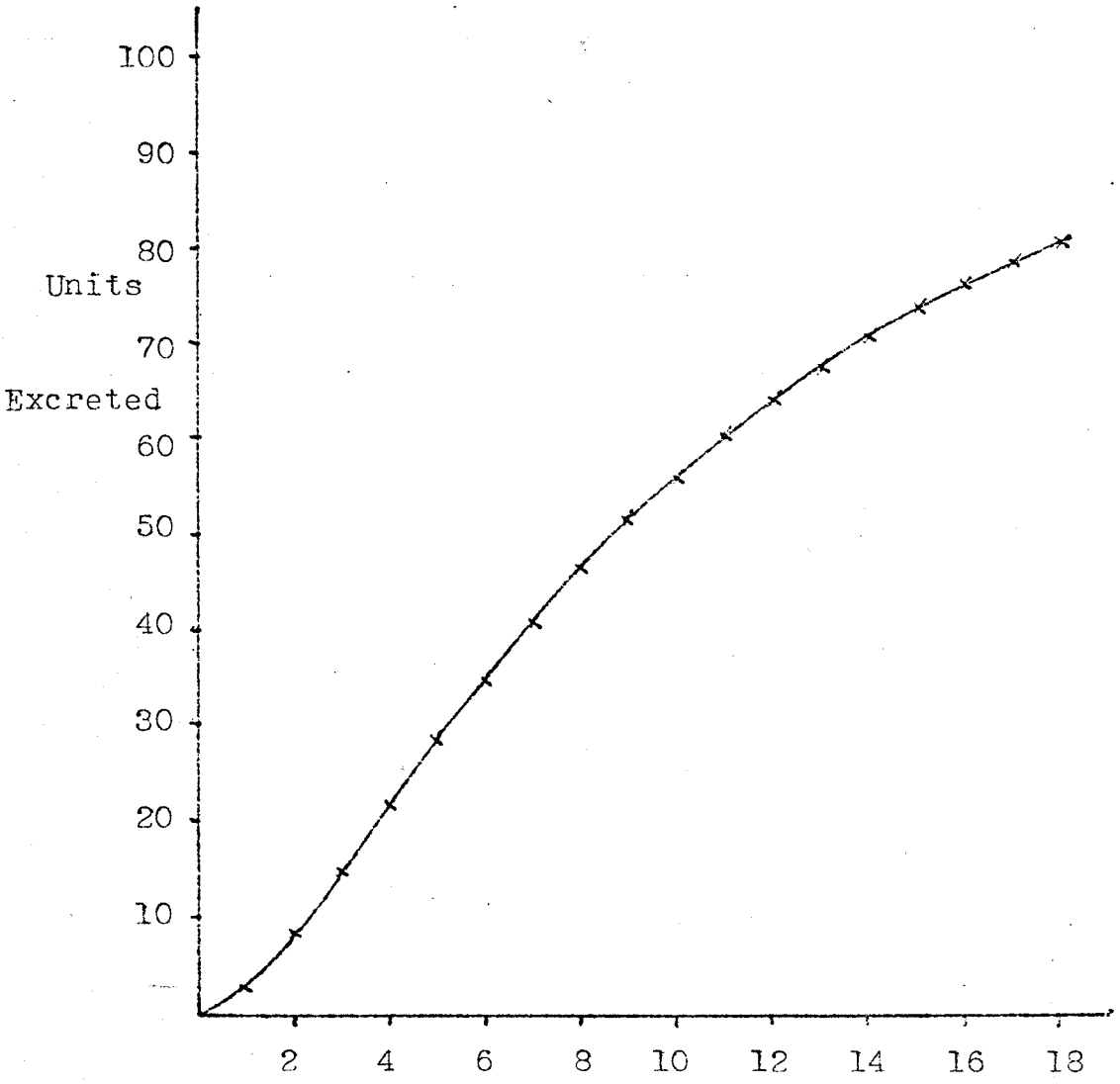
The standard deviations of the parameters were small except in simulation set 6. The original points and computer curve corresponding to simulation set 6 are shown in Fig.6.3. The poor estimates of the parameters and large standard deviations are reflected in the deviation of the original points from the computer curve.

Fig.6.2 shows the original points and computer curve corresponding to simulation set 8. Here, the accuracy of the parameter estimates and the small standard deviations are reflected in the lack of deviation.

The computer program used, employs an iterative technique and twenty iterations are recommended as sufficient for reasonably accurate estimations. In the

Plot of cumulative amounts of drug excreted into the urine versus time. Simulation 4.

(Fig.6.4.)



X - indicates original points.

Line indicates computer generated curve.

above cases, upto sixty iterations were used.

In simulation sets 1, 2, 3, 5 and 7 only reasonable estimates of the original parameter values were obtained.

In simulation set 4, the computer has not drastically changed the input values of the parameters, the standard deviations are extremely small and there is no deviation between the original points (experimental points) and the computer curve.(Fig.6.4.) The computed parameters are, however, grossly inaccurate. In such a case, with experimental data these results would be acceptable in that workers would not know the real values of the parameters. Also, workers tend to have faith in their computer programs and only allow a limited number of iterations. This again could lead to gross inaccuracies.

Chiou and Riegelman(1969) and Jusko and Levy(1970) stated that the constants r_1, r_2 and k_a could be obtained from experimental data as in Fig.6.5. The results cast some doubt on the accuracy of the parameters obtained from experimental curves of a triexponential nature.

Chapter 7.

Summary.

The metabolism and pharmacokinetics of sulfadimidine and the effects of various routes of administration have been studied in the rat. All routes gave similar K values from urinary excretion rate data. An unusual, initial slow excretion rate was noted after I.V. injection and so various experiments were attempted to discover the cause, viz;

1) The dose was increased and decreased to 30mg. and 7.5mg.; showing that there was no apparent saturation of binding sites.

2) The dose was administered in a larger dose volume; showing that there was no crystallisation of the drug in the blood. (The dose was also administered after four hours of water loading to increase diuresis; showing that there was no apparent crystallisation of the drug in the kidney.)

The dose was administered without anesthetic; showing the effect was not due to the anesthetic.

The blood level data gave no indication of the cause of the initial slow excretion rate after I.V. injection. These data also showed that there was very little metabolite in the blood and that the drug obeyed a two compartment open model.

The data were fitted using the "NONLIN" program and the IBM 360/65 digital computer.

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