I

THE PHARMACOKINETIC OF SULPHADIAZINE SODIUM INJECTION IN A RABBIT (INTRAVENEOUSLY) BY SINGLE DOSE

BY

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D E D I C A T I O N

This work is dedicated to my parents Mr and Mrs Aduvaga and the entire Family.

M. A. ADUVAGAH

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M. A. ADUVAGAH

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ABSTRACT

This investigation determined the pharmacokinetic parameters of sulphadiazine following rapid intravenous injection into rabbits. It also determined the type of compartment mode and the effect of administering different doses in increasing order on the pharmacokinetic parameters.

In both cases blood samples were collected at regular intervals after an i/v hours to administration and sulphadiazine analysed in each sample by calorimetric method. It was done for each dose.

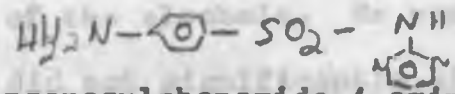
Plot of log concentration of plasma sulphadiazine against time was done to enable determination of pharmacokinetic parameters eg half-life, elimination constant total clerance and apparent volume of distribution and area under the curve. This was also done for each dose.

To establish the effect of increasing dose on pharmacokinetic parameters a plot of area under the curve against dose was done.

Results of these investigation showed that the plasma profile of sulphadiazine in rabbit was best described by one compartment model. It was also established that there was a linear relationship between the dose and the area under the curve suggesting that increase in dose did not have significant effect on the half-life and elimination rate constant.

INTRODUCTION

Sulphadiazine sodium injection is a sterile solution of sulphadiazine sodium in water for injection.



Benzenesulphonamide-4-amino-N-2 Pyrimidyl monosodium salt. It is under the sulphonamides.

A knowledge of the relationship between plasma concentration of a drug and time will provide information about the intensity and duration of pharmacological effects.

The concentration a drug in the body after its administration depends of many factors eg dose route of administration, rate and limitation of absorption, distribution in the body and rate of degradation and excretion(1).

Before carrying out the pharmacokinetic study of sulphadiazine (Sulphonamide) it is important to consider the various factors that influence the observed plasma concentration ie the time course of a drug in thd body is subject to the process shown in the schematic diagram of drug absorption, distribution and elimination.

Absorption:

Except for enteric sulphonamides thic class of drugs is rapidly and adequately absorbed from gastro-intestinal tract. The small intestines is the major site of absobption but some of the drug is absorbed from the stomach.

Absorption of sulphonamides may be influenced by concurrently administered drug or compounds Hayton W.L(2) studied the effect of several alcohols on the absorption of sulphapyridine using 0.5% solution of the alcohols. He was able to show that ethanol did not significantly affect drug absorption. Both butanol and hexanol reduced the rate of absorption of sulphapyridine.

Bioavailability of sulphonamides is also influenced by the dissolution behaviour of the sulphonamides. Mathur et al (3) usihg human subjects investigated the relationship between bioavailability and dissolution behaviour of sulphadiazine significant dissolution was obtained between maximum plasma concentration of sulphadiazine and % sulphadiazine dissolved in 30 mnutes.

Distribution:

Transfere of drug from blood to extravascular fluid and tissues drug distribution is frequently a rapid process having associated with it relatively large constants. It is further characterised by the fact that it is reversable. Sulphonamides in man are distributed throughout all tissues and body fluids. The drug readily enters pleural, peritoneal, peritoneal, synovial and ocular fluids. The protein content of such fluids is usually low and the drug mostly exists in the unbound active form. The sulphonamides readily pass across the placenta and reach the foetal circulation. The concetrations attained in the foetal tissues are

sufficient to cause toxic effects. Goodman L.S and Gilman A (1) determined the foetal blood level of Sulphadiazine to be 50 - 90% of that in the maternal circulation.

PROTEIN BINDING

All sulphonamides are bound in varying degrees to plasma proteins. The extent of binding is almost directly proportional to the concentration of albumin in the plasma: In hypoalbunaemiathe percentage boubd is much decreased (Anton 1968)(4). The bound drug is usually considered to be pharmacologically inactive (5) and can not cross biological membranes; also only the unbound fraction can be eliminated by glomerular filtration. This drug-protein binding may result in a prolonged residence of drug in the body In general acetylated sulphonamides are bound to a greater extent than their corresponding free form. The extend of protein binding that any given sulphonamide exhibit will influence the plasma concentration measured after its administration Levy G et al (6) investigated the effect of serum protein binding on sulphisoxazole distribution, metabolism and exretion in rats. Serum protein binding was found to be a major determinant of the intersubject differences in sulphonamide excretion and biotransformation kinetics.

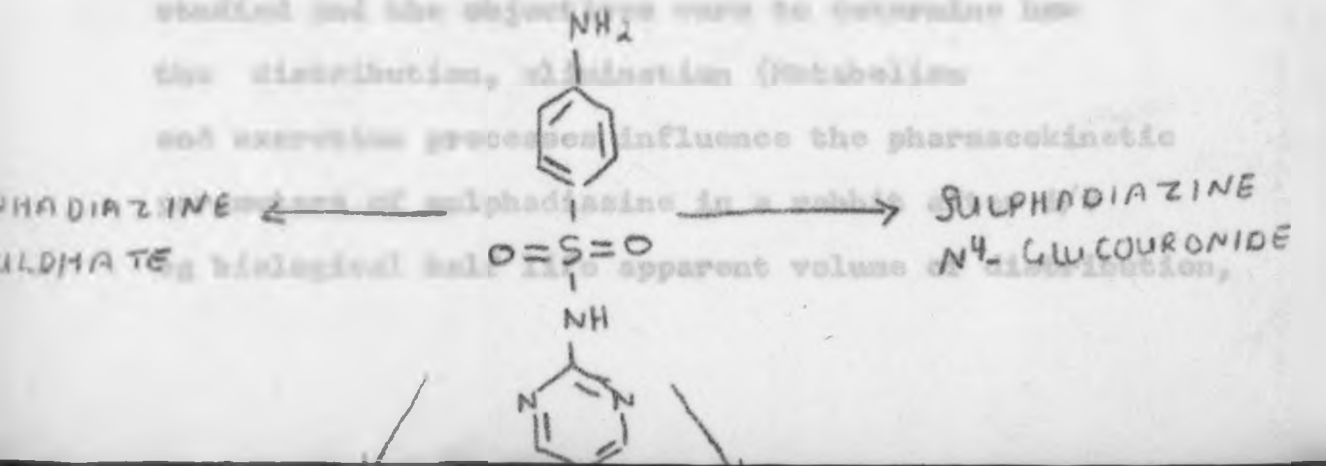
The influence of degree of protein binding on rate of excretion and therefore the frequency of dosage

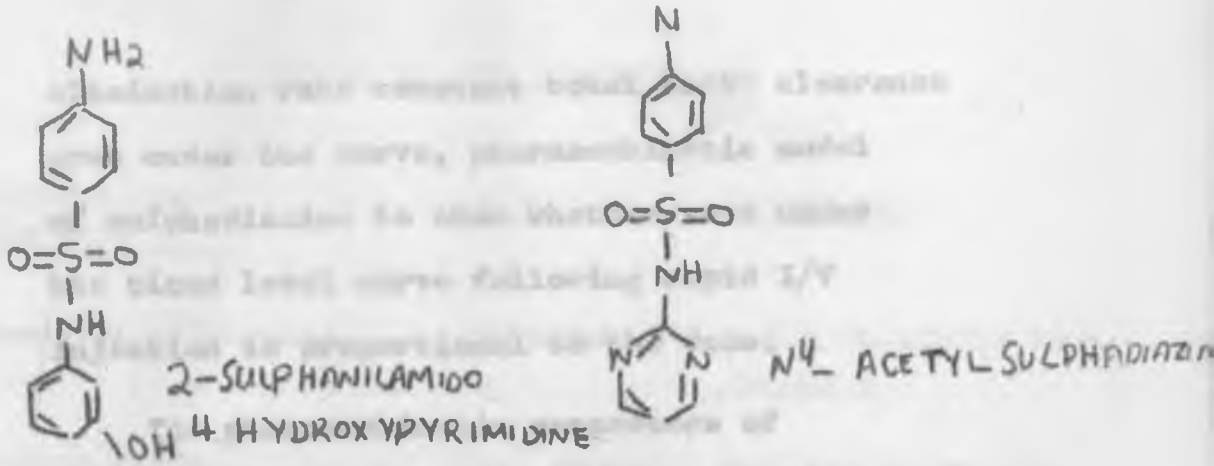
is shown by sulphadiazine (55% protein bound) and sulphamethoxypyridizine (90% protein bound) sulphadiazine is readily excreted in the urine and enters cerebrospinal fluid within four hours of a single dose sulphamethoxypyridi zine has a half life five times longer than the most "soluble sulphonamide(7). It is slowly excreted by kidney.

METABOLISM

The rate of metabolism will also determine the plasma level achieved after administration of a standard dose of sulphonamide. Routes of metabolisms of sulphadiazine in ruminants are acetylation and oxidation. Oxidation is the most importa-nt metabolic reaction (8). The oxidative products of sulphadizine include 2 sulphanilamide u-Hydroxy-pyrimidine while the acetylated products - N acetylsalphadiazine. Other metabolites include sulphadiazine N - sulphate and sulphadiazine N Glucuronide(8) metabolism of sulphadiazine in a goat

METABOLISM OF SULPHADIAZINE IN A GOAT (RUMMINA





Most of the sulphodiazine is eliminated as unchanged sulphadiazine principally by glomerular filtration(8).

Excretion:

The rate of excretion influences the drug plasma level because sulphadiazine is largely excreted in urine mostly as unchanged drug and to a smaller extent as metabolic products shown above. Unchanged sulphadiazine is also the major excretory product in urine.

The relative amount of N^4 - acetyl sulphadiazine excreted in urine seems however to be species depended as Smith and Williams (1948)(9) reported that 75% of Sulphadiazine was N^4-acetylated in rabbit urine while Galligen (1945)(10) found about 30% N^4 acetylated sulphadiazine in human urine.

In the present work sulphadiazine has been studied and the objectives were to determine how the distribution, elimination (Metabolism and excretion processes influence the pharmacokinetic parameters of sulphadiazine in a rabbit after i/v eg biological half life apparent volume of distribution,

elimination rate constant total body clearance area under the curve, pharmacokinetic model of sulphadiazine to show whether area under the blood level curve following rapid I/V injection is proportional to the dose.

The pharmacokinetic parameters of sulphadiazine were estimated by measuring plasma concentration achieved after administration at regular interval of time, the samples were assayed by a calorimetric method for analysis of sulphadiazine.

CHEMICALS AND REAGENTS

CHEMICAL OR REAGENT	GRADE	SUPPLIER
Ammonium sulphamate	Lab.Grade	S.D.S Chemicals Ltd (Bombay)
Sodium Nitrite	Lab. Grade	May and Baker Ltd.
Sodium Oxalate	Lab. Grade	B.D.H Chemicals Ltd
Trichloroacetic acid	Lab. Grade	E. Merck Darmstadt
Sodium chloride	Lab. Grade	B.D.H Chemicals Ltd
Glucose	Lab. Grade	May and Baker
Potassium chloride	Lab. Grade	Hopkins & Williams
Magnesium chloride	Lab. Grade	May & Baker
Sodium bicarbonate	Lab. Grade	May & Baker

Human blood from National Public lab.

GLASSWARE AND OTHER MINOR APPARATUS

1. Conical flasks
2. 0.1ml Pipettes
3. 10ml Burrettes
4. Sterile syringes and needles
5. Centrifuging tubes

EQUIPMENT	SUPPLIER
Bausch & Lomb SP. 21	KLSC Ltd.
Centrifuging machine	MSE Ltd.

EXPERIMENTAL

200mg of Sulphadiazine powder was weighed accurately on analytical balance and dissolved in 0.5N Sodium Hydroxide. Sulphadiazine went in solution as it is a weak acid forming a salt of sodium. The solution was made to one litre with distilled water This was the stock solution from which a series of dilution was made such that the concentration shown below were obtained.

Vol. of stock solution taken (ml)	Concentration of Standard solution (Mg/ml)
0.5	0.01
1.0	0.02
2.0	0.04
3.0	0.06
4.0	0.08

The volumes of stock solution used to make the concentration were made up to 10mls in 10ml volumetric flasks, using human plasma.

The use of plasma was to animilate the experimental conditions. The blood was centrifuged at 9 for 10 minutes from which plasma was obtained.

The assay of sulphadiazine in each sample was done by the method of Ritschel (14).

1:2: Animal preparation

Rabbits weighing between 1.52 - 1.98Kg were used in the blood level kinetics studies.

The rabbits were anaesthetised with 1.75g/Kg of urethane and placed on the animal table on which the limbs were tied. The skin between the left leg and midline was cut open to expose a femoral artery which was isolated from femoral vein and femoral nerve. The femoral artery and marginal vein were cunnulated with polythylene tubing. The inserted end of the cannular and the vein and artery were tied together to keep the cannular in vein and artery.

Doses of sulphadiazine injection were administered as bolus through marginal ear the vein catheter. Blood samples were withdrawn from femoral artery cannular at regular selected times after administration, in heparinized centrifuging tubes and centrifuged.

The intraveneous infusion was introduced through the marginal ear vein to minimise chances of the animal getting shock hence die before the experiment ends.

Doses of 0.1Kg/Kg, 0.2g/Kg and 0.5g/Kg of sulphadiazine sodium were administered to two rabbits per dose as an I/V bolus through marginal ear vein catheter. Blood samples 3-5mls were withdrawn from the femoral artery cannular at various time after dosing.

1.3: ASSAY OF SULPHADIAZINE

This was done by a variation of the method of Ritschel (14) as follows:

To a mixture of 2.0mls of plasma, 30mls of water (distilled) was added in a 50ml conical flask. The plasma and water were mixed by shaking gently and leaving them for 2 minutes. 8.0mls of Trichloroacetic acid solution was added using 10ml burrette and the mixture shaken vigorously and intermittently for 5 minutes. The precipitated proteins was filtered. Precipitation of proteins was eventually to release any of the sulphadiazine that was bound to it.

10mls of the filtered solution was transfered to a suitable container (100ml conical flask) using a 10ml burrette. 1.0ml of sodium nitrite solution was added using 1.0ml pipette, shaken and left for 3 minutes. 1.0ml of ammonium sulfomate

solution was added to destroy the excess nitrous acid and the solution shaken intermittently for 2 minutes and then 1.0ml of 0.05% W/V N-(1-naphthyl) ethylene diamine dihydrochloride was added using a 1.0ml pipette and the resulting solution shaken.

The colour was fully developed after 5 minutes. The optical density was measured at 545 nm on SP 21 in Klinnex tubes. The spectronic 21 was standardized by a blank which was of the same composition as the above solutions except there was no drug. The results are given in Chapter 2 (Page).

RESULTS

2.1: Values used to plot the standard curve shown in Fig 1.

The following table shows values for optical density and concentration of sulphadiazine sodium used to plot the standard curve (Fig I). for determination of sulphadiazine in plasma.

Table 1

The Concentration of Sulphadiazine mg/ml	Optical density at 545nm on SP 21
0.01	0.06
0.02	0.14
0.04	0.29
0.06	0.41
0.08	0.53

2.2: Results of the determination of Sulphadiazine in Plasma of rabbits

Tables 2 - 6 show the concentration of Sulphadiazine sodium attained in plasma at various times after drug administration in five rabbits with different doses i.e two rabbits per dose.

NB the concentrations were read directly from the standard curve after determining the optical density for each sample.

0.2
0.4
0.6
0.8
1.0
1.20
1.40
ABSORBANCE
STANDARD CURVE

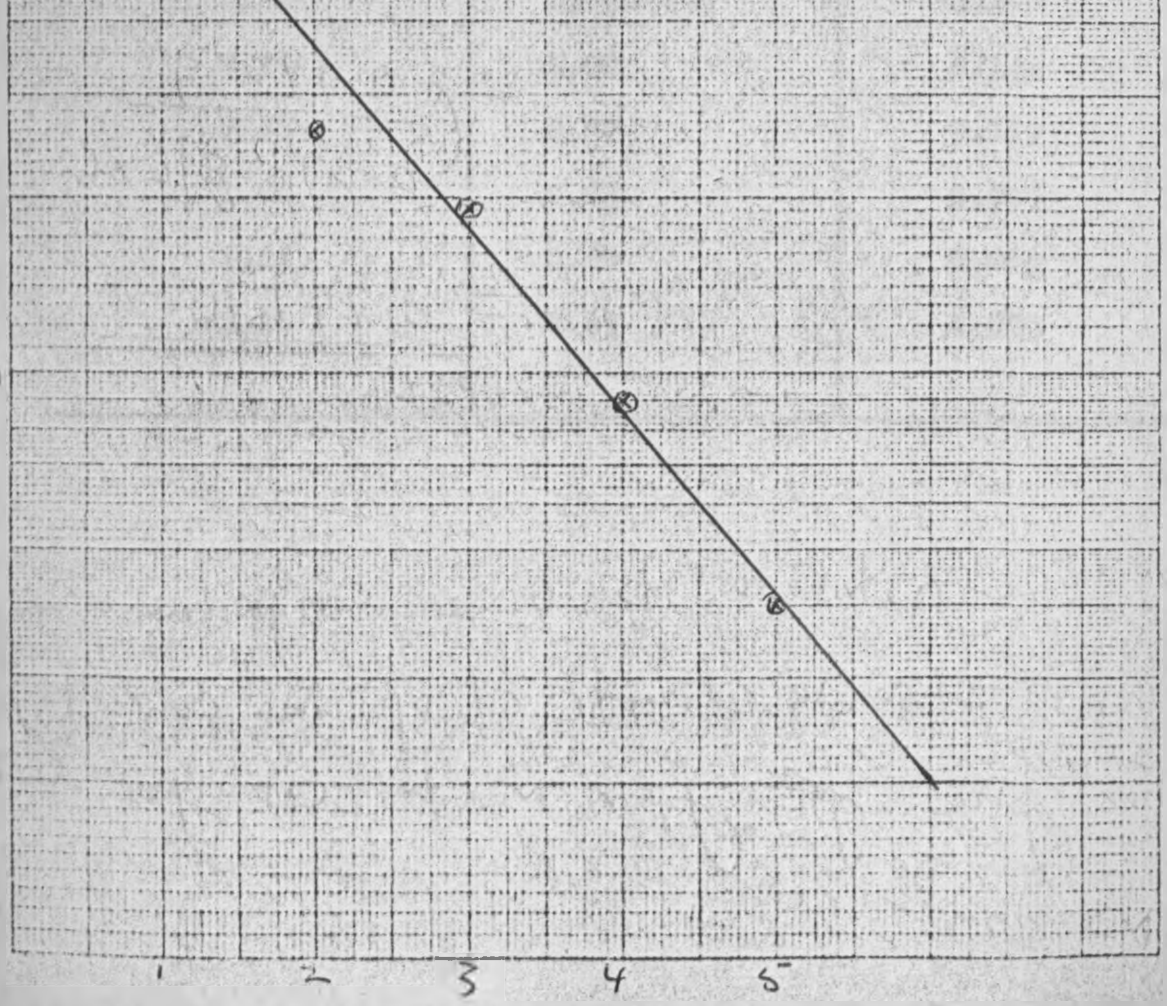
Table 2

Dose 0.1g Kg

Rabbit: One

Weight 1.82Kg.

Time after Drug Administration(Hrs)	Optical Density at 545nm	Conc. of Sulphadiazine Mg/ml
0.17	1.30	0.190
0.25	0.98	0.140
0.33	0.70	0.105
0.58	0.66	0.949
0.75	0.56	0.079
1.00	0.40	0.057
2.00	0.19	0.026
3.00	0.13	0.019
4.00	0.06	0.009
5.00	0.045	0.004


1
2
3
4
5
TIME (HOURS) →

RABBIT ONE: WEIGHT = 1.82 kg

DOSE = 0.1 g/kg ⇒ 0.182 g given.

P.T.O

Table 3

Dose: 0.1g/Kg

Rabbit: Two

Weight 1.521Kg

Time after drug administration(Hrs)	Optical Density at 545nm	Conc. Sulphadiazine Mg/ml
0.083	0.825 (2)	0.244
0.33	0.650 (2)	0.192
0.50	1.00	0.144
0.83	0.80	0.119
1.00	0.65	0.096
1.25	0.60	0.089
1.50	0.49	0.072

Rabbit two
weight 1.521 Kg.
dose 0.1g/Kg.

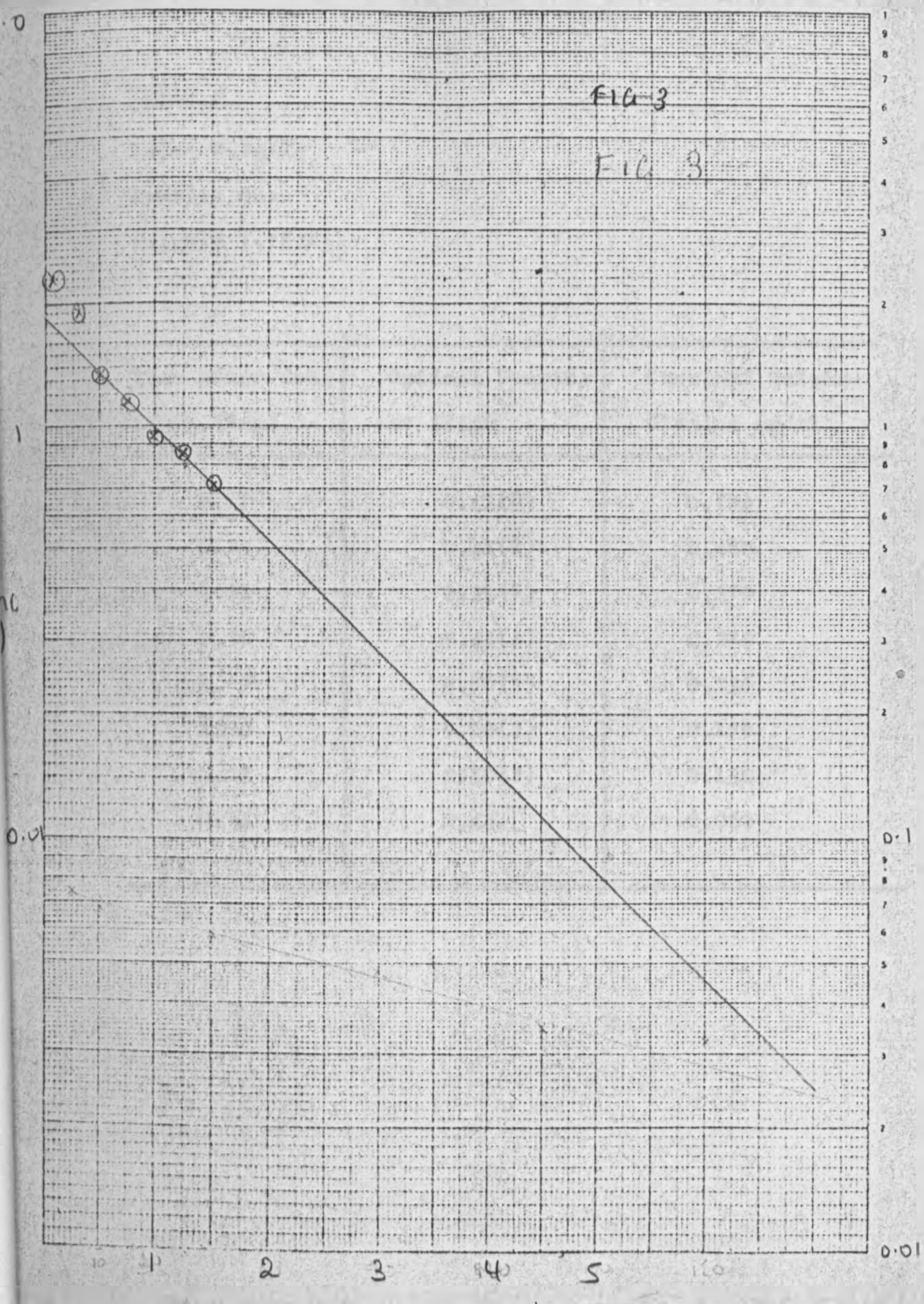


TABLE 4

Dose: 0.2g/Kg

Rabbit: One

Weight: 1.782Kg.

Time after drug admin.(Hrs)	Optical Density at 545nm	Conc. of Sulphadiazine mg/ml
0.17	0.43(8)	0.504
0.25	0.41(8)	0.480
0.33	0.31(8)	0.370
0.50	0.44(4)	0.270
0.67	0.80(2)	0.230
1.00	0.60(2)	0.170
1.25	0.50(2)	0.150
2.25	0.44	0.062

Rabbit ONE WEIGHT = 1.782 Kg.
DOSE 0.29/Kg ⇒

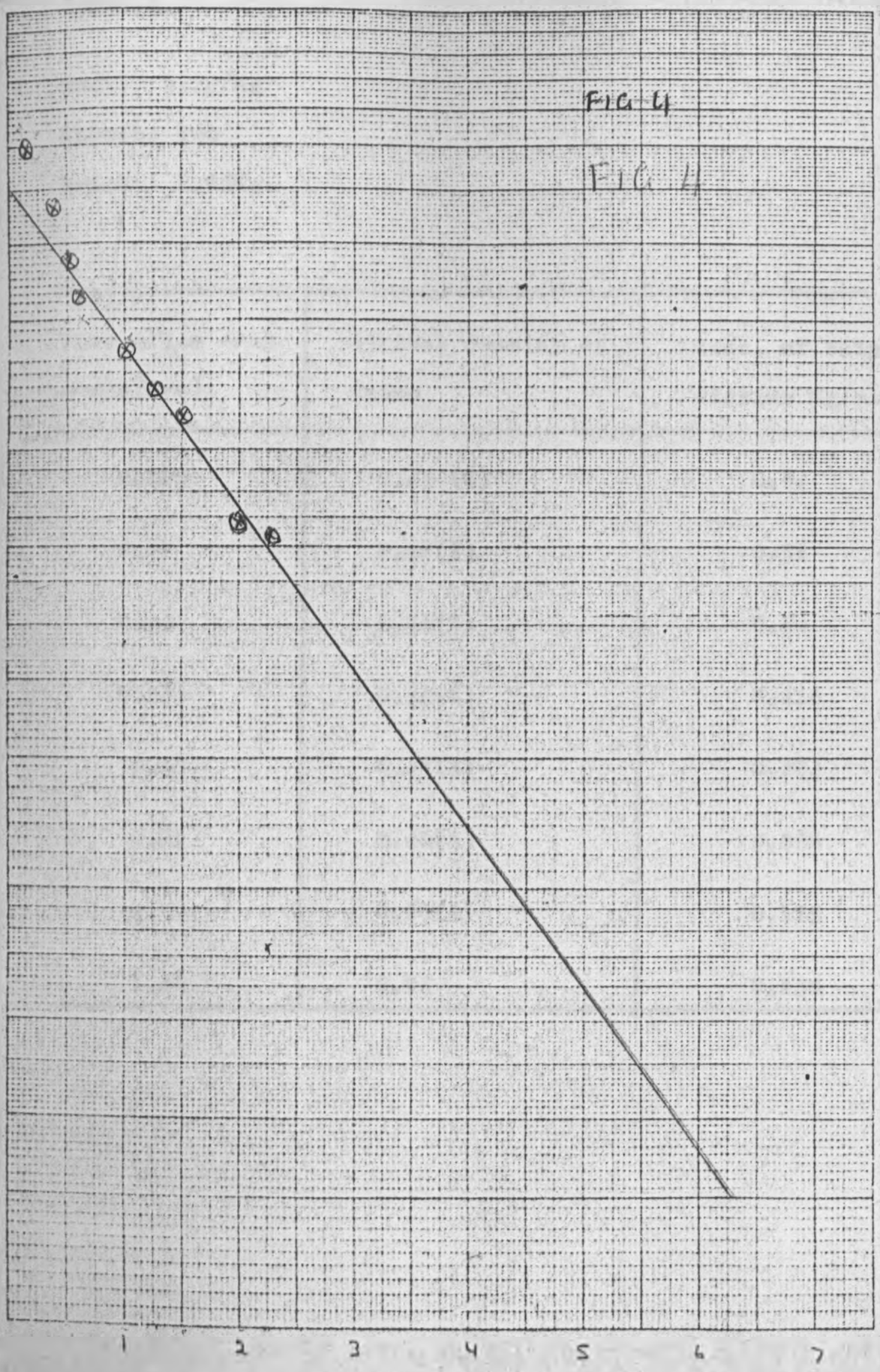


TABLE 5

Dose: 0.2g/Kg

Rabbit: Two

Weight: 1.63Kg.

Time after drug admin(Hrs)	Optical density at 545nm	Conc. of Sulphadiazine Mg/ml
0.083	0.45 (8)	0.64
0.17	0.48(8)	0.56
0.25	0.46(8)	0.54
0.33	0.35(8)	0.416
0.50	0.48(4)	0.284
0.67	0.40(4)	0.236
1.00	0.60(2)	0.175
1.50	0.95	0.130

Rabbit TWO WEIGHT = 1·63Kg
DOSE = 0·2g/Kg.

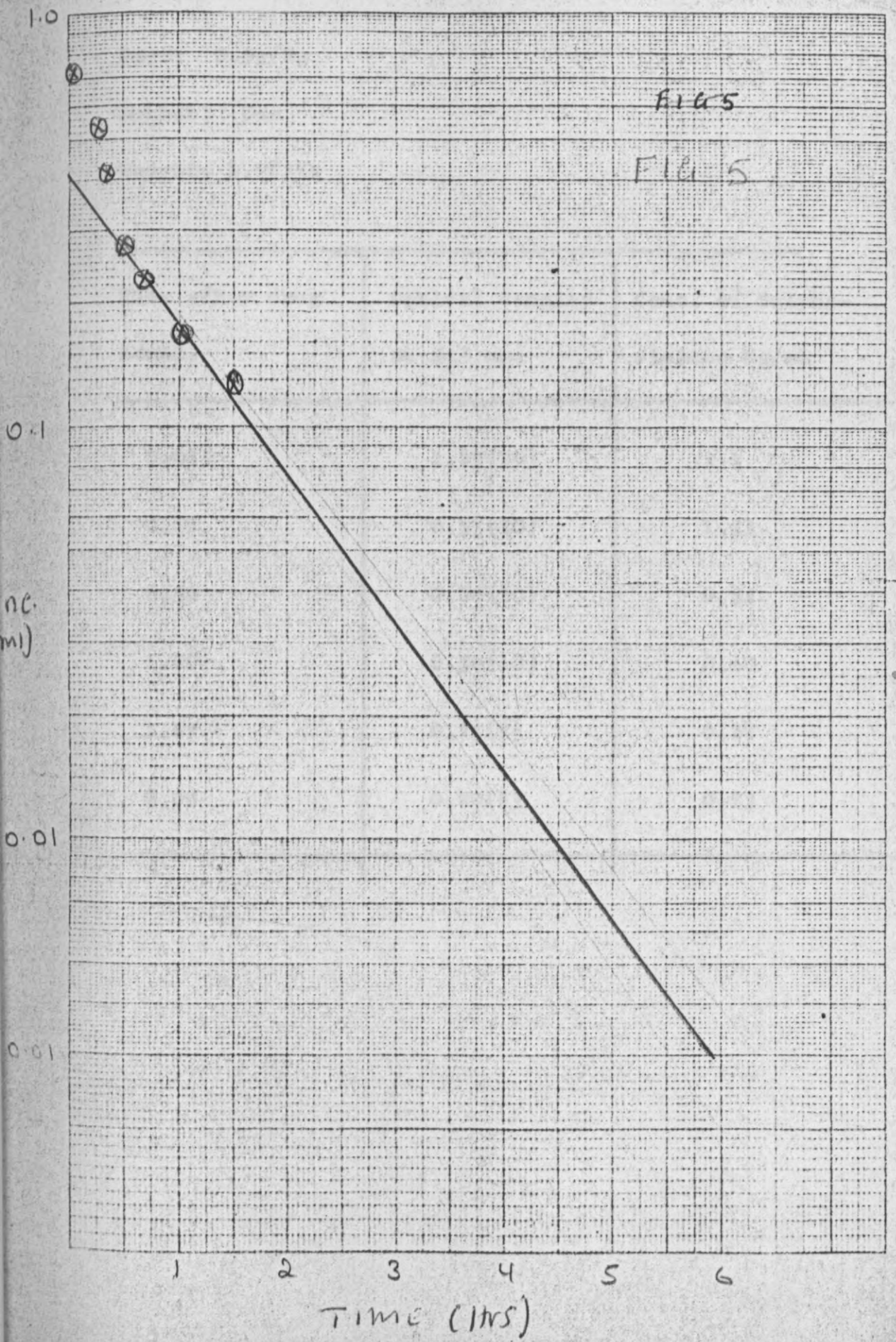


FIG 5

FIG 5

Dose: 0.5g/Kg

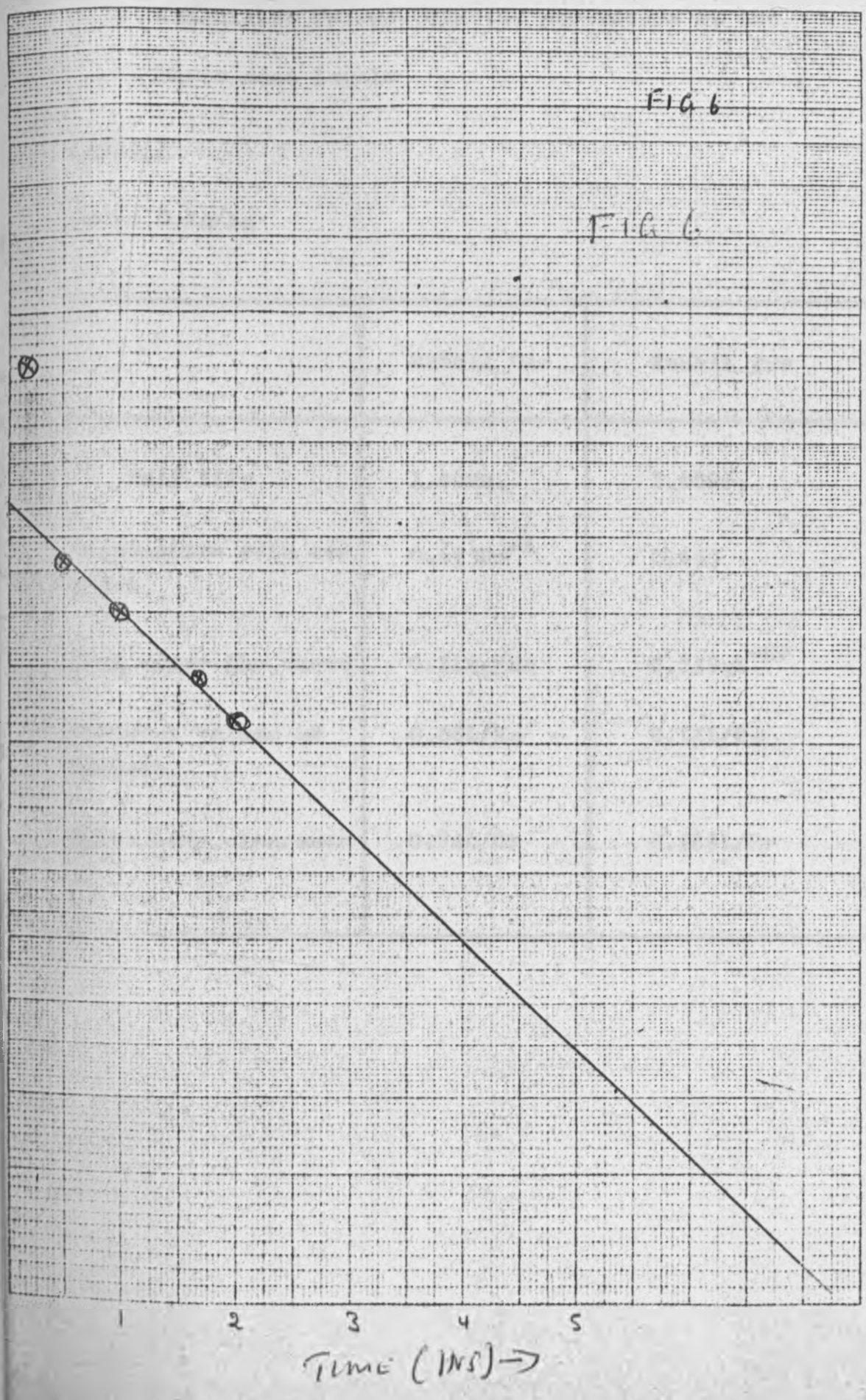
Rabbit: One

Weight: 1.983Kg

Time after drug admin.	Optical density at 545 nm.	Conc. of Sulphadiazine Mg/ml
0.083	1.25(20)	3.1
0.17	0.55(20)	1.54
0.50	0.20(20)	0.54
1.00	0.30(10)	0.40
1.67	0.25(8)	0.28
2.00	0.20(8)	0.23

Rabbit ONG. Weight - 1.983 kg.
Dose 0.5 g/kg.

FIG 6



2.3: Calculated Pharmacokinetic parameters at the three dose levels

Table 7

Dose: 0.1g/Kg

	Rabbit One	Rabbit Two
Half life	1.00Hr.	1.00Hr.
Elimination rate constant	$0.693Hr^{-1}$	0.693
Area under the curve	0.216g/Hr	$0.259gL^{-1}Hr$
Apparent volume of Distrib.	0.66L/Kg	0.55L/Kg
Total body clearance	0.84L/Hr	0.589L/Hr

Table 8

Dose: 0.2g/Kg		
	Rabbit One	Rabbit Two
Half Life	0.87Hrs	0.81
Elimination rate constant	$0.796Hrs^{-1}$	$0.760Hr^{-1}$
Area under the curve	0.502g/LHr	0.504g/LHr
Apparent volume of Distrib.	0.50L/Kg	0.49L/Kg
Total body clearance	0.71L/Hr	0.76L/Hr.

Table 9

Dose: 0.5g/Kg

	Rabbit One	Rabbit Two
Half life	1.3Hrs	
Elimination rate constant	$0.53Hr^{-1}$	
Area under the curve	1.32g/LHr	
Apparent volume of Distrib.	0.674Kg	
Total body clearance	0.846L/Hr.	

Table 10

Table for doses VS corresponding average area under curve

Dose (g/Kg)	Average area under the curve
0.1	0.24
0.2	0.51
0.5	1.32

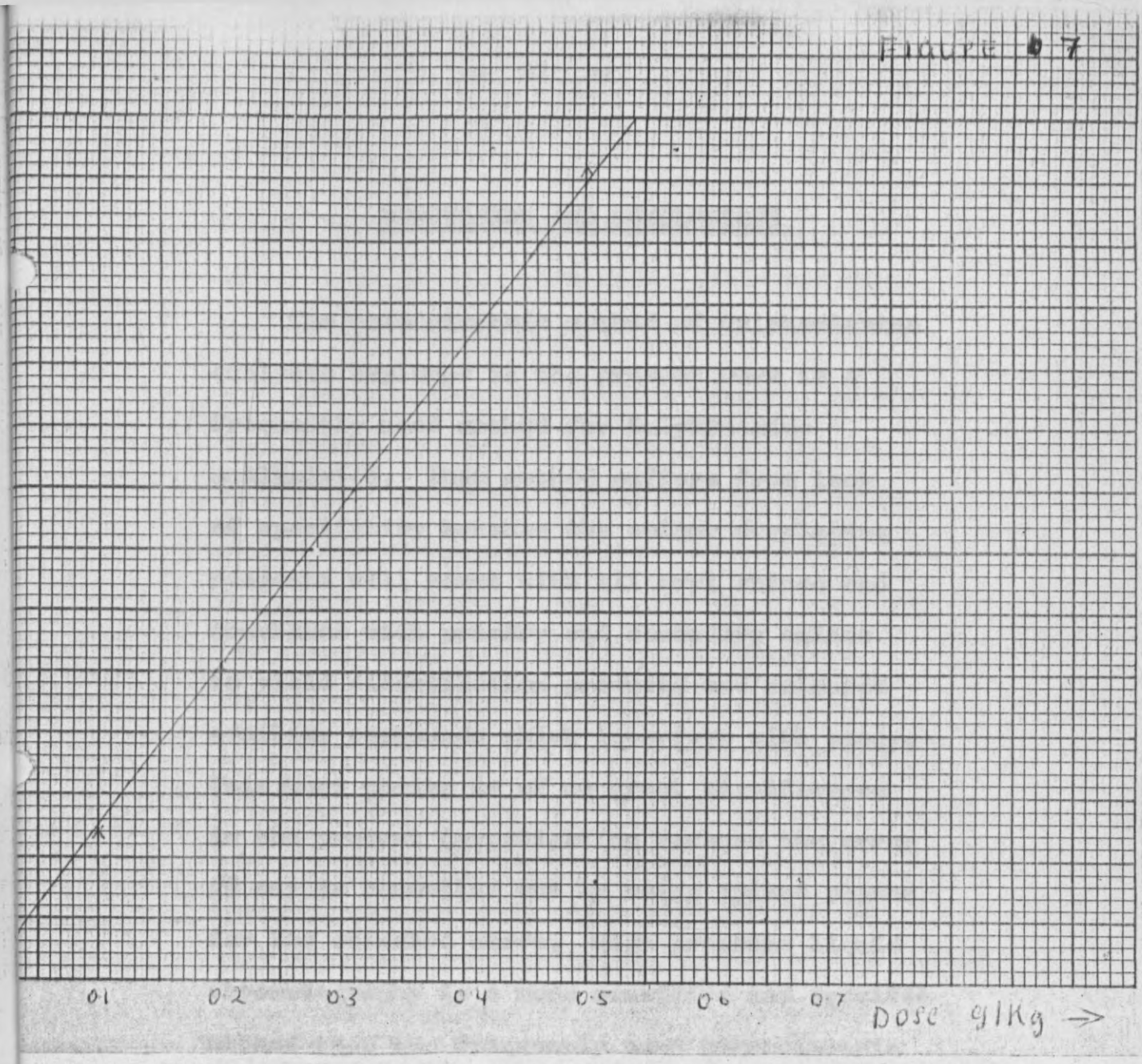


Figure 6.7
0·1
0·2
0·3
0·4
0·5
0·6
0·7
Dose g/kg →

DISCUSSION AND CONCLUSIONS

The colourimetric method of Sulphadiazine analysis employed in the present work is a frequently used method for Sulphonamide analysis(6). This method suffers from lack of specificity because the colour developing reagents will react with all aryl amines and sometimes with primary and secondary amines to yield diazotization products and coloured addition compounds which interfere with assay. This short coming is of no great significance in the present investigation because the error if any is cancelled out by using spiked plasma for the standard curve. High pressure liquid chromatography is a more sensitive and specific method than the frequently used colourimetric methods.

In this study arterial blood rather than venous blood was used. In conventional pharmacokinetic analysis no arterial venous differences was assumed (11) and therefore no significant differences were expected from the calculation of pharmacokinetic parameters based on either arterial or venous plasma data. However arterial venous differences (12) showed that the calculated

steady state volume of distribution and body elimination profile were quite different from the predication based on conventional theories.

The use of arterial blood samples rather than venous was due to the fact that it was easier to collect blood from the artery as it gushed out under greater positive pressure from the heart. Arterial plasma levels are prefered for correlation with their pharmacological activities since it has been shown that the arterial blood and not the peripherol venous blood carries a drug to different parts of the body for distribution and elimination (13). Moreover it has been shown that the use of instanteneous input principle as implied in central compartment analysis to calculate total venous plasma area (AUC) would result in an overestimation. This aspect has been recently evaluated for several drugs.

Examination of the first-order plot of plasma level data on the graphs figure (2-6) at the three different doses (0.1g/Kg, 0.2g/Kg and 0.5g/Kg) showed that the alpha phase was almost non existent i.e the distribution of the drug throughout the body was very rapid relative to the rate of elimination ie one compartment model was evident at the three doses.

Allowing for small variation in half life among the different rabbits used in the experiment, since individual variation affects half life among other factors, it was found that the half life range 0.81 - 1.3 Hrs (average 0.99 Hrs) was fairly constant so that increase in dose did not have much significant effect. This is proved further by the linear relationship between area under the curve against dose (Figure 7).

Figure 7 shows a plot of dose against area under the curve to determine whether Sulphadiazine manifests dose dependent elimination. The linear relationship between the area under the curve and the dose conforms that it was not dose dependent. Elimination at least within the dose range used (0.2 - 0.5g/Kg). Drugs which manifests dose-dependent elimination present considerable problems in therapeutics management because steady state concentration change disproportionately with changes in dose.

The apparent volume of distribution ranges between 0.49 - 0.67L/Kg with an average of 0.57L This suggest that the drug was fairly uniformly distributed in total body water.

CONCLUSION

The biological half life of Sulphadiazine in rabbit was found to vary between 0.81 - 1.3 Hrs. In man biological half life varied between 8 - 16Hrs (15). It means the rate of elimination of Sulphadiazine in rabbits is far much greater than in humans.

The apparent volume of distribution of the rabbit (0.49 - 0.67L/Kg) with average of 0.57L/Kg compared well with that in the dog (0.50 - 0.65) with average of 0.52(16) ie the extent of Sulphadiazine distribution in both the rabbit and dog was similar.

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