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ESTABLISHMENT OF PHARMACOKINETICS PARAMETERS THROUGH COMPARTMENTAL ANALISYS OF GENTAMICIN SULPHATE LABELLED WITH ^{99m}Tc. *

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ABSTRACT

Gentamicin sulphate is an aminoglycoside antibiotic type specifically used for treatment of infections produced by gram-negative bacterias but at the other hand it presents ototoxic reactions as a serious side effect. The main purpose of labelling gentamicin with ^{99m}Tc was to obtain a radioactive tracer for compartmental analysis of this antibiotic. The plasma decay curve of ^{99m}Tc gentamicin was obtained and the half-lives calculated. Furthermore the apparent volume of distribution was determined and the residual radioactivity in the body determined too, the biological half-life and total drug clearance were obtained. The distribution of ^{99m}Tc gentamicin in rats was set in a two-compartments in addition to a retention one for the 24 hours time interval studied.

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* Paper presented at "5th Congress of the World Federation of Nuclear Medicine and Biology" - August 26-31, 1990 - Montreal - Canadá.

ESTABELECIMENTO DE ALGUNS PARÂMETROS FARMACOCINÉTICOS COM O AUXÍLIO DA ANÁLISE COMPARTIMENTAL DO SULFATO DE GENTAMICINA MARCADO COM ^{99m}Tc.*

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RESUMO

Sulfato de gentamicina é um antibiótico do tipo aminoglicosídico usado especialmente contra as infecções causadas por bactérias Gram-negativas, e que apresenta a ototoxidez como efeito colateral mais grave. A marcação da gentamicina com ^{99m}Tc, tem por finalidade propor um traçador radioativo para análise compartimental deste antibiótico. Dileneou-se a curva de decaimento plasmático de gentamicina ^{99m}Tc, calculando-se então, as respectivas meias-vidas. Foram determinados também, o volume de distribuição aparente, a atividade residual do corpo, a meia-vida biológica do corpo inteiro e o valor da depuração total da droga. O modelo de distribuição da gentamicina ^{99m}Tc em ratos, se constitui num modelo compartimental com um de retenção para o intervalo de 24 horas em que foi estudado.

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* Trabalho apresentado no "5th Congress of the World Federation of Nuclear Medicine and Biology" - de 26 a 31 de agosto de 1990, em Montreal - Canadá.

I. INTRODUCTION.

The gentamicin sulphate is an antibiotic from the aminoglycoside type derived from the microorganisms of the Micromonospora Purpurea.

This antibiotic is mainly used for the treatment of severe infections produced by Gram-negative bacterias, some of these infections are: bacteremia, meningitis, pneumonia, otitis and piritonitis.

The most important and dangerous colateral effect of gentamicin is the irreversible ototoxicity due to progressive destruction of the vestibular sensory cells of the internal ear, highly sensible and of the acustic system having extention to the cochlear cells.

The main purpose of this work are:

a. Determinations of the half-life, the apparent volume of distribution and the total clearance drug.

b. Proposal of pharmacokinetics model to determine the gentamicin fraction transferred from a compartment to another one by time unit.

II. MATERIALS AND METHODS

II.1 - Reagents.

Gentamicin Sulphate: Sigma Chemical Company, USA
Urethane p.a. : Merck, Germany
"Liquemine" : 5000 ul/ml of heparin: La Roche, Brazil
^{99m}Tc was obtained by IPEN-CNEN/SP Generator: Brazil

II.2 - Methods.

14 groups of 6 rats each was used to study the uptake of gentamicin sulphate labelled with ^{99m}Tc by the organs, muscle and also for the evaluation of the plasmatic levels.

Initially, the rats were weighted and anesthetized with urethane (100 mg/100g weight). Samples with 0.075 ml of ^{99m}Tc-gentamicin solution were adjust to a pH 6,5 with a saline solution 0.9%. The specific activities of these samples were between 7992 and 11692 Kq/mg. These samples were injected in the penis dorsal arteria of each animal. Followed that, blood samples of 2 ml each were obtained by cardiac output after 3; 10; 15; 20; 30; 45; 60 and 90 minutes and also after

2; 3; 4; 6; 16; 20 and 24 hours. The blood were collected in glass flasks with liquemine. Part of these samples were used for hematocrit determinations. Plasma were obtained by centrifugation (2.500 rpm for 30 minutes) counted after volume determination.

The radioactivity measurements were performed and calculated in relation to the standard that had the same geometry of the counting system and counting time.

II.2.1 - Measurements in the whole body.

These measurements were performed in groups of 5 rats each, treated as described and having the same specific activities. These rats were holding in a metabolic cages and the radioactivity measurements were made after 1; 3; 4; 6; 30 and 24 hours.

II.2.2 - Computer Program Used For Statistical Studies.

These studies were based in mathematical analysis using a IBM/4341 computer and a SAS, CSMP Program[6,9].

III. RESULTS.

The experimental plasma data given in % of dose per ml, numerically adjust by the SAS Program[6], can be analytical handle by an exponential expression of two terms, such as:

$$P(T) = A_1 E^{-B_1 T} + A_2 E^{-B_2 T} \qquad (I)$$

VARIANCE ANALYSIS

Variance sources	L.G.	Sum Square	Mean Square
Model	4	107.8780	26.9695
Residue	10	0.2930	0.0293
Total (No corrected)	14	108.1710	
Total (Corrected)	13	79.3705	

PARAMETERS ESTIMATION

Parameter	Estimated Value	Standard Desviation	Confidence Interval(95%) Low Limit	High Limit
A_1	14.7821	0.5044	13.6583	15.9059
A_2	0.6866	0.1109	9.4395	0.9337
B_1	0.1725	0.0095	0.1514	0.1936
B_2	0.0021	0.0011	0.0004	0.0046

With these experimentals values, we have the curve.

$$P(T) = 14.7821 \quad E^{-0.1725T} + 0,6866 \quad E^{-0.0021T} \qquad (II)$$

At T=0, the initial concentration Co is:

$$Co = A_1 + A_2 = 14.7821 + 0.6866 = 15.468\% \text{ /ml}$$

DECAY ANALYSIS OF THE PLASMATIC CURVE OF ^{99m}Tc GENTAMICIN.

It was verified that the plasmatic curve had two exponential terms, which $T_{1/2}$ can be calculated from the expression (II)

$$T_{1/2} = \frac{1_N 2}{B_1} = \frac{0.693}{0.1725} = 0.07 \text{ hours}$$

$$T_{1/2} = \frac{1_N 2}{B_2} = \frac{0.693}{0.0021} = 5.5 \text{ hours}$$

DETERMINATION OF THE APPARENT VOLUME DISTRIBUTION.

It was determined from the definition.

$$V_D = \frac{DOSE}{Co}$$

$$V_D = \frac{100}{15.4687} = 6.4647 \text{ ml}$$

$$V_D = 6.4647 \text{ ml}$$

HALF-LIFE DETERMINATION.

The interpretation of the experimental data of the whole body measurements were performed using the SAS Program[6] with the following fitting model: l_N-Bt.

VARIANCE ANALYSIS.

Variance Source	L.G	Sum Square	Mean Square	Value F.	Prob > F
Model	1	0.5849	0.5849	48.580	0.200
Residue	2	0.0241	0.0120		
Total (Correct)	3	0.6090			

Square of the correlation coeficient R^2 = 0.9605

R^2 is defined as the quotient between the squares of the model and the residue.

PARAMETERS ESTIMATION

PARAMETER	ESTIMATED VALUE	STANDARD DESVIATION	T FOR HO PARAMETER=0	PROB>|T|
$l_N A$	4.2560	0.0773	55.075	0.0003
B	0.0007	0.0001	-6.970	0.0200

For the parameters estimation, the Student test (T) was applied, therefore we have T for HO given parameter = 0, that means that T for Hypothesis test, the parameter equal to zero, the values given in column prob > |T| , mean that when such values were small, the probability that these parameters were different from zero are bigger and therefore they are significant for the choose model.

$$T_{1/2} = \frac{0.693}{B}$$

$$T_{1/2} = \frac{0.693}{0.0007} = 990 \text{ min.} = 16{,}5 \text{ h}$$

DETERMINATION OF THE TOTAL DEPURATION.

The total depuration is given as the product of the total depuration constant (B) times the apparent volume distribution (V_D).

In this way, we have:

$$D^T = B \times V_D$$

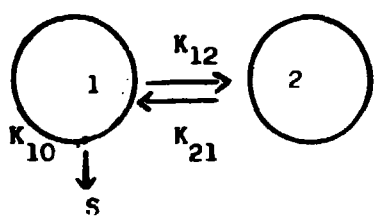
$$D^T = 0.0007 \times 6.4647$$

$$D^T = 0.0045 \text{ ml/min.}$$

COMPARTMENT MODEL FOR GENTAMICIN SULPHATE AFTER INTRAVENOUSLY INJECTION IN WISTAR RATS.

B_1 - COMPARTMENT MODEL

The data of plasma concentration as a function of time were fitted as a sum of two exponential terms. Therefore we have an open model with two compartments[3], esquematically given in Fig. 3.1.



1 → Intravascular Compartment

2 → Extravascular Compartment

S → Exit

Fig. 3.1 - Open model of two compartments.

The determination of the transfer constants K_{12}, K_{21}, and the elimination constant K_{10}, were made using the code SAAM 25[1].

We have:

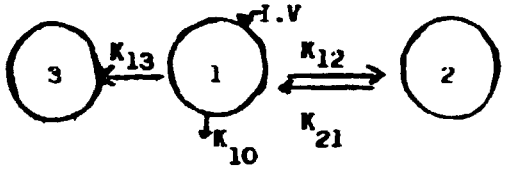
$$K_{12} = 0.1225 \pm 0.0068 \text{ (min.}^{-1}\text{)}$$

$$K_{21} = 0.0113 \pm 0.0007 \text{ (min.}^{-1}\text{)}$$

$$K_{10} = 0.0382 \pm 0.0010 \text{ (min.}^{-1}\text{)}$$

B_2 - COMPARTMENT MODEL WITH A RETENTION ONE.

In fig 3.3 is given a model with a intravascular (1) and extravascular (2) compartments and a retention one[3].



1 - Intravascular Compartment

2 - Extravascular Compartment

3 - Retention Compartment

K_{10} - E. nation Constant

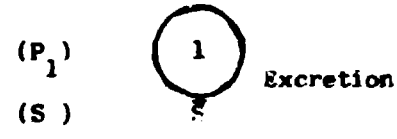
K_{13} - Transfer constant of the drug to the retention compartment (during experimental observation).

Fig. 3.3 - Model of 2 compartments and 1 retention.

The constant K_{10} was calculated with the experimental plasma data and those of the total excretion The last values were obtained from the data of the whole body measurements.

Total Excretion = 100 - % Whole Body

Considering compartment 1 as the direct eliminator of the excretion, we have the following model:



That is:

$$\frac{dS}{dT} = K_{10} \; P_1 \; (T) \quad \text{with } S(T = 0) \qquad (I)$$

Integrating Equation (I) between T=0 and $T=T_1$, we have:

$$S\,(T_1) = K_{10} \int_0^{T_1} P_1\,(T)\,DT \qquad (II)$$

But:

$$P_1(T) = A_1 E^{-B_1 T} + A_2 E^{-B_2 T}$$

Therefore:

$$\int_0^{T_1} P_1(T)\, dT = \frac{A_1 E^{-B_1 T}}{B_1} + \frac{A_2 E^{-B_2 T}}{B_2} \quad \text{(III)}$$

Putting these values in (II), we have:

$$K_{10}\ (T=T_1) = \frac{S(T_1)}{\frac{A_1(1-E^{-B_1 T_1})}{B_1} + \frac{A_2(1-E^{-B_2 T_1}}{B_2}}$$

Using the numerical values already known, we set $K_{10} = 0.0307 \pm 0.0026$ (min.$^{-1}$).

Making used of the "Continous System Modelling Program"[9] there were made several simulations and it was verified, comparing the whole body and excretions curves, that the best fittet values for K_{10} was 0.0285 min.$^{-1}$, this obtained value for K_{10} is contained in the registered interval of the standard desviation.

Through computer data process the following values for the transfer coefficients were obtained:

$K_{12} = 0.1225$ (min.$^{-1}$)

$K_{21} = 0.0113$ (min.$^{-1}$)

$K_{10} = 0.0285$ (min.$^{-1}$)

$K_{13} = 0.0097$ (min.$^{-1}$)

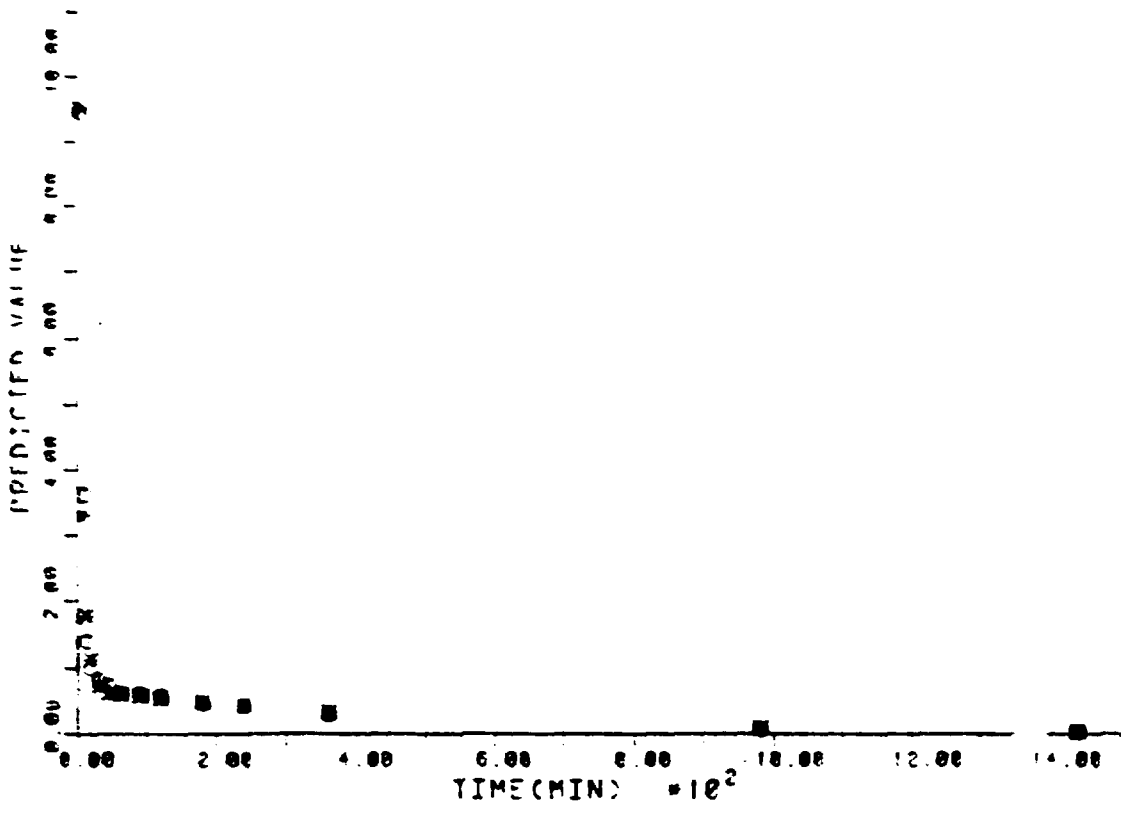


FIGURE 3.5 - RADIOACTIVITY X PLASMA (ML) - STATISTICS ANALYSIS
PREDICTED VALUE X TIME * -> SIMULATED VALUE
@ -> EXPERIMENTAL VALUE

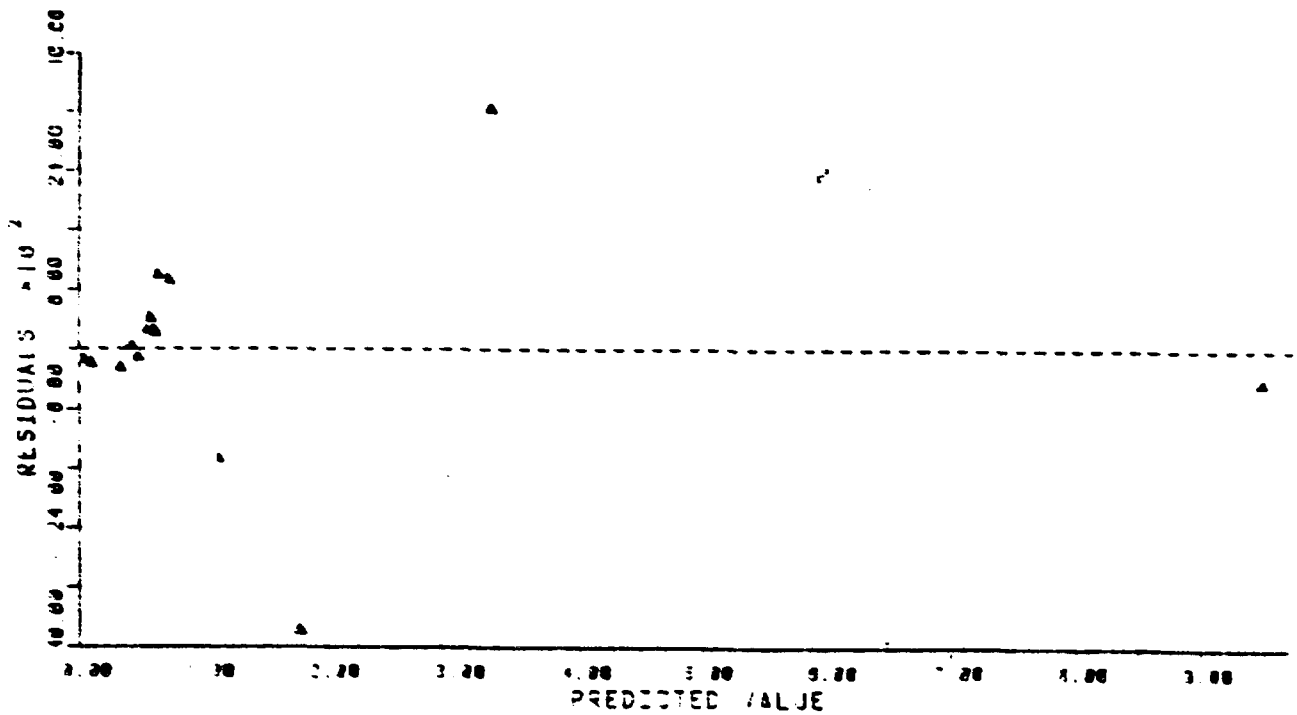


FIGURE 3 5: RADIOACTIVITY X PLASMA (ML) - STATISTICS ANALYSIS
RESIDUALS X PREDICTED VALUE

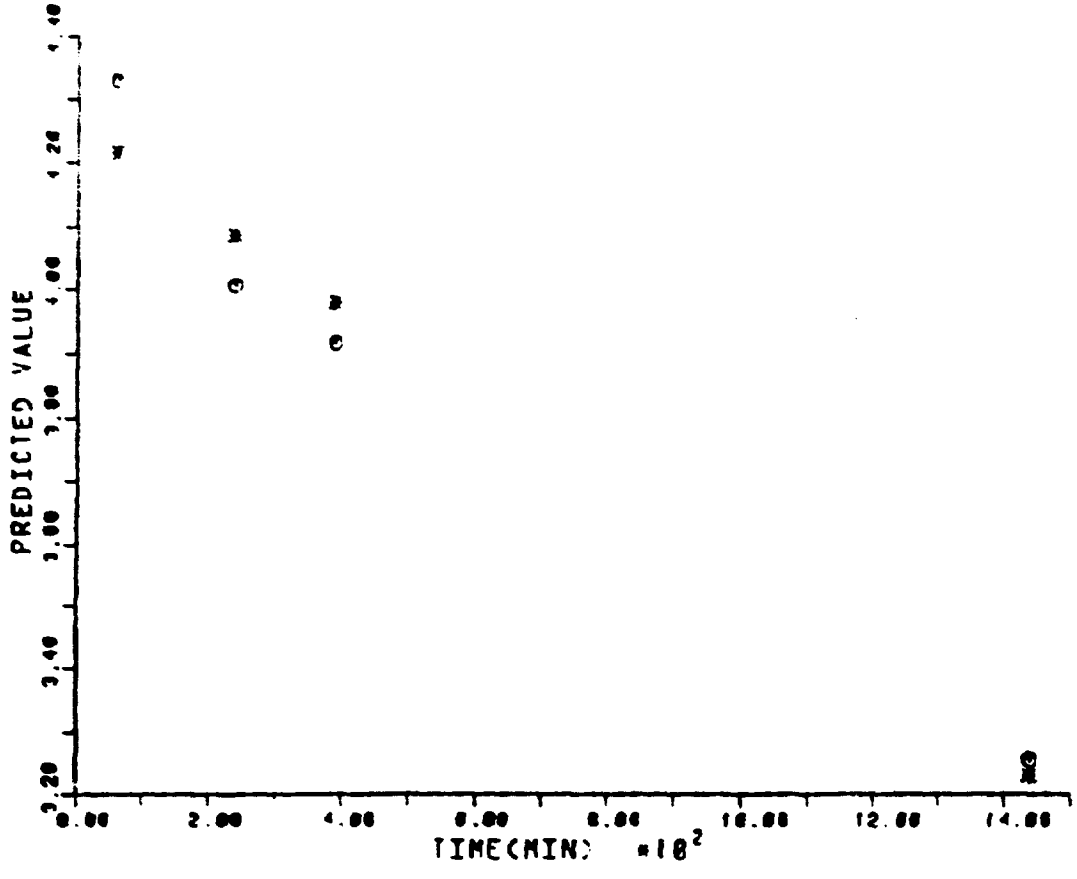


FIGURE 3.7: WHOLE BODY RADIOACTIVITY (% DOSE) - STATISTICS ANALYSIS
PREDICTED VALUE X TIME
* -> SIMULATED VALUE
⊙ -> EXPERIMENTAL VALUE

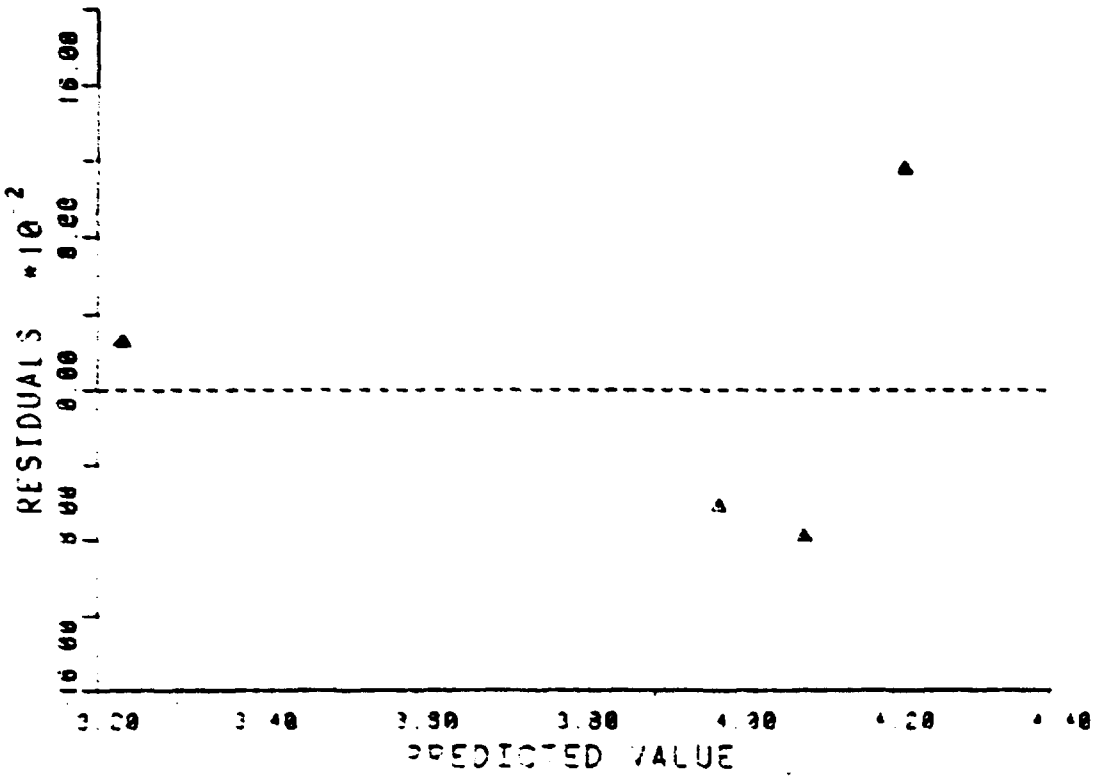


FIGURE 3.8 WHOLE BODY RADIOACTIVITY - STATISTICS ANALYSIS
RESIDUALS X PREDICTED VALUE

IV. DISCUSSION AND CONCLUSION.

PHARMACOKINETICS STUDY OF ^{99m}Tc - GENTAMICIN SULPHATE BY INTRAVENOUSLY VIA.

From the experimental data of plasmatic decay, obtained in the range of 24 hours, we can consider its behavior expressed by a mathematical relation given by the sum of 2 exponential terms, which can be modelled by using two compartments, one the intravascular and the other the extravascular[3].

From the variance analysis, related to the numerical fitting of the experimental data of plasmatic levels, it was established that the sum of the square of the model and of the residue give a well differentiated values: 107.8780 and 0.2930 respectively, which means that the fitting of the experimental data and the model choose was satisfactory. In Fig. 3.5, we can see the good agreement of 10 values of the simulated and experimental data of the plasmatic levels as a function of time. Furthermore, in Fig. 3.6, shows a satisfactory stastical distribution of the experimental values of the residues.

The apparent volume distribution was calculated from the curve of plasmatic levels of ^{99m}Tc gentamicin sulphate. The value obtained experimentally, for a normal Wistar rat of 275 grams. This means that in the first stage the drug, after it was applied, had an uniform distribution all over the animal organism.

The Fig. 3.7 and 3.8 show the given values of whole body in relation with time and the residues respectively.

These curves give the values used in the variance analysis (Fischer Test or T Test)

From these tests we get for R^2, the value 0,9605. This means that about 100% of the data are explained by the model.

The total depuration of the drug in the organism was determined to be 0,0045 ml/min, which it is mainly related with established renal depuration.

A detailed analysis of the plasmatic decay curve of ^{99m}Tc gentamicin was made using a computer data process system. This analysis shows the existence of two compartments with $T_{1/2}$ of 0.07 and 5.5 hours.

In the first phase, considered as distribution in which the radiopharmaceutical self flows freely in the blood stream, interacting in a reversible way with plasmatic proteins from which it is liberated through glomerular filtration[4,5], with a fast half-life of 0,07 hours.

The second phase with $T_{1/2}$ of 5.5 hours can be explained as the labelled gentamicin being transported by the plasmatic proteins to a specific site where, it is well fixed on the receptor cells and from this reason the great value for $T_{1/2}$

The results of this kinetc study are in good agreement with the behavior of gentamicin in human therapeutical treatment[7,8,10] in which a microbiological detection method of RIE was used.

The half-life of 16.5 hours obtained from whole body measurements, can be considered as the gentamicin activity in its site of major affinity.

As the study of pharmacokinetics was based on drug transfer between two compartments with a mathematical model, we can quantitative calculate the transfer coefficients of gentamicin in the animal organism. These data give the fraction of radiopharmaceutical that it goes through the membrane in a unit time.

Two hypothesis were made the choose this model:

1. At initial time all the drug was in the compartment 1, intravascular.
2. The labelled drug elimination had to obey a first order process, that is the amount of substance leaving a compartment per a unit time had to be proportional to the amount of substance in the compartment.

From the results obtained from blood and whole body it was shown that some changes had to be made in the first kinetic model.

A new compartment, retention, had to be included with this, it was satisfactory explained the difference between the data from the curves of excretion and whole body simulation with those experimental obtained.

With these data, a simulation was made using a model of two compartments plus a retention one. The coefficient K_{10} is used for the elimination of the labelled drug outside the biological environment and K_{13} for the transfer of ^{99m}Tc gentamicin to the retention compartment.

An explanation for the existence of a retention compartment is based on the absorption of the ^{99m}Tc gentamicin sulphate in the gastric - intestinal tract and also by the accumulation of the antibiotic in the renal cortex and in the carcass during the observed time.

V. CONCLUSION.

A plasmatic decay curve obtained with experiments data for the ^{99m}Tc gentamicin had a bi-exponential form, corresponding to two compartments: the intravascular and the extravascular with the calculated value for the apparent volume distribution, we can conclude that at the begining, after the gentamicin was injected by intravenously via, the antibiotic was spread all over the animal organism, being fixed only by the similar organs.

The renal depuration coefficient showed that the gentamicin was an antibiotic that was eliminated at a slow rate.

The choosed compartment model to study the pharmacokinetic behavior during 24 hours, shows that at the begining the drug diffuses through the whole body and it is preferentially eliminated by the kidneys with a half-life of 16.5 hours and that the 25% of residues represents the amount of drug that remain fixed in the renal cortex, carcass and in the gastric-intestinal tract, the last two ones probably fix in minor proportional.

The transfer coefficient from intravascular to extravascular compartment, K_{12} was more quickly than the reverse, so that $K_{12} > K_{21}$.

During the studies performed in 24 hours time, it was determined the existence of another small constant, K_{13}, attributed to a retention compartment.

Also during study carried out in 24 hours time, it was not confirmed the return of the ^{99m}Tc gentamicin sulphate to the intravascular compartment.

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