CHAPTER 3

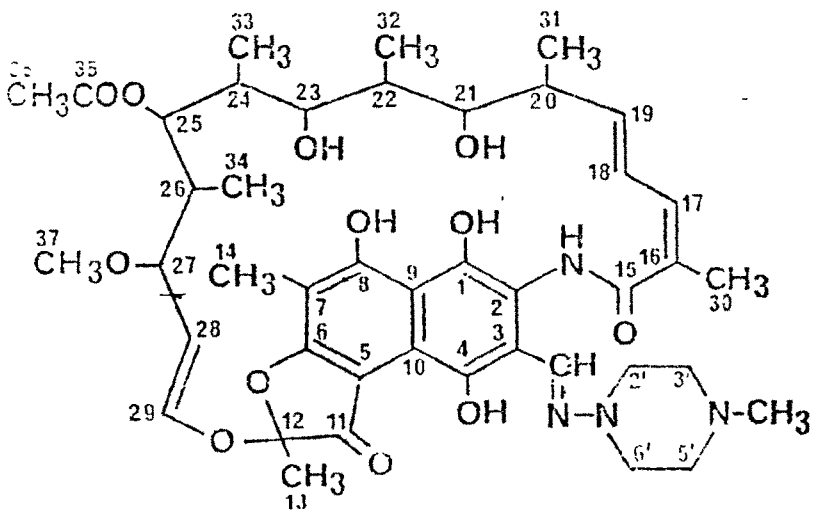
DRUGS AND THEIR PROFILES

- Rifampicin
- Gentamicin Sulphate
- Analytical profile

3.1. Science About the Drug - Rifampicin (RIFA)

3.1.1 OFFICIAL COMMANDINGS

Rifampin (rifampicin) is a synthetic derivative of a natural antibiotic rifamycin B produced by streptomyces mediterranei and belongs to the class of naphthalenic rifamycins.[133,134] Its principal uses are for the treatment of tuberculosis and leprosy.



$C_{43}H_{58}N_4O_{12}$ Mol.Wt.= 822.95

MolecularWeight : 822.95

Molecular Formula : $C_{43}H_{58}N_4O_{12}$

Chemical Name : 3-[{(4-Methyl-1-Piperazinyl) imino}methyl] rifamycin.

Appearance, Colour and Odour

Rifampin is a red-orange, odorless, crystalline powder.

PHYSICAL PROPERTIES

Ultraviolet Spectroscopy

The UV spectrum of rifampin, recorded on a Perkin Elmer model 4000-A spectrophotometer in aqueous phosphate buffer pH 7.38[135], exhibits the absorption maxima given in Table I.

INFRARED SPECTROSCOPY

The infrared absorption spectrum of Rifampin $CDCl_3$ Solution exhibits the following bands which are consistent with its structure:

Wave number (cm^{-1})	Assignment
3480	vOH-bonded
2970,2930 & 2880	vCH_3
2820	vCH_30
2800	vCH_3-N
3300-2300	vNH and vNH-bonded and vOH-bonded
1715	vCo acetyl
1640	vCO furanone (in solution intra H-bonded)
1620	amide I
1570	vC=C
1540 and 1520	amide II
1255, 1040 and 1020	vC-O-C acetyl

NMR SPECTROSCOPY

The NMR spectrum of Rifampin in deuterium oxide is consistent with its structure:

Chemical Shift	Multiplicity	Asignment
11.96[b)]	Singlet	NH
8.22	Singlet	CH=H
2 9-3.3	Multiplet	CH_2-2',6'
2.4-2.8	Multiplet	CH_2-3',5'
2 34	Singlet	N-CH_3
11.4-14.0[b)]	Broad Signal	OH-1
		OH-8
13.16 [b)]	Singlet	OH-4
1.82	Singlet	CH_3-13
2 22	Singlet	CH_3-14
6.3-6.8	Multiplet	H-17
		H-18
5 92	Doublet of doublets	H-19
2.26	Doublet of doublets of quarters	H-20
3 78	Doublet of doublets	H-21
3 2-4.2 [b)]	Broad Signal	OH-21
		OH-23
1.70	Doublet of doublets quarters	H-22
3 04	Doublet of doublets	H-23
1.52	Doublet of doublets of quarters	H-24
4.96	Doublet of doublets	H-25
1.22	Doublet of doublets of quarters	H-26
3.58	Doublet of doublets	H-27
5.00	Doublet of doublets	H-28
6.20	Doublet	H-29
2.10	Singlet	CH_3-30
0.88	Doublet	CH_3-31
1.01	Doublet	CH_3-32
0 58	Doublet	CH_3-33
-0.33	Doublet	CH_3-34
2.06	Singlet	CH_3-36
3.05	Singlet	CH_3-37

b = it exchanges with D_2O

MASS SPECTROMETRY

The most characteristic peaks correspond to M^+,$[M-CH_3OH]^+$ and to the chromophoric ions. The spectrum was only partially characteristic as the compound decomposes in the ion source and M^+,$[M-CH_3OH]^+$were absent, while the peak at m/e 398 corresponded to the chromophoric ion. The mass spectrum of rifampin was also obtained under field desorption ionization conditions and it exhibited the molecular ion at m/e 822 and the (M+1) peak corresponded to the isotopic contribution. No fragmentation peaks were observed.

POLAROGRAPHY

The polarographic behaviour of rifamycins has been described and the presence of an oxidation or reduction wave at about 0 volts vs. SCE indicates the hydroquinone or quinone (14,33-35) system respectively. Rifampin in Methanol-acetate buffer solution, pH 5.9. shows an oxidation wave with $E^{1/2}$=+0.10 volt vs. SCE, attributed to the hydroquinone system[135], and in aqueous phosphate buffer, pH 6.88, there is also a reduction wave with $E^{1/2}$=-1.66 volts vs. SCE .

OPTICAL ROTATION

The optical rotation for rifampin was reported to be $[\alpha]^{25}_d$= +10.6° (0.5%) in $CDCl_3$.

CRYSTAL PROPERTIES:

X-Ray Diffraction:

Single -crystal X-ray diffraction was used to deduce the structure of the p-iodoanilides of rifamycin B and rifamycin Y.

Thermal Analysis

Melting Range:

Rifampin melts with decomposition at 183-188°C.

Differential Scanning Calorimetry:

The heating curve of rifampin was determined using, Differential Thermal Analyzer and it was found that it melts and decomposed at 240°C

3.1.2 SOLUBILITIES OF RIFAMPIN

Solvent	mg/ml	Temperature	Reference
Chloroform	349	25°C	136
Dichloromethane	216		
Ethyl acetate	108		
dioxane	39		
methanol	16		
acetone	14		
n-hexane	0 43		
petroleum ether	0.33		
water pH 7.3	2.5		
water pH 4.3	1.3		
water pH 7.5	2.8	room	137
water pH 2.0	99.5		
0.1N HCl	200.0	37°C	138
Phosphate buffer pH 7.4	9.9		

DISTRIBUTION COEFFICIENT

Distribution coefficients in various PH and organic phases vs aqueous phase at room temperature are given below :

Organic Phase	Aqueous Phase	$K = \frac{Cog}{Caq}$
N-octanol	Phosphate buffer	K=15.6
Benzene	Aqueous Buffers	K=9.0 (pH 7.5)
		K=4.6 (pH 8.0)

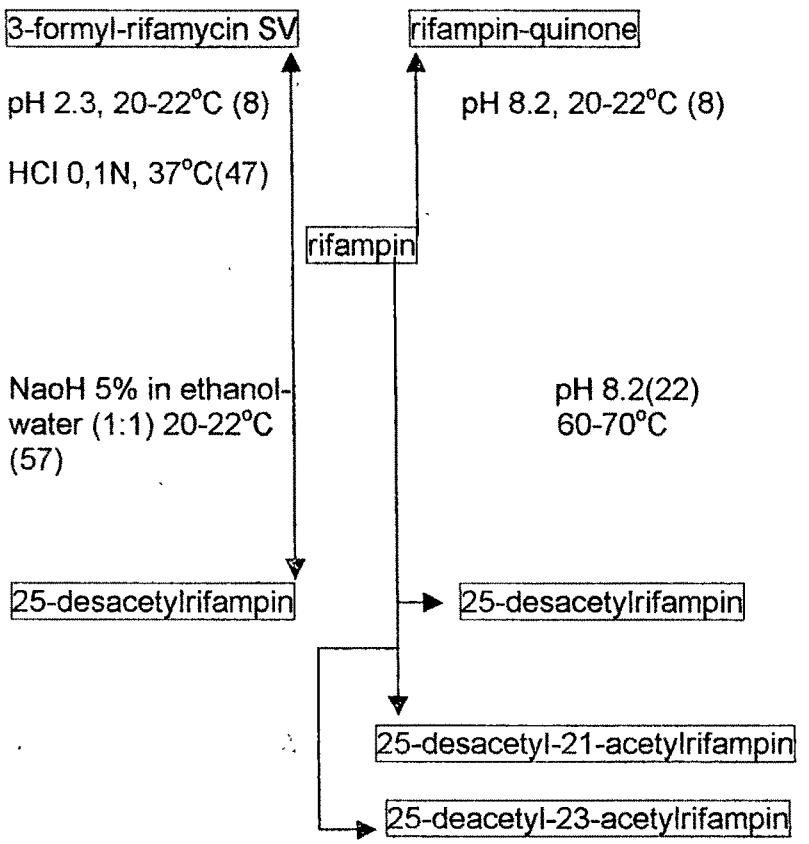
3.1.3 STABILITY

STABILITY AS POWDER

Rifampin is very stable in the solid state in sealed containers at room termperature. Rifampin in the solid state is stable also at temperatures up to 70°C, as reported by Sano et al.[136]

STABILITY IN SOLUTION

STABILITY OF RIFAMPIN IN AQUEOUS SOLUTIONS



3.1.4 METHODS OF ANALYSIS

SPECTROPHOTOMETRIC METHODS

SPECTROPHOTOMETRIC ASSAY ON BULK PRODUCT

The VIS maximum at 475 nm in aqueous phosphate buffer solution pH 7.38 with an absorptivity (g/1) value of 18.7 enables rifampin to be quantitatively assayed.

FLUOROMETRIC DETERMINATION

Rifampin was determined fluorometrically by transforming it with hydrogen peroxide into a fluorescent product. The maximum fluorescence develops in an aqueous carbonate-bicarbonate buffer, pH 9 2 at 480 nm, when the exitation wavelength is 370 nm. The relative fluorescence intensity is linear with concentrations of rifampin in the range 0.1 to 10 µg/ml.

VOLUMETRIC METHODS:

Rifampin in capsules was determined by a volumetric method the antibiotic was oxidized with an excess of ferric chloride,which was then titrated iodometrically.

CHROMATOGRAPHIC METHODS

THIN LAYER CHROMATOGRAPHY:

A reverse-phase partition TLC procedure has been described for the determination of rifampin: silanized Silicagel plates were used as stationary phase, with phosphate buffer, pH 7 containing 0.1% sodium

ascorbate as mobile phase. The coloured spot was eluted and measured spectrophotometrically. Reverse-phase partition TLC has been used for structure-activity correlations.

HIGH PRESSURE LIQUID CHROMATOGRAPHY:

Rifampin has been frequently cited as an example of the usefulness of HPLC Rifampin, 25-desacetylrifampin, rifampin quinone and 3-formyl rifamycin SV were separated[139] under the following conditions: DuPont 820 Chromatograph equipped with UV detector,ODS Permaphase column at 50°C and 1,000 psi, water to methanol with linear gradient 8 %/min. as mobile phase and 1ml/min flow rate.

MICROBIOLOGICAL METHODS:

Microbiological methods have been widely described for the determination of rifampin potency in bulk products, in pharmaceutical formulations and in body fluids, as listed in the review by Binda et al. These methods can be classified as a) diffusion plate methods, b) serial tube dilution methods and c) turbidimetric methods.

3.1.5 PHARMACOKINETICS AND METABOLISM IN MAN

The Pharmacokinetics of rifampin has been widely studied and the serum levels of rifampin after single and repeated administration of different doses were determined by various investigators and have been reviewed[140]. To summarize, after oral administration, rifampin is well-observed with the maximum plasma levels at 1.5-3 hr over a wide range

of single dose, i.e., 0.1 to 1.2 g. The levels are still appreciable (5-10% of the peak level) after 12hr only at the high doses.

Rifampin is widely diffusible in the tissues and in the various body fluids[137], this is related to the high lipotropism of rifampin.

Rifampin elimination, which must be considered slow, occurs mainly through the bile but also through the urine. The total amount of rifampin eliminated in the bile is not proportional to the dose administered while the urinary elimination increases with the dose. In an investigation carried out over 6 days, about 22% was eliminated in the faeces and about 26% in the urine. In another study, however, Riess[141] found that 96 hrs. after administration of 300mg of labeled rifampin, 94% of the total radioactivity had been recovered in equal percentages in the faeces and urine.

The main metabolite of rifampin in man was identified, after isolation from bile and urine, as 25-desacetyl rifampin. This compound is much less lipophilic than rifampin and it is easily excreted in urine and not reabsorbed. Sano et al.[142] reported that in addition to 25-desacetyl rifampin, a second metabolite is 3-formyl rifamycin SV.

3.1.6 TOXICOLOGY:

Acute and subacute toxicity tests in rodents shows good tolerance at well above therapeutic doses. The LD_{50} in the mouse following oral administration in approximately 1250 $mg.kg^{-1}$in 24h. In the rat the LD_{50} values are1700 $mg.kg^{-1}$ for oral administration . 550 $mg.kg^{-1}$ for intraperitonial administration and 330 $mg.kg^{-1}$ for intravenous

administration were given 50 and 100 mg.kg^{-1} daily for 26 weeks showed no notable toxicity but at doses over 100 mg.kg^{-1} there were dose-related histological changes in the liver. Rabbits also given doses over 100 mg kg^{-1} for 4 weeks or more showed progressive hepatotoxicity including jaundice and fatty changes at 400 mg.kg^{-1}. Dose-related minor histological changes were also served in the liver of monkeys given 40-80 mg.kg^{-1} for 2-4 weeks.

Teratogenic effects have been seen in rats and mice at doses of 150 mg kg^{-1}and above, with some skeletal malformations and spina bifida.

3.1.7 THERAPEUTIC USE:

Indications:

1. Tuberculous infections at all sites
2. Tuberculous infections in HIV-infected individuals.
3 Prophylaxis in tuberculin-positive children.
4. Opportunist mycobacterial infections
5. Leprosy.
6. Prophylaxis of meningococcal meningitis.

3.1.8 ADVERSE REACTIONS:

Acute overdosage:

29 overdose cases have been reviewed, in which the drug produced cutaneous pigmentation, described as the 'red-man syndrom'. In addition to pigmentation, periorbital or facial edema is described, as well as pruritus, nausea, vomiting and abdominal tenderness. In adults,

cardiovascular/pulmonary arrest may occur after a total dose of 14g. There are no specific antidotes or resuscitative measures, but rifampin is dialyzable.

Severe or irreversible adverse effects:

Severe reactions to rifampin are not common and when they occur, are usually related to sensitization or enzyme induction effects on the liver and consequent effects on the metabolism of other drugs. Sensitivity to rifampin is commoner with intermittent therapy and this may result in the 'flu syndrome' (fever, chills, and headache) or produce thrombocytopenic purpura. If purpura occurs during rifampin administration, the drug should be discontinued and not administered again. A shock-like syndrome has been described, as have acute hemolytic anemia and acute renal failure. Risk can be minimized by reintroducing the drug gradually, in small increasing daily doses.

Hepatic reactions may occur in patients withchronic liver disease, including alcobolism and such patients need careful monitoring. Associated drugs such as isoniazid and pyrazinamide may aggravate the situation.

An increased risk of venous thrombosis in patients receiving rifampin has also been reported. Ulcerative colitis has been reported in association with rifampin therapy for tuberculosis. Cutaneous vasculitis has also been reported.[153]

Symptomatic adverse effects:

Gastrointestinal reactions, including anorexia, nausea and

abdominal discomfort, occur from time to time, and occasionally vomiting may be severe. Taking rifampin with meals usually reduces these effects, although not always. Diarrhea is less frequent.

Patients should be warned about highly colored (red, orange or pink) urine and pink tears while on rifampin. The high concentration in tears may tint some varieties of contact lenses

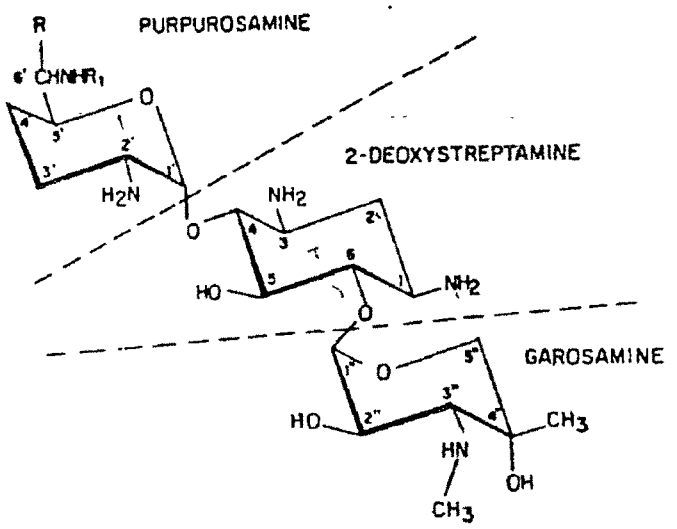
3.2 SCIENCE ABOUT THE DRUG

GENTAMICIN SULPHATE(GENTA)

3.2.1 OFFICIAL COMMANDINGS:

Gentamicin sulfate is an aminoglycoside antibiotic. For about a decade after its discovery in 1964, gentamicin was the preferred drug for the tratment of serious Gram-negative aerobic bacterial infections. Since the mid-1970s, its usefulness has decreased in some areas because of the emergence of bacterial resistance.

CHEMICAL NAME:



II. Molecular Formula:

GENTAMICIN C_1 $R=R_1=CH_3$	$C_{21}H_{43}N_5O_7$(M.W.477.6)
GENTAMICIN C_2 $R=CH_3$ $R_1=H$	$C_{20}H_{41}N_5O_7$(M.W.463.6)
GENTAMICIN C_{10} $R=R_1=H$	$C_{19}H_{39}N_5O_7$(M.W.449.5)

APPEARANCE COLOUR, ODOUR:

Gentamicin sulfate is a white to buff coloured, odourless, hygroscopic powder.

PHYSICAL PROPERTIES:

Infrared Spectroscopy.

The infrared absorption Spectrum of Gentamicin Sulphate exhibits the following bands which are consistent with its structure.

WAVE NUMBER (cm^{-1})	ASSIGNMENT
3500-2500 (s, vbr)	OH, NH_3^+ NH_2^+ stretch
1620(m)	NH_3^+, NH_2^+ symmetric bend
1525(m)	NH_3^+, NH_2^+ symmetric bend
1150-1000(vs, br)	C-O, HSO_4- stretch
610(s)	SO_2 bend

Notation: (s-strong, m-medium, vs-very strong, br-broad, vbr-very broad)

ULTRAVIOLET SPECTROSCOPY:

The Gentamicin complex does not possess ultraviolet light absorbing properties; both the free base and sulfate show end absorption only.

THERMAL PROPERTIES (TGA, DSC)

Thermo Gravimetric Analysis (TGA)

The thermogravimetric analysis of the USP Reference Standard indicates loss of approximately 12% water from ambient to 125^0 C. Decomposition starts at 220^0 C and proceeds stepwise until 330^0C above 330^0C additional de-composition occurs, yielding a final residue of about 30% which is attributable to the sulfate salt.

DIFFERENTIAL SCANNING COLORIMETRY (DSC):

The differential scanning calorimetry curve of the USP Reference Standard has a broad endothermic peak around 75^0 C due to loss of water and a large endotherm at 250^0 C corresponding to melting decomposition

OPTICAL ROTATION:

Allowable limits for the specific rotation of gentamicin sulfate are $+107^0$ to $+121^0$ The specific rotation of the USP Gentamicin Sulfate Reference Standard was found to be +115.9° when measured as a 0.3% aqueous solution.

X-Ray Diffraction

As it is an amorphous substance, no x-ray diffraction bands were observed

3.2.2 BIOSYNTHESIS

Gentamicins differ from the neomycins, kanamycins, and paromomycins in that they contain both C-methyl and N-methyl substituents, most studies on gentamicins have been aimed at

determining the source of the methyl groups. Studies carried out by Lee et al.[143] indicate a high efficiency of L-methionine incorporation into gentamicins. Labelling experiments using 13C-methyl methionine and ^{2}H-methyl methionine have shown that all of the methyl groups in gentamicin are derived from methionine. Additional work by Lee et al.[144] shows that when 13C methyl-methionine was added at the onset of biosynthesis of the gentamicin components, incorporation of label into the minor components preceded incorporation into the major components. Degradation occurred when 14C-methyl gentamicin major components were added to the gentamicin-producing culture medium and shaken.

Isolation and Purification Processes

Isolation of the gentamicin complex using ion-exchange chromatography[171]. A commonly used procedure is to adjust the whole broth to pH 2 with sulfuric acid, followed by filtration. After adjustment to pH 7, the neutralized filtrate is passed through an IRC-50 resin column in the ammonium cycle, and the antibiotic is then eluted with 2N aqueous ammonia. The gentamicin C complex may be isolated from co-produced minor components using a Dowex 1X2 column (OH-form).

3.2.3 STABILITY:

Gentamicin sulfate powder is very stable when stored in tightly closed containers at room temperature for long duration. Gentamicin sulfate is stable for atleast five years with respect to potency, specific rotation and pH and also in aqueous and alkali solutions. More recent

studies on gentamicin confirm its excellent stability in moderately acid to strongly basic aqueous media. Under highly stressed conditions (heating in IN sulfuric acid for 5 days at 60°C), approximately a 30% loss in potency was found.[172] Gentamicin sulfate exhibits excellent stability in various pharmaceutical dosage forms.

3.2.4 METHODS OF ANALYSIS:

IDENTIFICATION

Gentamicin in conveniently identified by thin-layer chromatography (TLC). Gentamicin is resolved into its 3 components and also can be separated frommost other related antibiotics using TLC. Silicagel TLC plate developed using equal volumes of chloroform, methanol and concentrated aqueous ammonia. The spots are visualized with ninhydrin reagent or with iodine vapors. Paper chromatography is also useful for identification and the solvent system, chloroform: methanol: concentrated aqueous ammonia: water (10:5:3:2) is used along with ninhydrin spray detection.

MICROBIOLOGICAL ASSAY

The current official microbiological assay procedure described in the U.S. Code of Federal Regulations (CFR) for the substance and dosage forms is a cylinder plate assay using Staphylococcus epidermidis ATCC 2228 as the test organism[173]. The British

Pharmacopoeia (1973) utilizes a cylinder plate assay and Bacillus Pumilus NCTC 8241 as ther test organism.

The minimum potency required by both the CFR and BP for acceptance of bulk commercial gentamicin sulfate is 590 mcg per mg on the dried (anhydrous) basis.

ION-EXCHANGE CHROMATOGRAPHY

An ion-exchange separation has been suggested as a quantities measure of C component ratios and content in gentamicin samples.

GAS CHROMATOGRAPHY:

Since gentamicin has relatively low volatility, all gas chromatographic analyses involve derivatization.

HIGH PRESSURE LIQUID CHROMATOGRAPHY:

A major obstacle to gentamicin HPLC assays has been the problem of detection. Gentamicin exhibits no significant UV bands above 190nm and has no native fluorescence. In addition, assay levels are generally too low for the use of the refractive index detector. Hence, the several methods which have been developed involve derivatization of the drug[174].

ELECTROPHORESIS:

Electrophoresis has been used to separate gentamicin from other antibiotic drugs in serum and in urine.

RADIOIMMUNO ASSAY

Radioimmunoassay for gentamicin is based on the binding of the antibiotic by an appropriate antibody. By adding both antibody and radio-labelled drug to a sample containing gentamicin, one establishes an equilibrium between the bound and free labelled and unlabelled gentamicin molecules. Following separation of the bound and free fractions, counting of the bound gentamicin provides a measure of the proportion of bound drug which is radioactively labelled. This measurement leads to the calculation of the amount of gentamicin in the original sample.

RADIOENZYME ASSAY

Determination of gentamicin in serum by radioenzyme assay is based on the enzyme-catalyzed derivatization of the drug with a labelled substituent group. Derivatized gentamicin is adsorbed onto some stationary medium to separate it from unreacted components. The radioactivity of the absorbent plus gentamicin thus provides a measure of the amount of drug present in the sample.

3.2.5. DRUG METABOLISM AND PHARMACOKINETICS.

Gentamicin shares with many other aminoglycoside antibiotics the important property of being stable in biological systems. When administered to man or animals the major portions is excreted in the urine by glomerular filtration. Gentamicin is not absorbed in appreciable amounts from the intact gastrointestinal tract.

After intramuscular administration, peak serum concentrations usually occur between 30 and 60 minutes and serum levels are measurable for six to eight hours. When gentamicin is administered by intravenous infusion over a two-hour period, the serum concentrations are similar to those obtained by intramuscular administration. Protein binding studies have indicated that the degree of gentamicin binding is low between 0 and 30%.

3.2.6 TOXICOLOGY

In rabbits, daily intraventricular gentamicin doses of 0.25-0.5 $mg.kg^{-1}$ were associated with ventriculitis, ventricular dilatation, abnormal postural reflexes, and ataxia.[145]

The safety of gentamicin during pregnancy has not been established The drug crosses the placenta but the risk of ototoxicity to the fetus is not known.

The $L.D_{50}$ of acutely administered intravenous gentamicin is 51.6 in mice [146]

3.2.7 THERAPEUTIC USE

Indications:

1 Septicemia and other severe infections due to aerobic Gram-negative bacilli.

2 Severe Gram-negative sepsis in children.

3 Urinary tract infections.

4 Bacterial endocarditis, usually in combination with a ß-lactam.

5. Chemoprophylaxis in abdominal surgery.

6. Biliary tract infections.

7 Other serious infections.

3.2.8 ADVERSE DRUG REACTIONS:

Potentially life-threatening effects:

Nephrotoxicity and neuromuscular blockade, described fully below, may occasionally be life-threatening. Anaphylactic shock has rarely been reported and may be due to sulfites in parenteral formulations.

Ototoxicity:

Gentamicin may affect the vestibular and auditory branches of the eighth cranial nerve, giving rise to loss of hearing, vertigo, and tinnitus which may be permanent. This effect usually occurs only if high doses are used or if patients have abnormal renal function and may be related to persistently high trough levels.

Nephrotoxicity:

Gentamicin is selectively concentrated in renal cortical cells and it may cause proximal tubular damage and glomerular dysfunction.

Nerumuscular blockade:

As result of its neuromuscular blocking effect, gentamicin may unmask or aggravate myasthenia gravis and cause postoperative respiratory distress.

Other severe reactions:

Infrequent effects reported include anemia, purpura and convulsions Central neurotoxicity, including encephalopathy, convulsions and hallucinations, has been reported in association with gentamicin therapy, but this is extremely rare.