CHAPTER-IV

R E S U L T S

(A) Animal studies

Pharmacokinetics of rifampicin/rifampicin + INH in normally nourished and undernourished rats

I. Influence of undernutrition and/or drug treatment on the growth pattern of rats

i) Effect of post-weaning undernutrition on body weights

At 17 weeks of age, the mean body weights of rats fed 22% casein diet (normal protein; NP) and those fed 7% casein diet (low protein; LP) were 238.3 and 161.5 g respectively. Thus, 14 weeks of post-weaning undernutrition produced significant decrease in mean body weight.

Following the administration of rifampicin 50 mg/kg, twice weekly for 6 weeks to rats of 11th to 17th week of age, the mean body weight was 264.9 g. The comparable value for LP group was 187.8 g which was significantly lower than that of the former group (Table-16, Fig.1).

Following a six week long concurrent administration of INH (50 mg/kg daily) and rifampicin (50 mg/kg, twice weekly), the mean body weights in NP and LP groups were 225.1 g and 157.2 g respectively (Table-16, Fig.1).

Table-16 : Mean body weights (kg ± SEM) of undernourished and normally nourished rats and effect of drug treatment thereon

A. Effect of undernutrition on mean body weights of rats (on dietary treatment)

Effect of undernutrition and/or drug treatment on mean body weights of rats (drug treatment for 6 weeks)

Group	Weeks of dietary treatment						Weeks of dietary treatment					
	I		III		IV		IX		XII		XV	
	NP	LP	NP	LP	NP	LP	NP	LP	NP	LP	NP	LP
Untreated n = 40	44.0 ±0.7	42.5 ±0.2	82.2 ±1.9	62.4 ±1.2	119.7 ±2.5	95.1 ±2.1	152.9 ± 3.9	110.1 ± 1.7	206.3 ± 4.0	132.7 ± 2.1	238.3 ± 4.5	161.5 ± 3.8
P	N.S.		0.001		0.001		0.001		0.001		0.001	
Rifampicin n = 20	47.7 ±1.1	45.4 ±3.5	85.7 ±2.5	65.8 ±1.8	123.5 ± 2.6	97.7 ±1.9	178.8 ± 3.6	118.2 ± 2.3	224.6 ± 2.2	151.3 ± 3.1	264.9 ± 3.2	187.8 ± 3.8
P	N.S.		0.05		0.001		0.001		0.001		0.001	
Rifampicin + INH n = 20	42.5 ±1.0	41.8 ±0.6	77.9 ±1.9	61.8 ±1.4	112.6 ± 1.4	84.5 ±2.0	135.1 ± 1.3	105.5 ± 1.6	195.6 ± 3.1	133.8 ± 3.8	225.1 ± 3.8	157.2 ± 5.0
P	N.S.		0.05		0.05		0.001		0.001		0.001	

Fig.1

Effect of undernutrition and/or drug treatment on mean body weights of rats. Treatment time in weeks is depicted on the abscissa and mean body weight in gms on the ordinate. Upper panel and lower panel show data of rats fed normal protein and low respectively. The drug treatment was started at the end of eighth week of the dietary treatment as pointed out by the arrow. Unshaded histograms (), vertically hatched histograms () and diagonally hatched () histograms depict mean values of untreated, rifampicin treated and rifampicin + INH treated groups, respectively. Vertical bars represent the SEM. Figures in parentheses indicate the number of rats.

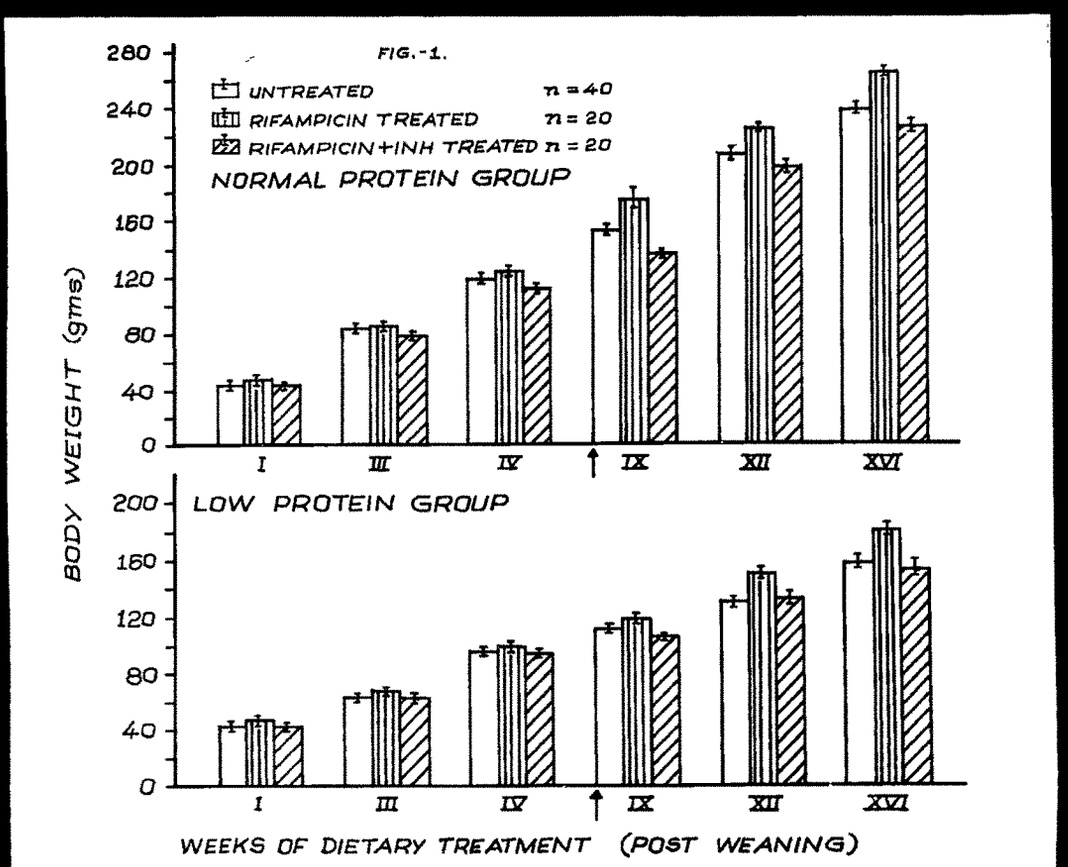


FIG.-1.
UNTREATED n = 40
RIFAMPICIN TREATED n = 20
RIFAMPICIN+INH TREATED n = 20
NORMAL PROTEIN GROUP
LOW PROTEIN GROUP
BODY WEIGHT (gms)
280
240
200
160
120
80
40
0
I
III
IV
IX
XII
XVI
WEEKS OF DIETARY TREATMENT (POST WEANING)

ii) Effect of undernutrition and/or drug treatment on the tissue weights of rats

Table-17 presents the mean weights of liver, lung and kidney, before and after the drug treatment given to NP and LP groups.

At 15th week of the dietary treatment, the mean liver weight of the NP group was 7.8 g compared to that of the LP group which was 4.5 g ($P < 0.01$). Post-weaning protein deficiency did not produce any significant decrease in the mean lung or kidney weights.

Under similar experimental conditions, drug treatment, either with rifampicin, or rifampicin + INH, did not induce any changes in the tissue weights in NP or LP groups.

II. In vitro absorption study

The amount of rifampicin absorbed by the intestinal loops during all the time brackets was significantly higher in loops obtained from NP groups (Table-18a, Fig.2, $n = 8$). The total amount of rifampicin absorbed in 4 hours was significantly higher in NP group than that observed in LP group (40.7 ± 1.5 and 23.5 ± 2.3 ug/ml in NP and LP groups, respectively).

Table-17 : Mean tissue weights (gm ± SEM) of undernourished and normally nourished rats and effects of drug treatment thereon

	LIVER		LUNG		KIDNEY	
	NP	LP	NP	LP	NP	LP
A. Without treatment						
	7.8 ± 0.9	4.5 ± 0.5*	1.3 ± 0.6	1.1 ± 0.7	1.8 ± 0.1	1.5 ± 0.1
P	0.01		N. S.		N. S.	
B. Chronic treatment with rifampicin alone						
	7.3 ± 0.1	5.0 ± 0.4*	1.3 ± 0.1	1.1 ± 0.2	1.8 ± 0.1	1.5 ± 0.1
P	0.01		N. S.		N. S.	
C. Chronic treatment with rifampicin + INH						
	7.0 ± 0.3	4.9 ± 0.2*	1.3 ± 0.1	1.1 ± 0.1	1.8 ± 0.1	1.5 ± 0.1
P	0.01		N. S.		N. S.	

* $P < 0.01$

Table-18a: In vitro absorption of rifampicin in isolated intestinal loops of rats

Group	Mean body weight (gms)	Rifampicin concentration ug/ml						Total ug absorption
		15	30	60	90	120	240	
NP	212.0 ± 3.7	7.0 ±0.5	8.7 ±0.6	7.9 ±0.5	6.6 ±0.5	6.3 ±0.8	4.1 ±0.3	40.7 ±1.5
LP	133.1 ± 3.1	3.8* ±0.4	4.6* ±0.8	5.3* ±0.6	4.8* ±0.4	3.1* ±0.7	1.9 ±0.5	23.5* ±2.3

* P < 0.01 compared with the corresponding NP group

Table-18b: In vitro absorption of rifampicin [illegible] in isolated intestinal loops of rats

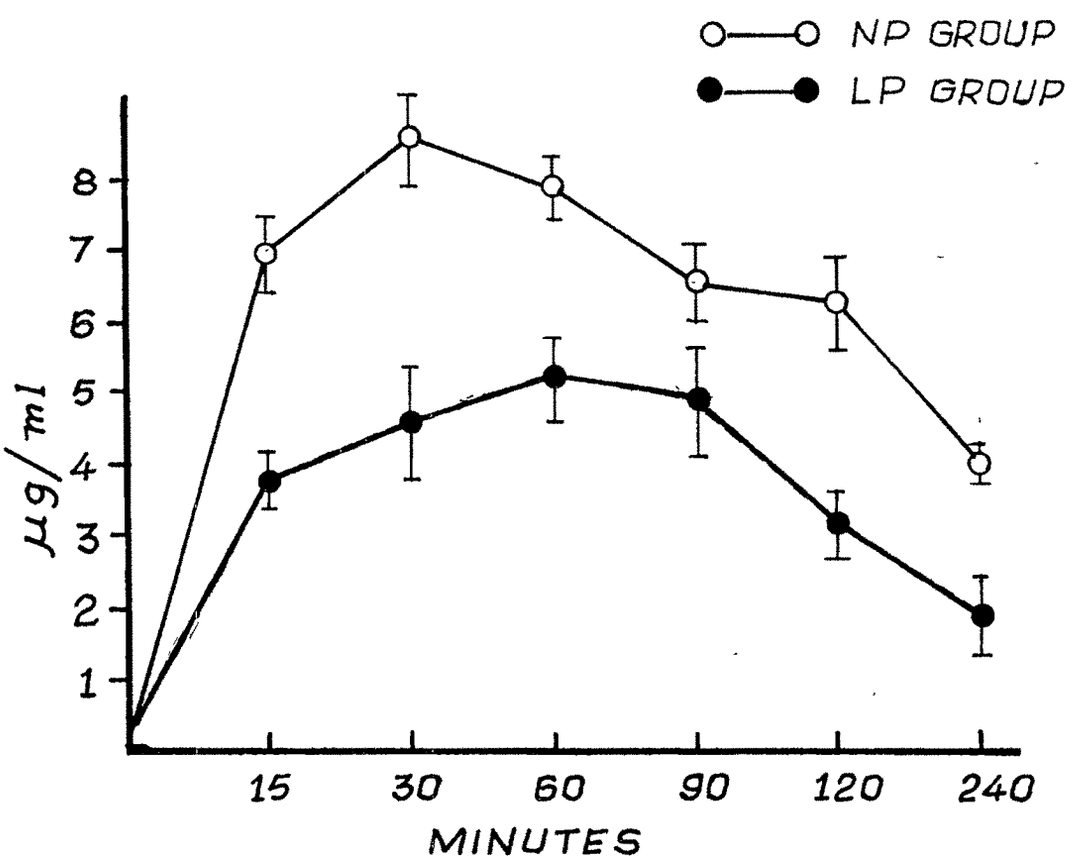
Group	Mean body weight (gms)	Rifampicin concentration ug/ml/cm^2						Total ug absorbed
		15	30	60	90	120	240	
NP	217.5	1.7 ±0.1	2.1 ±0.1	2.0 ±0.1	1.6 ±0.2	1.6 ±0.1	1.4 ±0.1	10.2
LP	158.7	1.0* ±0.1	1.3* ±0.2	1.3* ±0.3	1.2 ±0.2	0.7* ±0.2	0.4* ±0.1	6.0* ±1.1
P <		0.01	0.05	0.01	N.S.	0.01	0.05	

* P < 0.01 compared with the corresponding NP group

Fig.2

Effect of undernutrition on *in vitro* absorption pattern of rifampicin in intestinal loops of rats. Time in minutes is depicted on the abscissa and mean concentration of rifampicin (ug/ml) is depicted on the ordinate. Lines joining open circles (⊖——⊖) and closed circles (●——●) represent normal protein and low protein groups respectively. Vertical bars represent the SEM (n = 8).

FIG.-2



In order to eliminate the bias of available surface for absorption, in four additional experiments, the absorption of rifampicin was expressed as ug/ml/sq cm absorbing surface. The amount of rifampicin absorbed per sq cm in all the time brackets except at 90 min was significantly higher in the NP group (Table-18a, n = 4).

III. Effect of undernutrition on the serum rifampicin concentration in rats

(i) Acute treatment with rifampicin alone

From the data obtained at various intervals following one dose of rifampicin as described in "Materials and Methods", the following results were observed -

The maximum serum rifampicin concentration was observed at 2 hours in both the NP and LP groups and the maximum concentration reached was significantly ($P < 0.01$) higher in the NP group (24.2 ± 1.7) than in the LP group (15.5 ± 0.5) (Table-19, Fig.3A, upper panel).

The rate of absorption in the NP group was significantly greater than in LP group resulting in increased AUC (0-20) in the NP group, although it was not statistically significant (212.8 ± 5.6 and 196.6 ± 7.1 in NP and LP groups, respectively). Blood levels at 1 and 2 hours indicate the rate of absorption.

Table-19 : Effect of undernutrition on the serum rifampicin concentration (mean ug/ml ± SEM)

Dietary group		Hours after drug administration									
		1		2		5		10		20	
		NP	LP	NP	LP	NP	LP	NP	LP	NP	LP
Drug treatment											
Rifampicin alone	Acute	14.4 ± 0.8	12.8 ±0.7	24.2 ±1.7	15.5 ±1.5	13.2 ±0.8	12.9 ±0.8	11.1 ±0.6	11.7 ±0.6	4.4 ±0.4	4.0 ±0.6
	P	N.S.		0.01		N.S.		N.S.		N.S.	
	Chronic	11.6 ±3.5	10.4 ±1.0	19.9 ±2.7	16.3 ±1.0	12.7 ±1.3	9.5 ±0.8	10.3 ±0.7	6.4 ±0.9	5.6 ±0.4	2.7 ±0.7
	P	N.S.		N.S.		N.S.		0.05		0.01	
Rifampicin + INH	Acute	11.0 ±1.0	8.8 ±0.6	18.8 ±1.6	12.5 ±1.3	11.5 ±0.9	7.6 ±1.3	10.5 ±0.5	6.2 ±1.1	6.0 ±1.0	4.5 ±1.0
	P	N.S.		N.S.		N.S.		N.S.		N.S.	
	Chronic	8.4 ±1.2	7.4 ±0.9	14.4 ±0.8	12.9 ±0.8	12.2 ±0.8	11.9 ±0.1	8.9 ±0.5	11.1 ±0.7	3.5 ±0.6	7.5 ±1.0
	P	N.S.		N.S.		N.S.		N.S.		N.S.	
	P_1	N.S.	N.S.	N.S.	N.S.	N.S.	0.05	N.S.	0.01	N.S.	0.05
	P_2	N.S.	N.S.	N.S.	N.S.	N.S.	0.05	N.S.	0.01	N.S.	0.05
	P_3	N.S.	0.01	N.S.	N.S.	N.S.	0.05	N.S.	0.01	N.S.	N.S.
	P_4	N.S.	0.05	N.S.	0.05	N.S.	N.S.	N.S.	0.01	N.S.	0.01

P = NP vs LP

P_1 = Acute vs chronic, rifampicin alone

P_2 = Acute vs chronic, rifampicin + INH

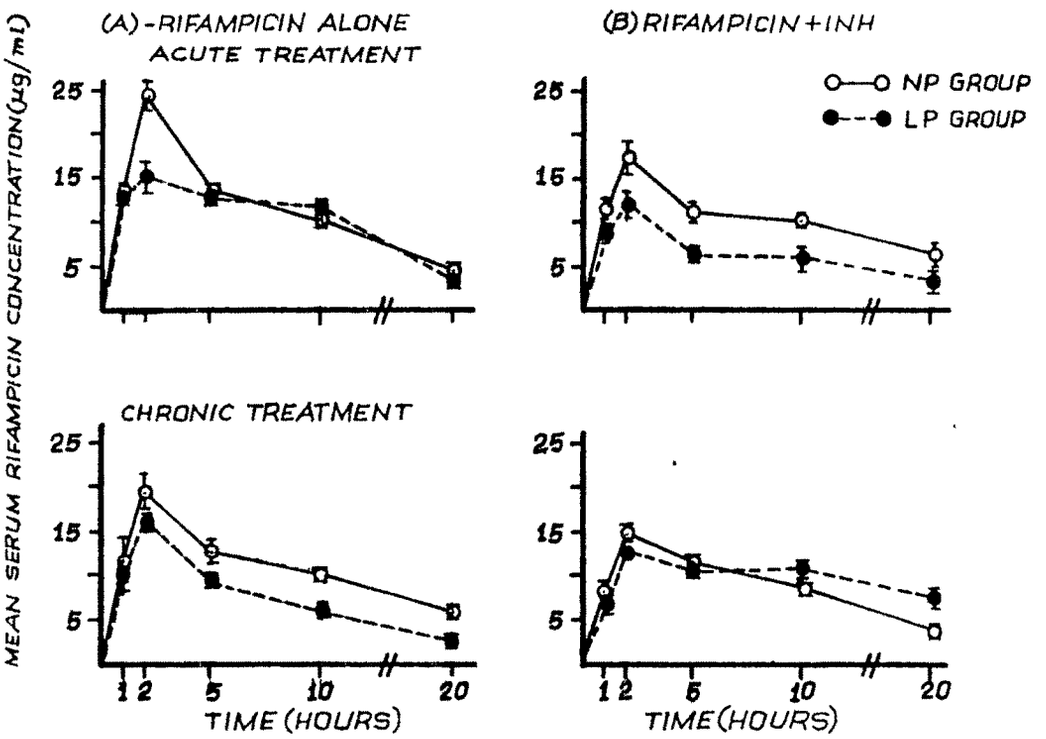
P_3 = Acute rifampicin alone vs rifampicin + INH

P_4 = Chronic treatment with rifampicin vs rifampicin + INH

Fig.3

Effect of undernutrition on the serum concentrations of rifampicin (ug/ml) at different time points (hrs). Time in hours is depicted on the abscissa and mean serum levels of rifampicin (ug/ml) are depicted on the ordinate. Panel A shows data of rats treated with rifampicin alone and panel B shows data of rats treated with rifampicin + INH. The upper and lower panels represent data of rats following acute and chronic drug treatment respectively. The lines joining open circles (⊖—⊖) and closed circles (●—●) represent mean serum concentrations of rifampicin for normal protein and low protein groups respectively. Vertical bars represent the SEM (n = 4).

FIG.-3


(A)-RIFAMPICIN ALONE
ACUTE TREATMENT
(B) RIFAMPICIN+INH
NP GROUP
LP GROUP
CHRONIC TREATMENT
MEAN SERUM RIFAMPICIN CONCENTRATION(μg/ml)
25
15
5
1 2 5 10 20
TIME (HOURS)

From 5 to 20 hours, nearly identical serum levels were found in the NP and LP groups. Levels at 10 hour sample time were too high to be considered as the 'tail-end' of the curve. Thus, correct elimination rate constant could not be calculated.

(ii) Chronic treatment with rifampicin alone

On perusal of the data obtained at various times (1,2,5,10 and 20 hours), following the last dose administration, the maximum serum rifampicin concentration was observed to occur at 2 hours, and there was no significant ($P > 0.05$) difference between NP (19.9 $\pm$ 2.7) and LP groups (16.3 $\pm$ 1.0; Table-19, Fig.3A, lower panel).

Although, the rate of absorption in NP group was greater than in LP group, this difference was statistically not significant. In a separate series of experiments done on NP and LP groups after a single dose of rifampicin and blood sample collected at 72 hours, it was found that serum levels in both groups were extremely low indicating that hardly any rifampicin was left over to produce a cumulative concentration from previous doses. Thus, the difference in blood levels at 12 hours appears to be clearly due to the effect of 12th dose feeding with almost no contribution due to previously accumulated rifampicin. It would have been better to study rifampicin blood levels at 72 hours

after the last but one dose to determine the true contribution of blood levels of rifampicin at the last dose.

The AUC (0-20) was significantly higher in the NP group than in the LP group (202.3 ± 6.6 and 137.2 ± 10.2 in NP and LP groups, respectively; $P < 0.01$).

Due to a limited number of blood serum sampling time points, correct elimination rate constants could not be calculated. Levels at 10 hour sample time were too high to be considered as the 'tail-end' of the curve. Looking at the 'tail-end' of the time-concentration curves, it however, appears that elimination rates are identical between NP and LP groups. Blood levels of rifampicin at 10 and 20 hour post-rifampicin administration were significantly higher in NP group compared to LP group.

On chronic rifampicin administration, serum rifampicin concentrations tended to be lower in both NP and LP groups than those after acute administration. However, statistically significant differences between the two groups were noted in LP group after drug administration at 5, 10 and 20 hours.

(iii) Treatment with single dose of rifampicin + INH

Following a single oral dose of rifampicin (50 mg/kg) along with INH (50 mg/kg), the maximum serum rifampicin concentrations were observed at 2 hours (18.8 ± 1.6 and

12.5 ± 1.3 in NP and LP groups, respectively). In the NP group, the serum rifampicin concentrations were higher than those of LP group at all the time points studied, although the differences were statistically insignificant (Table-19, Fig.3B; upper panel).

The relative AUC (0-20) of NP group was 223.6 ± 18.5 and that of LP group was 202.8 ± 13.4. The difference with respect to AUC was also statistically insignificant. In comparison with the result following single oral dose of rifampicin alone, concurrent administration of INH along with rifampicin decreased the serum rifampicin levels at all the time points in the LP group except at 2 and 20 hours where the levels were not significantly higher. In the NP group, although administration of INH decreased the serum rifampicin concentration compared to that found after rifampicin treatment alone, the difference was not significant.

(iv) Chronic treatment with rifampicin + INH

Six weeks of chronic treatment with rifampicin (50 mg/kg, twice weekly) and INH (50 mg/kg, daily) resulted into a variable pattern of serum rifampicin concentration, with apparently higher but statistically insignificant levels in NP group at 1, 2 and 5 hour and in LP group higher levels at 10 and 20 hours. In both the groups, the maximum

concentration was found at 2 hours. The relative AUCs (0-20) were 206.7 ± 14.2 in NP and 197.3 ± 6.6 in LP group ($P > 0.05$) respectively.

In comparison to the rifampicin chronic treatment alone, when rifampicin was administered with INH chronically in LP group, significantly lower concentration was observed at 1 and 2 hours and higher concentrations were observed at 10 and 20 hours. No such differences were observed in the NP group (Table-19, Fig.3B, lower panel).

In the LP group, chronic treatment with rifampicin + INH resulted in significantly higher serum rifampicin concentrations at 5, 10 and 20 hours than those found after a single dose treatment with rifampicin + INH. There was no statistically significant difference in the NP group at any of the time points studied.

IV. Effect of undernutrition on the tissue distribution of rifampicin tissue levels

Tissue distribution of rifampicin after drug administration, acute or chronic, with or without INH is divided under three headings -

a) Deeper compartments - liver, kidney and heart
b) Highly vascular central compartment - lung
c) Miscellaneous compartment - spleen and skeletal muscle.

i) Acute treatment with rifampicin alone

a) Rifampicin levels in the liver, kidney and heart

In the LP group, the concentrations in the liver at 1,5,10 and 20 hours were significantly higher (Table-20, Fig.4).

In the kidney, significantly higher levels were observed at 2,5, 10 and 20 hours. However, considering the low rate of absorption (as seen in blood levels at 1 hour) there appeared to be a remarkable concentration even at 1 hour (Table-21, Fig.4).

In the heart, the concentrations at 1,2,10 and 20 hours were significantly higher (Table-22, Fig.4).

b) Rifampicin levels in lung

Being a highly vascular organ, the lung is considered to be in the central compartment. The pattern of lung rifampicin levels was similar to that of blood, peak concentration occuring at 2 hours. Inspite of low peak serum concentration in LP group peak lung levels were similar in NP and LP groups. On the whole, the graph exhibited pattern like that of serum concentration and the pattern of higher tissue concentration in the LP group (Table-23).

Table-20: Effect of undernutrition on liver rifampicin concentration (mean ug/100 mg tissue ± S.E.M.)

Dietary group		Hours after drug administration									
		1		2		5		10		20	
		NP	LP	NP	LP	NP	LP	NP	LP	NP	LP
Drug treatment											
Rifampicin alone	Acute	9.6 ±0.9	13.2 ±0.7	11.8 ±0.8	12.5 ±0.8	13.7 ±1.2	22.2 ±0.8	11.3 ±0.8	17.4 ±0.6	2.4 ±0.4	3.4 ±0.5
	P	0.005		N. S.		0.01		0.01		0.05	
	Chronic	11.2 ±0.7	13.2 ±1.2	14.0 ±1.8	15.3 ±1.5	24.2 ±1.5	21.2 ±0.9	21.4 ±1.2	16.7 ±1.9	9.2 ±1.0	11.7 ±0.8
	P	N. S.		N. S.		N.S.		N.S.		N. S.	
Rifampicin + INH	Acute	9.1 ±0.5	10.5 ±0.5	13.6 ±0.6	12.8 ±1.5	14.0 ±0.4	18.2 ±1.7	10.8 ±2.1	15.6 ±1.2	2.4 ±0.5	5.7 ±0.6
	P	N. S.		N.S.		N. S.		N. S.		0.01	
	Chronic	10.5 ±0.9	11.9 ±2.1	13.0 ±1.6	14.4 ±1.9	15.6 ±2.2	19.9 ±2.4	11.3 ±1.1	15.1 ±1.4	2.8 ±0.6	13.2 ±2.9
	P	N. S.		N.S.		N. S.		N.S.		0.05	
	P_1	N.S.	N.S.	N.S.	N.S.	0.01	N.S.	0.01	N.S.	0.001	0.001
	P_2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.05
	P_3	N.S.	0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.05
	P_4	N.S.	N.S.	N.S.	N.S.	0.05	N.S.	0.001	N.S.	0.01	N.S.

P_1 = Rifampicin treatment acute vs chronic

P_2 = Rifampicin + INH treatment acute vs chronic

P_3 = Acute treatment rifampicin vs rifampicin + INH

P_4 = Chronic treatment rifampicin vs rifampicin + INH

Table-21: Effect of undernutrition on kidney rifampicin concentration (mean ug/100 mg tissue ± SEM)

Dietary group		Hours after drug administration									
		1		2		5		10		20	
		NP	LP	NP	LP	NP	LP	NP	LP	NP	LP
Drug treatment											
Rifampicin alone	Acute	10.3 ±0.9	7.8 ±0.8	9.6 ±0.8	15.6 ±1.3	7.9 ±0.7	19.5 ±0.3	2.4 ±0.3	10.4 ±0.7	0.9 ±0.0	4.4 ±0.8
	P	N. S.		0.01		0.001		0.001		0.05	
	Chronic	10.2 ±0.5	12.3 ±1.1	11.4 ±1.0	18.2 ±1.0	9.4 ±0.4	14.5 ±0.4	10.6 ±0.7	12.7 ±0.1	6.8 ±0.5	8.2 ±0.4
	P	N. S.		0.05		0.001		0.05		N. S.	
Rifampicin + INH	Acute	8.7 ±1.0	4.7 ±0.9	14.2 ±2.5	11.6 ±0.9	10.7 ±0.9	13.3 ±1.3	4.8 ±0.5	7.5 ±0.5	0.8 ±0.2	1.1 ±0.3
	P	0.05		N. S.		N. S.		0.001		N. S.	
	Chronic	6.8 ±1.2	10.9 ±0.8	11.2 ±1.1	15.2 ±1.0	9.6 ±1.4	12.5 ±1.4	4.0 ±1.2	7.8 ±0.7	1.2 ±0.2	6.0 ±0.8
	P	N. S.		0.05		N. S.		N. S.		0.001	
	P_1	N.S.	0.01	N.S.	N.S.	N.S.	0.001	0.001	0.01	0.001	0.01
	P_2	N.S.	0.001	N.S.	0.05	N.S.	N.S.	N.S.	N.S.	N.S.	0.001
	P_3	0.01	0.05	N.S.	N.S.	N.S.	0.01	0.01	0.05	N.S.	0.05
	P_4	0.05	N.S.	N.S.	N.S.	N.S.	N.S.	0.01	0.001	0.001	0.05

P_1 = Rifampicin treatment acute vs chronic

P_2 = Rifampicin + INH treatment acute vs chronic

P_3 = Acute treatment rifampicin vs rifampicin + INH

P_4 = Chronic treatment rifampicin vs rifampicin + INH

Table-22: Effect of undernutrition on heart rifampicin concentration(mean ug/100 mg tissue ± SEM)

Dietary group		Hours after drug rifampicin									
		1		2		5		10		20	
		NP	LP	NP	LP	NP	LP	NP	LP	NP	LP
Drug treatment											
Rifampicin alone	Acute	1.6 ±0.2	2.3 ±0.2	4.0 ±0.3	6.0 ±0.6	4.0 ±0.4	4.9 ±0.3	2.1 ±0.3	5.0 ±0.7	2.6 ±0.2	4.2 ±0.4
	P	0.05		0.01		N.S.		0.01		0.05	
	Chronic	4.2 ±0.2	4.9 ±0.6	7.9 ±0.2	8.3 ±0.8	18.5 ±0.6	8.0 ±0.6	18.0 ±1.3	6.7 ±0.3	4.9 ±0.3	5.7 ±0.3
	P	N.S.		N.S.		N.S.		N.S.		N.S.	
Rifampicin + INH	Acute	1.2 ±0.2	1.7 ±0.2	3.2 ±0.3	4.0 ±0.1	4.4 ±0.3	3.3 ±0.2	2.5 ±0.3	1.6 ±0.2	2.0 ±0.2	1.7 ±0.3
	P	N.S.		0.05		0.05		N.S.		N.S.	
	Chronic	1.7 ±0.5	2.4 ±0.2	3.1 ±0.3	4.4 ±0.6	4.3 ±0.9	2.8 ±0.2	3.4 ±1.4	1.9 ±0.2	1.9 ±1.0	0.8 ±0.1
	P	N.S.		N.S.		N.S.		0.05		N.S.	
	P_1	0.001	0.001	0.001	N.S.	N.S.	0.01	0.01	N.S.	0.001	0.05
	P_2	N.S.	0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.05
	P_3	N.S.	N.S.	N.S.	0.05	N.S.	0.01	0.01	0.01	N.S.	0.01
	P_4	0.01	0.01	0.001	0.01	N.S.	0.001	0.05	0.001	0.05	0.001

P_1 = Rifampicin treatment acute vs chronic

P_2 = Rifampicin + INH treatment acute vs chronic

P_3 = Acute treatment rifampicin vs rifampicin + INH

P_4 = Chronic treatment rifampicin vs rifampicin + INH

Table-23: Effect of undernutrition on lung rifampicin concentration (mean ug/100 mg tissue ± SEM)

Dietary group		Hours after drug administration: 1 NP	1 LP	2 NP	2 LP	5 NP	5 LP	10 NP	10 LP	20 NP	20 LP
Drug treatment											
Rifampicin alone	Acute	16.5 ±1.4	8.4 ±0.4	19.2 ±0.9	18.6 ±0.4	15.4 ±1.0	15.7 ±0.5	7.4 ±1.0	12.8 ±0.6	4.9 ±1.1	9.4 ±0.3
	P	0.01		N.S.		N.S.		0.05		N.S.	
	Chronic	13.9 ±0.8	11.9 ±1.1	20.3 ±2.7	16.2 ±1.3	14.8 ±1.8	18.7 ±1.6	12.5 ±1.9	14.7 ±1.8	7.6 ±0.7	10.2 ±0.6
	P	N.S.		N.S.		N.S.		N.S.		0.05	
Rifampicin + INH	Acute	12.3 ±1.0	9.0 ±0.9	17.2 ±1.7	14.5 ±0.3	13.6 ±1.8	11.5 ±0.5	6.2 ±1.2	10.0 ±0.9	3.4 ±0.3	5.6 ±0.5
	P	0.05		N.S.		N.S.		0.05		0.001	
	Chronic	10.9 ±1.0	6.8 ±0.7	15.9 ±2.2	10.9 ±0.5	13.2 ±1.5	7.0 ±0.8	5.0 ±0.8	7.2 ±0.5	2.0 ±0.4	0.5 ±0.1
	P	0.05		N.S.		0.01		0.05		0.05	
	P_1	N.S.	0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.01
	P_2	N.S.	N.S.	N.S.	0.01	N.S.	0.05	N.S.	0.05	0.05	N.S.
	P_3	0.05	N.S.	N.S.	0.01	N.S.	0.001	N.S.	N.S.	N.S.	N.S.
	P_4	N.S.	0.01	N.S.	0.01	N.S.	0.001	0.01	0.01	0.001	0.001

P_1 = Rifampicin treatment acute vs chronic

P_2 = Rifampicin + INH treatment vs chronic

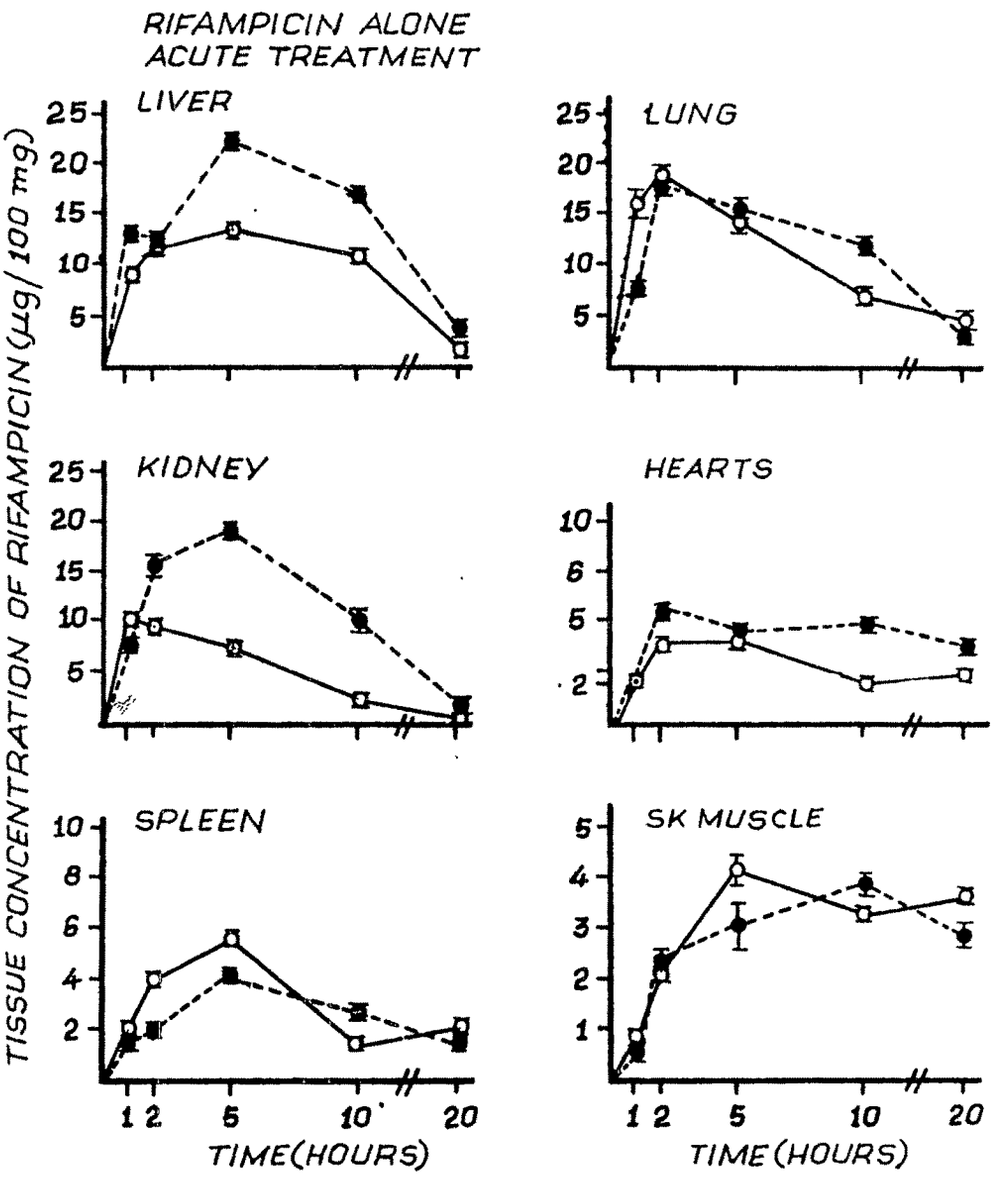
P_3 = Acute treatment rifampicin vs rifampicin + INH

P_4 = Chronic treatment rifampicin vs rifampicin + INH

Fig.4

Effect of undernutrition on the tissue concentrations of rifampicin following acute treatment with rifampicin. Time in hours is depicted on the abscissa and mean tissue concentrations of rifampicin (ug/100 mg) are depicted on the ordinate. The lines joining open circles (⊖——⊖) and closed circles (●——●) represent tissue concentrations of rifampicin for the normal protein and low protein groups respectively. Vertical bars represent the SEM (n = 4).

FIG.-4


RIFAMPICIN ALONE
ACUTE TREATMENT
LIVER
LUNG
KIDNEY
HEARTS
SPLEEN
SK MUSCLE
TISSUE CONCENTRATION OF RIFAMPICIN (µg/100 mg)
TIME (HOURS)
TIME (HOURS)

Rifampicin levels in spleen and muscle

It appears that spleen (Table-24) and muscle (Table-25) are probably deeper compartments. As in the case of heart muscle, much less spleen concentration of rifampicin was observed. However, a clear pattern did not emerge between LP and NP groups. Peak levels were obtained at 5 hour interval in both the tissues.

ii) Chronic treatment with rifampicin alone

There was an apparent increase in AUC values of rifampicin in all the tissues after chronic treatment. However, all were statistically insignificant ($P > 0.05$). There was no significant difference in the concentrations of rifampicin in the liver of either NP or LP groups at any time point. In the liver, the maximum rifampicin concentration was observed at 5 hour after the administration of the last dose in both the groups (24.16 $\pm$ 1.5 and 21.2 $\pm$ 0.87 in the NP and the LP groups, respectively; Table-20).

The LP group had higher kidney levels of rifampicin at all the five time points studied (1,2,5,10 and 20 hours) with statistically significant difference at 2,5 and 10 hours only (Table-21, Fig.5). As in the liver, in the heart also there was no significant difference in the concentration of rifampicin in either group (Table-22, Fig.5).

Table-24:Effect of undernutrition on spleen rifampicin concentration (mean ug/100 mg tissue ± SEM)

Dietary group		Hours after drug administration									
		1		2		5		10		20	
		NP	LP	NP	LP	NP	LP	NP	LP	NP	LP
Drug treatment											
Rifampicin alone	Acute	2.0 ±0.1	1.9 ±0.2	3.9 ±0.2	2.0 ±0.1	5.4 ±0.3	4.2 ±1.3	1.5 ±0.2	2.8 ±0.2	2.1 ±0.2	1.6 ±0.1
	P	N.S.		N.S.		0.001		0.05		0.05	
	Chronic	1.2 ±0.2	2.3 ±0.3	1.8 ±0.1	4.1 ±0.3	1.9 ±0.2	4.1 ±0.5	2.0 ±0.1	2.6 ±0.2	0.7 ±0.1	0.8 ±0.2
	P	0.001		0.001		0.01		0.001		0.05	
Rifampicin + INH	Acute	1.7 ±0.2	1.8 ±0.4	3.5 ±0.3	2.8 ±0.3	4.2 ±0.5	2.4 ±0.4	2.9 ±0.3	2.8 ±0.5	2.0 ±0.2	1.3 ±0.3
	P	N.S.		N.S.		0.05		N.S.		0.01	
	Chronic	1.9 ±0.5	2.4 ±0.3	3.6 ±0.7	4.0 ±0.2	2.8 ±0.4	3.7 ±0.2	2.0 ±0.5	2.8 ±0.2	0.6 ±0.1	1.8 ±0.3
	P	N.S.		N.S.		N.S.		N.S.		N.S.	
	P_1	0.01	N.S.	0.001	0.001	0.001	N.S.	0.05	N.S.	0.001	0.001
	P_2	N.S.	N.S.	N.S.	N.S.	0.05	0.05	N.S.	N.S.	0.01	N.S.
	P_3	N.S.	N.S.	N.S.	N.S.	N.S.	0.05	0.05	N.S.	N.S.	N.S.
	P_4	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.05

P_1 = Rifampicin treatment acute vs chronic

P_2 = Rifampicin + INH treatment acute vs chronic

P_3 = Acute treatment rifampicin vs rifampicin + INH

P_4 = Chronic treatment rifampicin vs rifampicin + INH

Table-25: Effect of undernutrition on skeletal muscle rifampicin concentration (mean ug/100 mg tissue ± SEM)

Dietary group		Hours after drug administration									
		1		2		5		10		20	
		NP	LP	NP	LP	NP	LP	NP	LP	NP	LP
Drug treatment											
Rifampicin alone	Acute	0.8 ±0.1	0.5 ±0.1	2.1 ±0.1	2.3 ±0.3	4.2 ±0.4	3.2 ±0.4	3.3 ±0.2	3.9 ±0.3	3.6 ±0.2	2.9 ±0.4
	P	N.S.		N.S.		N.S.		N.S.		N.S.	
	Chronic	1.3 ±0.2	1.7 ±0.1	1.7 ±0.1	2.2 ±0.3	3.4 ±0.8	2.9 ±0.5	3.5 ±0.4	3.0 ±0.6	1.7 ±0.1	2.4 ±0.2
	P	N.S.		N.S.		N.S.		N.S.		0.05	
Rifampicin + INH	Acute	0.6 ±0.2	0.8 ±0.1	1.7 ±0.2	1.5 ±0.5	3.3 ±0.3	3.4 ±0.3	2.7 ±0.4	3.2 ±0.3	1.7 ±0.2	2.3 ±0.4
	P	N.S.		N.S.		N.S.		N.S.		N.S.	
	Chronic	0.8 ±0.2	1.4 ±0.3	1.2 ±0.3	1.7 ±0.7	1.7 ±0.3	2.0 ±0.3	1.0 ±0.2	1.6 ±0.4	1.0 ±0.2	1.3 ±0.2
	P	0.01		N.S.		0.05		N.S.		N.S.	
	P_1	0.05	0.001	0.01	N.S.	N.S.	N.S.	N.S.	N.S.	0.001	N.S.
	P_2	N.S.	N.S.	N.S.	N.S.	0.01	0.05	0.01	0.001	0.001	0.05
	P_3	N.S.	0.05	N.S.	N.S.	0.05	N.S.	N.S.	N.S.	0.001	N.S.
	P_4	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.001	0.05	0.01	0.001

P_1 = Rifampicin treatment acute vs chronic

P_2 = Rifampicin + INH treatment acute vs chronic

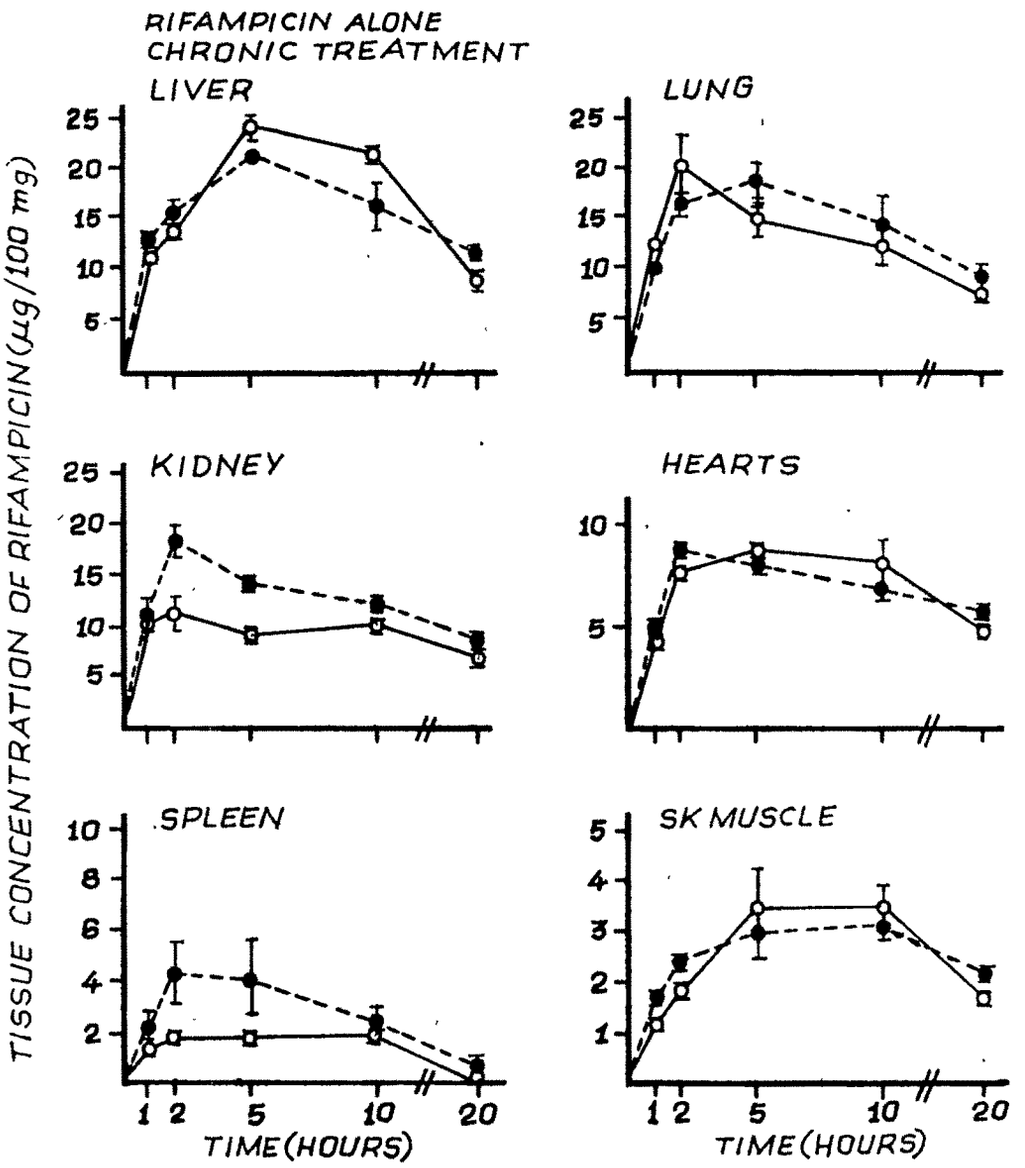
P_3 = Acute treatment rifampicin vs rifampicin + INH

P_4 = Chronic treatment rifampicin vs rifampicin + INH

Fig.5

Effect of undernutrition on the tissue concentrations of rifampicin following chronic treatment with rifampicin. Time in hours is depicted on the abscissa and mean tissue concentrations of rifampicin (ug/100 mg) are depicted on the ordinate. The lines joining open circles (⊙—⊙) and closed circles (●—●) represent tissue concentrations of rifampicin for the normal protein and low protein groups respectively. Vertical bars represent the SEM (n = 4).

FIG.-5


RIFAMPICIN ALONE
CHRONIC TREATMENT
LIVER
LUNG
KIDNEY
HEARTS
SPLEEN
SK MUSCLE
TISSUE CONCENTRATION OF RIFAMPICIN (µg/100 mg)
TIME (HOURS)
TIME (HOURS)

Lung being a highly vascular organ followed the pattern of serum rifampicin concentration. There was no significant difference in the concentrations of rifampicin in either group following chronic treatment or single dose of rifampicin (Table-23, Fig.5).

The spleen rifampicin concentrations were significantly lower in the NP group as compared to LP group at all the time points (Table-24, Fig.5). In the skeletal muscle, the differences were insignificant (Table-25, Fig.5).

iii) Acute treatment with rifampicin + INH

a) In the liver, rifampicin concentrations in the LP group were higher at 1,2,5,10 and 20 hours (significant difference at 20 hours). The maximum concentrations were found at 5 hours (Table-20, Fig.6). In the kidney, the LP group had significantly higher concentrations at 10 hours. The maximum concentration was observed at 2 hours in the NP group and at 5 hours in the LP group (Table-21, Fig.6).

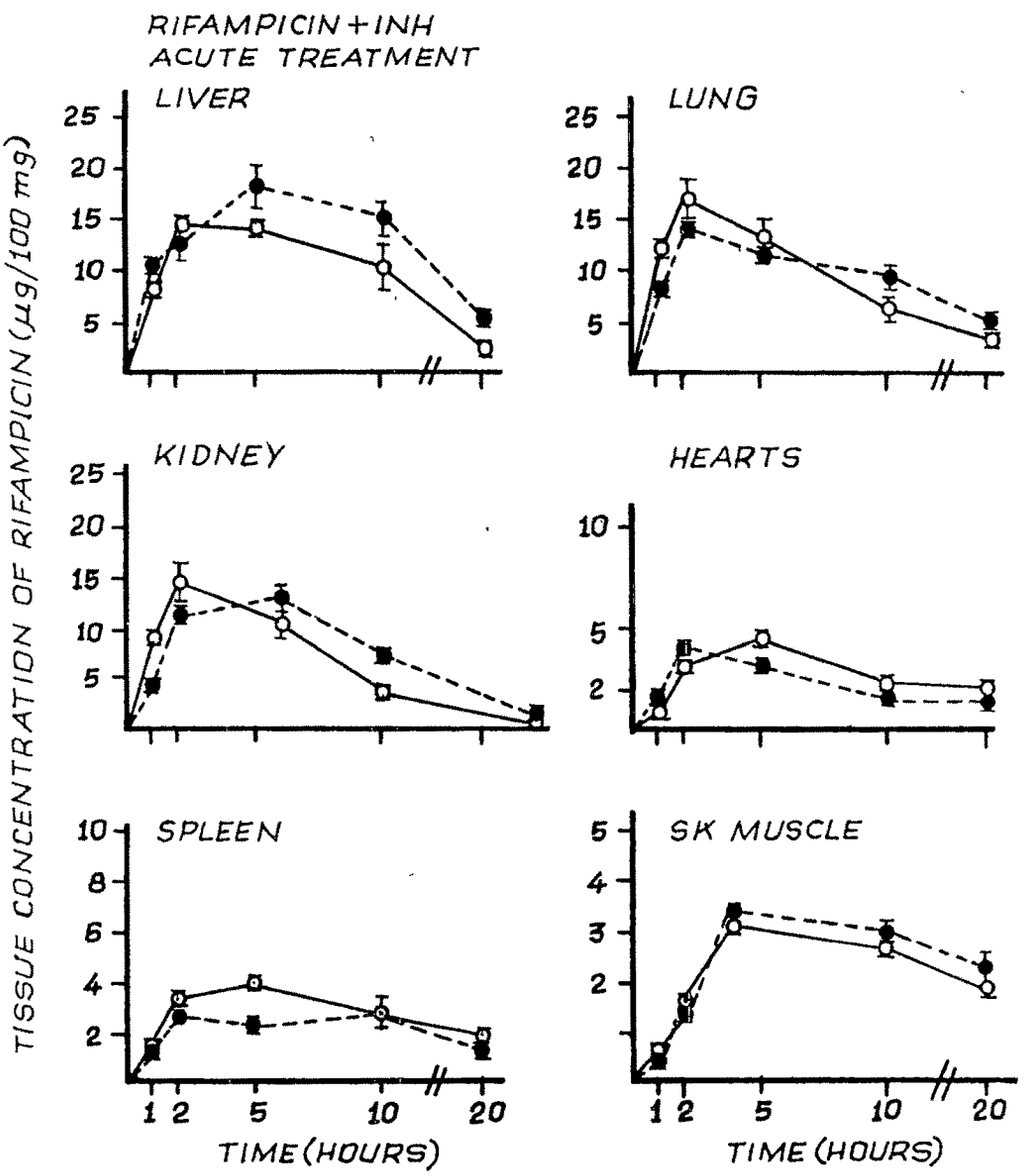
In the heart, the concentration was significantly higher at 2 hours in the LP group. The NP group showed the maximum rifampicin concentration at 5 hours (Table-22, Fig.6).

b) In the lung, the concentration resembled that found in serum. The peak was reached at 2 hours in both NP and LP

Fig.6

Effect of undernutrition on the tissue concentrations of rifampicin following rifampicin + INH acute treatment. Time in hours is depicted on the abscissa and mean tissue concentrations of rifampicin (ug/100 mg) are depicted on the ordinate. The lines joining open circles (⊙——⊕) and closed circles (●——●) represent tissue concentrations of rifampicin for the normal protein and low protein groups respectively. Vertical bars represent the SEM (n = 4).

FIG.-6


RIFAMPICIN + INH
ACUTE TREATMENT
LIVER
LUNG
KIDNEY
HEARTS
SPLEEN
SK MUSCLE
TISSUE CONCENTRATION OF RIFAMPICIN (µg/100 mg)
TIME (HOURS)
TIME (HOURS)

groups and there was no significant difference between these values. The LP group had higher rifampicin concentration at 10 and 20 hours (Table-23, Fig.6).

c) In the spleen (Table-24, Fig.6) and skeletal muscle (Table-25, Fig.6), much less tissue concentrations were obtained as in the heart muscle; however, there was no significant difference between the concentration in the NP and the LP groups except 5 and 20 hour.

iv) Chronic treatment with rifampicin + INH

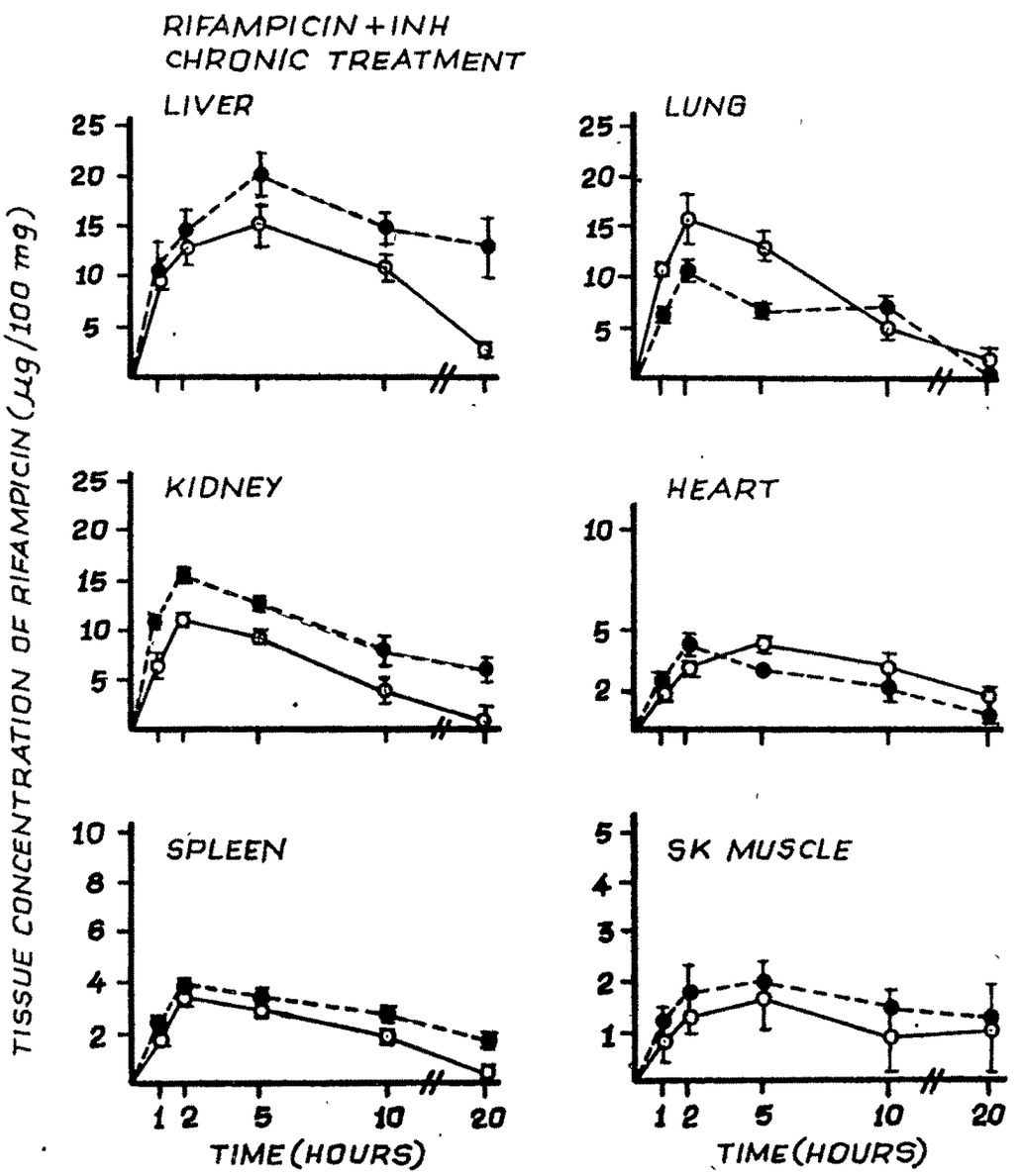
a) In the liver and kidney, the LP group showed higher rifampicin concentrations at all the time points studied. For the liver the differences were statistically significant at 20 hours (Table-20, Fig.7) and for the kidney at 2 and 20 hours (Table-21, Fig.7). In the liver, the maximum concentration was reached at 5 hours while in kidney, it was at 2 hours. In the heart, the concentration was significantly higher in the NP group at 10 hours. The maximum concentration was reached at 5 hours in the NP group and at 2 hours in the LP group (Table-22, Fig.7).

b) At sampling points, 1,2,5 and 20 hours, the NP group had higher lung rifampicin levels. The NP group showed significantly higher levels for the lung at 1,5 and 20 hours (Table-23, Fig.7).

Fig.7

Effect of undernutrition on the tissue concentrations of rifampicin following rifampicin + INH chronic treatment. Time in hours is depicted on the abscissa and mean tissue concentrations of rifampicin (ug/100 mg) are depicted on the ordinate. The lines joining open circles (⊖—⊖) and closed circles (●—●) represent tissue concentrations of rifampicin for the normal protein and low protein groups respectively. Vertical bars represent the SEM (n = 4).

FIG.-7


RIFAMPICIN + INH
CHRONIC TREATMENT
LIVER
LUNG
KIDNEY
HEART
SPLEEN
SK MUSCLE
TISSUE CONCENTRATION OF RIFAMPICIN (µg/100 mg)
TIME (HOURS)
TIME (HOURS)

c) In the spleen (Table-24, Fig.7) and skeletal muscle (Table-25, Fig.7) like that in the liver and kidney, the LP group showed higher rifampicin concentrations at 1,2,5 10 and 20 hours; however, they were statistically insignificant. In the skeletal muscle, the maximum concentration was reached at 5 hours and in the spleen at 2 hours.

The tissue concentrations obtained in brain and intestine are excluded because of inconsistency of results.

V. Effect of undernutrition on the urinary excretion of rifampicin

i) Acute treatment with rifampicin alone

At all the elimination time points of 12, 24, 48 and 72 hours, the urinary excretion of rifampicin (as percentage of the dose administered) was significantly greater in the LP group than that in the NP group (Table-26, Fig.8). However, when expressed as ug/ml/kg, the urinary excretion was found to be significantly higher in the NP group than in the LP group (Table-27, Fig.9).

ii) Chronic treatment with rifampicin alone

At 12, 24, 48 and 72 hours, the urinary excretion of rifampicin (as percentage of the dose administered) was significantly greater in the LP group than that in the NP group (Table-26, Fig.8). The pattern of urinary excretion

Table-26: Effect of undernutrition on urinary excretion of rifampicin (% of dose administered)

Dietary group		Hours after drug administration							
		12		24		48		72	
		NP	LP	NP	LP	NP	LP	NP	LP
Type of drug treatment									
Rifampicin alone	Acute	5.8 ±0.3	6.8 ±0.4	7.7 ±0.4	9.2 ±0.4	9.5 ±0.3	11.1 ±0.3	12.0 ±0.4	13.5 ±0.5
	P	0.05		0.05		0.01		0.05	
	Chronic	4.9 ±0.2	5.6 ±0.3	6.3 ±0.2	7.3 ±0.2	7.3 ±0.2	8.9 ±0.2	8.3 ±0.3	10.2 ±0.3
	P	0.05		0.01		0.001		0.001	
Rifampicin + INH	Acute	3.7 ±0.1	5.2 ±0.3	6.4 ±0.1	7.9 ±0.4	8.6 ±0.2	10.4 ±0.5	10.2 ±0.3	12.4 ±1.4
	P	0.001		0.01		0.01		0.001	
	Chronic	3.1 ±0.2	4.5 ±0.2	5.4 ±0.4	6.6 ±0.2	6.9 ±0.2	8.4 ± 0.2	8.2 ±0.3	10.8 ±0.5
	P	0.001		0.001		0.001		0.01	
	P_1	0.01	0.05	0.01	0.001	0.001	0.001	0.001	0.001
	P_2	0.05	0.05	0.05	0.05	0.001	0.01	0.001	0.05
	P_3	0.001	0.001	0.01	N.S.	0.05	N.S.	0.01	N.S.
	P_4	0.001	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Fig.8

Effect of undernutrition on the urinary excretion of rifampicin. Time in hours is depicted on the abscissa and excretion of rifampicin as percentage of dose administered is depicted on the ordinate. Panel A shows data for rats treated with rifampicin alone and panel B shows data for rats treated with rifampicin + INH. The upper panel and the lower panel represent data of rats following acute and chronic drug treatments respectively. The lines joining open circles (⊙—⊖) and closed circles (●—●) depict mean values of rifampicin in normal protein and lower protein groups respectively. Vertical bars represent SEM.

FIG.-8

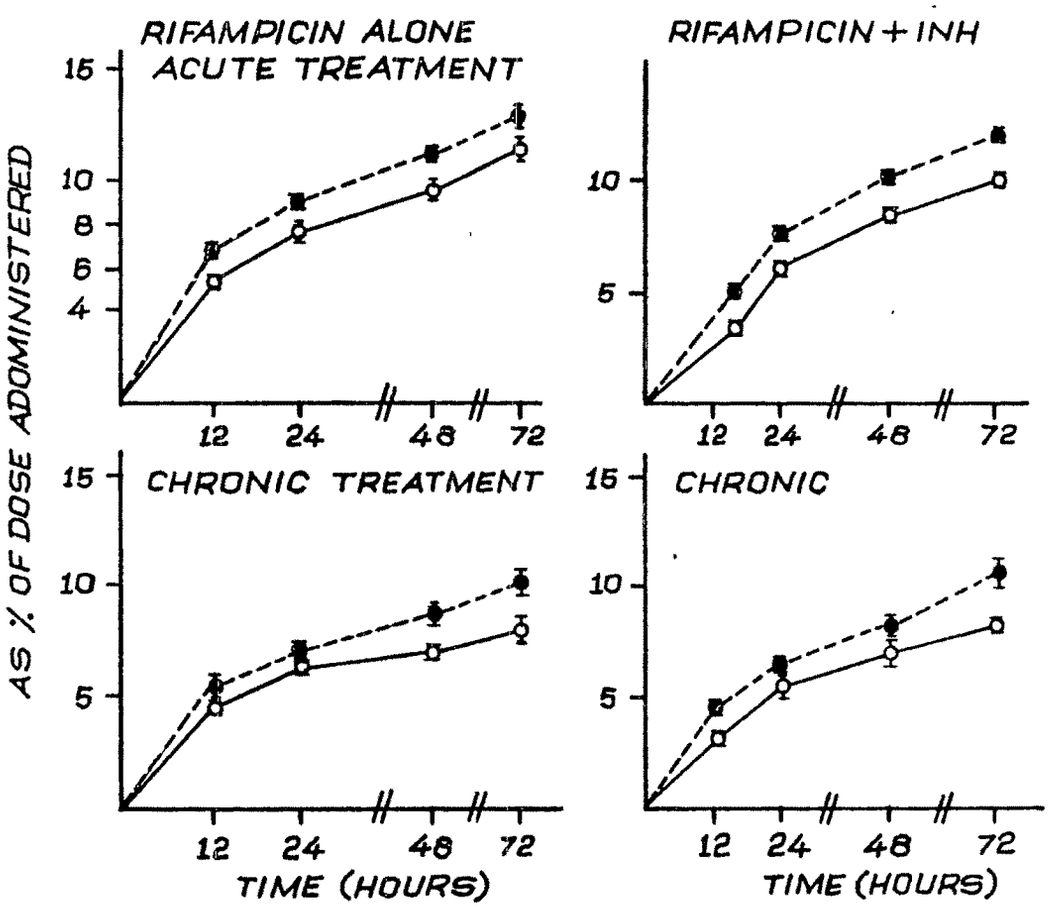

RIFAMPICIN ALONE
ACUTE TREATMENT
RIFAMPICIN + INH
CHRONIC TREATMENT
CHRONIC
AS % OF DOSE ADOMINISTERED
TIME (HOURS)
TIME (HOURS)
15
10
8
6
4
5
12
24
48
72

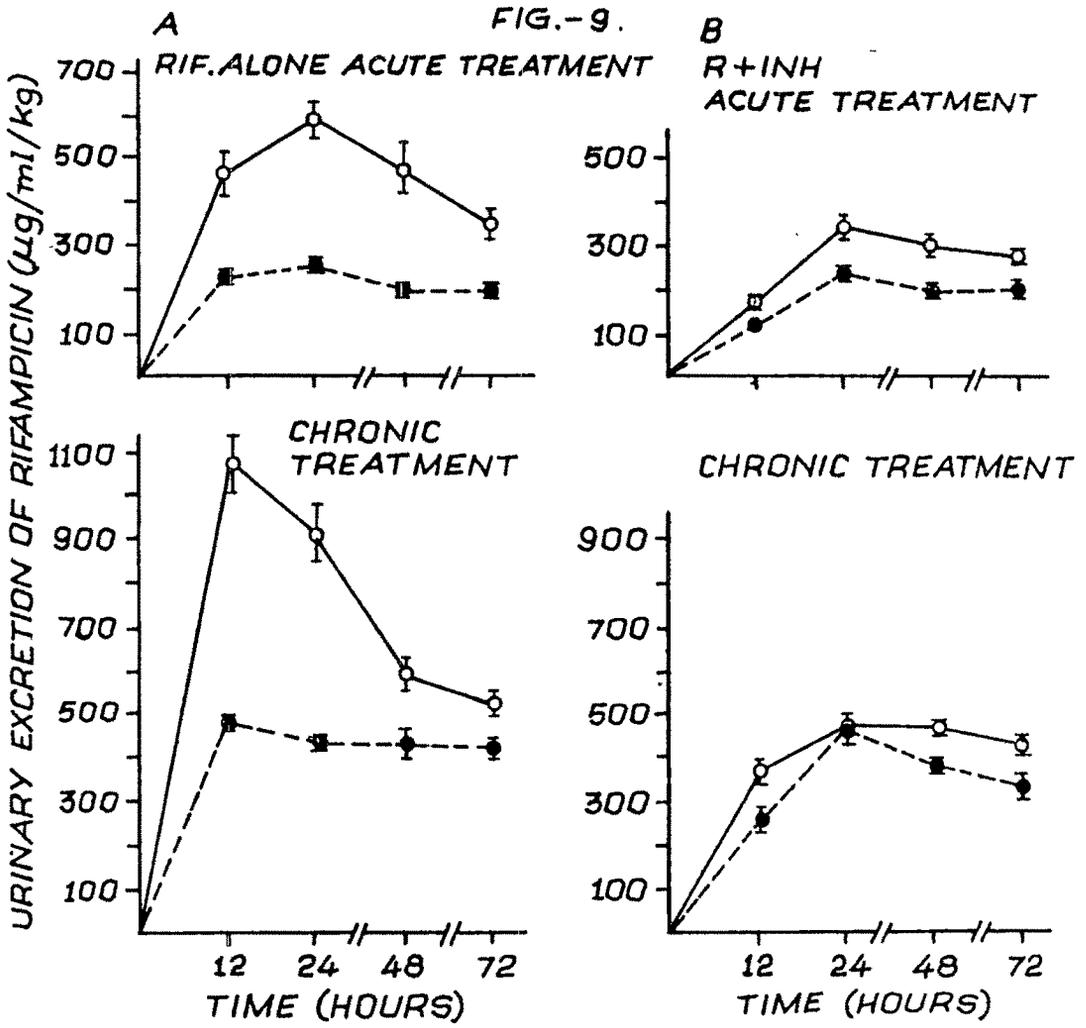
Table-27: Effect of undernutrition on the urinary excretion of rifampicin (mean ug/ml/kg ± SEM)

		Hours after drug administration							
		12		24		48		72	
Dietary group		NP	LP	NP	LP	NP	LP	NP	LP
Drug treatment									
Rifampicin alone	Acute	468.8 ±38.6	231.6 ± 8.57	593.9 ±37.6	254.7 ±13.2	484.3 ±69.7	200.6 ± 9.5	355.6 ±32.7	196.0 ±8.2
	P	0.001		0.001		0.01		0.001	
	Chronic	1094.0 ± 77.4	499.3 ±35.63	937.3 ±68.6	447.5 ±12.6	597.0 ±48.0	348.8 ±28.4	538.0 ±46.1	335.0 ±19.3
	P	0.001		0.001		0.001		0.01	
Rifampicin + INH	Acute	178.1 ±34.5	132.3 ±16.1	349.5 ±51.8	234.2 ±18.1	306.5 ±55.6	203.6 ±24.5	283.0 ±32.4	206.5 ±22.1
	P	N.S.		N.S.		N.S.		N.S.	
	Chronic	350.9 ±56.0	268.3 ±34.5	491.2 ±45.1	468.3 ±52.7	472.3 ±46.1	393.9 ±55.5	448.3 ±48.1	360.4 ±63.7
	P	N.S.		N.S.		N.S.		N.S.	
	P_1	0.05	0.05	0.05	0.05	N.S.	0.05	0.05	0.05
	P_2	0.05	0.05	N.S.	0.05	N.S.	0.05	0.05	N.S.
	P_3	0.05	0.05	0.05	0.05	N.S.	N.S.	N.S.	N.S.
	P_4	0.05	0.05	0.05	N.S.	N.S.	N.S.	N.S.	N.S.

Fig.9

Effect of undernutrition on the urinary excretion of rifampicin. Time in hours is depicted on the abscissa and excretion of rifampicin as ug/ml/kg is depicted on the ordinate. Panel A shows data for rats treated with rifampicin alone and panel B shows data for rats treated with rifampicin + INH. The upper panel and the lower panel represent data of rats following acute and chronic drug treatments respectively. The lines joining open circles (⊖——⊖) and closed circles (●——●) depict mean values of rifampicin in normal protein and lower protein groups respectively. Vertical bars represent SEM.

FIG.-9.


A
RIF. ALONE ACUTE TREATMENT
B
R+INH
ACUTE TREATMENT
CHRONIC
TREATMENT
CHRONIC TREATMENT
URINARY EXCRETION OF RIFAMPICIN (µg/ml/kg)
700
500
300
100
1100
900
700
500
300
100
500
300
100
900
700
500
300
100
12
24
48
72
TIME (HOURS)
12
24
48
72
TIME (HOURS)

remained the same even when expressed as ug/ml/kg (Table-27, Fig.9).

iii) Acute treatment with rifampicin + INH

At 12, 24, 48 and 72 hours, the urinary excretion of rifampicin expressed as percentage of the dose administered was significantly higher in LP group (Table-26, Fig.8). Although the values were higher for the NP group than the LP group when expressed as ug/ml/kg, they were statistically insignificant (Table-27, Fig.9).

iv) Chronic treatment with rifampicin + INH

The excretion of rifampicin expressed as percentage of the dose administered was significantly higher in the LP group than in the NP group (Table-26, Fig.8). When expressed as ug/ml/kg, the urinary excretion was higher in the NP than in the LP group (but statistically insignificant; Table-27, Fig.9).

The urinary excretion of desacetyl rifampicin was decreased after chronic treatment with rifampicin. After chronic treatment, the excretion of desacetyl rifampicin as percentage of the total rifampicin excretion was higher in the LP group of rats at 12, 24 and 48 hours (Table-28).

Table-28 : Excretion of desacetylated rifampicin as percentage of total rifampicin excretion

Dietary group	n	a - acute treatment				b - chronic treatment			
		12	24	48	72	12	24	48	72
MP	10	20.2 ±0.9	23.3 ±2.1	35.4 ±3.2	36.4 ±3.4	10.3 ±0.5	14.3 ±1.2	22.6 ±1.3	34.6 ±2.2
EP	10	21.1 ±1.7	26.4 ±1.6	43.9 ±2.3	35.6 ±3.3	16.8* ±1.4	20.0* ±1.3	37.8* ±3.4	33.1 ±3.1

* $P < 0.05$

v) Effect of undernutrition and/or drug treatment on hepatic microsomal enzyme system

Neither undernutrition nor the drug treatment could modify the liver or the microsomal protein content significantly (Table-29).

Protein deficiency reduced the cytochrome P-450 concentration to 36.54% of the control value (Table-30a). Cytochrome P-450 concentration was apparently increased in both NP and LP groups following drug treatment but the increase was not significant ($P > 0.05$). Protein deficiency also lowered the cytochrome b_5 concentration to 0.41 ± 0.01 from 1.18 ± 0.16 umoles/mg microsomal protein in the untreated group and to 0.49 ± 0.04 from 1.19 ± 0.04 umoles/mg microsomal protein in the treated group.

Although, in both the NP and LP groups, there was increase in the cytochrome b_5 content, it was statistically insignificant (Table-30b).

(B) Human studies

I. Studies in patients of pulmonary tuberculosis

The main focus of the present study was to see the effect of nutritional status on the rifampicin pharmacokinetics. We found that more than 95% patients of pulmonary tuberculosis attending the Government hospitals covered in

Table-29 : Effect of undernutrition and drug treatment on the microsomal and liver protein of rat

Group	Liver protein mean ± SEM (mg/g liver)		Microsomal protein mean ± SEM (mg/g liver)	
	Untreated	treated	Untreated	treated
Normally nourished NP group	166.0 ± 7.4	167.0 ± 5.1	5.3 ±0.7	5.4 ±0.3
Undernourished LP group	177.0 ± 8.1	170.0 ± 4.6	6.1 ±0.3	6.0 ±0.2
P	N.S.	N.S.	N.S.	N.S.
P_1	N. S.		N. S.	
P_2	N. S.		N. S.	

P = NP vs LP

P_1 = Np group untreated vs treated

P_2 = LP group untreated vs treated

Table-30a : Effect of undernutrition and drug treatment on the cytochrome P-450 content of rat liver

Group	Cytochrome P-450 content umoles/mg microsomal protein (mean ± SEM)	
	Untreated	Treated
Normally nourished NP group	1.53 ± 0.2	1.69 ± 0.1
Undernourished, LP group	0.57 ± 0.10	0.95 ± 0.20
P	0.001	0.001
P_1	N. S.	
P_2	N. S.	

Table-30b : Effect of undernutrition and drug treatment on the cytochrome b_5 rat liver

Group	Cytochrome b_5 umoles/mg microsomal protein (mean ± SEM)	
	Untreated	Treated
Normally nourished, NP group	1.18 ± 0.16	1.19 ± 0.04
Undernourished, LP group	0.41 ± 0.01	0.49 ± 0.04
P	0.001	0.001
P_1	N. S.	
P_2	N. S.	

P = NP vs LP

P_1 = NP untreated vs treated

P_2 = LP untreated vs treated

this study had nutritional score below 0.18. Therefore, it became imperative to divide the available patients into two subgroups for comparison.

A. patients having nutritional score 0.14 or below 0.14 i.e. comparatively more undernourished

B. patients having nutritional score above 0.14 i.e. comparatively less undernourished.

Table-31a presents the anthropometric data of undernourished patients (n = 12) of pulmonary tuberculosis. There was no significant correlation between nutritional score and total proteins (r = 0.5, 't' = 1.82). There was significant direct correlation between nutritional score and serum albumin (r = 1.05, 't' = 3.32).

A. Relationship between nutritional score and serum rifampicin concentration (n = 12).

Table-31b presents serum rifampicin concentrations (ug/ml) at different time points in undernourished patients of pulmonary tuberculosis (n = 12). In this preliminary study, it became evident that as the nutritional score decreased, it took longer for individual serum rifampicin levels following single oral dose (10 mg/kg) to reach the maximum. Apparently the values of Cmax kept on decreasing with decreasing nutritional score. Although, there was high degree of variability in the serum rifampicin concentrations

Table-31a : Anthropometric profile of undernourished patients of pulmonary tuberculosis (n = 12)

Sr. No.	Rifampicin administered (mg)	Age (years)	Weight (kg)	Height (cms)	Nutritional score	Total proteins (g/100 ml)	Albumin (g/100 ml)
1.	440	32	44	160	0.166	5.8	3.1
2.	410	50	41	157	0.166	6.1	3.4
3.	390	42	39	154	0.164	5.6	3.2
4.	360	29	36	154	0.152	5.4	3.2
5.	380	30	38	160	0.148	6.0	3.5
6.	380	47	38	162	0.145	5.8	3.0
7.	360	51	36	158	0.145	5.4	3.0
8.	340	36	34	155	0.142	5.5	3.1
9.	390	43	39	166	0.141	6.2	3.6
10.	360	32	36	160	0.141	5.8	3.4
11.	360	46	36	161	0.139	6.0	3.5
12.	330	45	33	155	0.137	5.4	3.0
Mean	375.0	40.3	37.5	147.0	0.15	5.8	3.3
S.E.	± 8.8	±2.3	±0.9	±12.0	±0.003	±0.1	±0.06

Table-31b : Serum concentration of rifampicin at different time points in undernourished patients of Table-31a

Sr. No.	Serum rifampicin concentration (ug/ml)				
	1	2	4	6	8
1.	17.5	25.0	23.1	15.0	10.8
2.	11.7	18.8	15.0	9.2	6.8
3.	13.3	20.8	10.8	8.3	5.8
4.	6.7	11.7	9.2	5.4	3.3
5.	8.3	15.0	14.2	8.3	1.7
6.	8.8	10.3	11.7	6.7	3.3
7.	3.8	9.8	7.5	4.2	1.7
8.	4.2	8.1	9.2	5.8	2.7
9.	9.8	12.5	14.2	12.9	6.7
10.	2.8	7.5	13.3	12.5	6.8
11.	3.3	9.2	13.4	10.8	9.2
12.	2.5	6.3	13.3	11.7	5.8
Mean	7.7	12.9	12.9	9.2	5.4
S.E.	±1.4	±1.7	±1.2	±1.0	±0.8

reached, subjects having nutritional score above 0.14 showed preponderance for Tmax of 2 hours and those having nutritional score below 0.14 had Tmax of 4 hours.

B. Pharmacokinetic parameters

Anthropometric data of 25 patients where 15 patients (group A) were more undernourished with nutritional scores below 0.14 and 10 patients (group B) were comparatively less undernourished with nutritional score between 0.18 and 0.14, are presented in Table-32a.

The mean body weights of these two subgroups were significantly different from each other ($P < 0.001$); however, the serum total protein and albumin concentrations did not show significant difference between the two subgroups.

Among the pharmacokinetic parameters studied, serum half-life, maximum concentration reached and apparent volume of distribution (aVd in litres) were higher in group B than in group A; on the other hand, the Tmax and elimination rate constant were higher ($P < 0.05$) in group A as compared to group B (Table-32b).

Table-32 shows the urinary excretion of rifampicin following single oral dose (10 mg/kg) in group A and group B. The urinary volume was higher in group A than in group B ($P < 0.05$). The urinary excretion of rifampicin (mg/day)

Table-32a : Anthropometric profile of patients of pulmonary tuberculosis (mean ± SEM values)

Group	n	Rifampicin administered (mg)	Age (years)	Weight (kg)	Nutritional score	Total proteins (g/100 ml)	Albumin (g/100 ml)
A	15	316.9 ±15.8	37.5 ±2.8	31.8 ±1.3	0.12 ±0.003	6.1 ±0.1	3.1 ±0.1
B	10	407.0 ±34.9	41.3 ±3.9	40.7 ±3.2	0.16 ±0.02	6.1 ±0.04	3.6 ±0.7

Table-32b : Pharmacokinetic profile of rifampicin in patients of pulmonary tuberculosis (mean ± SEM values)

Group	n	Co	$aVd_{(e)}$	Cmax (ug)	Tmax (hr)	$t_{1/2}$	Elimination rate constant
A	15	18.2 ±1.2	18.1 ±1.5	10.4 ±1.0	2.7 ±0.2	2.6 ±0.5	0.4 ±0.0
B	10	14.9 ±1.9	26.7 ±1.5	12.9 ±2.1	2.1 ±0.2	4.9 ±0.1	0.2 ±0.0

Table-32c : Urinary excretion of rifampicin in patients of pulmonary tuberculosis administered rifampicin

Group	n	Urine volume (ml, mean ± SEM)	mg/day (mean ± SEM)	Excretion rate (mg/ml/24 hours) (mean ± SEM)
A	15	450.0 ±20.9	154.8 ± 3.3	0.35 ±0.02
B	10	332.0 ±15.1	121.5 ± 3.8	0.37 ±0.01

was higher in group A than in group B ($P < 0.05$). When the data were expressed as mg/kg/day, the same pattern was maintained. The excretory rate in either group was not statistically different from each other.

C. Drug interaction

Tables-33, 34 and 35 present the effect of concurrently administered antitubercular drugs on rifampicin pharmacokinetics. a, presents anthropometric data; b, mean serum rifampicin levels and c, corresponding values of urinary excretion, at different time points, in different study protocols (i to iv).

There was no statistically significant difference in serum rifampicin concentrations at various time points when rifampicin was administered alone (study-i) or with INH (study-ii), or when the third drug was streptomycin (study-iii) or ethambutol (study-iv) or pyrazinamide (study-v) (b of Tables-33, 34 and 35).

The urinary excretion of rifampicin (mg/day) and the excretion rate in all the above studies was also not statistically significant (c of Tables-33, 34 and 35).

II. Studies in healthy volunteers

Table-36 presents data of normally nourished, healthy volunteers (n = 6). a, presents anthropometric data; b, serum rifampicin concentration (ug/ml) at different

Table-33a: Anthropometric data of patients of pulmonary tuberculosis (mean ± SEM values, n = 10)

Rifampicin administered (mg)	Age (years)	Weight (kg)	Nutritional score	Total proteins (g/100 ml)	Albumin (g/100 ml)
379.0 ±19.3	44.0 ±3.2	37.9 ±2.0	0.15 ±0.8	5.9 ±0.1	3.2 ±0.0

Table-33b : Serum rifampicin concentration in patients of pulmonary tuberculosis (ug/ml, mean ± SEM)

Study No.	Hours after drug administration					
	½	2½	4	8	12	24
i) Rifampicin alone	6.1 ±0.6	10.9 ±1.3	10.7 ±1.9	7.2 ±1.0	3.5 ±0.8	1.3 ±0.5
ii) Rifampicin + INH	6.7 ±0.6	11.7 ±0.9	9.5 ~~8.3~~ ±0.8	5.8 ±0.9	3.3 ±0.5	1.2 ±0.2
iii) Rifampicin + INH + streptomycin	4.5 ±0.5	10.6 ±0.6	9.9 ±1.1	6.1 ±0.8	2.6 ±0.4	1.1 ±0.1

Table-33c : Urinary excretion of rifampicin

Study No.	Urine volume (ml, mean ± SEM)	mg/day (mean ± SEM)	Excretion rate (mg/ml/24 hours) (mean ± SEM)
i) Rifampicin alone	690.0 ±15.3	141.5 ± 3.8	0.2 ±3.0
ii) Rifampicin + INH	731.0 ±20.9	146.3 ± 3.9	0.2 ±2.2
iii) Rifampicin + INH + streptomycin	686.0 ±27.6	145.5 ± 4.3	0.2 ±3.1

Table-3a: Anthropometric data of patients of pulmonary tuberculosis (n = 8) (mean + SEM values)

Rifampicin administered (mg)	Age (years)	Weight (kg)	Nutritional score	Total proteins (g/100 ml)	Albumin (g/100 ml)
371.3 ±14.2	37.1 ±1.9	37.1 ±1.9	0.15 ±0.005	6.4 ±0.1	3.2 ±0.1

Table-34b: Serum rifampicin concentration in patients of pulmonary tuberculosis (ug/ml mean ± SEM)

Study No.	Hours after drug administration			
	2½	8	12	24
i) Rifampicin alone	20.0 ±3.0	11.1 ±2.5	4.3 ±0.6	2.5 ±0.5
ii) Rifampicin + INH	16.7 ±2.3	9.3 ±2.0	3.3 ±0.8	2.0 ±0.8
iii) Rifampicin + INH + ethambutol	17.9 ±3.0	8.0 ±2.0	3.4 ±0.8	0.9 ±0.2

Table-34c : Urinary excretion of rifampicin in patients of pulmonary tuberculosis

Study No.	Urine volume (ml, mean ± SEM)	mg/day (mean ± SEM)	Excretion rate (mg/ml/ 24 hours) (mean ± SEM)
i) Rifampicin alone	532.5 ±21.0	140.0 ± 7.4	0.3 ±0.01
ii) Rifampicin + INH	482.3 ±30.5	146.4 ± 7.7	0.3 ±0.03
iii) Rifampicin + INH + ethambutol	518.8 ±14.7	143.3 ± 7.4	0.3 ±0.01

Table-35a: Anthropometric data of patients of pulmonary tuberculosis (mean ± SEM values, n = 8)

Rifampicin administered (mg)	Age (years)	Weight (kg)	Nutritional score	Total proteins (g/100 ml)	Albumin (g/100 ml)
386.3 ±11.7	41.9 ±5.3	39.3 ±1.4	0.2 ±0.007	6.2 ±0.1	3.0 ±0.6

Table-35b: Serum rifampicin concentration in patients of pulmonary tuberculosis (ug/ml, mean ± SEM)

Study No.	Hours after drug administration			
	2½	8	12	24
i) Rifampicin alone	17.0 ±3.0	9.5 ±2.5	6.4 ±1.8	2.0 ±0.6
ii) Rifampicin + INH	15.7 ±2.9	9.5 ±2.5	6.1 ±2.1	1.6 ±0.4
iii) Rifampicin + INH + pyrazinamide	17.5 ±2.4	8.4 ±1.7	4.0 ±1.4	0.6 ±0.1

Table-35c : Urinary excretion of rifampicin in patients of pulmonary tuberculosis

Study No.	Urine volume (ml, mean ± SEM)	mg/day (mean ± SEM)	Excretion rate (mg/ml/24 hours) (mean ± SEM)
i) Rifampicin alone	516.3 ±59.7	136.2 ± 6.7	0.3 ±0.02
ii) Rifampicin + INH	488.8 ±57.6	126.9 ± 6.3	0.3 ±0.01
iii) Rifampicin + INH + pyrazinamide	503.1 ±45.2	134.3 ± 5.9	0.3 ±0.03

Table-36a: Anthropometric data of healthy volunteers

(mean ± SEM values, n = 6)

Rifampicin administered (mg)	Age (years)	Weight (kg)	Nutritional score	Total proteins (g/100 ml)	Albumin (g/100 ml)
572.5 ±40.9	29.7 ±2.1	57.3 ±4.7	0.2 ±0.01	7.3 ±0.1	3.8 ±0.1

Table-36b: Serum rifampicin concentration of healthy volunteers

(mean ± SEM values)

Study No.	Hours after drug administration						
	½	1	2	4	8	12	24
i) Rifampicin alone	9.5 ±0.7	11.5 ±0.9	16.1 ±1.2	10.8 ±1.0	7.5 ±1.0	4.7 ±0.3	2.6 ±0.3
ii) Rifampicin + INH	9.2 ±0.5	11.6 ±0.7	14.8 ±1.1	10.5 ±0.7	5.6 ±0.7	3.4 ±0.2	2.0 ±0.4
iii) Rifampicin + INH + ethambutol	10.5 ±1.0	11.6 ±0.7	15.0 ±0.6	10.6 ±0.3	5.2 ±0.3	3.4 ±0.2	2.0 ±0.2

Table-36c : Urinary excretion of rifampicin of healthy volunteers

Study No.	Urine volume (ml, mean ± SEM)	mg/day (mean ± SEM)	Excretion rate (mg/ml/24 hours) (mean ± SEM)
i) Rifampicin alone	796.7 ±62.3	165.8 ±16.0	0.2 ±0.02
ii) Rifampicin + INH	801.2 ±55.1	149.8 ±11.6	0.2 ±0.01
iii) Rifampicin + INH + ethambutol	963.3 ±90.1	166.3 ±15.1	0.2 ±0.02

time points; c, urinary excretion of rifampicin following single oral dose (10 mg/kg). There were no significant differences among studies i, ii or iii in either serum rifampicin concentration or urinary excretion of rifampicin.

Table-37 presents data of undernourished healthy volunteers (n = 5). a, presents anthropometric data; b, serum rifampicin concentration (ug/ml); c, urinary excretion of rifampicin following single oral dose (10 mg/kg). There were no significant differences among studies i, ii or iii in either serum rifampicin concentration or urinary excretion. The mean AUCs of normally nourished, healthy volunteers were significantly higher than mean AUCs of undernourished volunteers (Table-38).

Table-39 and figure-10 present comparison of pooled data of all groups studied namely -

a) undernourished patients of pulmonary tuberculosis (n = 15)
b) comparatively better nourished patients of pulmonary tuberculosis (n = 10)
c) normally nourished healthy volunteers (n = 6)
d) undernourished healthy volunteers (n = 5).

III. Percentage binding of rifampicin to serum proteins

The type of serum protein fraction that binds rifampicin in particular was investigated. Amongst the five

Table-37a : Anthropometric data of undernourished volunteers (mean ± SEM values, n = 5)

Rifampicin administered (mg)	Age (years)	Weight (kg)	Nutritional score	Total proteins (g/100 ml)	Albumin (g/100 ml)
390.0 ± 7.0	30.4 ±4.5	39.0 ±0.8	0.2 ±0.004	7.6 ±0.2	4.2 ±0.1

Table-37b: Serum rifampicin concentration in undernourished volunteers (ug/ml mean ± SEM)

Study No.	Hours after drug administration						
	½	1	2	4	8	12	24
i) Rifampicin alone	5.6 ±0.9	7.8 ±1.3	11.2 ±1.1	8.9 ±1.0	5.8 ±0.8	3.0 ±0.6	1.9 ±0.5
ii) Rifampicin + INH	5.4 ±0.8	8.5 ±1.1	10.7 ±1.4	8.6 ±0.8	5.8 ±0.6	3.1 ±0.4	1.8 ±0.1
iii) Rifampicin + INH + ethambutol	5.4 ±0.8	7.5 ±0.9	10.8 ±1.0	8.8 ±0.6	5.5 ±0.6	3.1 ±0.2	1.9 ±0.3

Table-37c : Urinary excretion of rifampicin of under-nourished volunteers

Study No.	Urine volume (ml, mean ± SEM)	mg/day (mean ± SEM)	Excretion rate (mg/ml/24 hrs, mean ± SEM)
i) Rifampicin alone	534.0 ±48.1	110.8 ± 5.7	0.2 ±0.03
ii) Rifampicin + INH	536.0 ±39.7	109.8 ± 3.8	0.2 ±0.03
iii) Rifampicin + INH + ethambutol	574.0 ±40.7	124.0 ± 6.8	0.2 ±0.02

TABLE-38

AUCs in volunteers

Study No.	Undernourished	Well nourished
i) Rifampicin alone	32.9 ±3.5	46.7 ±3.5
ii) Rifampicin + INH	31.8 ±3.3	43.6 ±2.8
iii) Rifampicin + INH + ethambutol	31.9 ±2.8	44.2 ±1.6

Table-39a : Pharmacokinetic data as mean ± SEM

Group	n	Nutritional score	Serum albumin (g/100 ml)	Peak serum concentration	Tmax (hours)	A U C	Excretion rate
a) Healthy volunteers	6	0.22 ±0.01	3.8 ±0.12	16.1 ±1.2	2.0 ±0.1	46.7 ±3.5	0.18 ±0.02
b) Undernourished volunteers	5	0.17 ±4.23	3.2 ±0.17	11.2 ±1.1	2.0 ±0.1	32.9 ±3.5	0.22 ±0.03
c) Patients (less undernourished)	10	0.16 ±0.02	3.6 ±0.7	12.9 ±2.1	2.1 ±0.2	29.0 ±5.7	0.37 ±0.14
d) Patients (more undernourished)	15	0.12 ±0.003	3.1 ±0.06	10.4 ±1.0	2.7 ±0.2	21.4 ±3.2	0.35 ±15.5

Table-39b : Mean serum concentration of rifampicin single oral dose (10 mg/kg ± SEM) in healthy volunteers and patients of pulmonary tuberculosis

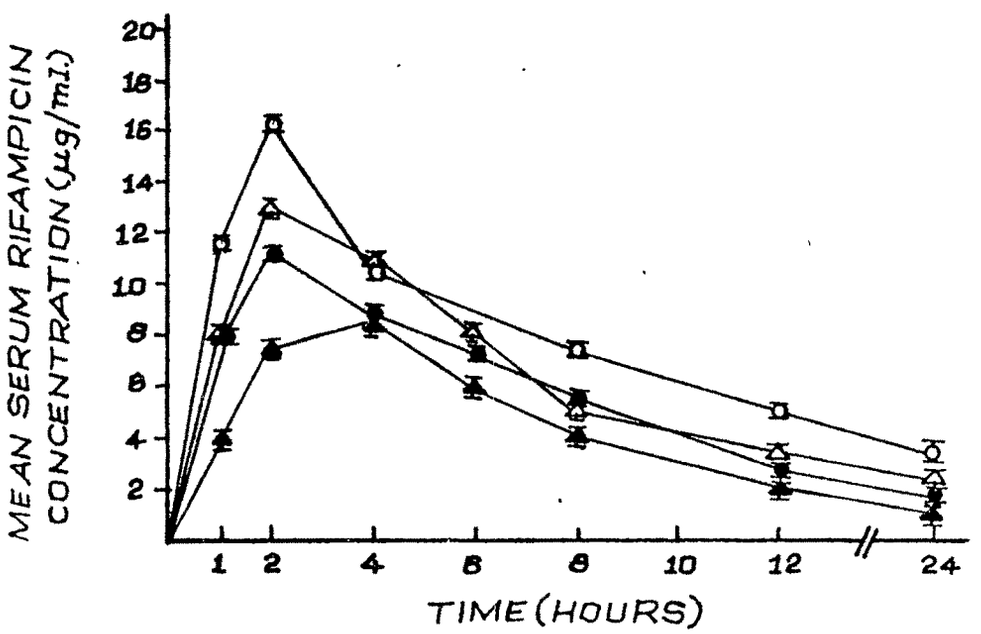
Group	n	Mean serum concentrations (hrs)					
		1	2	4	8	12	24
Healthy volunteers	6	11.5 ±0.9	16.1 ±1.2	10.8 ±1.0	7.5 ±1.0	4.7 ±0.3	2.6 ±0.3
Undernourished volunteers	5	7.8 ±1.3	11.2 ±1.1	8.9 ±1.0	5.8 ±0.8	3.0 ±0.5	1.9 ±0.5
Comparatively better nourished patients (nutritional score more than 0.14 and less than 0.18)	37	8.1 ±0.8 (25)	13.3 ±1.2 (25)	10.9 ±1.0 (37)	6.5 ±0.7 (25)	4.2 ±0.6 (27)	2.1 ±0.4 (17)
Comparatively more nourished patients (nutritional score 0.14)	26	3.4 ±0.5 (17)	7.1 ±0.8 (17)	8.3 ±0.9 (23)	4.9 ±0.8 (26)	3.3 ±0.5 (9)	1.0 ±0.2 (9)

Note: figures in parentheses show the number of observations for each time-point.

Fig.10

Effect of undernutrition on the mean serum concentrations of rifampicin at different time points. Time in hours is depicted on the abscissa and mean serum levels of rifampicin (ug/ml) are depicted on the ordinate. The lines joining open circles (○——○) and closed circles (●——●) represent serum concentrations of rifampicin for normally nourished (n = 6) and undernourished volunteers respectively and those joining open triangles (△——△) and closed triangles (▲——▲) represent serum concentrations of rifampicin for comparatively less undernourished (n = 10) and comparatively more undernourished (n = 15) patients of pulmonary tuberculosis. Vertical bars represent the SEM.

FIG.-10


MEAN SERUM RIFAMPICIN CONCENTRATION (µg/ml.)
20
18
16
14
12
10
8
6
4
2
1
2
4
6
8
10
12
24
TIME (HOURS)

serum protein fractions, viz. albumin, $alpha_1$, $alpha_2$, beta and gamma, merely albumin and $alpha_2$ fractions were involved in the binding of the antibiotic. The data suggested that, in vivo at 4 hour interval, 4.67 ± 0.1 gms/dl of albumin binds to 424 ug of rifampicin (Table-40a).

Moreover, in vitro at the same time interval, rifampicin was exclusively bound to albumin and not to $alpha_2$. In vivo, the percentage of rifampicin bound to $alpha_2$ protein fraction was 79.9 ± 1.6.

At 24 hour interval, there was no binding of rifampicin either to albumin or to $alpha_2$ protein fractions. However, there was binding to albumin at 24 hours in in vitro experiments. Thus, 48.89 gms of rifampicin was bound to 4.55 gm/dl of albumin. Furthermore, the binding concentrations of rifampicin was relatively higher with $alpha_2$ protein fraction than with albumin (Table-40b, Fig.11).

The rifampicin binding to fibrinogen was found to be variable, ranging from 3 to 18%.

TABLE-40

Percentage binding of rifampicin to albumin and $alpha_2$ protein fraction concentration in gm/dl of

Study	Albumin (gm/dl)	$Alpha_2$ (gm/dl)	4 hours after drug administration concentration of rifampicin ug/dl bound to				24 hours affecting concentration of rifampicin ug/dl bound to			
			Albumin	%	$Alpha_2$	%	Albumin	%	$Alpha_2$	%
a) In vivo	4.7 ±0.1	0.5 ±0.0	423.8 ±36.8	20.0 ±1.6	1698.9 + 90.7	79.9 ±1.6	-	-	-	-
b) In vitro	4.5 ±0.19	0.52 ±0.04	59375.8	-	-	-	48890.6	23.04 ±2.36	67930.0	76.95 ±2.36

FIG.-11

ELECTROPHORETIC PATTERN OF SERUM-RIFAMPICIN.