

# Kinetics of rifampicin and isoniazid administered alone and in combination to normal subjects and patients with liver disease

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**SUMMARY** The possible existence of kinetic interactions between rifampicin and isoniazid and the effect of the concomitant presence of an impaired liver function were investigated in man.

In a first study normal healthy subjects and patients with chronic liver disease received, on three different occasions, a single dose of 600 mg rifampicin or isoniazid and of rifampicin and isoniazid associated in randomized sequences. The results have shown that in both groups the serum levels, half-life values, and urinary excretion of each drug given alone are not significantly different from those observed when the other drug is associated. Serum levels and half-life of rifampicin and isoniazid were significantly higher in patients with chronically impaired liver.

In a second study, rifampicin and isoniazid were given in combination at the same doses as in the first study over a period of one week. The results have shown a trend to decrease in the serum levels of rifampicin of the healthy subjects and a trend to increase in the patients with chronic liver disease on day 7 of treatment. In both groups a reduction in the half-life of rifampicin was also observed. No changes in serum isoniazid concentrations were observed between day 1 and day 7 in the healthy subjects, whereas a significant increase was observed in the patients. No significant changes in the half-life of isoniazid were observed.

The results of a large series of therapeutic trials carried out in different countries have indicated that the combined treatment with rifampicin and isoniazid can be regarded as a very important new acquisition in the therapy of human tuberculosis (*Lancet*, 1969; *New England Journal of Medicine*, 1970).

It is now widely recognized that in the case of treatment with drug combinations special studies are needed with the aim of assessing the pharmacokinetics of the combined drugs, which cannot always be predicted on the basis of the knowledge of each individual drug employed. Possible interactions may in fact involve problems of tolerance and/or efficacy.

The present study was undertaken in order to assess the possible existence of kinetic interactions between rifampicin and isoniazid in normal healthy subjects and in patients with impaired liver function.

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## Material and Methods

### SUBJECTS

Twelve healthy male subjects and 13 male patients, of adult age, for whom cirrhosis of the liver had been diagnosed on the basis of clinical and laboratory data, participated in the trial. Since in the latter group histological evidence in support of such a diagnosis was not available, they will be referred to as patients with chronic liver disease.

### TREATMENT

Rifampicin and isoniazid were administered orally at a fixed daily dose of 600 mg after an overnight fast. Capsules containing 300 mg of rifampicin and tablets containing 100 mg isoniazid were used. No food or beverages were permitted for two hours following the administration.

#### EXPERIMENTAL DESIGN

Two studies were carried out, the first with a single dose, the second with repeated administration of the combination. In both studies each patient acted as his own control. Blood samples were taken two, four, eight, and 12 hours after administration; urine studies were carried out on 24-hour samples.

##### *Single dose*

Six normal subjects and seven patients with liver disease received, on three different occasions separated by a four-day interval, a single oral dose of 600 mg rifampicin, of 600 mg isoniazid, and of the combination of the same dose of the two drugs. The sequences of administration of the three treatments were randomized. The following measurements were carried out on the serum of each blood sample (when appropriate according to the treatment received by the patients): concentration of rifampicin ( $\mu\text{g/ml}$ ), concentration of total and free isoniazid ( $\mu\text{g/ml}$ ), concentration of total bilirubin ( $\text{mg}/100 \text{ ml}$ ).

The recovery in urines of rifampicin and its metabolites and of total and free isoniazid was calculated when appropriate according to the treatment schedule.

##### *Repeated administration of the combination*

Six normal subjects and six patients with liver disease were treated over a period of seven days with a daily oral dose of the combination rifampicin 600 mg and isoniazid 600 mg. Serum concentrations and urine recovery of the two drugs and serum bilirubin were assessed on days 1 and 7 of treatment, as in the single dose experiment.

#### METHODS OF ASSAY

##### *Rifampicin*

The agar plate method was used for the determinations of rifampicin, *Sarcina lutea* ATCC 9341 being employed as test organism (Fűrész, Scotti, Pallanza, and Mapelli, 1967). Rifampicin metabolic derivatives were assayed by column chromatography.

##### *Isoniazid*

Total and free isoniazid concentrations in serum and urine were determined according to the method of Maher, Whitney, Chambers, and Stanonis (1957).

##### *Bilirubin*

Serum total bilirubin levels were determined according to the method of Malloy and Evelyn (1937).

#### STATISTICAL ANALYSIS

Analysis of variance of each set of serum levels and recoveries in urines was carried out considering the

subjects as randomized blocks. The biological half-life values of each drug, calculated according to standard methods (Laidler, 1965), were submitted to a joint analysis of variance of factorial ( $4 \times 2$ ) type.

#### Results

##### SINGLE DOSE

##### *Serum levels*

The mean serum concentrations of rifampicin and of total isoniazid are reported in Figs. 1 and 2 respectively. As can be seen, the values found when each drug was administered alone did not differ appreciably from those observed after administration of the combination, either in the healthy subjects or in the patients. Within each of the two groups the differences between the serum levels obtained at each time interval are in fact far from significant. The same holds true for the levels of free isoniazid. Out of 13 subjects tested, only one healthy subject was a rapid acetylator, and acetyl-isoniazid accounted for 48 to 89% of the total isoniazid present in his serum at the different time intervals; all the others were slow acetylators, and acetyl-isoniazid accounted, on the average, for 18 to 59% of the total in the remaining five healthy subjects and for 15 to 59% in the seven patients. In these two groups of subjects the mean values found in the patients were significantly higher than those in the healthy subjects, both for rifampicin ( $p < 0.01$ ) and for isoniazid ( $p < 0.05$ ).

##### *Recovery in urine*

The mean rifampicin recovery ( $\text{mg}/24 \text{ hr}$ ) in the group of healthy subjects was  $89.7 \pm 6.7$  and  $88.0 \pm 12.2$  after the administration of the antibiotic alone and combined with isoniazid respectively. The figures in the patient group were  $101.4 \pm 19.2$  and  $93.4 \pm 17.7$ . No differences in the relative proportions of metabolized rifampicin derivatives were found. The mean isoniazid recovery ( $\text{mg}/24 \text{ hr}$ ) was  $299.8 \pm 59.2$  (45% free) and  $365.4 \pm 37.9$  (50% free) in the healthy subjects after administration of the drug alone and in combination with rifampicin, respectively. The proportion of free isoniazid in the five slow acetylators was, on the average, 45% when the drug was given alone, 50% when it was given in combination; in the rapid acetylator it was 15 and 19% respectively. In the patient group the recovery was  $291.3 \pm 28.2$  (50% free) and  $298.7 \pm 32.2$  (51% free) after administration of isoniazid alone and combined with rifampicin, respectively. None of the differences found for these parameters in the different groups are statistically significant.

##### *Serum bilirubin*

The time course of the mean serum total bilirubin

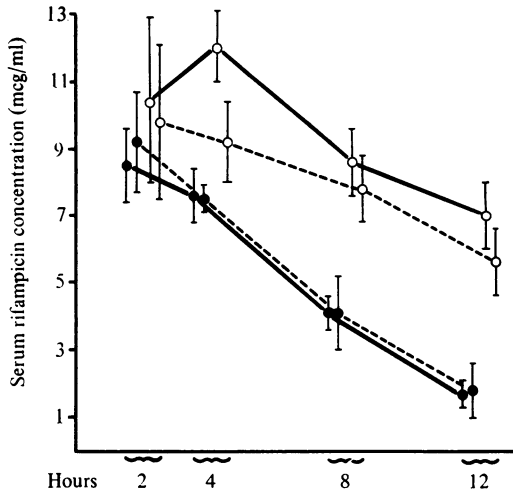


Fig. 1 Serum rifampicin concentrations ( $\mu\text{g}$ ) in six normal subjects (●) and seven patients with liver disease (○) after a single (600 mg) oral dose of rifampicin alone (—) and of rifampicin in combination with isoniazid (-----). Mean values  $\pm$  SE.

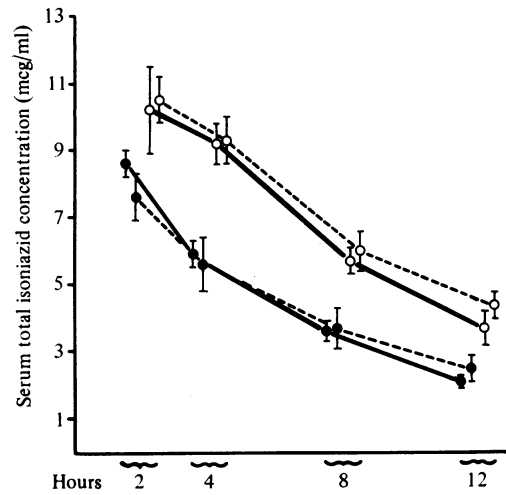


Fig. 2 Serum total isoniazid concentrations in six normal subjects (●) and seven patients with liver disease (○) after a single (600 mg) oral dose of isoniazid alone (—) and isoniazid in combination with rifampicin (-----). Mean values  $\pm$  SE.

in the two groups is summarized in Figures 3a and 3b. In both the administration of rifampicin, either alone or combined with isoniazid, was accompanied by a transient and statistically significant increase in the serum bilirubin levels. No appreciable modification of the serum bilirubin levels was observed

in connexion with the administration of isoniazid.

REPEATED ADMINISTRATION OF THE COMBINATION

Serum levels

The mean serum rifampicin levels observed on the

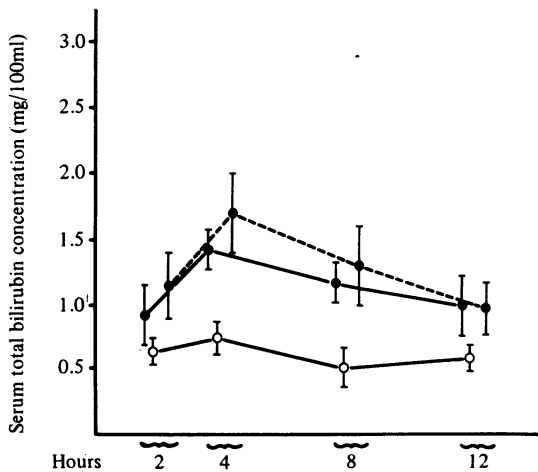


Fig. 3a

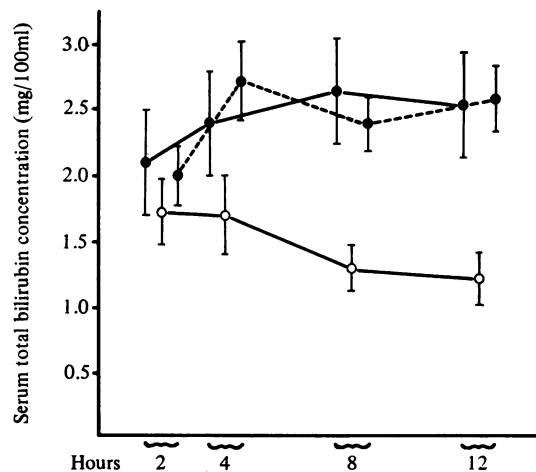


Fig. 3b

Fig. 3 Serum total bilirubin levels in six normal subjects (3a) and seven patients with liver disease (3b) after a single (600 mg) oral dose of isoniazid alone (○—○), of rifampicin alone (●—●), and of isoniazid combined with rifampicin (●-----●). Mean values  $\pm$  SE.

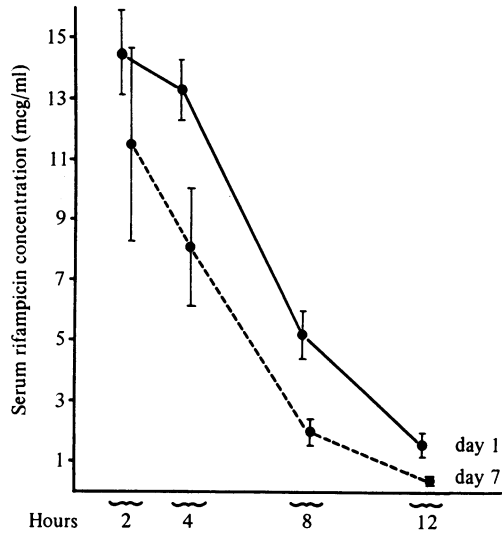


Fig. 4 Serum levels of rifampicin in six normal subjects on the first and seventh days of treatment with rifampicin 600 mg in combination with isoniazid 600 mg. Mean values  $\pm$  SE.

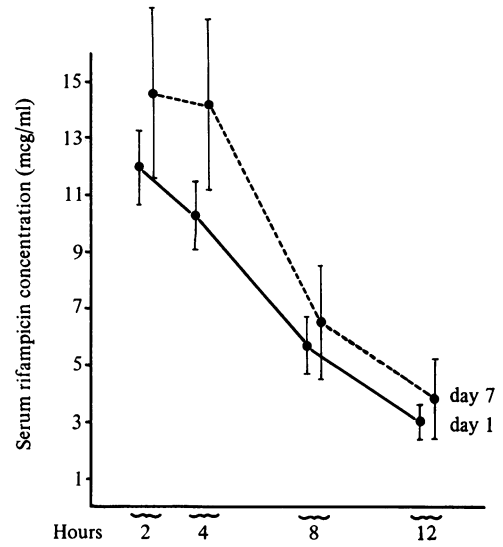


Fig. 5 Serum levels of rifampicin in six patients with liver disease on the first and seventh days of treatment with rifampicin 600 mg in combination with isoniazid 600 mg. Mean values  $\pm$  SE.

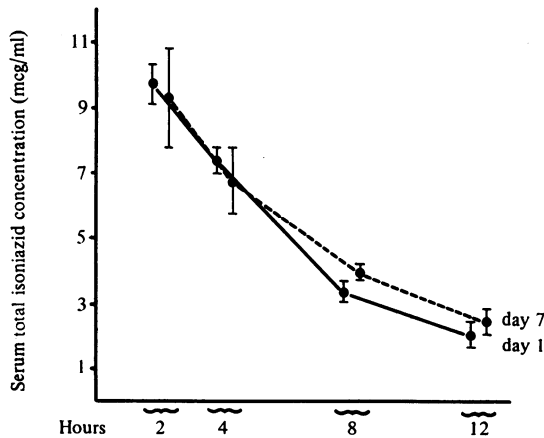


Fig. 6 Serum levels of total isoniazid in six normal subjects on the first and seventh days of treatment with rifampicin 600 mg in combination with isoniazid 600 mg. Mean values  $\pm$  SE.

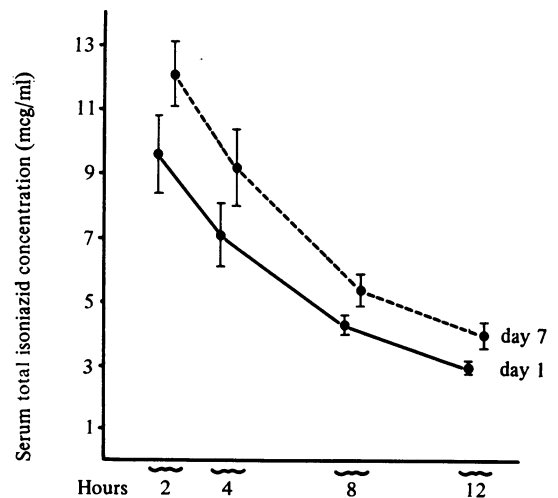


Fig. 7 Serum levels of total isoniazid in six patients with liver disease on the first and seventh days of treatment with rifampicin 600 mg in combination with isoniazid 600 mg. Mean values  $\pm$  SE.

first and seventh days of treatment in the two groups of subjects are reported in Figs. 4 and 5. As can be seen, in the healthy subjects the mean concentrations on day 7 were somewhat lower than on day 1; in the patients, on the contrary, some increase in the mean values was seen; in neither case, however, did the differences reach the significance level.

The mean serum levels of total isoniazid are reported in Figs. 6 and 7 respectively for the healthy subjects and the patients. As can be seen, almost superimposable serum level curves were obtained in the two days of study in the healthy subjects, whereas a significant ( $P < 0.001$ ) increase in the serum levels was observed in the patients on day 7 compared with day 1.

The same results were obtained by evaluating the levels of free isoniazid. All the six healthy subjects were slow acetylators, and acetyl-isoniazid accounted on the average, at the different time intervals, for 50 to 88% of the total isoniazid on day 1 and for 49 to 85% on day 7. Of the six patients, two were rapid acetylators, and acetyl-isoniazid accounted, on average, at the different hours, for 50 to 97% of total isoniazid in serum on day 1 and for 55 to 99% on day 7; the remaining four were slow acetylators and acetyl-isoniazid accounted, on the average, for 40 to 85% of total isoniazid on day 1 and for 45 to 80% on day 7.

#### Recovery in urine

The mean rifampicin recovery (mg/24 hr) in the healthy subjects was  $123.5 \pm 22.1$  and  $75.8 \pm 14.8$  respectively on days 1 and 7. The differences between the two series of values did not reach the significance

level. In the patients the mean recovery was  $59.8 \pm 11.6$  on day 1 and  $66.8 \pm 11.8$  on day 7, the differences being, again, not significant. In both groups and on both days of study the relative proportions of non-metabolized and metabolized derivatives of rifampicin did not show modification of relevance.

The mean isoniazid recovery in the healthy subjects was  $332.8 \pm 23.3$  on day 1 and  $377.6 \pm 37.8$  on day 7. In the patients the values were respectively  $343.0 \pm 38.1$  and  $373.5 \pm 23.3$ . The mean percentages of free isoniazid in the urine on days 1 and 7 were found to be 59 and 56 respectively in the healthy subjects and 55 and 63 respectively in the four patients who were slow acetylators; in the two patients who were rapid acetylators they were 15 and 16% respectively.

#### Serum bilirubin

The mean serum bilirubin levels observed in the groups of subjects on day 1 and 7 are reported in Figure 8. As can be seen, in the healthy subjects the levels increased on day 1, returned to almost normal values on day 7. The levels in the patients did not show appreciable differences in the two days of study.

#### BIOLOGICAL HALF-LIFE

The biological half-life values of rifampicin in the patients with chronic liver disease were found to be significantly higher ( $P < 0.01$ ) than in the healthy subjects in all the experimental conditions tested, the grand average and standard error being, respectively,  $5.42 \pm 0.55$  and  $2.80 \pm 0.22$  hours. No significant differences were found, in either group, between the

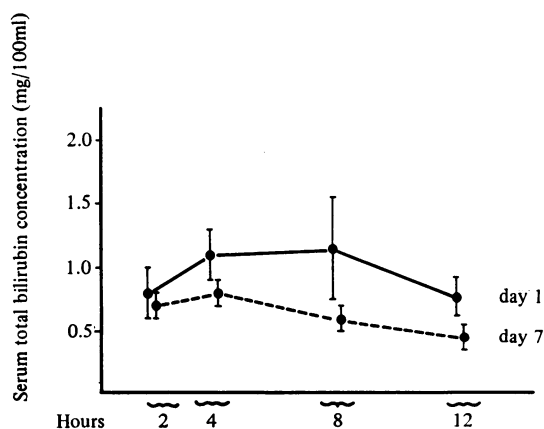


Fig. 8a

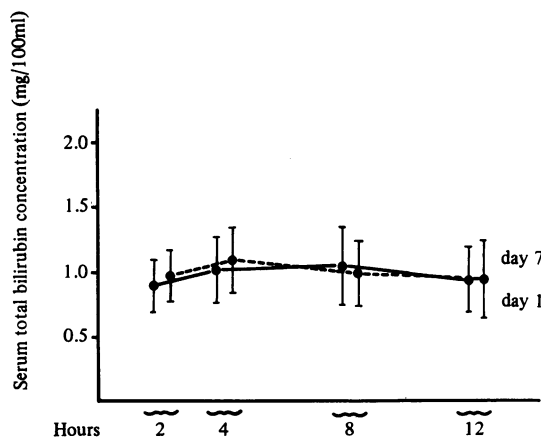


Fig. 8b

Fig. 8 Serum total bilirubin levels observed in six normal subjects (8a) and in six patients with chronic liver disease (8b) on the first and seventh days of treatment with rifampicin 600 mg in combination with isoniazid 600 mg.

mean values obtained when the drug was given alone and when it was given in combination, and between those obtained in the single-dose studies and after the first administration in the repeated-dose studies. Some reduction was seen following one week of treatment, both in the patients (from  $4.70 \pm 0.95$  to  $3.19 \pm 0.71$  hours) and in the healthy subjects (from  $2.90 \pm 0.49$  to  $1.83 \pm 0.05$  hours), the overall difference being significant ( $P < 0.01$ ).

Also for total isoniazid, the half-life values were found to be significantly higher in the patients with chronic liver disease than in the healthy subjects, the grand average and standard error being, respectively,  $6.74 \pm 0.33$  and  $3.24 \pm 0.14$  hours. Within each group, no significant differences were seen between the different sets of values, obtained for the drug given alone and in combination, and for the first and seventh days of treatment. Similar results were obtained for free isoniazid, the grand average and standard error being  $4.24 \pm 0.29$  hr for the patients with chronic liver disease and  $3.38 \pm 0.12$  hr for the healthy subjects.

## Discussion

The studies reported here were designed as within-subjects comparisons of the kinetics of the two drugs given alone and in combination and of the kinetics of the combination over a treatment period of seven days. The results obtained do not provide any evidence in favour of the hypothesis of kinetic interactions between rifampicin and isoniazid. In fact, the pattern of absorption, metabolism, and excretion of each drug administered alone was not found to be different from that observed when the other drug was administered simultaneously, both in normal subjects and in patients with liver disease.

After administration of the combined drugs over a period of seven days, the serum levels of rifampicin showed a decrease in the biological half-life of the drug which was more evident in the healthy subjects, in agreement with the decrease demonstrated in previous studies in healthy subjects given rifampicin alone at the same dose. The serum levels of total and free isoniazid remained practically unchanged in the healthy subjects, while in the patients with liver disease a moderate but significant increase was seen, without significant changes in half-life.

The apparent absence of interactions between the two drugs may be due to the involvement in their metabolism of different structures and mechanisms.

For isoniazid, a process of acetylation is regarded as an essential step in its metabolic fate, probably mediated by a non-microsomal enzyme present in the liver cell (Hughes, 1953), acetyl-isoniazid being the main metabolic product also at the dose level

employed in this study (Peters, Miller, and Brown, 1965).

Rifampicin, on the other hand, has been shown to undergo a process of desacetylation in animals (Tenconi and Beretta, 1970) and man (Maggi, Fürész, Pallanza, and Pelizza, 1969), the desacetyl derivative showing an increased polarity as compared with the parent compound. The desacetyl derivative accounts for all the antibiotic activity present in the bile a few hours after administration and is also present in serum and urines (Tenconi and Beretta, to be published). Although the site(s) and mechanism(s) of this transformation have not been fully elucidated there is evidence that the administration of rifampicin is accompanied by functional and morphological evidence of proliferation of the smooth endoplasmic reticulum and of activation of microsomal enzyme systems in the hepatocyte (Cohn, 1969; Acocella, Pagani, Marchetti, Baroni, and Nicolis, 1971; Acocella, Lamarina, Nicolis, Pagani, and Sepre, 1971; Michot, Bürgi, and Büttner, 1970; Curci, Ninni, and Fabbrocini, 1970; Jezequel, Orlandi, and Tenconi, 1971).

The adoption of a fixed dose scheme in the present study, selected in order to reproduce the conditions of clinical treatment of the patients, makes the comparisons between the serum levels in different groups of subjects affected, to some extent, by differences in body weight, and thus much less accurate than within-subject comparisons. However, for both antibiotics, alone or combined, the serum levels were generally higher in the patients than in the healthy subjects: the differences at the various time intervals were clear-cut in the single-dose studies, much less so after the first administration in the multiple-dose studies. The half-life values, on the other hand, are probably less affected by differences in body weight, and their analysis has shown for both drugs, given alone or in combination, a consistent increase in the patients with chronic liver disease in comparison with healthy subjects.

In connexion with these kinetic differences, it may be observed that the interference of liver function in the kinetics of rifampicin was known (Capelle, Mora, Feldman, Berthelot, and Fauvert, 1970), whereas conflicting data have been reported for isoniazid. In fact, in some studies the presence of an impaired liver function appeared to be without effect on the kinetics and metabolism of this drug (Leménager and Morel, 1960; Mitchell and Bell, 1957; Jenne, 1960; Bourgeois, Dubois-Verlière, Ledouarec, and Gandon, 1960), while in others (Levi, Sherlock, and Walker, 1968) a prolonged half-life was seen in patients with liver disease. The results of this study are in agreement with the latter. No evidence has been obtained in our study suggesting

that the increased isoniazid levels are due to an altered acetylating capacity of the diseased liver as had been previously suggested (Manzini, Cenciotti, and Turchetto, 1957).

The higher levels and longer half-life of rifampicin and isoniazid observed in patients with chronic liver disease and the significant increase of the isoniazid levels demonstrated in these patients after one week of treatment certainly deserve attention in the therapeutic use of the combination. In this connexion, one should recall that signs of adverse reactions, either in the form of elevations of serum transaminase levels or as histological evidence of liver damage, were observed in a number of subjects undergoing treatment with isoniazid (Scharer and Smith, 1969); furthermore, as a high intake of alcohol is not infrequent in many populations of tuberculous patients, it may be recalled that the LD<sub>50</sub> of isoniazid is influenced by alcohol in the mouse (Glass, Gossow, and Mallach, 1964; Glass and Mallach, 1965) and that its half-life has been shown to be influenced by alcohol in man (Lester, 1964).

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