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ˣ To whom inquiries should be directed.

# Drug Interactions with Isoniazid Metabolism in Rats

**BARRY H. THOMAS ˣ and GNANAPRAKASAM SOLOMONRAJ**

**Abstract** □ $^{14}$C-Isoniazid (20 mg/kg po or iv) was administered alone or in combination with aspirin (100 mg/kg po), rifampin (30 mg/kg po), ethambutol (100 mg/kg po), or ethanol (3 g/kg po) to rats. In another experiment, phenobarbital sodium (40 mg/kg/day ip) was administered for 3 days prior to isoniazid. Aspirin and ethanol retarded the rate of isoniazid absorption from the GI tract. None of the drugs significantly altered the $^{14}$C-elimination rate from the blood over the first 4 hr. A tissue distribution study showed that changes in the blood levels produced by ethanol were reflected in the other tissues. When isoniazid was given intravenously, ethanol increased the amount of carbon-14 excreted in urine up to 24 hr after dosing; no other changes were observed in the total carbon-14 recovered in urine. Aspirin inhibited the conjugation of isonicotinic acid with glycine. Ethanol increased *N*-acetylisoniazid excretion and decreased isonicotinic acid excretion. None of the other treatments had more than a slight effect on isoniazid metabolism. Acute doses of isoniazid failed to produce any signs of hepatotoxicity, as judged by measurement of serum transaminase levels. The data do not suggest that any of the drugs studied are likely to potentiate the hepatotoxicity of isoniazid when administered acutely. Isoniazid metabolism in rats differed quantitatively from that reported for humans.

**Keyphrases** □ Isoniazid—absorption, tissue distribution, excretion, and metabolism, effect of aspirin, rifampin, ethambutol, ethanol, and phenobarbital, radiochemical analysis, rats □ Absorption—isoniazid, effect of various drugs, radiochemical analysis, rats □ Distribution, tissue—isoniazid, effect of various drugs, radiochemical analysis, rats □ Excretion—isoniazid, effect of various drugs, radiochemical analysis, rats □ Metabolism—isoniazid, effect of various drugs, radiochemical analysis, rats □ Radiochemistry—analysis, isoniazid, effect of various drugs on absorption, tissue distribution, excretion, and metabolism, rats □ Interactions, drug—isoniazid, absorption, tissue distribution, excretion, and metabolism, effect of aspirin, rifampin, ethambutol, ethanol, and phenobarbital, radiochemical analysis, rats □ Antibacterials, tuberculostatic—isoniazid, absorption, tissue distribution, excretion, and metabolism, effect of various drugs, rats

Interest in the metabolism and toxicity of isoniazid was aroused by the death of two patients and the development of clinical hepatotoxicity in 17 others in 1970 following an isoniazid chemoprophylactic program (1). Furthermore, the antituberculous drug rifampin increased the incidence of hepatotoxicity in humans when given with isoniazid (2). This same association was observed in rats (3).

Recently, it was shown that metabolism by the microsomal mixed function oxidase system is responsible for isoniazid hepatotoxicity (4). It was suggested that it is necessary to acetylate isoniazid before it can be a substrate for the toxication pathway. The purposes of this study were to screen drugs likely to be taken by tuberculous patients and to observe their influence on isoniazid metabolism as a clue to possible toxic drug interactions.

## EXPERIMENTAL

[$^{14}$C-Carboxyl]-isoniazid[1] had a specific activity of 11.3 mCi/mmole and a radiochemical purity of 96–97%, as determined in two paper chromatography and two TLC systems. Aspirin[2], ethambutol[3], rifampin[4], phenobarbital sodium[5], and absolute ethanol[6] were obtained commercially.

Male Wistar rats[7], 170–200 g, were deprived of food but not water for 16 hr prior to dosing and were housed in metabolism cages[8] that separated urine and feces. In most experiments, $^{14}$C-isoniazid was given orally at a dose of 20 mg/kg in 10 ml of water/kg. In one experiment, $^{14}$C-isoniazid was given intravenously into the saphenous vein at a dose of 20 mg/kg in 1.0 ml/kg. The dose of carbon-14 in all experiments was 25 μCi/kg.

Aspirin (100 mg/kg) in 0.25% gum tragacanth, ethambutol (100 mg/kg), rifampin (30 mg/kg), and ethanol (3 g/kg) were given orally immediately prior to the dose of isoniazid in 10 ml of water/kg. Phenobarbital sodium, 40 mg/kg/day ip, was administered for 3 days prior to isoniazid. Control animals were dosed similarly with saline for 3 days.

Duplicate 10-μl blood samples were collected from the tail at 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 12 hr after dosing using calibrated capillary tubes. The total carbon-14 in the samples was determined by digestion and

[1] Amersham/Searle, Oakville, Ontario, Canada.
[2] J. T. Baker Chemical Co., Phillipsburg, N.J.
[3] Myambutol, Cyanamid of Canada, Montreal, Quebec, Canada.
[4] Rimactane, Ciba-Geigy Canada Ltd., Dorval, Quebec, Canada.
[5] B.D.H. Canada Ltd., Toronto, Ontario, Canada.
[6] Consolidated Alcohols Ltd., Toronto, Ontario, Canada.
[7] Woodlyn Farms, Guelph, Ontario, Canada.
[8] Model 4-640-000, Acme Research Products, Cincinnati, Ohio.

**Table I—Urinary Excretion of Isoniazid Metabolites in Groups of Five Rats Dosed Orally with $^{14}$C-Isoniazid (20 mg/kg) and Either Saline (Control) or Aspirin (100 mg/kg) [a]**

| Metabolite | Hours | Treatment | |
|---|---|---|---|
| | | Control | Aspirin |
| I | 0–6 | 5.08 ± 0.49 | 6.26 ± 0.79 |
| | 0–12 | 5.19 ± 0.51 | 6.49 ± 1.96 |
| | 0–24 | 5.22 ± 0.52 | 6.76 ± 0.64 |
| II | 0–6 | 3.99 ± 0.68 | 0[b] |
| | 0–12 | 4.51 ± 0.69 | 0.16 ± 0.17[b] |
| | 0–24 | 4.90 ± 0.70 | 0.16 ± 0.17[b] |
| III | 0–6 | 14.55 ± 1.05 | 14.52 ± 2.79 |
| | 0–12 | 17.21 ± 1.22 | 19.74 ± 1.85 |
| | 0–24 | 17.72 ± 1.27 | 21.14 ± 1.81 |
| IV | 0–6 | 32.75 ± 2.58 | 29.64 ± 6.43 |
| | 0–12 | 40.11 ± 2.10 | 42.33 ± 4.06 |
| | 0–24 | 42.02 ± 1.71 | 44.98 ± 3.80 |

[a] The proportion of each metabolite is expressed as a percentage of the isoniazid dose. Values are means ± *SE*. [b] $p < 0.001$ (compared to control).

liquid scintillation counting as previously described (5). Urine was collected at 6, 12, and 24 hr after dosing, and the total carbon-14 was determined (5). The metabolites of isoniazid were separated by paper[9] chromatography using 2-propanol–water (85:15) as the solvent as described previously (6, 7). The location and quantity of each metabolite were determined by serially sectioning the chromatograms into 1-cm strips and counting in a liquid scintillation counter.

The tissue distribution of carbon-14 was studied in groups of rats killed by decapitation at either 0.5 or 3 hr after dosing. The liver, kidneys, lungs, brain, skeletal muscle (sample from the hindleg), and blood were collected. The concentration of carbon-14 was estimated in these tissues as previously described (5).

In a small-scale study of acute hepatotoxicity, isoniazid (50, 200, and 500 mg/kg) was administered orally to groups of three rats. Blood samples (0.2 ml) were withdrawn from the tail at 6, 12, and 18 hr after dosing for estimation of serum glutamic–oxaloacetic transaminase and serum glutamic–pyruvic transaminase. The transaminases were analyzed by a spectrophotometric method (8) using an automatic enzyme analyzer[10].

Differences between treatment groups were analyzed by the unpaired Student *t* test. When there were several treatments and only one control, the significance of the *t* values was evaluated using Dunnett's method (9). The half-lives ($T_{1/2}$) of elimination of carbon-14 from blood were determined using the regression analysis equations from the literature (18G) (10) on a minicomputer[11]. These same equations were used to test for a change in $T_{1/2}$ between control and treatment groups.

## RESULTS

**Blood Profiles and Urine**—Analysis of the blood data indicated the presence of two compartments, but it was not possible to define the later phase because of insufficient data points. Estimation of carbon-14 in this later phase was difficult due to the low counts. Since the urine data showed that over 50% of the drug had been excreted in the first 6 hr, this period is the most important phase of elimination.

Paper chromatography of the urine separated the radioactivity into three major peaks, one small peak, and several variable minor peaks. All corresponded in terms of $R_f$ with reported values (6). On the basis of the published $R_f$ and concurrently run authentic specimens, one major peak was identified as isonicotinic acid (III). On the basis of $R_f$ values, the other two major peaks were tentatively identified as *N*-acetylisoniazid (IV) and isonicotinoylglycine (II) and the small peak was tentatively identified as α-ketoglutaric isonicotinoylhydrazone (I) (Table I). Isoniazid itself was present in only trace quantities, as were the two spots due to pyruvic acid isonicotinoylhydrazone.

**Aspirin**—The blood profile shown in Fig. 1 indicates little effect of aspirin on the elimination of carbon-14 from blood. Analysis of the regression lines between 1 and 4 hr showed no significant differences between the two groups, and the calculated $T_{1/2}$ values[12] over this period were 1.09 (0.96–1.25) hr for the control group and 1.28 (1.12–1.50) hr for the aspirin-treated group.

Elimination of carbon-14 in urine was rapid and constituted the major excretion route (control: 0–6 hr, 57.25 ± 4.09%; 0–12 hr, 67.97 ± 8.69%; 0–24 hr, 70.93 ± 3.00%; and aspirin: 0–6 hr, 50.56 ± 8.69%; 0–12 hr, 66.92 ± 5.64%; 0–24 hr, 73.24 ± 5.08%). No significant treatment effects were observed. Only one of the four metabolites estimated was affected; II was markedly inhibited by aspirin.

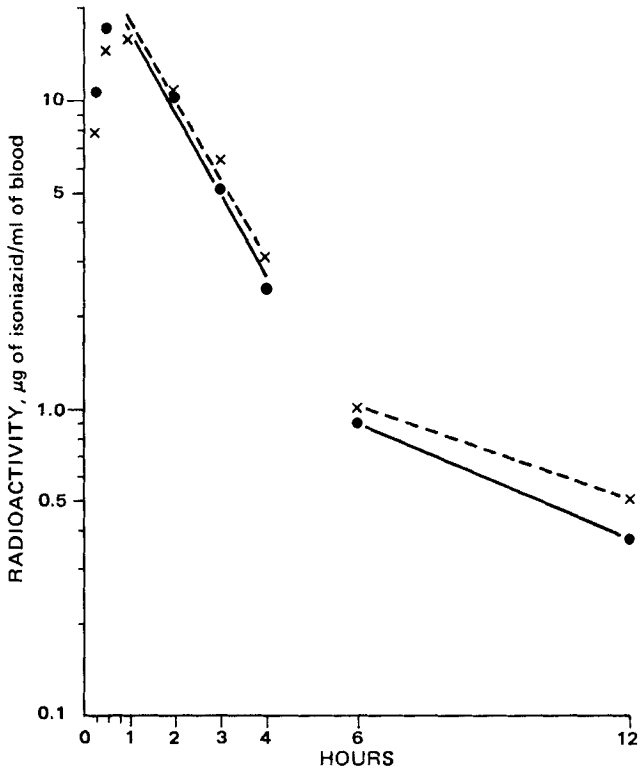


**Figure 1**—*Effect of aspirin on the blood carbon-14 concentration, expressed as micrograms of isoniazid per milliliter of blood. Values are means from groups of five rats dosed orally with $^{14}$C-isoniazid (20 mg/kg). Key: ●, isoniazid alone; and ×, isoniazid and aspirin (100 mg/kg).*

**Rifampin and Ethambutol**—These two antitubercular drugs are considered together because they were studied on the same day against one control group. The blood profiles (Fig. 2) were all similar, with the calculated $T_{1/2}$ values between 1 and 4 hr being 1.23 (1.14–1.33) hr for the control group, 1.31 (1.22–1.44) hr for the rifampin group, and 1.40 (1.28–1.54) hr for the ethambutol group. Neither treatment had a significant effect on the $T_{1/2}$.

Elimination of carbon-14 was not affected at any of the times studied, and the excretion of isoniazid metabolites (Table II) was only slightly changed by one treatment. Ethambutol reduced the excretion of III at 6, 12, and 24 hr.

**Ethanol and Oral Isoniazid**—The linear portion of the blood profile shown in Fig. 3 is shorter than in the other interactions studied, probably

**Table II—Urinary Excretion of Isoniazid Metabolites in Groups of Three Rats Dosed Orally with $^{14}$C-Isoniazid (20 mg/kg) and Saline (Control), Rifampin (30 mg/kg), or Ethambutol (100 mg/kg) [a]**

| Metabolite | Hours | Treatment | | |
|---|---|---|---|---|
| | | Control | Rifampin | Ethambutol |
| I | 0–6 | 1.45 ± 0.30 | 2.27 ± 0.51 | 1.38 ± 0.07 |
| | 0–12 | 1.72 ± 0.24 | 2.41 ± 0.55 | 1.48 ± 0.05 |
| | 0–24 | 1.88 ± 0.34 | 2.46 ± 0.50 | 1.56 ± 0.05 |
| II | 0–6 | 6.68 ± 0.82 | 6.14 ± 0.33 | 9.14 ± 0.92 |
| | 0–12 | 6.99 ± 0.76 | 7.92 ± 0.87 | 9.15 ± 0.93 |
| | 0–24 | 7.64 ± 0.80 | 8.61 ± 0.94 | 9.15 ± 0.93 |
| III | 0–6 | 12.52 ± 0.94 | 10.53 ± 0.94 | 9.20 ± 0.68[b] |
| | 0–12 | 14.51 ± 0.95 | 11.28 ± 1.02 | 11.01 ± 0.22[b] |
| | 0–24 | 16.18 ± 0.76 | 12.22 ± 1.39 | 11.56 ± 0.28[b] |
| IV | 0–6 | 27.54 ± 3.02 | 27.72 ± 2.51 | 29.38 ± 3.82 |
| | 0–12 | 33.30 ± 4.16 | 32.73 ± 3.18 | 37.23 ± 4.13 |
| | 0–24 | 37.67 ± 3.71 | 34.83 ± 3.47 | 38.54 ± 3.87 |

[a] The proportion of each metabolite is expressed as a percentage of the isoniazid dose. Values are means ± *SE*. [b] $p < 0.05$ (compared to control).

[9] Whatman No. 1.
[10] Abbott bichromatic analyzer, ABA-100.
[11] Datagen Nova 800.
[12] The 95% confidence limits are given in parentheses.

**Table III—Urinary Excretion of Isoniazid Metabolites in Groups of Five Rats Dosed Orally with $^{14}$C-Isoniazid (20 mg/kg) and Either Saline (Control) or Ethanol (3 g/kg) [a]**

| Metabolite | Hours | Treatment | |
|---|---|---|---|
| | | Control | Ethanol |
| I | 0–6 | 0.97 ± 0.17 | 2.13 ± 0.52 |
| | 0–12 | 0.98 ± 0.14 | 2.33 ± 0.50[b] |
| | 0–24 | 1.05 ± 0.13 | 2.35 ± 0.50[b] |
| II | 0–6 | 9.00 ± 1.09 | 3.52 ± 0.80[b] |
| | 0–12 | 10.44 ± 1.07 | 5.64 ± 0.60[b] |
| | 0–24 | 11.97 ± 1.04 | 6.21 ± 0.61[b] |
| III | 0–6 | 14.19 ± 0.79 | 6.98 ± 0.57[b] |
| | 0–12 | 15.70 ± 0.80 | 9.99 ± 0.81[b] |
| | 0–24 | 17.02 ± 0.73 | 11.22 ± 0.83[b] |
| IV | 0–6 | 35.58 ± 3.05 | 40.35 ± 4.34 |
| | 0–12 | 38.85 ± 3.21 | 56.37 ± 3.53[b] |
| | 0–24 | 42.20 ± 2.40 | 59.27 ± 3.78[b] |

[a] The proportion of each metabolite is expressed as a percentage of the isoniazid dose. Values are means ± *SE*. [b] $p < 0.05$ (compared to control).

**Table IV—Urinary Excretion of Isoniazid Metabolites in Groups of Four Rats Dosed Intravenously with $^{14}$C-Isoniazid (20 mg/kg) and Orally with Either Saline (Control) or Ethanol (3 g/kg) [a]**

| Metabolite | Hours | Treatment | |
|---|---|---|---|
| | | Control | Ethanol |
| I | 0–6 | 1.01 ± 0.12 | 1.62 ± 0.41 |
| | 0–12 | 1.16 ± 0.15 | 1.71 ± 0.39 |
| | 0–24 | 1.26 ± 0.15 | 1.72 ± 0.39 |
| II | 0–6 | 3.38 ± 1.59 | 4.82 ± 0.60 |
| | 0–12 | 5.06 ± 1.87 | 5.67 ± 0.65 |
| | 0–24 | 5.88 ± 1.94 | 6.24 ± 0.66 |
| III | 0–6 | 14.75 ± 2.01 | 10.87 ± 0.34 |
| | 0–12 | 17.18 ± 1.81 | 12.09 ± 0.36[b] |
| | 0–24 | 18.31 ± 1.91 | 12.94 ± 0.40[b] |
| IV | 0–6 | 34.04 ± 1.23 | 47.27 ± 1.37[b] |
| | 0–12 | 42.55 ± 2.50 | 53.20 ± 1.05[b] |
| | 0–24 | 44.87 ± 2.29 | 55.59 ± 1.00[b] |

[a] The proportion of each metabolite is expressed as a percentage of the isoniazid dose. Values are means ± *SE*. [b] $p < 0.02$ (compared to control).

because of a marked effect during the absorption phase. The concentration of carbon-14 was significantly lower in the ethanol-treated group at 0.25, 0.5, and 1 hr, suggesting that ethanol reduced the isoniazid absorption rate. Over the linear 2–4-hr period, the $T_{1/2}$ values were 1.26 (1.09–1.48) hr for the control group and 1.22 (1.05–1.44) hr for the ethanol-treated group. The difference was not significant. At 12 hr, the blood concentration of carbon-14 was significantly higher in the ethanol group.

Elimination of carbon-14 into urine was once more unaffected by the treatment, but changes were observed in the proportions of the isoniazid metabolites (Table III). Both II and III were inhibited by ethanol treatment, but I and IV were increased. These opposing shifts in metabolism canceled each other out, resulting in no overall change in renal elimination.

The tissue distribution of carbon-14 at 0.5 and 3 hr after dosing is shown in Fig. 4. At 0.5 hr, the highest concentration was observed in the kidneys followed by the lungs. The liver and muscle were close to the blood level, and the brain was less than the blood level. In all tissues except the lungs, ethanol reduced the concentration significantly. At 3 hr, the effect of ethanol was essentially the same, but the reduction was then significant in the lung and not in the kidney tissues. All tissue concentrations were substantially lower at 3 than 0.5 hr, except for the brain where the decline was not as great.

**Ethanol and Intravenous Isoniazid**—Since some significant changes were observed with ethanol, most likely including an effect on absorption, it was decided to give isoniazid intravenously. The blood profile (Fig. 5) shows little indication of any effect of ethanol on elimination. The $T_{1/2}$ values over 1–4 hr were 1.19 (1.07–1.33) hr for the controls and 1.18 (1.08–1.29) hr for the ethanol group. At 2 hr, the carbon-14 concentration was significantly higher in the ethanol group than in the controls, but this effect was the only significant one.

Urinary elimination was similar in magnitude to that observed after oral dosing (controls: 0–6 hr, 51.45 ± 9.02%; 0–12 hr, 71.42 ± 2.26%; 0–24 hr, 75.92 ± 2.11%; and ethanol treated: 0–6 hr, 64.17 ± 6.70%; 0–12 hr, 79.79 ± 2.11%; 0–24 hr, 83.68 ± 2.68%), but in this case ethanol significantly ($p < 0.05$) increased the amount excreted at 12 and 24 hr. This effect may be related to a significant increase in urine volume in the ethanol-treated group during 0–6 hr (controls: 1.78 ± 0.17 ml; and ethanol treated: 3.64 ± 0.27 ml; $p < 0.001$). However, by 12 hr, the difference was

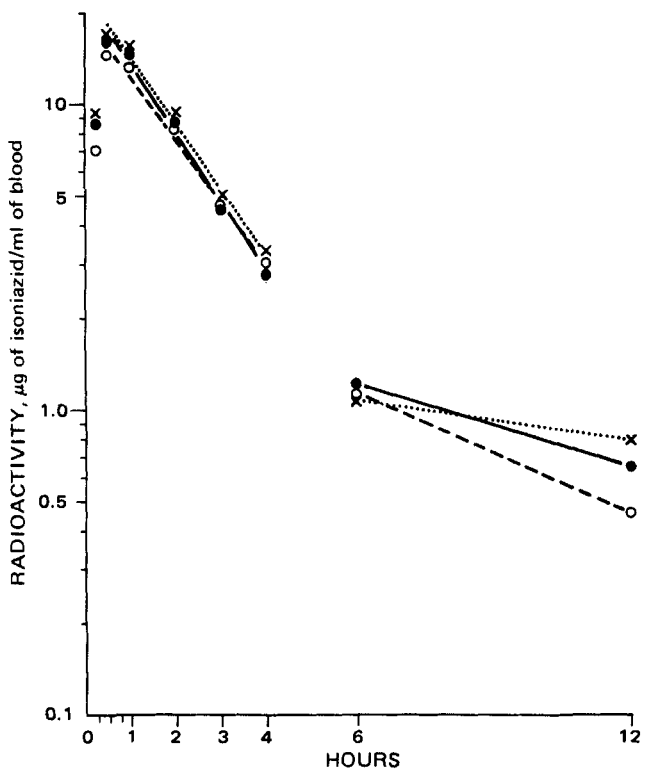


**Figure 2**—*Effect of rifampin and ethambutol on the blood carbon-14 concentration, expressed as micrograms of isoniazid per milliliter of blood. Values are means from groups of five rats dosed orally with $^{14}$C-isoniazid (20 mg/kg). Key: ●, isoniazid alone; ×, isoniazid and rifampin (30 mg/kg); and ○, isoniazid and ethambutol (100 mg/kg).*

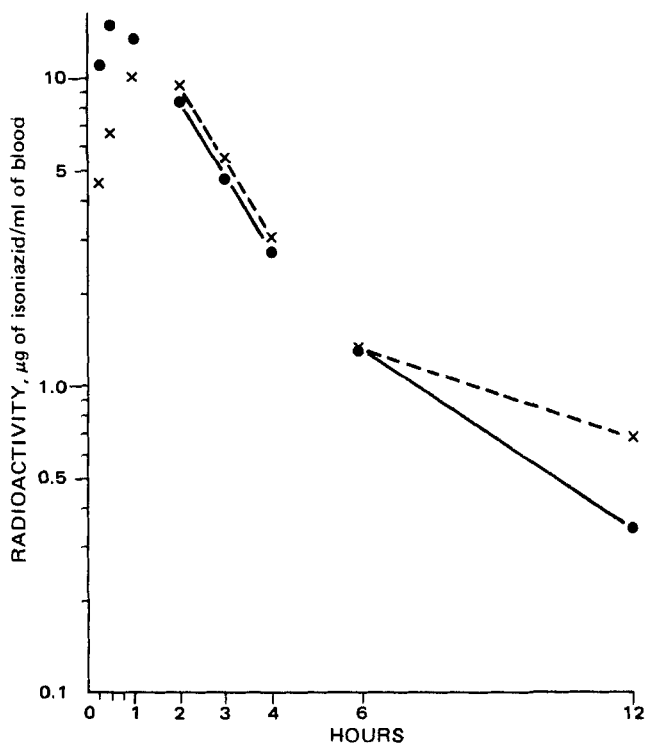


**Figure 3**—*Effect of ethanol on the blood carbon-14 concentration, expressed as micrograms of isoniazid per milliliter of blood. Values are means from groups of five rats dosed orally with $^{14}$C-isoniazid (20 mg/kg). Key: ●, isoniazid alone; and ×, isoniazid and ethanol (3 g/kg).*

**Table V—Urinary Excretion of Isoniazid Metabolites in Six Rats Dosed with Phenobarbital Sodium (40 mg/kg ip) for 3 Days and in Four Control Rats that Received Similar Doses of Saline; All Rats Were Dosed Orally with $^{14}$C-Isoniazid (20 mg/kg) on the Following Day [a]**

| Metabolite | Hours | Treatment | |
|---|---|---|---|
| | | Control | Phenobarbital |
| I | 0–6 | 1.72 ± 0.71 | 1.01 ± 0.44 |
| | 0–12 | 2.00 ± 0.73 | 1.36 ± 0.50 |
| | 0–24 | 2.05 ± 0.71 | 1.38 ± 0.50 |
| II | 0–6 | 3.55 ± 1.12 | 2.61 ± 1.05 |
| | 0–12 | 7.20 ± 2.32 | 9.41 ± 0.92 |
| | 0–24 | 8.80 ± 2.45 | 10.97 ± 1.06 |
| III | 0–6 | 12.62 ± 1.92 | 5.16 ± 1.57[b] |
| | 0–12 | 17.80 ± 2.65 | 13.19 ± 1.53 |
| | 0–24 | 19.84 ± 2.68 | 14.80 ± 1.62 |
| IV | 0–6 | 21.80 ± 2.56 | 15.09 ± 3.77 |
| | 0–12 | 31.54 ± 3.84 | 27.34 ± 1.41 |
| | 0–24 | 37.50 ± 2.60 | 33.68 ± 3.06 |

[a] The proportion of each metabolite is expressed as a percentage of the isoniazid dose. Values are means ± *SE*. [b] $p < 0.025$ (compared to control).

no longer significant. The metabolite data in Table IV confirm that ethanol inhibited III excretion and increased IV excretion, but in this case the increase in IV was sufficient to increase overall excretion.

**Phenobarbital**—Treatment with phenobarbital for 3 days to induce the microsomal enzyme system had no significant effect on the elimination of carbon-14 from blood (Fig. 6). The $T_{1/2}$ values over 1–6 hr were 1.41 (1.28–1.57) hr for the controls and 1.59 (1.47–1.73) hr for the phenobarbital-treated group, and the difference was not significant. None of the individual blood values was significantly different.

Renal excretion was unaffected by the pretreatment, and only at 6 hr was the excretion of isoniazid metabolites changed (Table V). At this one time, less III was excreted by the pretreated rats.

**Serum Transaminases**—Isoniazid was not hepatotoxic in the rat when given as single doses, even in large amounts (Table VI). In no case was there a significant increase in the transaminase levels.

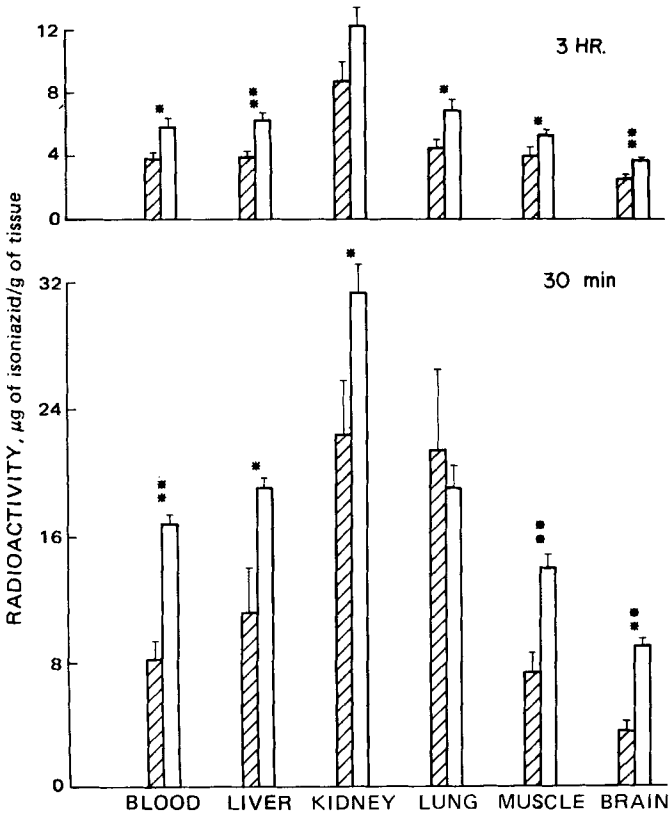


**Figure 4**—*Effect of ethanol on the tissue distribution of radioactivity, expressed as micrograms of isoniazid per milliliter of blood. Values are means and standard errors from groups of five rats dosed orally with $^{14}$C-isoniazid (20 mg/kg) and killed at 0.5 or 3 hr. Key: □, isoniazid alone; ▨, isoniazid and ethanol (3 g/kg); *, p < 0.05; and **, p < 0.01.*

**Table VI—Serum Glutamic-Oxaloacetic Transaminase (V) and Serum Glutamic-Pyruvic Transaminase (VI) Activities in Groups of Three Rats Administered Three Different Doses of Isoniazid [a]**

| Treatment, mg/kg | Hours after Dosing | | | | | |
|---|---|---|---|---|---|---|
| | 6 | | 12 | | 18 | |
| | V | VI | V | VI | V | VI |
| Control | 106 ± 9 | 34 ± 1 | 136 ± 11 | 30 ± 1 | 97 ± 4 | 73 ± 3 |
| Isoniazid | | | | | | |
| 50 | 100 ± 6 | 33 ± 4 | 112 ± 6 | 29 ± 5 | 96 ± 7 | 61 ± 6 |
| 200 | 75 ± 3 | 33 ± 1 | 90 ± 3 | 27 ± 1 | 84 ± 3 | 52 ± 2[b] |
| 500 | 42 ± 1 | 34 ± 2 | 70 ± 10 | 24 ± 2 | 93 ± 6 | 44 ± 3[b] |

[a] The activities of each enzyme are expressed as international units. Values are means ± *SE*. [b] $p < 0.05$ (compared to control).

## DISCUSSION

Isoniazid was eliminated rapidly by rats. The $T_{1/2}$ of carbon-14 in the first phase of elimination, during which over half of the dose was excreted in the urine, had a mean value of about 1.2 hr in the control animals. Due to this extremely rapid elimination, it was not possible to define the second phase, which commenced at about 6 hr after dosing. None of the drugs significantly changed the calculated $T_{1/2}$. Grassi and Grassi (11) also observed that acute doses of ethanol in rats did not affect the serum isoniazid level but that chronic doses of 5 g of ethanol/kg/day did significantly increase serum isoniazid levels. Aspirin and ethanol probably retarded the isoniazid absorption rate, because the blood carbon-14 concentration was lower in the rats treated with these drugs during the first 0.5 hr after dosing.

The tissue distribution study showed that isoniazid and/or its metabolites can enter the brain; but since the level was less than that in the blood, it was inferred that there was some barrier to the passage from blood to brain. The highest concentration of carbon-14 at both 0.5 and 3 hr was observed in the kidneys, while in the control animals the other tissues resembled the blood level. The results are in accord with those reported for rats (12) when the smaller dose and different sampling times are taken into consideration.

The changes in blood level produced by ethanol, which are attributed

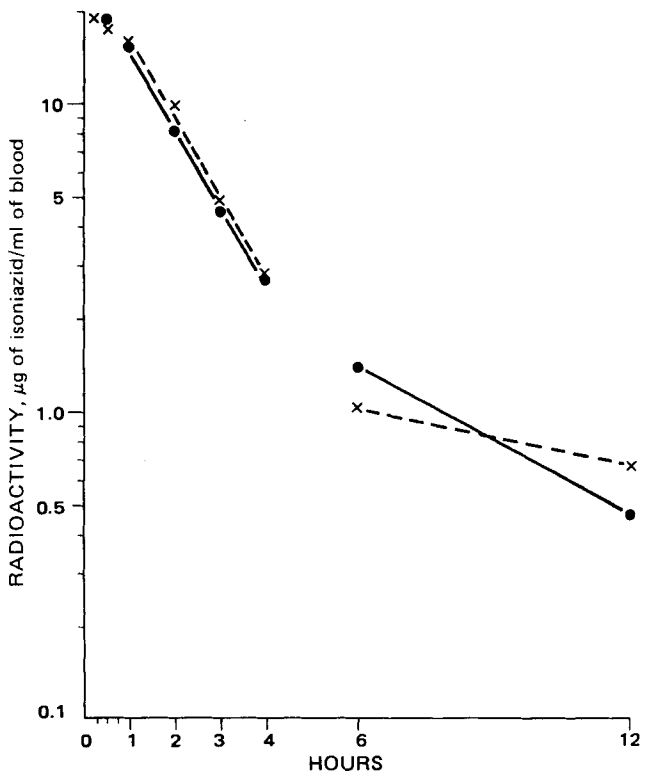


**Figure 5**—*Effect of ethanol on the blood carbon-14 concentration, expressed as micrograms of isoniazid per milliliter of blood. Values are means from groups of five rats dosed intravenously with $^{14}$C-isoniazid (20 mg/kg). Key: ●, isoniazid alone; and ×, isoniazid and ethanol (3 g/kg).*

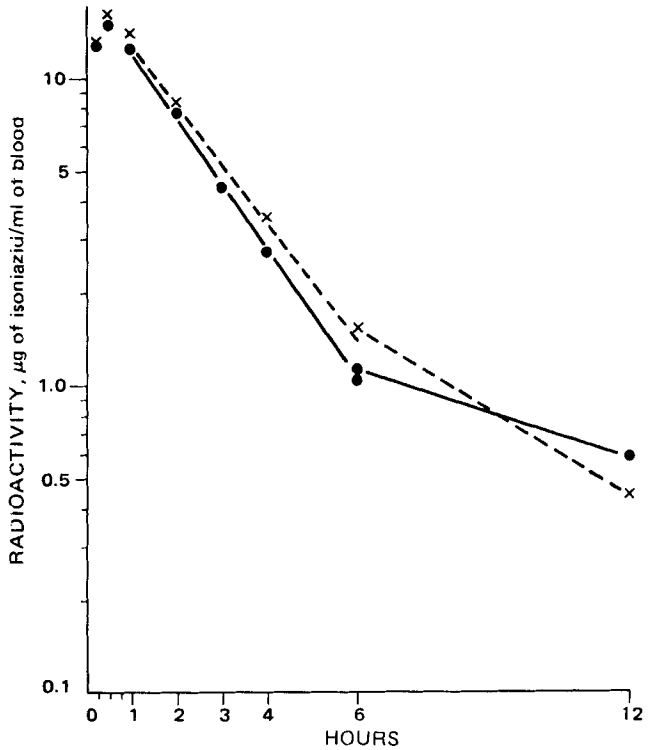


**Figure 6**—*Effect of phenobarbital pretreatment on the blood carbon-14 concentration, expressed as micrograms of isoniazid per milliliter of blood. Values are means from a group of six rats pretreated with phenobarbital sodium (40 g/kg/day ip) for 3 days and a group of four control rats that received saline. All rats were dosed orally with $^{14}C$-isoniazid (20 mg/kg). Key: ●, saline-dosed controls; and ×, phenobarbital-pretreated rats.*

mostly to an effect on absorption, were generally reflected in the tissues. Hence, estimation of the blood carbon-14 level is a good indication of the situation in the whole body. One possible exception is that at 0.5 hr the lung level of carbon-14 was significantly ($p < 0.05$) higher than the blood in the ethanol-treated rats. This effect was not apparent at 3 hr, where the lung level reflected the blood level with a significantly lower level in the ethanol-treated rats.

Renal excretion was the major route of isoniazid elimination, with about 70–80% of the dose of carbon-14 being recovered in the urine in 24 hr. Only ethanol affected the amount of carbon-14 excreted and then only when isoniazid was administered intravenously. In this case, ethanol treatment significantly increased the amount of carbon-14 excreted, which could have been due to the diuretic effect of ethanol.

Most treatments produced some change in the proportions of the isoniazid metabolites excreted in urine. Aspirin almost completely inhibited II excretion, probably due to salicylate competing for the glycine pool since salicylglycine is an aspirin metabolite (13). Rifampin did not produce any significant changes, and ethambutol caused only a slight decrease in the amount of III excreted. Phenobarbital pretreatment produced a similar effect but only up to 6 hr after dosing.

Ethanol produced the most marked interaction. When isoniazid was given orally, all four metabolites estimated were affected: II and III were decreased and I and IV were increased. However, when isoniazid was administered intravenously, only III was decreased and IV was increased. If the theory of Peters *et al.* (14) that isoniazid is acetylated prior to being hydrolyzed to isonicotinic acid is true, then ethanol is blocking the cleavage of *N*-acetylhydrazine. Since it may be at this stage that hepatotoxicity is caused, there is reason to believe that acute doses of ethanol will not potentiate isoniazid-induced liver damage. Nevertheless, prolonged ethanol abuse will no doubt increase the danger of liver damage and isoniazid may potentiate this effect. At present, it is not possible to explain why ethanol has this effect on isoniazid metabolism, but it is similar to other cases where acute doses of ethanol block drug metabolism (5).

Measurement of serum transaminase levels in rats dosed with isoniazid showed that the rat is not susceptible to an acute hepatotoxic reaction to this drug. Mitchell and Jollow (4) also reported that hepatic necrosis could not be produced in the rat even after lethal isoniazid doses. However, their observation (4) that very little III is formed after isoniazid administration to rats is contrary to the present results, in which ~25% of the isoniazid dose was metabolized to II and III. Hence, the resistance of the rat to the hepatotoxicity of isoniazid remains unexplained.

One possibility is that the metabolic activation of *N*-acetylhydrazine (15) is poorly developed in the strain of rats used. Comparison of the total amounts of II, III, and IV with recent human data (16) shows that the amount of IV in the rat is about the same as in rapid acetylator humans but that the amount of III in the rat is less than in either slow or rapid acetylator humans. These results could indicate that the rat is not able to cleave acetylhydrazine from IV as rapidly as the human. This also could explain the low hepatic toxicity in the rat.

It is concluded that, of the drugs studied, only ethanol had a significant interaction with isoniazid metabolism. Nevertheless, this effect did not change the elimination rate from the blood. Based on current knowledge of the mechanism of isoniazid-induced hepatotoxicity, it is not likely that acute doses of ethanol potentiate isoniazid toxicity. The rat is not a good model for the study of isoniazid-induced hepatotoxicity and its metabolism of isoniazid does differ quantitatively from that observed in humans.

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[x] To whom inquiries should be directed.