Technical Papers

Some Pharmacologic Characteristics of Isonicotinyl Hydrazide (Pyricidin®), A New Antituberculosis Drug

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Recent reports that heterocyclic thiosemicarbazone derivatives are effective in the treatment of experimental tuberculosis (*1, 2*) have stimulated the search for superior drugs. This search (*3*) has led to the synthesis of isonicotinyl hydrazide (Pyricidin®).[2] The compound is not new, having originally been synthesized 40 years ago (*4*). Only recently, however, it has been reported to be markedly effective in the treatment of both experimental (*5*) and human tuberculosis (*6*). It is of interest, therefore, to present in this preliminary report our findings on the pharmacology of isonicotinyl hydrazide.

Acute toxicity: The acute oral, intraperitoneal, and intravenous toxicity of isonicotinyl hydrazide was determined in 17–22-g male albino mice (CF-1 strain); oral and intraperitoneal toxicity was determined in 150–200-g male rats (CF), and in 300–400-g male guinea pigs (CF). Toxic signs consisted of spasmodic tremors, salivation, convulsions, terminal tetanic spasm, and respiratory arrest. Immediate postmortem examination revealed actively beating auricles, and in some instances, ventricles. There were no gross abnormalities. The LD_{50}s were computed graphically (*7*) and are expressed as mg/kg, orally (p. o.), intraperitoneally (i. p.) and intravenously (i. v.): *Mouse*: LD_{50} p. o., 205; i. p., 159; i. v., 150. *Rat*: p. o., 650, i. p., 380. *Guinea pig*: p. o., 280; i. p., 200. The limits of error, based on 10–20 animals/dose point, were generally less than ±10%. The results indicate that isonicotinyl hydrazide had relatively low acute toxicity in all three species. It will be noted that the drug was distinctly less toxic in the rat than in the mouse or guinea pig. It is of interest that the oral LD_{50} in the rat was nearly twice the parenteral value. In the mouse and guinea pig, however, the oral and parenteral values were nearly the same. This finding may be interpreted as indicating that isonicotinyl hydrazide was rapidly absorbed from the intestinal tract in the latter species. The reduced toxicity in the rat may be attributable to a slower rate of absorption. In addition to the studies in small animals, isonicotinyl hydrazide was injected intravenously into unanesthetized mongrel dogs in large dosage (25 mg/kg). Except for salivation, the animals appeared normal.

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[2] Pyricidin® is a registered trademark of the Nepera Chemical Co., Inc.

Blood pressure, respiration, and intestinal motility: Mongrel dogs were anesthetized with pentobarbital, and blood pressure, respiration, and intestinal movements were recorded kymographically. None of these functions was affected following the intravenous injection of isonicotinyl hydrazide in single doses of up to 100 mg/kg, or in multiple doses totaling 146 mg/kg.

Isolated tissues (ileum and uterus): Isolated guinea pig ilea and uteri were suspended in a 100-ml bath of oxygenated physiologic solution and attached to levers for kymographic recording of spontaneous movements. Isonicotinyl hydrazide produced no perceptible change in the normal functioning of these tissues at concentrations as high as 100 μg/ml bath fluid. (*Heart*): The isolated guinea pig, rat, and rabbit heart was attached by an aortic cannula to a modified Langendorff apparatus for coronary perfusion at 37° C under constant pressure. Ventricular movements were recorded kymographically. Aqueous solutions of isonicotinyl hydrazide (2.5, 5.0, 10.0, and 100.0 μg) injected into the perfusion system immediately proximal to the cannula had no effect on the rate and amplitude of ventricular contractions or on the coronary perfusion rate.

Irritation (cilia): The action of isonicotinyl hydrazide on mammalian cilia was studied by means of a method developed in these laboratories (*8*). The procedure, in brief, consisted of sectioning the rat trachea into rings which were then immersed in Locke-Ringer solution containing isonicotinyl hydrazide. Loss of ciliary motility and the formation of "globoid bodies" were taken as end points. The results indicate that isonicotinyl hydrazide had no effect on tracheal cilia at concentrations as high as 10%. (*Gastric mucosa*): We were able to demonstrate that isonicotinyl hydrazide produced no observable gastric irritation in the rat at high dosage (200 mg/kg, as a 10% solution). In these studies the drug was introduced retrograde through the duodenum into the gastric lumen. Ligatures were then placed at the cardiac and pyloric orifices, and, after 1 hr, the animals were sacrificed and the stomachs removed and examined grossly. Control rats, pretreated with physiologic saline, were subjected to the same operative procedure. The gastric mucosa obtained from rats pretreated with 10% isonicotinyl hydrazide or physiologic saline appeared normal and were indistinguishable. It is noteworthy that isonicotinyl hydrazide, unlike many of the earlier agents employed in the chemotherapy of tuberculosis, has striking water solubility, and that the pH of even concentrated solutions is well within the physiologic range.

Antidotes: Graded doses of phenobarbital and chloral hydrate were administered orally to groups of 10 male albino mice (CF-1, 17–22 g). Thirty min later, a lethal dose ($2 \times LD_{50}$) of isonicotinyl hydra-

zide was injected intraperitoneally into the pretreated and also into nonpretreated (control) mice, and the number of surviving animals was recorded after 24 hr. The doses protecting 50% of the animals (ED_{50}) were: chloral hydrate, 650 mg/kg; phenobarbital, 61 mg/kg. The therapeutic indices (LD_{50}/ED_{50}) were 1.7 and 5.3, respectively. It is clear that both drugs conferred protection but, because of its favorable therapeutic index, phenobarbital was preferred.

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Absorption of Nutrients by Stems and Branches of Woody Plants[1]

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Although roots are the principal nutrient absorbing parts or organs of plants, it has been shown that foliage is also capable of absorption (*1–3*). Deficiencies of magnesium have been corrected by foliar applications of Epsom salts (*4, 5*), proper nitrogen levels in fruit trees have been maintained by foliage sprays of urea (*6*), and as much as 7% of the phosphorus requirement of developing fruits of young tomato plants has been supplied through a single application to the foliage (*7*). The question naturally arises as to the absorption of nutrients by stems and branches.

Radioactive isotopes lend themselves well to such studies. K^{42} as potassium carbonate applied in a 6-in. band of cotton gauze around dormant branches of bearing apple trees (*Malus domestica* var. R. I. Greening) in midwinter (February) was detected 24 hr later in both the phloem and the xylem 18 in. above the point of application and 18 in. below, although the air temperature was below freezing during the period and reached a minimum of −3° F. Radiopotassium was detected likewise in branches rising vertically from horizontal branches of apple trees to which it was applied. With the Elberta variety of peach (*Prunus persica*) activity was detected within 48 hr in dormant branches of bearing trees both 6 in. beyond and 6 in. below the point of application.

Potted dormant 2-year-old trees of the McIntosh apple and the Elberta peach were treated with P^{32} *o*-phosphoric acid in a band 2 in. wide on the bark 6 in. above the soil and placed in the greenhouse at 70° F. Radiophosphorus was detected within 28 hr, not only in the stem above the point of application but in the roots as well.

Finally, measurements were made of urea hydrolysis from applications of C^{14} urea made to the branches in full leaf of apple, peach, and cherry (*Prunus cersus* var. Montmorency). Plants were placed in a chamber in the dark, and the atmosphere was circulated at a slow rate in a closed system containing a Geiger-Muller counter and a continuous recorder, as described by Hinsvark, Wittwer, and Tukey (*8*). Applications were made to leaves, to the bark of branches from which leaves had been removed, and to the bark of branches from which leaves had not been removed.

The data are given in Table 1 as enzymatic rate constants (zero order) derived from the initial slopes of the activity time curves. Hydrolysis of urea occurred in all cases following bark applications. The rate of hydrolysis varied with the parts and condition of the plant treated. Most rapid hydrolysis occurred when urea was applied to the bark of branches in full leaf, followed by application to a comparable leaf surface on a newly developed leaf, and the slowest rate of hydrolysis occurred when the urea was applied to the bark of branches from which the leaves had been removed. Activity of the bark and leaves of the apple appears to be approximately three times that of the peach.

TABLE 1

ENZYMATIC RATE CONSTANTS (ZERO ORDER) DERIVED FROM THE INITIAL ACTIVITY-TIME OF THE HYDROLYSIS OF C^{14} UREA, APPLIED TO THE BARK AND LEAVES SEPARATELY OF APPLE, CHERRY, AND PEACH TREES

Point of application	Rate constants (Counts/hr)		
	Apple	Cherry	Peach
Leaves	42	15	15
Bark, leaves removed	30	10	8
Bark, leaves not removed	130	32	20

To determine the concentration of nutrients that could be applied to dormant trees without visible injury, applications were made of calcium chloride, *o*-phosphoric acid, potassium nitrate, and urea in 2, 4, 8, 16, and 32% solutions and slurries to 2-year-old McIntosh apple trees and 1-year-old Montmorency cherry trees. Injury in the form of bud killing and delayed bud start resulted from concentrations greater than 8% calcium chloride, *o*-phosphoric acid, and urea. On the other hand, no injury to dormant trees was observed from potassium nitrate at any of the

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