Preliminary Observations on a New Derivative of Isoniazid (Ro 2-4969) (20624)

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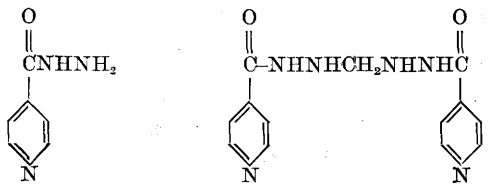
The effectiveness of isoniazid in the treatment of experimental tuberculosis in animals was first reported by Grunberg and Schnitzer(1). These investigators have since found that Ro 2-4969, a new derivative of isoniazid, has similar anti-tuberculosis properties(2). Chemically, Ro 2-4969 is 1,1-'Methylenebis (2-isonicotinylhydrazine). The structural relationship between Ro 2-4969 and isoniazid is represented as follows:



Isoniazid Ro 2-4969

Benson *et al.*(3) found that the subacute toxicity of Ro 2-4969 in rats is less than that of isoniazid; acute toxicity in other species also compares favorably with that of isoniazid. According to Schnitzer and Grunberg(2), the chemotherapeutic ratio (LD_{50}/PD_{50}) of Ro 2-4969 in mice is greater than 260; the comparative value for isoniazid is 44. In view of these promising reports the present study was undertaken to determine the effects of Ro 2-4969* on tuberculosis in man. Particular attention was paid to the absorption of Ro 2-4969 because of its low solubility.

Methods. The concentration of Ro 2-4969 in blood plasma was determined according to the method of Rubin *et al.*(4). This technic is based on the determination of isonicotinic acid. All results of determinations of plasma levels are therefore expressed as micrograms of isonicotinic acid per ml. It is not known in what form Ro 2-4969 exists in the blood. Most of the subjects used in this study were patients at the Lakeview Sanatorium. We are indebted to Dr. John K. Shumate, Superintendent and Medical Director of Lakeview, for his generous cooperation and assistance in the care and study of these patients.

Results. Fig. 1 presents the plasma concentrations following a single oral dose of Ro 2-4969 compared with a similar dose of isoniazid in the same subject. The rapid rise and decline following isoniazid is typical of the drug. No detectable concentration was found 24 hours after the administration of isoniazid. Ro 2-4969, on the other hand, reached its peak more slowly, a significant level was present at the end of 12 hours and a detectable concentration at the end of 24 hours.

The presence of discernible quantities at the end of 24 hours led to an examination of the possible accumulation of Ro 2-4969 if it were given over a period of several days. The plasma concentrations of 3 patients who were given the drug daily for 8 days were observed. Ro 2-4969 was administered for the first 4 days at a total dose of 5 mg/kg/day and then increased to 10 mg/kg/day for the remaining 4 days. The divided doses were given at 8:00 A.M., noon, and 5:00 P.M.

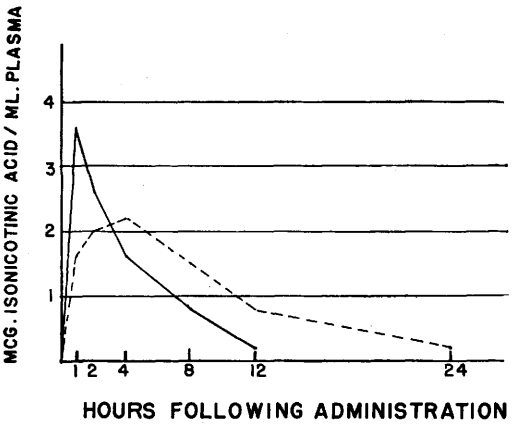


FIG. 1. Plasma concentrations in the same subject following oral administration of 200 mg each isoniazid (solid line) and Ro 2-4969 (broken line).

* Made available for this study by Dr. M. J. Schiffrin, Hoffmann-La Roche, Inc., Nutley, N. J.

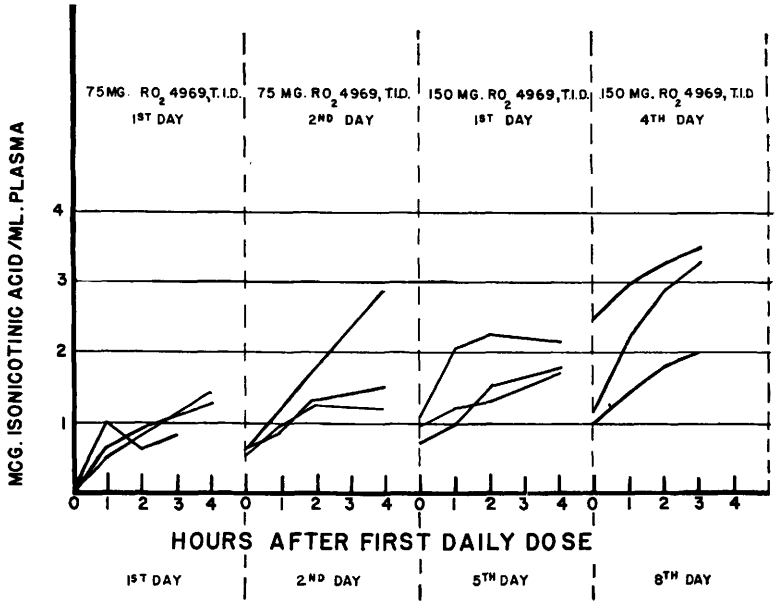


FIG. 2. Plasma concentrations in 3 subjects receiving Ro 2-4969 daily in divided doses for a period of 8 consecutive days. Figures at top refer to day of administration at either the 75 mg or 150 mg level. Days listed at bottom of figure refer to the experimental days.

Plasma levels were determined before the morning administration and at one, 2, and 4 hours after the first dose. A definite cumulative tendency was observed throughout the 8-day period (Fig. 2).

The possibility of accumulation over longer periods was studied in 10 patients who received a single daily dose of Ro 2-4969, 10 mg/kg, over a period of 9 weeks. Plasma concentrations were determined once weekly just prior to the administration of the daily dose, hence 24 hours following the previous dose. These data are presented in Table I. There was a definite tendency toward establishment of a continuous blood concentration at a high therapeutic average level of 1.0-1.5 μg/ml plasma from the third week on. In addition to these 10 patients, 16 patients were given the same dosage but without regular determinations of plasma concentrations.

Discussion. It is evident that when Ro 2-4969 is administered orally, a derivative of isonicotinic acid appears in the plasma. The peak concentration appears later and is lower than that following the administration of isoniazid; however, measurable quantities persist longer than with isoniazid.

At the 10 mg/kg/day dosage, there is a cumulative effect even when Ro 2-4969 is given as a single daily dose, so that after 2 or 3 weeks clinically significant concentrations (one or more μg/ml) are present 24 hours following each dose. It is reasonable to assume that such significant levels persist throughout the entire 24-hour period.

A detailed report of the clinical observations made during the treatment of these patients will appear subsequently. In general, the clinical, x-ray and bacteriological response in 26 patients treated for 2 months or longer have paralleled those seen with isoniazid therapy. As with any new agent, the final assessment of the value of Ro

TABLE I. μg Isonicotinic Acid per ml Plasma.

	Weeks of treatment							
	1	2	3	4	5	6	7	8
B.	.2	1.0	1.1	1.0	.8	1.6	.8	.8
C.	.6	1.0	1.2	1.4	1.5	1.1	—	1.1
G.	—	.6	.8	.8	.9	.9	—	1.1
H.	.5	1.3	1.9	—	2.5	1.1	—	1.6
W.	.6	1.0	1.6	—	1.7	1.4	—	—
A.W.	.6	.7	.9	1.1	1.3	1.4	1.6	1.2
J.B.	.3	1.2	.8	—	1.9	.9	1.2	1.2
F.	.5	.8	.8	1.2	1.6	1.1	—	1.1
S.	.7	.7	.8	—	1.4	.8	—	—
K.	.4	1.0	—	1.1	.9	—	1.7	.8
Avg	.5	.9	1.1	1.1	1.5	1.1	1.3	1.1

2-4969 in the treatment of human tuberculosis must await prolonged clinical trial. It is significant, however, that no toxic reactions to Ro 2-4969 were observed.

Summary. 1. Ro 2-4969, a compound of low solubility, related to isoniazid, has been administered experimentally to human subjects to determine the rate and extent of absorption from the gastrointestinal tract. 2. The compound or its derivatives appeared in the plasma and, probably because of slow absorption, detectable and clinically significant amounts remained for a longer period than when the highly soluble isoniazid was administered. 3. The drug administered in a single daily dose of 10 mg/kg/day was found to maintain drug concentrations of one μg/ml of plasma without dangerous cumulative effect. 4. No toxic reactions have been observed in 26 patients treated for 2 months or more.

1. Grunberg, E., and Schnitzer, R. J., *Quart. Bull. Sea View Hosp.,* 1952, v13, 3.

2. Schnitzer, R. J., and Grunberg, E., personal communication.

3. Benson, W. M., Roe, M. D., and Mermod, C., personal communication.

4. Rubin, S. H., Drekter, L., Scheiner, J., and De Ritter, E., *Diseases of the Chest,* 1952, v21, 75.

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The Effect of Adrenalectomy on Propulsive Motility of the Small Intestine in Rats. (20625)

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Digestive disturbances occur in Addison's disease and in adrenal insufficiency in animals (1,2), but relatively few direct studies of gastro-intestinal behavior after adrenalectomy have been reported. Anorexia, nausea, vomiting, either constipation or diarrhea, and abdominal pain are symptoms in Addison's disease; congestion, diffuse hemorrhage of the intestine and bloody diarrhea are common in adrenalectomized animals. Vogt(3) found that the circular muscle coat of the isolated intestine of the adrenalectomized rabbit frequently developed powerful, spasmotic, rhythmic contractions, while Auerbach's plexus often failed to respond to normally effective stimuli. Since adrenal extract did not restore normal activity, it was concluded that the abnormal intestinal behavior was due to secondary effects of adrenal removal.

Methods. Propulsive motility of the small intestine was studied by the method of Van Liere *et al.*(4) in 82 young male, albino Wistar rats, weighing from 90 to 150 g. Thirty adrenalectomized rats were tested 4 to 7 days after operation. Since two types of control animals (intact and sham-operated) are necessary, 15 intact and 19 sham-operated were tested. Purina Laboratory Chow and tap water was supplied *ad libitum,* and daily food intake was measured. Since adrenalectomized rats lose their appetite, controls for the effect of relative starvation were performed by feeding an additional 6 intact and 12 sham-operated rats a daily amount of food equal to the average amount eaten by adrenalectomized rats and testing them after periods of reduced food intake equal to that after adrenalectomy. In all groups, the rats were starved for 24 hours prior to testing for intestinal propulsion.

Results. The results are presented in Table I as percent of the total number of rats in each experimental group falling within each 5% range of the length of the small intestine traversed by the test meal of charcoal. No essential difference occurred between the intact and sham-operated rats. Partial starvation of both normal and sham-operated rats produced some increase in intestinal motility. However, no significant differences were found

* Many of the experiments were performed by the following graduate students: Milton Stern, Harold Carlin, and Mario M. Boschetti.