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The Distribution of C^{14} Labeled Isonicotinic Acid Hydrazide in Normal Mice.* (19942)

L. J. ROTH AND R. W. MANTHEI. (Introduced by E. M. K. Geiling.)
From the Department of Pharmacology, University of Chicago.

Isonicotinic acid hydrazide and its isopropyl derivative are now being subjected to widespread clinical evaluation throughout this country(10). The promise that these drugs have shown warrants a further study of their pharmacological properties, whether or not these drugs do become accepted generally for use in the treatment of tuberculosis. This paper is the first report of our group on a series of investigations to determine such properties of isonicotinic acid hydrazide. As a basis for interpreting future work, we have studied the absorption, distribution and excretion of the drug in normal mice.

The isotope tracer technic was used in this problem since it is a rapid yet sensitive technic well suited to determining micro quantities of a drug and its metabolite in many tissues. We have been able to obtain isotopically labeled isonicotinic acid hydrazide through the cooperation of the Health Division, Los Alamos Scientific Laboratory, Los Alamos, New Mexico. The drug which was synthesized by Murray and Langham(1) is labeled with C^{14} in the carboxyl position and has an activity of 0.045 mc/mg.†

Materials and methods. A saline solution of the labeled isonicotinic acid hydrazide diluted with crystalline normal drug was made up to contain 400 micrograms per milliliter with an activity of 2.54×10^6 cpm. The refrigerated solution was found by paper chromatography to be stable for longer than one month. Forty-two female mice of the CF_1 strain weighing approximately 20 g each were used for this study. Each mouse received a subcutaneous injection in the right hind leg of 0.5 ml of the above solution (10 mg/kg). The mice were kept in all-glass metabolism chambers(2) throughout the experimental period and the exhaled CO_2 trapped in towers of sodium hydroxide solution. At the end of each experimental period, the mice were sacrificed by cervical fracture, the heart rapidly exposed and 0.25 to 0.5 ml of blood withdrawn by cardiac puncture. The tissues were dissected out, weighed and aliquots plated directly in duplicate(3) on copper discs as 10% water homogenates. Three mice were used for each of the shorter experimental periods and four or more for each of the longer ones. All tissue samples were counted in windowless gas-flow counters(4) with a counter efficiency of 65%. Standard corrections were applied for geometry, resolving time, and self-absorption before the average of the duplicate plates was taken. In view of the high activity of the injected drug, statistically significant counts could still be detected in some tissues as long as one week after injection. Since this represented only a relatively small amount of drug, only values greater than twice the standard deviation of the counting background were considered as significant. Thus counts equivalent to less than 0.0075 μg of drug or metabolite per gram of tissue are

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† The C^{14} used for the synthesis of this compound was obtained on allocation from the Isotope Div., U.S. A.E.C.

TABLE I. Tissue Distribution of Isonicotinic Acid Hydrazide and Its Metabolites.

Tissue	.5 hr	1 hr	4 hr	8 hr	24 hr	2 day	4 day	7 day
Blood	5.7	4.1	.96	tr	tr	tr	tr	tr
Brain	4.4	3.2	2.1	.20	0	0	0	0
Lung	6.6	5.2	2.2	.32	.19	tr	tr	tr
Skin	5.4	3.6	2.6	.57	.41	.39	0–tr	tr–1.6
Muscle*	5	2.7	.66	0	0	0	0	0
Kidney	11.9	8	1.9	.21	tr	tr	tr	0–tr
Liver	10.6	8.9	4.1	2.3	.62	.42	.23	.14
Bile†	.09	.14	.19	.07	0	0	0	0
Feces	3.5	6.6	19.6	14	.25	0	0	0

* Adrenals, abdominal fat, heart, lymph nodes, sex organs and spleen distribution pattern similar to muscle.

† Bile reported as μg equivalents aspirated from gall bladder. All other data reported as μg equivalents/g of tissue or ml of blood. Each value represents avg of 3 or more mice, the variation in tissue concentration between mice was less than 10%.

considered as insignificant while values up to 0.015 μg are reported as trace quantities.

The total radioactivity measured in the tissues represents both that due to the drug and to its metabolites. However, since the metabolites have not all been characterized, the data are reported in terms of microgram equivalents of injected drug. A microgram equivalent is defined as the tissue radioactivity equivalent to one microgram of isonicotinic acid hydrazide.

In order to fractionate metabolites of the drug, chromatograms of tissues and excreta were run on Whatman No. 1 paper strips with a solvent system of butanol saturated with water. It was found that extraction of the urine with isobutyl alcohol yielded the same number of metabolites on the chromatograms as did direct application of the urine to the paper strips. Since they could not be applied directly, homogenates of tissues were filtered and extracted with isobutyl alcohol before being applied to the strips. The $C^{14}O_2$ was measured by means of an ionization chamber and vibrating reed electrometer(5). Current readings were converted to dps and then to cpm as measured by the windowless gas-flow counters.

Results. The data obtained in the distribution study are presented in Table I. Total recoveries from tissues and from excreta of injected activity ranged from 88-97%. Following subcutaneous injection, the drug is rapidly absorbed and distributed throughout the body but is retained in significant amounts by only a few tissues after 8 hours.

The activity in the blood was found both in plasma and in the saline washed cellular elements. Peak concentrations of activity were obtained in the blood shortly after subcutaneous injection of the labeled drug. After 8 hours only trace amounts of activity were detectable but these levels were maintained in the blood for as long as one week.

The lung contained more activity per gram of tissue than the blood per milliliter at each experimental period. Approximately 0.5% of the injected dose, or 1.1 μg equiv., was concentrated in the lung within the first hour. By 8 hours only 0.04% of the dose was present but trace amounts of activity were detectable for as long as one week.

During the first hour, the brain had less activity per gram of tissue than the blood per milliliter; however, it retained relatively more activity than the blood during the next 7 hours indicating a movement of drug or metabolite from blood into brain tissue. By 24 hours after injection, however, the brain no longer contained any detectable activity. Chromatograms of brain homogenates showed that both isonicotinic acid hydrazide and isonicotinic acid are present. Preliminary evidence obtained in these studies indicates that other metabolites containing the labeled carboxyl group do not enter the brain.

The liver took up large amounts of the drug and retained more on a per gram basis than any other organ throughout the experimental period. 9.8% of the injected dose was present in the liver after one-half hour while 1.8% was present by 8 hours. At the end of

a week 0.9% of the injected activity was still detectable in the liver.

The skin sample was taken from the back of the neck to eliminate the possibility of contamination from the injection site. Similar results to those reported were obtained with mice receiving intraperitoneal injections of the drug. The results obtained with skin samples were less consistent during the longer experimental periods than they were during the first 48 hours. Only one mouse in four showed more than trace activity in the skin at 4 days, while two out of four showed more than trace activity at one week after administration of the drug.

Shortly after injection such highly vascular organs as the spleen and the adrenal glands contained practically equal amounts of activity as the blood on a gram-ml basis; however, tissues such as striated muscle, heart, and abdominal fat were usually considerably lower. The activity detectable in the lymph nodes during the first few hours after injection was consistently lower than that of any other tissue. Muscle taken from near the injection site was equal in activity to other muscle tissue by 4 hours.

The data on the feces are reported on a per gram basis of the formed feces found in the lower bowel. The highest concentration of activity was noted during the 4 to 8 hour period. Direct plates of bile aspirated from the gall bladder show the highest activity during the 1- to 4-hour period. Excretion studies revealed that from 2-8% of the injected dose is excreted through the gastro-intestinal tract during the first 24 hours while from 1-3% is excreted during the following 48 hours.

The high activity detected in the kidneys during the first hour reflects the early excretion of the drug by this organ. Trace amounts of activity were detectable in the kidney throughout the experimental period. It was found that from 75-90% of the injected activity was excreted in the urine during the first 24 hours. Radioautographs made of chromatograms of the urine revealed the presence of unchanged drug plus 5 other metabolites. The distribution of activity on the chromatograms indicated that approximately 26% of the radioactivity in the urine results from unchanged drug while 55% is due to isonicotinic acid.

TABLE II. CPM per 100 ml Exhaled CO_2.

1–8 hr	8–16 hr	16–24 hr
25.6	17.9	14.3

When the exhaled CO_2 was precipitated as $BaCO_3$ and plated directly(6), no significant count could be detected with the windowless gas-flow counters. However, when larger volumes of CO_2 were counted in the ionization chamber, the count shown in Table II was obtained. If one considers the average CO_2 exhalation of a 20 g mouse as 0.05 mole per 24 hours(7), then less than 1% of the injected radioactivity was expired through the lungs during this period.

Discussion. The distribution and excretion studies indicate that labeled isonicotinic acid hydrazide is rapidly absorbed following subcutaneous injection, transported via the blood stream and excreted chiefly through the kidneys.

In view of the therapeutic use of isonicotinic acid hydrazide, it is highly significant that the lung always contains more activity per gram of tissue than any but the kidney, liver and skin. Since this drug has been found to be bacteriostatic *in vitro* against *M. tuberculosis* H37Rv at a concentration of 0.015 μg/ml(8), it is also noteworthy that the lung retains activity approximately equivalent to this amount of drug per gram of tissue for as long as a week after injection. The observation that the lung contains significant amounts of activity even with low blood levels during the period from 8 to 24 hours bears further investigation.

The apparent stimulation of the central nervous system by this drug(9) could be associated with the presence in the brain itself of the unchanged drug or its major metabolite. A more precise localization of the activity in the brain is now being studied both by dissection and by radioautograph of brain slices.

The localization of the drug in the skin presents a problem for further study in other species. In view of the relative size of the

skin as a body organ, the results given would suggest that the skin serves as a major storage depot for the drug. Whether the drug is retained here by combination with some fatty element or by the relatively high water content of the skin is at present unknown.

The gradual increase in the activity noted in the formed feces in the bowel, with the peak concentration at about four hours, results from both the increased output of activity through the bile during the previous two hours and the constipating effect of the drug. The mice generally held back both urine and feces during the first 4 hours following subcutaneous injection of the drug.

Summary. 1. Tissue distribution studies of labeled isonicotinic acid hydrazide in mice indicate that the drug is localized chiefly in the liver, skin, lung, brain and kidneys. 2. The lung contains significant amounts of activity even after the blood level has fallen to trace amounts. 3. Both isonicotinic acid hydrazide and its major metabolite isonicotinic acid are found in brain tissue. 4. The skin may serve as a major storage depot for the drug. 5. The urinary system is the major pathway for the excretion of the drug. 6. Less than 1% of the injected drug is converted to carbon dioxide.

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