of the various substances is illustrated further by the fact that mercaptosuccinate is not protective, in contrast to cysteine, cysteinamine, glutathione and BAL(4). In perhaps the the same sense, it may be recalled that cystinamine, $(S\text{–}CH_2\text{–}CH_2\text{–}NH_2)_2$, is an effective protective agent under certain circumstances but that cystine is inactive. The effect of cystinamine, unlike cysteinamine or cysteine, is restricted to the intact animal. This suggests that an *in vivo* interconversion to an active form is necessary. Cystinamine, for example, does not modify the radiosensitivity of pea roots(7) or reticulocytes of dog's blood *in vitro*(8). Protection of the whole animal may, perhaps, be attributed to its reduction to cysteinamine(6) or to the rapid formation of histamine(7). Large doses of histamine and other amines have been shown to enhance the survival of irradiated mice perhaps because of altered tissue oxygen tension resulting from vascular effects(5,7). Cysteinamine, or the other hand, does not liberate histamine(7) and presumably exerts a more direct action as a consequence of its sulfhydryl group, an action comparable to that of cysteine. This is, in fact, suggested by the apparent additivity of the 2 compounds.

Summary. 1. The observation of a post-irradiation effect of cysteinamine in rats with the liver area shielded has not been verified. 2. Cysteinamine is about 5 times as effective as cysteine when given to mice intravenously in equimolar amounts prior to x irradiation. However, maximally tolerated doses of each give equivalent protection. 3. Cysteinamine and cysteine appear to be additive in their protective effect against acute radiation lethality. This is suggestive of a similar mechanism of action.

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Rapid Colorimetric Method for Determination of Isonicotinic Acid Hydrazide in Blood Plasma. (20759)

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(Introduced by B. J. Olson.)

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The observation that isonicotinic acid hydrazide (isoniazid*) and its isopropyl and glucose derivatives have activity against tuberculosis in experimentally infected laboratory animals and against human tuberculosis (1) has raised the need for methods of assay of these compounds in body fluids. Reports of 3 methods appeared simultaneously as follows: S. Rubin and his coworkers(2) described an oxidation procedure and assay of the resulting isonicotinic acid with cyanogen bromide and ammonia. The degree of sensitivity was not stated. B. Rubin and collaborators (3) employed a rapid extraction method, sensitive to 5 $\mu g \pm 10\%$ per ml of blood plasma, which involved estimation of the ultraviolet absorption of an isoamyl alcohol extract. Kelly and Poet(4) extracted hydrazide from alkalinized plasma or urine by the addition of ammonium sulfate and an isoamyl alcohol-

* See footnote 5, *Am. Rev. Tuberc.,* 1952, v65, 754.

ether mixture, and after acidification, determined the ultraviolet absorption of the extract or the color with p-dimethylaminobenzaldehyde. The latter was reported to be sensitive to one μg $\pm$ 5% per ml of plasma.

The present report describes a procedure for colorimetric determination of isoniazid in blood plasma. The reaction results from condensation of isoniazid with glutaconic aldehyde formed by alkaline hydrolysis of 4-pyridylpyridinium dichloride. The intensity of the yellow color developed varies logarithmically with the concentration of isoniazid. This reagent has been described by Feigl(5) as useful in detecting small amounts of primary aromatic amines, but to our knowledge has not been used for measuring hydrazine and hydrazides.

Method. The determination is carried out as follows: Two ml of isoniazid solution containing 3% (w/v) aqueous trichloracetic acid are pipetted into a 5 ml volumetric flask. Five-tenths ml of 4-pyridylpyridinium dichloride reagent (1% aqueous solution of recrystallized Eastman product) is added, followed by 0.7 ml 2 normal sodium hydroxide, and 0.5 ml 2 normal hydrochloric acid. In the absence of trichloracetic acid, 0.5 ml of 2 normal sodium hydroxide is sufficient to liberate the glutaconic aldehyde. After adding distilled water up to volume and shaking, the mixture is held at room temperature for 15 minutes to assure maximum color development. The color is stable for at least an hour. The yellow solution is then transferred to a Fisher electrophotometer microcell and read against distilled water with the 425 mμ filter. A blank of the reagents and a standard solution containing 10 μg/ml of isoniazid are tested with each series of determinations.

Ten determinations were made of each of the concentrations such as might be found in body fluids, namely, 2, 4, 8, 12, 16, 20 μg. From the results in Table I, it is evident that the color intensity is proportional to the concentration of isoniazid, in accordance with Lambert-Beer's law, and that it is reproducible and sensitive. (2 μg per ml $\pm$ 3.2% in duplicate determinations).

Recovery from plasma. For *in vitro* recovery tests, varying amounts of isoniazid were added to normal guinea pig plasma containing ACD† as anticoagulant and to normal horse serum. Two ml of plasma or serum, diluted with 6 ml of distilled water, were mixed with 2 ml of 15% trichloracetic acid and held at room temperature for 15 minutes. After filtration, 2 ml portions of filtrate were measured into 5 ml flasks and the above procedure followed. Recovery of 10 μg of isoniazid added to one ml of guinea pig plasma was found to vary from 9.6 μg to 10.0 μg, 96 to 100%, and with one ml of normal horse serum averaged 9.5 μg or 95%.

TABLE I. Standardization and Reproducibility of Color Reaction of Isoniazid and Glutaconic Aldehyde from 4-Pyridylpyridinium Dichloride.

Isoniazid, μg	Optical density* Range of 10 determinations	Avg of 10 determinations	Sample avg minus blank avg
0	13.1-13.4	13.2	0
2	15.1-15.4	15.24	2.04
4	17.1-17.4	17.25	4.05
8	21.1-21.3	21.19	7.99
12	25.0-25.3	25.18	11.98
16	29.1-29.3	29.21	16.01
20	32.9-33.3	33.26	20.06

* Figures represent 100 $\times$ E/425 mμ, measured in Fisher electrophotometer 3 ml microcell.

Determination of isoniazid in blood plasma of animals under treatment. Each of 120 DBA mice, weighing 20 to 25 g, was given an oral dose of 5 mg of isoniazid. Then in groups of 15 the animals were bled ½, 1, 1½, 2, 3, 4, 7, and 18 hours after the drug administration. Using ACD as anticoagulant, the bloods were pooled from each group of 15 mice and the plasma separated. Blood plasma from 15 untreated mice served as control. Duplicate portions of the trichloracetic acid filtrates from the plasma were assayed for isoniazid. The readings were corrected for the slight amount of chromogen found in the untreated mouse plasma. The averages of results from 2 series of experiments were plotted against corresponding times as shown in Chart 1. The curve reveals that the maximum plasma peak of 6.5 mg per 100 ml was reached in ½ hour, with a sharp decline in 4 hours, and a negligible amount or no drug at all in the 18-hour

† ACD contains 8 g citric acid, 22 g sodium citrate, 24 g dextrose in 1000 ml water; 0.5 ml solution is added to 5 ml blood.

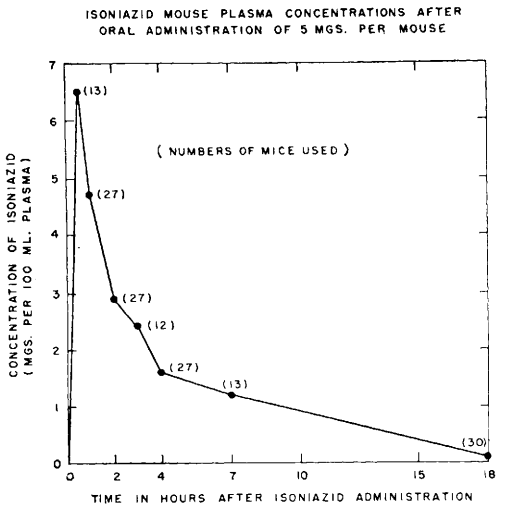

CHART 1

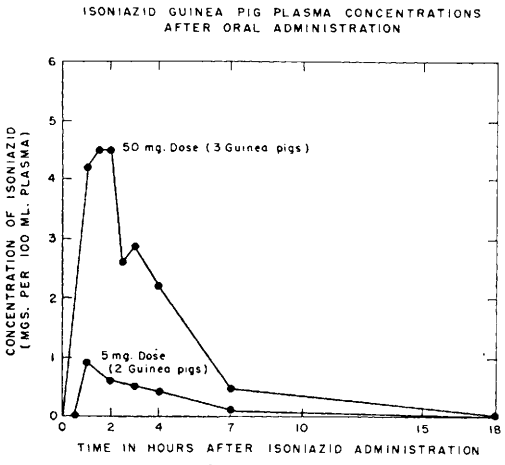

CHART 2

sample. Determinations of one-hour plasma levels were repeated in 2 additional experiments with results of 5.1 and 4.7 mg per 100 ml. These results approximate the average figure of 4.4 mg per 100 ml reported by Rubin (3) as present one-half hour after administration of the drug in mice.

Guinea pig plasma levels were determined in the same manner on the pooled plasma from 2 animals, each of which had received a 5 mg oral dose of isoniazid, and also on plasma from 3 animals after a 50 mg oral dose. The results are plotted in the curves in Chart 2. As expected, higher levels were obtained after the 50 mg dose than were found after the 5 mg dose. In both instances the maximum concentration was reached within 2 hours after administration of the oral dose of isoniazid.

Discussion. Although other concentrations of reagent, and of acid, alkali, and protein precipitant have been tested, the optimum concentrations found for reproducibility in this test differ but slightly from those reported by Feigl for his aromatic amine spot test. Hence plasma levels of isoniazid may be increased by the presence of such aromatic amines as the sulfonamide drugs or p-aminosalicylic acid. Since normal animal plasma have shown small amounts of other chromogens, a control determination on the plasma before isoniazid administration is essential.

Summary. The color reaction between isonicotinic acid hydrazide (isoniazid) and glutaconic aldehyde liberated from 4-pyridylpyridinium dichloride as described conforms to Lambert-Beer's law and is the basis for a sensitive and rapid method for determination of hydrazides in blood plasma. Blood plasma levels in mice and guinea pigs after oral administration of respectively 5 and 50 mg of isoniazid have been found to reach a maximum within 30 minutes to 2 hours and to fall to low levels within 18 hours.

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