TABLE III. Specific Activities at 24 Hr.

	% Ca^{45}/g Ca	% Ra^{226}/g Ca	Ra^{226}/Ca^{45}
Blood plasma	18	9.8	.55
Skeleton	2.0	1.9	.96
Teeth	.84	.41	.49

calcium and radium has been studied in a young beagle dog. Ra^{226} left the blood more rapidly than Ca^{45}; the preferential excretion of radium by both kidney and gut is a factor in explaining the blood data, and also is the principal gross metabolic difference. The retention of both alkaline earths was greater than 90%, consistent with the fact that the skeleton was rapidly growing. Analysis of individual bones revealed no significant difference in the relative deposition of the two isotopes, but the teeth showed a preferential incorporation of calcium.

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Effect of Glycerine on Toxicity of Isoniazid in Mice. (22918)

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Isoniazid (INH) continues in widespread use as a therapeutic agent against human tuberculosis; but dosage is limited by its toxicity. This toxicity has been reduced in laboratory animals by simultaneous administration of appropriate amounts of sodium glucuronate and glycine with the INH(1). Some other chemicals, certain amino and alpha-keto acids, have also been found to be effective in increasing the tolerated dose of INH (2). In addition several organic solvents have now been investigated. One of the most promising is glycerine which has long been recognized as an effective bactericidal agent. As early as 1869 Mueller(3) prepared cowpox vaccine in approximately 75% glycerine solution, and found its activity to be unimpaired whereas extraneous microorganisms were destroyed. A thorough investigation of the antiseptic and germicidal properties of glycerine was reported by Rosenau(4), who concluded that, while 10% by volume favored growth, 50% glycerine inhibited all bacteria studied—some 10 pathogens and a few saprophytes. Locatelli and Bowden(5) and Wood (6) found that sulfa drugs were more effective for topical and surgical application respectively when dispersed in glycerine. Fisher(7) reported that the tuberculostatic activity of streptomycin was enhanced in Youman's medium containing 2% glycerine. Montestruc and Garcin(8) reported successful use of glycerine-suspended BCG to detect allergy in BCG-vaccinated individuals. From these studies it may be deduced that glycerine in suitable concentrations is useful as an adjuvant. Furthermore its low toxicity for human beings was observed by Johnson, Carlson, and Johnson(9) in a group of individuals given 110 g daily for 50 days with no adverse manifestations. However, to our knowledge evidence of its capacity to reduce the toxic action of chemotherapeutic agents in animals has not been presented.

The present study is an investigation of the effect of glycerine on the toxic action of INH in mice. The experiments include: (a) acute toxicity of glycerine solutions in DBA mice, (b) effect of glycerine on acute toxicity of INH in mice, (c) effect of repeated administration of various dosage combinations of INH and glycerine, (d) blood levels in mice following combined administrations, and (e) effect of glycerine on bacteriostatic activity

TABLE I. Acute Toxicity of Isoniazid in Glycerine Solutions.

Isoniazid, mg	Glycerine, % by vol	DBA mice No.	Survivors No.	Survivors %
0	25	20	20	100
0	35	30	29	97
0	50	38	34	89
0	75	27	21	77
	undil.	30	17	57
4	0	20	20	100
4	25	20	19	95
4	35	20	19	95
4	50	20	19	95
4	75	20	15	75
6	0	20	0	0
6	15	20	9	45
6	20	20	13	65
6	25	20	18	90
6	35	118	113	95
6	50	19	13	68
6	75	20	14	70
7	25	20	10	50
7	35	50	36	72
7	50	69	46	67
7	75	20	11	52
8	0	17	0	0
8	25	20	8	40
8	35	74	28	37
8	40	20	15	75
8	50	146	126	86
8	75	50	28	56
10	25	20	0	0
10	35	60	10	17
10	50	29	10	34
10	75	114	55	48
10	undil.	20	0	0
15	50	20	1	5
15	75	40	6	15
20	50	20	0	0
20	75	21	0	0

in vitro of INH on *Mycobacterium tuberculosis var. hominis.*

Materials. 1. *Isoniazid powder*—isonicotinyl hydrazide, obtained from Hoffmann-La Roche, Inc. The INH was dissolved in aqueous glycerine solution such that the desired concentration of each per 20-g mouse was contained in 0.5 ml volume for oral administration. 2. *Glycerine,* 95% reagent grade, Sp. gr. = 1.249. Two commercial products were used. Aqueous solutions of glycerine were prepared to give the desired concentration by volume; *e.g.,* "25% solution" indicates 0.312 g/ml of pure glycerol. 3. *DBA inbred strain of mice* maintained at National Institutes of Health.

Results. Acute Toxicity of Glycerine and INH-Glycerine Mixtures in Mice. A wide range of concentrations of INH in a series of glycerine solutions was tested by gavage in DBA mice. Table I shows the results of the control series without INH, and of combinations of 4 to 20 mg INH per 20-g mouse (200-1000 mg/kg), each tested with 2 or more concentrations of glycerine. It is evident that in the control significant numbers of mice succumbed when more than 50% glycerine was administered. Similarly with a sublethal dose of INH, 4 mg, the number of surviving animals closely followed the control series, even when a concentration of 75% glycerine was used. However, with a lethal dose of 6 mg INH, 35% glycerine permitted 95% of the animals to survive. This survival rate was confirmed in 6 replicate experiments with a total of 118 mice. The group given 8 mg INH demonstrated the endpoint, inasmuch as 50% glycerine concentration was needed to counteract the INH toxicity and this amount of glycerine *per se* was slightly toxic (11% mortality). Above this dose the toxicities of both the chemotherapeutic agent and the solvent were involved. This was evident in that with 10 mg INH in 75% glycerine only 48% of the mice survived. It would thus appear that in DBA mice a single dose of 6 mg INH in 35% glycerine, or as much as 8 mg INH in 50% glycerine was tolerated almost as well as the sublethal 4 mg dose of INH in water.

Effect of repeated administrations of INH in glycerine solutions. Two groups of experiments were then carried out to ascertain the mortality following repeated oral administrations of these optimal combinations of INH with glycerine. A. *Daily administration.* As seen in Table II, daily doses (except Sundays) of sublethal 4 mg amount of INH in glycerine and of mixtures containing 6, 8, and 10 mg INH in glycerine resulted in extended survival time. With the 10 mg dose evidently several resistant animals were included inasmuch as 83% of the 30 survived one dose, in contrast to the 48% survival rate shown in Table I. It was unexpected that the sublethal 4 mg dose in 35% glycerine

TABLE II. Effect of Repeated Oral Administrations of Isoniazid with Glycerine.

Isoniazid, mg	Glycerine, % by vol	No. of mice	No. of doses 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
			Daily administration (6 doses weekly)																								
4	0	19	18	18	18	18	17	16	16	16	16	15	15	15	14	13	13	13	13	11	9	8	7	6	6	6	6*
4	35	18	15	14	14	14	14	14	14	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13†
6	35	19	18	12	11	9	8	6	5	5	5	5	5	5	5	4	2	2									
8	50	20	16	16	13	12	6	5	2	—																	
10	75	30	25	18	15	10	9	8	7	5	4	—															
			Twice weekly administrations																								
0	35	19	18	18	18	18	18	16	14	12	11	10	10	10	10	10	10	—									
0	50	14	14	14	14	13	11	8	8	8	8	8	8	8	8	7	7	—									
6	35	9	9	9	9	9	8	8	7	4	3	3	3	3	3	3	2	—									
8	50	22	20	20	17	16	15	14	10	8	7	6	6	6	6	6	6	—									

* Survivors received 5 additional doses, with one death after the 28th and one after the 30th dose.
† " " " ; all survived.

was tolerated better than the corresponding aqueous solution; for 13 mice survived 30 doses, in contrast to only 4 mice of the latter group. In addition, these 13 surviving mice were much quieter than the control animals which became "jumpy" after 13 doses. B. *Two administrations weekly.* Employing the procedure of intermittent administration used in earlier studies(1), the other group of experiments demonstrated survival time when the same concentrations of INH and glycerine were given the mice twice weekly (Mondays and Thursdays). The 50% endpoint of survival was obtained with approximately double the number of administrations employed in the daily series. Here again it is to be noted that a content of 50% glycerine used with 8 mg INH was itself sufficiently toxic to influence the survival time of the mice. From these 2 series of experiments on repeated administrations, it is evident that the presence of glycerine in appropriate amounts appreciably increased the tolerated dose of INH in DBA mice. Optimal results were obtained with the sublethal dose of INH (4 mg/20-g mouse) in a 35% glycerine solution.

Blood levels of INH after administration in glycerine solutions. The INH concentration in blood plasma of DBA mice was measured at various time intervals—1, 2, 4, 7, and 24 hours—after a single administration of respectively: 4 mg in water, 4 mg in 35% glycerine, 6 mg in 35% glycerine, and 8 mg in 50% glycerine. Heart's blood, from groups of 10 mice, at each time for each dose, was drawn into tubes containing ACD* as anticoagulant. The separated plasma was assayed for INH concentration by the "pyridyl" method(10). Fig. 1 shows the resultant absorption curves. As might be expected the higher levels were obtained following 6 and 8 mg doses. With the 4 mg dose not only did the glycerine not interfere with absorption, but actually it enhanced the level throughout the curve in comparison to the control. This observation is of special interest in the

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

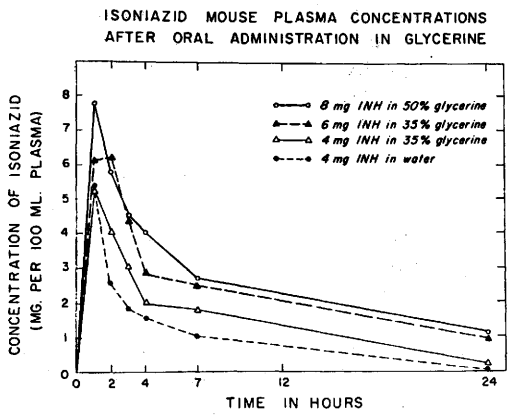

FIG. 1.

light of the promising results of the previous experiment on prolonged administration with this INH-glycerine combination. In addition, assay was made of the INH concentration in blood plasma from the mice sacrificed 2 hours and 24 hours after the 30th daily dose in the experiment described above. At the 2-hour interval, 3.1 mg% following administration of the aqueous solution is to be contrasted with the 4.3 mg% level following the glycerine solution. In the 24-hour bleeding of the latter group the INH persisted at 0.44 mg%. This is roughly comparable to the 24-hour level obtained when other INH detoxicants were tested(1) and somewhat higher than the 0.25 mg% of the control.

Effect of glycerine on in vitro inhibitory action of isoniazid on Mycobacterium tuberculosis. One procedure has been carried out to ascertain the effect of glycerine on the *in vitro* inhibitory action of INH on *Mycobacterium tuberculosis.* Following the technic described earlier(1), 2 human strains of tubercle bacilli have been used to determine INH inhibition endpoints with and without glycerine as solvent for the INH. The endpoints with INH alone were respectively: for strain H37Rv, 40 μg/ml; and for strain 198 ARB, 5 μg/ml. A 3% solution of glycerine as solvent did not change the INH inhibition endpoint with strain B103 (avirulent acid-fast). While there was questionable growth of this strain in control tubes containing 17.5% glycerine without INH, this glycerine concentration gave an endpoint for INH at 2.5 μg as contrasted with 10 μg in the absence of any glycerine. But in the presence of 25% glycerine no growth of any of these strains developed with or without INH. In other words, there apparently is bacteriostatic synergy of the INH and glycerine at the higher concentrations.

Discussion. As is well known, glycerine in lower concentrations is a nutrient for bacteria. Pertinent to the present study, tubercle bacilli have been successfully grown on media containing from 2 to 12% glycerine(11). Long and Finner's studies 1927(12) showed that 7.5% to 10% glycerol gave maximum yield, as measured by dry weight of tubercle bacilli, with no growth at 15% concentration of glycerol. The *in vitro* results reported above also show that it is in the higher concentrations (25% and higher) that bacteriostatic effect is noted.

The long term *in vivo* experiment with 4 mg INH in 35% glycerine solution gave two unexpected results: first, that the animals were apparently in better physical condition than those receiving the aqueous solution of INH; and second, that they gained in weight normally in contrast to the retarded gain or even loss in the latter. It should be noted that some animals may have succumbed due to the mode of treatment, inasmuch as it has been observed that similar handling of normal mice to administer saline over the same prolonged period caused as much as 20 to 30% mortality.

While the effectiveness may be due in part to its ability to prevent the inhibition of certain enzymes by INH(13), it is possible that the action of the glycerine may be physical due to its hygroscopic characteristics and its viscosity, or some other mechanism as yet not explored.

Summary and conclusions. The toxic effect of isoniazid (INH) in DBA mice has been reduced by solution in several appropriate concentrations of glycerine. A sublethal dose of 4 mg INH (200 mg/kg) in 35% by volume of glycerine (10.9 g glycerol/kg) was administered orally 30 times within 36 days with a higher survival rate and fewer toxic manifestations than the same dose of INH

dissolved in water. Doses of 6 or 8 mg INH, lethal *per se* in aqueous solution, gave 50% mortality after 7 intermittent (twice weekly) administrations when 35% or 50% glycerine solution respectively was employed as solvent. With larger amounts of INH the requisite amount of glycerine lay in the toxic range. The absorption curves indicate that significantly higher blood plasma levels of INH were obtained with the glycerine solutions than with aqueous solutions. In higher concentrations of glycerine there was apparently synergism with INH in the *in vitro* bacteriostatic action on 2 human strains of *Mycobacterium tuberculosis.*

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Formation of Porphyrin from Delta-Aminolevulenic Acid by Uterine and Liver Tissue from Laying Hens.* (22919)

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A variety of avian tissues and organs are capable of forming porphyrins, *in vitro,* from delta-aminolevulenic acid (ALA): namely, chicken erythrocytes(1,2), duck erythrocytes (2,3), pigeon liver and breast muscle(4). The uterus of the hen has not been similarly characterized, and would be a likely organ to form porphyrin from ALA, *in vitro,* as oöporphyrin is deposited on the egg shell within this organ.

Methods and materials. White Leghorns, which lay white eggs, and Crossbreeds and New Hampshires, which lay brown eggs of varying shade, were used in these experiments. At a time when no egg was in the uterus, birds were killed by decapitation and uterus, portions of liver and of breast muscle were removed immediately and placed on crushed ice. In a cold room (6°C), 1 g tissue was homogenized in a mortar containing coarse sand and 4 successive 5 ml aliquots of buffered saline solution, pH 7.2(5). After each grinding supernatant solution was decanted into a 25 ml graduate, and finally contents of the mortar were rinsed into the graduate with buffered-saline and diluted to 25 ml. The graduate was stoppered, the contents mixed and allowed to stand in crushed ice for 5-10 minutes. Aliquots of 0.2, 0.4, 0.8 and 1.6 ml (equivalent to 8, 16, 32 and 64 mg of tissue) from the upper half of the graduate were pipetted into tubes containing 1.4 ml of 0.008 M ALA in buffered-saline. One tube containing 1.6 ml of solution but no ALA, and another tube without the solution but with ALA were used as controls. All tubes were made up to 4.4 ml with buffered-saline, capped and incubated at 41°C for 24 hours. Each series of tubes was run in duplicate.

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