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Increase in Tolerated Dose of Isoniazid in Mice by Use of Cycloserine. (22865)

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Isoniazid continues to be in widespread use in the therapy of human tuberculosis. The toxicity of the drug is a limiting factor in dosage. In animal experiments with this compound, it has been found possible to increase the tolerated dose by the use of suitable detoxifying agents(1). Such agents were also employed to investigate the possible detoxication of the new antibiotic cycloserine,* D-4-amino-3-isoxazolidone, of current interest in the treatment of human tuberculosis(2-4). Unexpectedly, it was observed that simultaneous administration of isoniazid (INH) and cycloserine (CS) resulted in a slight reduction of the hyperactivity induced in mice by the CS, and marked reduction in the percentage mortality in mice given larger, otherwise lethal, doses of INH. This latter observation prompted the present detoxication study with these two chemicals.

Methods. Control toxicity tests of each chemical by gavage in DBA mice showed that (a) INH permitted 45% survival with a 5 mg dose and no survival with 6 mg (250 and 300 mg/kg respectively)(1); and (b) CS was tolerated by 6 of 7 mice each given 250 mg, but was 100% lethal with 300 mg (12.5 and 15 g/kg respectively). CS in amounts of 25 mg or more caused hyperactivity evidenced by continuous circling in the jar beginning about 30 minutes after administration of the chemical.

Results. Tolerance limits after a single dose of mixed chemicals. The effect of a single oral dose of mixtures of various concentrations of CS and INH on survival time of

* Kindly supplied as seromycin by Eli Lilly and Co., Indianapolis, Ind.

TABLE I. Effects of a Single Administration of Mixtures of Isoniazid and Cycloserine at Designated Concentrations in DBA Mice.

Isoniazid, mg	Cycloserine, mg	No. of mice	Survival of mice No.	Survival of mice %
0	100	25	25	100
0	250	7	6	86
0	300	4	0	0
4	0	10	10	100
5	0	49	22	45
6	0	10	0	0
8	25	18	8	44
8	50	18	13	72
8	75	15	15	100
10	50	14	11	79
10	75	16	13	81
10	100	22	22	100
15	100	11	7	63
15	200	11	4	36

DBA mice is reported in Table I. The 2 chemicals in appropriate amounts were mixed in a test tube and dissolved in water to such volume that the desired dose of each was contained in 0.5 ml volume. A single administration of 8 mg INH plus 75 mg CS, or of 10 mg INH plus 100 mg CS, per 20-gram mouse permitted 100% survival. As much as 15 mg INH with 100 mg CS were tolerated by 7 of 11 mice (63%), but with larger amounts of CS the mixtures were toxic.

Survival time after repeated doses of mixed chemicals. Repeated administration of a mixture of 10 mg INH with 100 mg CS was attempted on successive days; however, after the second dose, 5 of 6 mice succumbed. These same concentrations were then administered twice weekly (Mondays and Thursdays) for 10 weeks. All of the 6 mice tolerated 14 doses and 5 withstood 20 doses with no loss in weight but a normal gain over the period. This observation was confirmed in 2 similar experiments. As seen in Table II, the composite of the 3 experiments yielded a 73% survival after 20 doses. In one control experiment, 6 animals given CS only tolerated 17 doses; one died after the 18th dose, and all appeared "sick" after the 19th and 20th doses when the experiment was terminated. A second control group of 10 mice reached a 50% endpoint with 11 doses; the survivors, given 2 more doses, were observed

TABLE II. Survival Time after Repeated Doses of Mixed Chemicals, Isoniazid and Cycloserine, in DBA Mice. Administration twice weekly for 10 weeks.

Isoniazid, mg	Cycloserine, mg	No. of mice	No. of mice surviving following No. of doses 1	7	13	20
0	100	6	6	6	6	5
0	100	10	10	8	5*	
10	100	26†	23	23	22	19

* Discontinued after 13 doses; subsequent deaths occurred on 7th, 9th, 14th, and 21st day.
† Composite of 3 exp.

for 21 days. Four of the 5 mice died in that interval. It is significant that the hyperactivity due to CS, although not as marked as with this drug alone, was noticeable after each administration of the mixed drugs. Thus it would appear that CS permitted the mice to tolerate almost double the amount of INH which was lethal *per se*.

Blood plasma concentrations. At the end of the above long-term experiment, studies were made of blood plasma concentrations of the 2 chemicals. INH content was measured by the "pyridyl" method(5), and CS by the Jones method(6). It had been previously determined that there was no interference in these 2 methods. The plasma 72 hours after the 20th administration contained 0.38 mg % of INH and no detectable amount of CS. The CS control blood plasma level, 24 hours after the 20th dose, was 0.68 mg %. This figure is to be contrasted with the 24-hour level following a single injection: for from an absorption curve with this compound, it was found that 100 mg CS alone gave the following levels: at 2 hours, 11.2 mg %; at 4 hours, 8.7 mg %; at 7 hours, 5.0 mg %; and at 24 hours 0.1 mg %. Thus apparently repeated doses of CS alone resulted in a slight cumulative effect which might account for the "sick" appearance of the animals in the last week of the 70-day experiment. This was not observed in the mice given the mixture of INH with CS.

Effect on in vitro *Inhibition Tests.* One method has been employed for testing whether these proportionate amounts of CS and INH have any effect on the *in vitro* inhibitory activity of either compound against *Mycobacterium tuberculosis*. Following the

procedure described earlier(1), it was found in replicate experiments that as much as 200 μg per ml of CS alone had no inhibitory action on the growth of strain B103; INH alone inhibited in a dose of 5 to 10 μg per ml; and with the mixture, concentrations in the proportion of 100 μg CS and 10 μg INH per ml gave an inhibition endpoint the same as with INH alone. Similar experiments with 24-day growth of 3 other strains, 198ARB, H37Rv, and BCG, although demonstrating variations in response to CS alone (inhibition with 200, 20, 10 μg, respectively) were similar in their inhibition results with INH (5 to 10 μg) and with the mixture (10 μg INH). One may conclude from these *in vitro* tests that at least the presence of CS under the conditions used did not interfere with the inhibitory action of INH.

Summary. The acute toxicity of isoniazid (INH) in DBA mice by gavage, $LD_{50} = 5$ mg/20 g mouse, may be reduced by simultaneous administration of the antibiotic cycloserine (CS). Each of 22 mice survived a single dose of 8 mg INH with 75 mg CS (0.4 g and 3.75 g/kg respectively), or of 10 mg INH with 100 mg CS (0.5 g and 5.0 g/kg respectively). On repeated semi-weekly administration for 10 weeks, 19 of 26 mice, 73%, survived the latter mixture. Significant amounts of INH were detected in the blood plasma 72 hours after the 20th dose. In *in vitro* bacteriostatic tests with 4 strains of *Mycobacterium tuberculosis*, CS did not interfere with the activity of the INH.

Since submission of this manuscript for publication, a statement in a technical bulletin indicated that in clinical use "toxicity (of CS) is decreased and its efficacy increased" when administered with INH. (*Phys. Bull.*, 1956, v21, 227).

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Localization of Lactic Acid Dehydrogenase Activity in Serum Fractions. (22866)

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The enzyme lactic acid dehydrogenase (LDH) is known to catalyze the conversion of pyruvic acid to lactic acid in the presence of reduced diphosphopyridine nucleotide (DPNH)(1,2). More recently Vallee has shown that LDH is a zinc protein(3). Increased interest in the level of LDH in various disease states has followed reports that in patients with myocardial infarction and leukemia the serum level of LDH is increased significantly(4,5,6,7).

The main purpose of this paper is to describe 3 separate peaks of LDH activity in the electrophoretically separated fractions of the serum proteins.

Methods and materials. Twelve subjects, 3 with leukemia, 2 with myocardial infarction, 2 normal individuals and 5 patients with such various diseases as cirrhosis of liver, hemochromatosis and Wilson's disease, were employed. Three to 5 ml of fresh, non-hemolyzed serum were separated by zone electrophoresis using a starch or polyvinyl supporting medium in barbital buffer pH 8.6 ($\pi/2$