Summary and conclusions. 1. Immature female rats were treated with 1.0 mg of cortisone acetate daily for 2 or 4 weeks starting at 23 days of age and were maintained on pair or *ad libitum* feeding regimes. 2. Body weight, adrenal and thymus weights were reduced compared to control animals. These differences were less marked when animals were autopsied 3 weeks after cessation of treatment. 3. The weights of ovary and uterus were generally greater in the treated animals. The increased weight of the ovaries was due to a greater number of follicles and to an increased size of the antra. 4. Pituitary glands removed from animals treated with cortisone appeared to have a greater gonadotrophic potency than normal as indicated by the growth of the ovaries in immature rats injected with these glands. 5. Possible explanations for these changes are discussed.

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A Means of Increasing the Tolerated Dose of Isoniazid in Mice. (21201)

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(Introduced by B. J. Olson.)

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Isoniazid (INH) as a therapeutic agent in human tuberculosis is in widespread use; one limiting factor is its toxicity(1-5). Two well-known detoxifying agents, glycine and sodium glucuronate, administered simultaneously with the INH in mice, have shown a marked effect in increasing toleration to this therapeutic drug. Three types of experiments are reported here: 1) Survival of DBA mice (20-g weight) after single and repeated oral administrations of toxic amounts of INH with different quantities of the 2 chemicals; 2) blood plasma levels of INH after a single oral administration of the 3 chemicals; and 3) an *in vitro* test of the effect of the detoxifying chemicals on the bacteriostatic action of INH on tubercle bacilli.

Inasmuch as the toxicity data were available for white mice only, a preliminary determination of the toxic dose of INH in DBA mice was necessary. According to Grunberg and Schnitzer(6) the LD_{50} by gavage in white mice was found to be 203 mg/kg. In our laboratory this amount in white mice resulted in 57% survival, but in the DBA strain all the mice survived. However, an oral dose of 250 mg/kg (5 mg/20-g mouse) resulted in only 45% survival in a group of 49 DBA mice. In contrast, when 50 mg of sodium glucuron-

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TABLE I. Mouse Survival following Oral Administration of 8 mg Isoniazid with Sodium Glucuronate and Glycine. Single dose.

Sodium glucuronate, mg	Glycine, mg	Mouse survival (DBA strain) No. survivors / No. treated	% survival
0	0	2/70	3%
5	0	0/20	0
10	0	0/20	0
25	0	1/10	10
50	0	2/20	10
75	0	10/20	50
0	5	0/20	0
0	10	1/20	5
0	25	5/10	50
0	50	13/20	65
0	75	13/20	65
5	5	1/15	7
5	25	6/10	60
10	10	3/15	20
10	25	10/10	100
20	25	10/10	100
25	5	3/10	30
25	10	3/10	30
25	20	8/10	80
25	25	63/65	97
50	50	9/10	90
75	75	20/20	100

ate monohydrate† were added to the 5 mg of INH per mouse the survival of the 8 mice tested was 100%.

Mortality after a single administration of chemicals. In view of this preliminary observation with sodium glucuronate, a larger dose of INH, 8 mg (400 mg/kg), which alone had proved to be 100% lethal within 2 hours, was tested in combination with several concentrations of sodium glucuronate. At the same time, the effect of various amounts of glycine with and without the glucuronate was studied. The chemicals were weighed, combined in one tube, and dissolved in water to such a volume that the desired concentration of each was contained in 0.5 ml for oral administration. At least 10 DBA mice were used for each test solution. The data recorded in Table I include the results of a series of tests with the 3 chemicals in various proportions. Here it is seen that, whereas with 8 mg of INH alone only 2 of the 70 mice survived, the addition of glucuronate or glycine increased the survival rate. The optimum results were found when both chemicals were used. For with 25 mg of each chemical 97% of a group of 65 mice tolerated the 8 mg toxic dose. The smallest amounts of these chemicals necessary for 100% survival of 10 mice with the 8-mg dose of INH were found to be 25 mg of glycine and 10 mg of sodium glucuronate. In other words, the use of equimolar amounts of INH and sodium glucuronate plus 5 or 6 moles of glycine will enable the mouse to withstand an amount well above the minimal lethal dose of INH alone (6 mg/20-g mouse).

Mortality after repeated administration of chemicals. Two experiments show the mouse survival time following repeated administrations of a lethal dose of INH mixed with excessive amounts of glycine and glucuronate. In the first experiment, each of 20 DBA mice was given a mixture of 8 mg INH with 75 mg of sodium glucuronate and 75 mg of glycine on 5 successive days. The first toxic fatality in these mice occurred after the third dose and first outward symptoms of drug accumulation were apparent when a few animals became hyperactive and 4 succumbed after the fourth treatment. After the fifth and last daily dose all were hyperactive and only 4 mice survived more than 2 hours. Increased tolerance was definite inasmuch as only 2 of the 70 control animals survived one dose of 8 mg INH alone, and 95% of the treated animals survived 3 daily doses of the mixed chemicals. In the second experiment, with 10 mice for each dose, INH in concentrations of 10, 15, 20, 25, 30, and 40 mg was combined with the detoxifying chemicals in the amounts shown in Table II, and administered orally at 72-hour intervals. The treatments were continued until approximately 50% mortality was reached. Under these conditions, the 10 mg dose of INH could be tolerated for 15 times during a period of 45 days, for, with 2 accidental losses, only one mouse of the 10 succumbed to the drug after the 14th treatment. With 15 mg in each treatment, a 50% endpoint occurred after 5 doses, and with 20 mg, after 2 doses. Indeed it is amazing that, even with 30 mg of INH mixed with these

† Kindly supplied by the Corn Products Refining Co., New York.

TABLE II. Mouse Survival following Oral Administration of Isoniazid with Sodium Glucuronate and Glycine. Repeated administration at 72-hr intervals.

Isoniazid, mg	Sodium glucuronate, mg	Glycine, mg	Survivors of 10 DBA mice after treatments														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10	50	50	10	10	10	10	10	9*	9	9	9	9	9	9	8*	7	7
15	50	50	10	10	7	6	6	4	2								
20	75	75	10	9	4												
25	100	100	10	6													
30	125	125	4														
40	150	150	0														

* One mouse missing for 6th treatment; one killed accidentally during 13th treatment.

detoxifying chemicals, 4 of the 10 mice survived one dose, and only when the single dose was as much as 40 mg—more than 6 times the usual lethal amount—did the mortality reach 100%.

Blood plasma levels. Blood plasma levels were studied to ascertain the absorption of larger doses of INH in the presence of these additional chemicals. Each of 45 DBA mice was given orally a solution containing in 0.5 ml 8 mg INH, 25 mg sodium glucuronate, and 25 mg glycine. In groups of 15 the mice were sacrificed at 24, 48, and 72 hours after drug administration and the hearts' bloods from each group pooled. Assay of INH in the blood plasma was carried out by the Rubin method(7) and the "pyridyl" method (Prescott, Kauffmann, James(8)). For controls, blood plasma levels were assayed in the pooled bloods of 10 mice 24 hours after each was given 4 mg of INH, the maximum amount which permits 100% survival of DBA mice for this length of time. It was found that the control (4 mg) plasma yielded 0.13 mg % INH. On the other hand, the plasma from the mice given the 8 mg INH mixture, after 24 hours yielded 0.88 mg %, a 6-fold increase over the control; after 48 hours 0.45 mg %, and after 72 hours 0.18 mg %, approximately like the control at 24 hours. In 2 other tests, whereas the control (4 mg) plasma levels were 5.5 and 5.2 mg % at one hour and negligible at 24 hours, after a 10 mg dose of INH with 50 mg each of glycine and glucuronate, the plasma levels were 6.4 and 6.2 mg % at one hour, and 0.7 and 0.8 mg % at 24 hours. Thus, at least in DBA mice, appreciably higher plasma levels were maintained for a longer time following large doses of INH in combination with sodium glucuronate and glycine than with the 4 mg dose of INH alone.

Effect on bacteriostatic action of INH. One procedure has been employed to determine whether the presence of either sodium glucuronate or glycine, or both, has any appreciable effect on the *in vitro* bacteriostatic action of INH on tubercle bacilli. One mg of INH alone or in combination with the chemicals, in the proportions effective *in vivo,* was dissolved in 0.5 ml volume, and added to 9.5 ml of horse meat infusion broth. Dilutions were made in broth. The tubes were inoculated with 0.2 ml of a 6-day growth of the human strain B103 of tubercle bacilli and incubated at 37°C for 6 days. Macroscopic readings of growth were recorded on the third and sixth days. The inhibition endpoint of INH alone or in mixture was 0.01 mg/ml. The control sodium glucuronate or glycine in the doses used had no effect on this culture. Hence it may be concluded that these 2 chemicals, effective as detoxifying agents *in vivo,* were without effect on the bacteriostatic action of INH with this strain of tubercle bacilli.

Discussion. The metabolic changes involved in this detoxication are not at present established. Presumably the toxic manifestations following the use of INH are largely due to an imbalance in normal metabolism, possibly a blocking or exhaustion of normal detoxifying agents(9-11). The introduction of supplementary quantities of the 2 chemicals, glycine and sodium glucuronate, both of which are normally available in the body, permits mice to survive an amount of INH some 5 times larger than the otherwise toxic dose. The fact that the results are better when both chemicals are used, than with

either alone, suggests that the INH may be split into toxic products which are separately conjugated with the respective chemicals either directly or by enzyme action.

The recent report of Ragno and others(12), suggests the explanation that the amino acid combines with ammonia liberated from the INH. These investigators have used glutamic acid as a detoxifying agent with INH in patients with tuberculous meningoencephalitis. With such combined therapy they have been able to use larger oral doses of INH without toxic disturbances, and have obtained higher concentrations of the drug in the spinal fluid.

Summary. Attempts have been made to find a means of increasing the amount of isoniazid (INH) which mice will tolerate. In the DBA strain of mice, INH alone was lethal for 45% of the animals when 5 mg/20 g mouse (250 mg/kg) were given orally. With an 8 mg dose of INH, lethal *per se,* simultaneous administration of as little as 25 mg glycine and 10 mg sodium glucuronate monohydrate was sufficient to keep alive all the animals tested. By the use of appropriate amounts of the 2 detoxifying chemicals, all mice survived either a single oral dose of 25 mg INH, or 3 doses of 8 mg daily, or 13 doses of 10 mg each given at 72-hour intervals. Larger amounts were not tolerated. A study of the blood plasma concentrations of INH showed that, whereas 24 hours after a 4-mg dose alone only a negligible amount remained, appreciably higher concentrations, approximately 6-fold, persisted for a longer period following 8 or 10 mg INH in combined treatment. In the proportions tested, sodium glucuronate and glycine had no effect on the bacteriostatic action of INH *in vitro* on one human strain of tubercle bacilli.

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Nitrogen Requirements of *Glaucoma scintillans* and *Colpidium campylum.** (21202)

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The ciliated protozoans, *Glaucoma scintillans* and *Colpidium campylum* were isolated and established in axenic (bacteria-free) culture in 1941(1). They were grown in a medium containing yeast cell fragments, and because growth failed when the particles were withheld it was concluded that they were phagotrophic. Later Peterson(2) was able to grow *Colpidium* in a non-particulate medium containing yeast protein fractions. We have found that *Glaucoma* also is dependent not on particles but on peptides and it is our analysis

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