Means of Increasing the Tolerated Dose of Streptomycin in Mice. Steroids. (28698)

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The widespread use of the chemotherapeutic agents streptomycin, isoniazid and kanamycin separately or when given concomitantly are somewhat limited in usefulness by their toxicity. The importance of a chemotherapeutic compound of low or no toxicity for clinical use is obvious. The influence of certain adjuvants such as amino acids(1,2), vitamins(3,4) and certain organic solvents (5) on the detoxification of the chemotherapeutic drugs streptomycin and/or isoniazid has been described. It was also observed that the tolerated dose of these toxic chemotherapeutic agents could be increased in animals without loss in chemotherapeutic activity, making possible the administration of the drugs in mice in much larger doses than was previously possible. The present report extends the study to include certain steroids, in particular, bile acids in appropriate amounts as possible detoxifying agents. The approach has been similar to that used in increasing the tolerated dose of streptomycin (3), namely simultaneous administration of the chemotherapeutic agent with the various steroids.

The steroids studied as adjuvants included: cholic acid, sodium taurocholate, sodium glycocholate, sodium choleate and sodium glycotaurocholate. White mice and DBA mice of the strains maintained at Nat. Inst. of Health were used. For toxicity tests, the appropriate amount of adjuvant was mixed in a test tube with an aqueous solution of the required amount of chemotherapeutic agent in such volume that the desired amounts of adjuvant and drug were contained in and administered subcutaneously at the rate of 0.25 ml per 20 g mouse. When necessary, the hydrogen ion concentration was adjusted to pH 7.

Results. The data recorded in Table I include the results of a series of tests of a single subcutaneous dose of a mixture of steroids and streptomycin at various dosage levels in both white and DBA mice. Here it

TABLE I. Increased Tolerance in Mice to Streptomycin with Certain Steroids by Single Subcutaneous Administration.

Streptomycin (mg)	Steroid	Dose (mg)	Mouse survival: White mice Ratio	White mice %	DBA mice Ratio	DBA mice %
—	Sodium taurocholate	50	20/20	100	20/20	100
20	—	—	0/20	0	0/20	0
20	Sodium taurocholate	25	40/40	100	20/20	100
20	"	10	20/20	100	20/20	100
20	"	5	17/20	85	14/20	70
20	"	2.5	14/20	70	24/40	60
40	"	25	36/49	73	—	
40	"	20	18/26	69	—	
40	"	10	16/30	53	—	
—	Sodium glycocholate	25	20/20	100	20/20	100
20	"	25	20/20	100	20/20	100
20	"	10	20/20	100	20/20	100
20	"	5	16/20	80	18/20	90
20	"	2.5	7/10	70	3/20	15
—	Sodium glyco-tauro*	25	20/20	100	20/20	100
20	"	25	20/20	100	20/20	100
20	"	10	20/20	100	20/20	100
20	"	5	16/20	80	18/20	90
20	"	2.5	7/20	35	1/20	5

* Sodium glyco-taurocholate.

TABLE I-A. Increased Tolerance in Mice to Streptomycin with Certain Steroids by Single Subcutaneous Administration.

Streptomycin (mg)	Steroid	Dose (mg)	Mouse survival: White mice Ratio	White mice %	DBA mice Ratio	DBA mice %
—	Cholic acid	50	20/20	100	20/20	100
20	"	50	20/20	100	36/40	90
20	"	25	17/20	85	20/20	100
20	"	10	16/20	80	20/20	100
20	"	5	11/20	55	18/20	90
20	"	2.5	1/20	5	13/20	65
40	"	50	14/20	70	20/20	100
40	"	25	17/20	85	17/20	85
40	"	20	—		24/40	60
40	"	15	—		15/40	38
40	"	10	6/20	30	7/20	35

TABLE II. Effect of Steroids on Streptomycin-Isoniazid Mixtures in Mice by Single Subcutaneous Administration.

Streptomycin (mg)	Isoniazid (mg)	Steroid	Dose (mg)	Mouse survival: White mice Ratio	White mice %	DBA mice Ratio	DBA mice %
10	4	—	—	0/20	0	0/20	0
10	4	Cholic acid	50	19/20	95	—	
10	6	" "	"	18/20	90	—	
10	8	" "	"	3/20	15	—	
10	2	Sodium choleate	10	20/22	90	30/30	100
10	4	" "	"	24/24	100	24/30	80
10	6	" "	"	19/20	95	23/30	77
10	8	" "	"	6/20	30	24/30	80
10	10	" "	"	3/20	15	16/30	53
15	2	—	—	0/30	0	0/70	0
15	2	Sodium taurocholate	25	40/40	100	40/40	100
15	4	" "	"	40/40	100	40/40	100
15	6	" "	"	39/40	98	40/40	100
15	8	" "	"	39/40	98	39/40	98
15	10	" "	"	10/40	25	33/40	83
15	2	" "	10	—		32/40	80
15	4	" "	"	—		18/40	45
15	6	" "	"	—		6/40	15

is seen that with a 20 mg (1 g/kg) dose of streptomycin which is 100% lethal for both strains of mice, the 4 steroids tested in a 25 mg dose or more permitted 100% survival in both strains of mice. Sodium taurocholate seems to be the most effective in increasing tolerance since as little as 2.5 mg permitted 70% survival in white mice and 60% survival in DBA mice given 20 mg streptomycin. With the other steroids tested, concomitant injection of this amount of sodium glycocholate or cholic acid permitted 70% of white mice and 15% of DBA mice, respectively, to survive a 20 mg dose of streptomycin. Increasing the streptomycin dose to 40 mg still demonstrated the effectiveness of sodium taurocholate and of cholic acid. With this dose, maximum survival was 70 to 85% in both white and DBA mice when 25 mg of adjuvant was used. As previously observed, there was a difference in responses of the 2 strains of mice to the streptomycin in combination with the adjuvants. Thus, in a single subcutaneous administration, each of the steroids tested permitted the animals to tolerate at least twice the lethal dose of streptomycin.

Effect of steroids on streptomycin-isoniazid mixtures. In earlier detoxification studies, it had been observed that when mixtures of non-toxic amounts of streptomycin and isoniazid were given simultaneously, they became lethal for two-thirds of the animals tested. In contrast, the steroids in appropriate amounts in solutions of streptomycin and isoniazid were effective in increasing tolerance to such mixtures, permitting 90-100% survival in 2 strains of mice (Table II). Initial studies with the lethal combination of 10 mg streptomycin and 4 mg isoniazid when administered with 50 mg cholic acid indicated that the mixture was tolerated by 95% of the white mice tested. Ten mg sodium choleate permitted 100% survival of white mice. As in tests with other adjuvants, experiments were carried out employing increasing doses of the drugs with various amounts of adjuvant. In DBA mice, when 30 mg of streptomycin was combined with 2 or 4 mg of isoniazid and 25 mg of sodium taurocholate, the mixture was tolerated by 95% and 55% of the mice, respectively. Sodium glycotaurocholate was also effective. With 25 mg as adjuvant, there was 83% survival with a mixture of 20 mg streptomycin and 10 mg isoniazid.

Effect of repeated doses. Three steroids were further tested with streptomycin in daily repeated administrations (5 times weekly) until about 50% mortality was reached.

TABLE II-A. Effect of Steroids on Streptomycin-Isoniazid Mixtures in Mice by Single Subcutaneous Administration.

Streptomycin (mg)	Isoniazid (mg)	Steroid	Dose (mg)	DBA mice Ratio	%
20	2	Sodium taurocholate	25	39/40	97
20	4	"	"	37/40	93
20	6	"	"	32/40	80
20	8	"	"	30/40	75
20	10	"	"	21/40	52
20	2	"	10	18/40	45
30	2	"	25	38/40	95
30	4	"	"	22/40	55
15	2	Sodium glyco-tauro*	25	40/40	100
15	4	"	"	40/40	100
15	6	"	"	40/40	100
15	8	"	"	40/40	100
15	10	"	"	39/40	98
15	2	"	10	39/40	98
15	4	"	"	19/40	48
15	6	"	"	7/40	18
20	2	"	25	40/40	100
20	4	"	"	40/40	100
20	6	"	"	39/40	98
20	8	"	"	37/40	93
20	10	"	"	33/40	83

* Sodium glyco-taurocholate.

Controls of these adjuvants without streptomycin revealed absence of toxicity and normal gain in weight of mice during the test period. It should be noted that these tests were carried out in DBA mice which are somewhat more resistant to streptomycin than are white mice. Under these conditions, with 20 mg of streptomycin, only sodium glycocholate exhibited a protective effect, 6 of 10 mice surviving 10 doses (Table III). The first toxic fatalities in these mice occurred after the sixth dose, with outward symptoms of drug accumulation in survivors. Under the conditions of the test, a 60% endpoint was reached with sodium taurocholate and streptomycin after 8 doses and a 50% endpoint with sodium glycotaurocholate and streptomycin after 7 doses. In the mixture of 20 mg streptomycin and 2 mg isoniazid with sodium taurocholate as adjuvant, 8 of 10 mice survived 5 daily doses. Increased tolerance was definite inasmuch as 20 mg streptomycin was 100% lethal (Table I). Work is now in progress to test whether smaller doses of streptomycin and isoniazid mixtures can be tolerated for longer periods with the steroids as adjuvants.

Discussion. The metabolic changes involved in this detoxification are not established. One of the toxic manifestations following use of streptomycin over extended periods is largely due to an imbalance in normal calcium metabolism. The result of lowering ionized plasma calcium may cause an alteration in the normal metabolism of calcium and phosphorus which leads to an increase in total inorganic phosphate and a decrease in phosphates such as creatine phosphate, adenosine diphosphate and adenosine triphosphate thereby interfering with certain enzyme activities. From Keller's studies(7) and our findings(8), one might conclude that ionic calcium is effective in reducing the toxic manifestations caused by prolonged streptomycin treatment, since calcium is physiologically active in this form. Since bile acids are concerned in some way with normal calcium absorption and metabolism, it may be that administration of small amounts of certain steroids in mixtures of SM and/or INH simply prevent depletion of ionized calcium and the secondary inhibition

TABLE III. Effect of Repeated Administrations of Streptomycin-Isoniazid Mixtures with Certain Steroids in DBA Mice (Daily Subcutaneous Administrations).*

Streptomycin (mg)	INH (mg)	Steroid	Dose (mg)	No. mice	No. of mice surviving following No. of doses 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
20	—	Sodium taurocholate	25	10	10	10	10	10	9	8	8	6	—						
20	2	" "	25	10	10	10	10	10	8	7	3	—							
—	—	" "	25	10	10	10	10	10	10	10	10	10	10	10	10	9	9	9	9
20	—	Sodium glycocholate	10	10	10	10	10	10	10	10	7	7	7	6	—				
—	—	" "	10	10	10	10	10	10	10	10	10	10	9	9	—				
20	—	Sodium glyco-tauro†	10	10	10	10	9	8	8	7	5	—							
—	—	" "	10	10	10	10	10	10	10	10	10	10	8	7	—				

* Monday to Friday incl.

† Sodium glyco-taurocholate.

of certain enzymes by the drug. Reifenstein and co-workers(6) showed that certain steroids such as estradiol play an important role in regulating calcium and phosphorus levels and that negative balances are markedly improved by administration of certain steroids. Whatever the mechanism of the detoxification of SM and/or INH in mice, the use of appropriate amounts of bile salts as adjuvants permit tolerance of doses which are well above the lethal dose. It is pertinent that Marcus and Christopoulos(9) in their studies with steroids reported greater therapeutic effectiveness against tuberculosis infection with a combination of steroid and antimicrobial therapy.

Since submission of this manuscript for publication, it has come to our attention that salts of streptomycin and certain bile acids have been prepared and tested for chemotherapeutic activity(10).

Summary. Streptomycin was found to be 100% lethal in a dose of 20 mg (1 g/kg) by subcutaneous administration in white and DBA mice. By the use of various steroids as adjuvants, it was possible to protect mice against 2 to 3 times the lethal dose.

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Potentiating Actions of Indolealkylamines and Lysergic Acid Diethylamide On Reflexes Elicited by 5-Hydroxytryptamine. (28699)

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Indolealkylamines in general are known to inhibit actions of 5-hydroxytryptamine (5-HT) on isolated organs(1,2), and the structurally related lysergic acid diethylamide (LSD) is considered to be one of the most potent anti-5-HT agents(3,4). Since muscular receptors and nervous receptors of the autonomic nervous system sensitive to 5-HT (*e.g.*, in autonomic ganglia) exhibit marked differential selectivity to various 5-HT blocking agents(5), the interest arose as to whether indolealkylamines and LSD which are only weak antagonists of 5-HT on some nervous preparations (*e.g.*, inferior mesenteric ganglion of the cat)(6,7), show any antagonistic action to reflex actions elicited by 5-HT.

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Materials and methods. Cats under nembutal anesthesia (35 mg/kg i.p.) were used. Blood pressure and pulse rate were recorded from the femoral artery and respiration from the chest by a strain gauge on a polygraph. In 5 experiments spontaneous action potentials of afferent fibers in the vagus nerve were also recorded. The vagus was cut on one side, and the activities of single afferent nerve fibers have been recorded through monopolar platinum electrode on an oscilloscope. All injections were given into the femoral vein. Repeated doses of 5-HT (creatinine sulfate) were injected before, and one, 3 or 4, and sometimes 10 and 20 minutes following the test compounds. The following compounds have been tested against 5-HT in 3-7 experiments each: lysergic acid diethylamide (Sandoz); tryptamine- HCl,