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,

N,N-dimethyl tryptamine (Regis Co.); N,N-diethyl tryptamine (Argyll Co.); N,N-dipropyl tryptamine, N,N-dibutyl tryptamine, Psylocin (Light Ltd.); 6-methoxy tryptamine, 6-benzyloxy tryptamine, alpha-methyl tryptamine, and alpha-ethyl tryptamine. The compounds were given in 2 to 4 doses differing by a ratio of 1:2, 1:2.5 or 1:4. Doses were increased until the reflex effects of 5-HT have been changed to a measurable extent. (A change of at least 10 Hg mm in the 5-HT induced blood pressure drop, and at least 30% change in the bradycardic response and an alteration by at least 2 respiratory cycles of the 5-HT induced apnea have been chosen as criteria for significant response.)

Results. A. Several indolealkylamines and LSD not only failed to diminish or block the cardiovascular and respiratory responses elicited by intraveneously given 10-40 μg/kg of 5-HT, but in appropriate doses which by themselves did not show 5-HT-like effects enhanced the 5-HT induced reflex bradycardia, blood pressure fall, and apnea. The potentiating action of indolealkylamines was observed regularly in all experiments and the same phenomena could be repeatedly elicited on the same animal employing several times an effective potentiating dose. The action of these agents was short and reversible with a recovery period of 10 to 20 minutes. LSD had a longer lasting effect and in contrast to the indolealkylamines in the potentiating dose range altered considerably the blood pressure and heart rate; therefore, no attempt was made to administer this compound in repeated doses and beyond the 100 μg/kg dose level. Results of 2 typical experiments with LSD and N,N-diethyl tryptamine are shown in Fig. 1. The approximate minimal effective potentiating doses of the indolealkylamines studied ranged from 0.1 mg/kg to > 2 mg/kg. The most active was N,N-diethyl tryptamine, usually being effective in 0.1 mg/kg. N,N-dipropyl and N,N-dibutyl tryptamine were slightly less potent. The minimal effective potentiating doses of tryptamine and Psylocin were found at 0.4 mg/kg and for N,N-dimethyl tryptamine it ranged between 0.4 and 2.0 mg/kg; 6-methoxy tryptamine; and 6-benzyloxy tryptamine were ineffective up to 2 mg/kg. Alpha-methyl and alpha-ethyl tryptamine, which are known as potent inhibitors of monoaminoxidase (MAO) were tested only in one experiment each and were inactive up to 400 and 200 μg/kg respectively. This seems to indicate that a MAO inhibiting action is not important for the potentiating action. LSD which increased the reflex action of 5-HT in 40 and 100 μg/kg was more potent than any of the indolealkylamines tested. 2-bromo-LSD, a non-hallucinogenic structural analogue of LSD with marked musculotropic anti-5-HT properties, showed no potentiation to the reflex actions of 5-HT in the same doses.

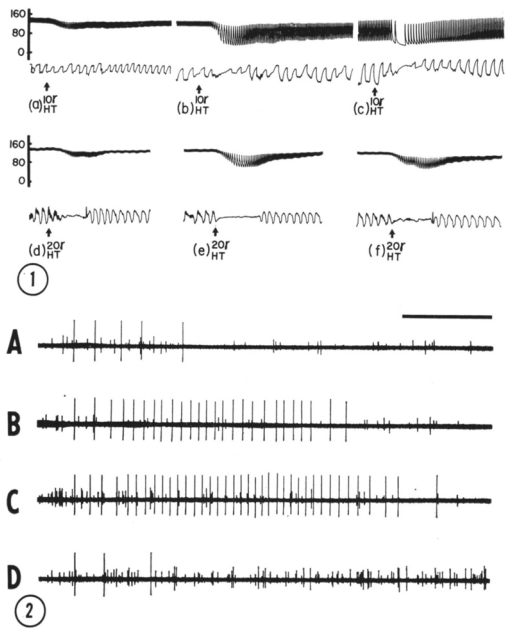

FIG. 1. Potentiation of cardiovascular and respiratory reflex actions of 5-HT by LSD and N,N-diethyltryptamine. Upper tracings: blood pressure; lower tracings: respiration. *a* and *d*: effects of 10 and 20 μg/kg 5-HT iv.; *b* and *c*: effects of 10 μg/kg 5-HT 1 and 4 min. after 100 μg/kg LSD iv.; *e* and *f*: effects of 20 μg/kg 5-HT 1 and 4 min. after 400 μg/kg N,N-diethyltryptamine.

FIG. 2. Potentiating action of tryptamine on afferent vagus nerve activity elicited by 5-HT. A, response of an afferent unit of vagus nerve to 30 μg/kg 5-HT i.v.; B, C and D responses to the same doses of 5-HT, 1, 4 and 10 min. after administration of 2 mg/kg tryptamine iv. Time scale: 1 sec. (right upper corner).

B. The above investigated reflexes are elicited by 5-HT through activation of viscerosensory chemosensitive receptors in the chest (8,9), and their afferent pathways run in the vagi. No other receptor elements with a proven 5-HT sensitivity are represented along the reflex pathways except autonomic ganglia, in which indolealkylamines and LSD exhibit only weak blocking action(6,7). In addition, the relations between 5-HT and indolealkylamines and LSD in chemical structure, as well as in their biological actions, indicated that the observed potentiation of reflex actions possibly is a phenomenon occurring at the viscerosensory receptors through an interaction between 5-HT and the agents mentioned. Accordingly the afferent peripheral portion of the reflex pathways has been investigated. In 5 cats displaying the action potentials of single afferent fibers of the vagus nerve, it was found that several types of afferent fibers in that nerve did not indicate stimulation by 5-HT:

1. Respiratory afferents were not affected by the conventional doses of 5-HT. (This is not in accordance with earlier findings(10).)

2. Constantly firing, fast conducting fibers in the vagus did not respond to 5-HT.

3. In one experiment a slow conducting unit with slow rhythmical bursts of discharge (probably a gastrointestinal afferent fiber) did not respond to 5-HT. The fibers which showed positive responses to 5-HT were those slow conducting types (as judged from the duration and size of the action potentials displayed) which had a tendency for irregular spontaneous activity, no spontaneous activity or which belonged to the group of typical cardiac afferents. Responses of 5-HT on these latter types of fibers have been tested before and after administration of 2 representative indolealkylamine compounds (tryptamine and N,N-diethyl tryptamine) and before and after LSD. The number of spikes elicited by 5-HT was greatly enhanced after 400 μg/kg N,N-diethyl tryptamine, 1 mg/kg tryptamine (Fig. 2) and to a lesser extent by 100 μg/kg LSD, indicating that the afferent impulses were potentiated at the 5-HT sensitive viscerosensory receptors. The possibility of facilitation at other sites of the reflex arc by these agents, although remote, is not yet excluded.

Discussion: Projection of these findings into the field of somatosensory functions does not seem to be irrelevant. There is evidence that 5-HT in minute doses is capable of activating somatosensory pathways(11,12), and certain findings with LSD indicate an enhancement of sensory inputs at receptor sites such as the retina(13) and at auditory pathways(14). These observations and the present results with indolealkylamines and LSD suggest that a significant pharmacological action of these agents, many of them possessing hallucinogenic properties(15), is a potentiating action on sensory input mechanisms which can be activated by agents like 5-HT.

Summary. Several indolealkylamines (*i.e.*, tryptamine, N,N-diethyl tryptamine, N,N-dipropyl tryptamine, N,N-dibutyl tryptamine, and Psylocin and lysergic acid diethylamide (LSD) potentiate the cardiovascular and respiratory reflex actions (blood pressure fall, bradycardia, apnea) of 5-HT in the cat. Since a potentiating action on afferent vagal fibers activated by 5-HT was also observed by the same type of agents, it is proposed that indolealkylamines and LSD influence 5-HT induced reflexes by facilitating the stimulant action of 5-HT on viscerosensory receptors.

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Production of a Polysaccharide by *Staphylococcus aureus*. II. Effect of Temperature and Antibiotics. (28700)

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A previous study(1) has shown that various strains of *Staphylococcus aureus* possess a polysaccharide component which can be released from the staphylococcal cells into the surrounding medium by treatment with heat or with sonic disruption. Although chemical characterization of the polysaccharide is incomplete, its depolymerization by testicular hyaluronidase and not by bacterial hyaluronidases suggested a material not unlike chondroitin sulfate. However, the salient observation reported was the occurrence of a marked accumulation of the polysaccharide within cells of *S. aureus* grown in the presence of subinhibitory concentrations of penicillin G or nafcillin.

This report describes experiments which give further insight into the nature of the polysaccharide response, particularly as it influences the structural entity of the cell wall of *S. aureus*. These include the effect of lysozyme and trypsin on the cell walls of organisms grown in the presence of nafcillin and the rate of synthesis of the polysaccharide following exposure of growing cells of *S. aureus* to subinhibitory levels of a number of antibiotics. Observations were made also on the effect of subinhibitory concentrations of nafcillin on the cell structure of the organism following thermal stress.

Materials and methods. The materials and methods were for the most part described previously(1). The essential information is summarized as follows: The experiments described were performed with a penicillin-resistant strain (CHP) of *S. aureus* a penicillinase producer which is highly resistant to penicillin G, but susceptible to semi-synthetic penicillins nafcillin, oxacillin and methicillin (2,3). The organism was grown at 37°C on the surface of an agar medium in which all constituents except casein hydrolysate were chemically defined. For the studies on the effect of various antibiotics on polysaccharide production stock solutions in sterile buffered 0.85% salt solution were stored in a freezer (—20°C), thawed and final dilutions were prepared and added to previously liquefied medium immediately before use. Commercial preparations of nafcillin, oxacillin, methicillin, novobiocin, chloramphenicol, chlortetracycline, vancomycin, triacetyloleandomycin, bacitracin and erythromycin were used.

Blake bottles containing 200 ml of the agar medium were seeded with an 18-24 hour culture and incubated for 18 hours at 37°C. The highest concentration of each antibiotic incorporated into the medium which just permitted good growth to occur was selected for study. Cells were washed from the agar surface with distilled water, centrifuged and washed twice with distilled water. They were suspended in saline, standardized to contain 8.0×10^{10} cells/ml, autoclaved at 120°C for 10 minutes, cooled and centrifuged at