ical or neural mechanism but must also explain the relative difficulty with which tolerance to mescaline is established.

To distinguish drug tolerance from "learning," the loss of impairment must be contingent upon the pattern of drug injections and not upon climbing experience. When we injected rats daily with the drug but omitted the climbing test on the second and third injection days, climbing was no longer impaired on the fourth injecion day. Conversely, when we injected rats daily with placebos and permitted them to climb daily, climbing was impaired with drug injection on the fourth day. Finally, rats in groups A, B, and C were permitted to lose tolerance; climbing was impaired with the first injection of the drug, and with daily injections the animals regained tolerance, each in its own characteristic pattern.

In a set of experiments on cardiac effects, we found that rats restrained in a holder displayed a tachycardia which was affected only slightly by single doses of LSD-25. In order to record bradycardia in rats, subcutaneous needle electrodes were attached to the limbs, allowing the animal freedom of movement during electrocardiographic recordings. Intraperitoneal placebo injections caused a tachycardia under these conditions, and injections of LSD-25 induced bradycardia, the pulse decreasing from a base of 415 to 300 per minute. Daily intraperitoneal injections of at least 175 μg of LSD-25 per kilogram were administered for as long as 12 days. Bradycardia was most marked within the first 30 minutes following injection and began to decrease at 90 minutes. Although the degree of bradycardia varied from day to day, we found no clear indications of the development of tolerance. Similarly, chronic experiments with mescaline-induced bradycardia in rats have failed to demonstrate tolerance (*5*). The bradycardia induced with LSD-25 has been thought to be due to a central mechanism, since, in the cat, LSD-25–induced bradycardia is abolished by spinal section (*6*). The peripheral anticholinesterase effects of LSD-25 would be minimal in the dosage range employed here (*2*).

Our findings and those of others suggest a pattern underlying the development of tolerance to the effects of LSD-25. No tolerance is manifest with respect to bradycardia and the respiratory arrest that occurs with high dosages (*7*); these two effects probably involve centers in the caudal brain stem. Pyrexia, mydriasis, and piloerection are autonomic effects of LSD-25 to which tolerance has been shown to develop (*6, 8*); more rostral brain-stem mechanisms have been implicated in the origin of these responses (*6*). Similarly, rostral mechanisms may be involved both in the behavioral effects and in the tolerance observed with respect to both psychosomimetic drugs. The rostral mechanisms which are involved in electroencephalographic and behavioral arousal and which show "habituation" to sensory stimulation (*9*) could as well show tolerance to chemical stimulation. This suggests that both neurochemical and electroencephalographic studies would be useful in investigating the basis of tolerance. Since tolerance may be a phenomenon characteristic of the entire group of psychosomimetic drugs, comparative studies of autonomic behavioral and electroencephalographic effects should be attempted.

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Antimicrobial Activity of Horny Corals

Recent studies (*1*) on the occurrence of antibiotic substances in marine organisms have revealed some interesting antimicrobial properties of gorgonian corals, belonging to the phylum Coelenterata. Corals were collected for this work from reefs located off the southern coast of Puerto Rico. For the assays of antibacterial activity in the various materials, many indicating marine bacteria were isolated from the same region and grown in Difco nutrient agar made with sea water. Other common test microorganisms were grown in ordinary nutrient agar made with distilled water. Small pieces of coral, or various extracts from different species of coral, were placed on nutrient agar plates, which had been inoculated with the appropriate indicating microbes. After incubation for about 16 hours, zones of microbial inhibition became conspicuous around the fragments of coral and paper discs containing extracts from active corals.

Among the corals which showed antibacterial action were the following species: *Antillogorgia turgida, A. americana, Rhipidogorgia flabellum, Briareum asbestinum, Plexaura homomalla, Plexaurella dichotoma,* and *Plexauropsis crassa.* The sea whip, *Antillogorgia turgida,* was especially striking in its action against numerous marine bacteria, *Clostridium feseri, Micrococcus aureus, Bacillus subtilis,* and *Escherichia coli.* Strains of penicillin-resistant *Micrococcus* were equally susceptible to inhibition by extracts from *Antillogorgia.* Unsusceptible organisms included *Lactobacillus casei, Candida albicans, Kloeckera brevis, Cryptococcus neoformans,* and *Saccharomyces cervisiae.* It was easily demonstrated that antimicrobial activity could be extracted from both fresh and dried materials of sea whips, sea fans, and plexaurid corals, by means of water or other common solvents. The active principle appears not to be located in the brown core of the horny corals, but it is present in the outer, gray-purple cortex. This suggests that the activity is probably not associated with halogenated gorgonin of the horny axis. Segments of the fine branches, as well as the large basal stems, showed very sharp zones of inhibition on agar plates containing marine bacteria. In contrast to these results with gorgonian corals, little or no antimicrobial activity could be detected in the species of stony corals that were tested. Examples of inactive species are *Acropora palmata, Porites porites, Millepora alcicornis,* and *Montastrea sp.*

It is not known whether the coral polyps or their associated zooxanthellae produce antibacterial substances. It is of interest to note that another large group of terrestrial symbionts, the lichens, commonly produce antibiotic substances (*2*). The increasing number of examples of naturally occurring chemical antagonism among numerous kinds of organisms lends support to the idea, expressed so well by Brian (*3*), that these phenomena are "not incompatible with the view that the capacity to produce antibiotics is a character conducive to fitness." Perhaps successful symbiosis may be enhanced by the antibiotic properties of the complex organization of fungi and algae in lichens or of animals and algae in gorgonian corals.

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Positive Reinforcement Produced by Stimulating Hypothalamus with Iproniazid and Other Compounds

There is a growing body of information on the distribution of possible transmitter substances in the brain and on factors which cause temporary or long-run changes in these concentrations (*1, 2*). No technique, however, has been available to demonstrate the excitatory or inhibitory function of these chemicals in the central nervous system, nor has there been any method to demonstrate their suspected capacity to act selectively on some functional and anatomical groups without affecting others.

Pharmacotherapy in mental illness has tended to emphasize the role of epinephrine, norepinephrine, and serotonin as transmitters because these tend to be concentrated in areas of the hypothalamus and tegmentum which have proved motivational functions, and because drugs which affect mental states also affect the concentrations of these substances (*1, 2*). These three supposed transmitters are believed to be broken down in the brain by monoamine oxidases which can be inhibited by iproniazid (*1*). Thus, the latter may have its effect by augmenting the action of one or more of these three substances.

Iproniazid, in doses of 50 mg three times daily, has recently been shown to have pronounced effects in relieving severe depressions (*3*). The therapeutic effect has been tentatively related to supposedly excitatory effects of serotonin (*3*).

In our experiments (*4*), chemicals were injected into the hypothalamus of the rat in microgram amounts to test for possible rewarding effects of chemical stimulation in areas where electric stimulation is highly rewarding (*5*). Iproniazid is shown by these experiments to be a rewarding stimulant in quantities of 1 to 2 μg. The rewarding effect is specific to the chemical and to the site of injection in the brain. Iproniazid appears to share this rewarding function with epinephrine.

In these experiments, a plastic holder was screwed to the skull of each rat. From it a pair of insulated silver electrodes and a No. 26 Huber pointed hypodermic needle penetrated into the brain. This method of implantation is our own version of a technique reported by Fisher (*6*), who had based his design on an electrode developed by one of us (J. O.) (*7*).

The strategy of the series of experiments was first to canvass a range of chemical agents on the basis of a habit established by electric reward and then, after finding agents which would sustain behavior by themselves, to train a new group of animals by means of the chemical reward alone.

In the first group of experiments, electrodes and pipettes were implanted in an area of the ventral posterior hypothalamus just in front of the mammillary body. This is the center of an extensive region in which electric stimulation is rewarding (*5*). Four animals were used in these tests.

After implantation and a 14-day recovery period, animals were trained to press a lever for electric stimulation. All animals achieved rates of self-stimulation of 2000 an hour or more; the technique used has been described elsewhere (*5*). At this point they were shifted to tests of self-injection.

In self-injection studies, a polyethelene tube was used to connect a microinjector to the rat's pipette. Each lever-pressing response caused 1/700 ml of solution to be injected into the hypothalamus. The following chemicals were tested, in solutions of 1 mg per milliliter of physiological saline: acetylcholine chloride, adenosine, triphosphate disodium salt, serotonin creatinine sulfate, epinephrine hydrochloride, norepinephrine bitartrate, and Marsilid phosphate (iproniazid). Each injection contained approximately 1.4 μg of the whole compound being tested. Also, for control, physiological saline was injected alone.

The results for acetylcholine and serotonin indicate that these do not have any rewarding effect but quickly cause the animal to lose muscular tone and apparently to go to sleep. Adenosine triphosphate and noradrenaline did not have any observable effects, in the concentrations used.

Adrenaline, on the other hand, appeared to produce approach behavior; however, in the concentrations used, it caused a loss of motor coordination, and self-injection was so slow as to leave some doubt about its rewarding effects.

In the case of iproniazid, there was striking self-injection behavior. Animals injected the chemical at rates of more than 300 self-injections an hour; this is far above the chance rate obtained with saline (see Fig. 1).

In the second set of tests, electrodes and pipettes were implanted to provide

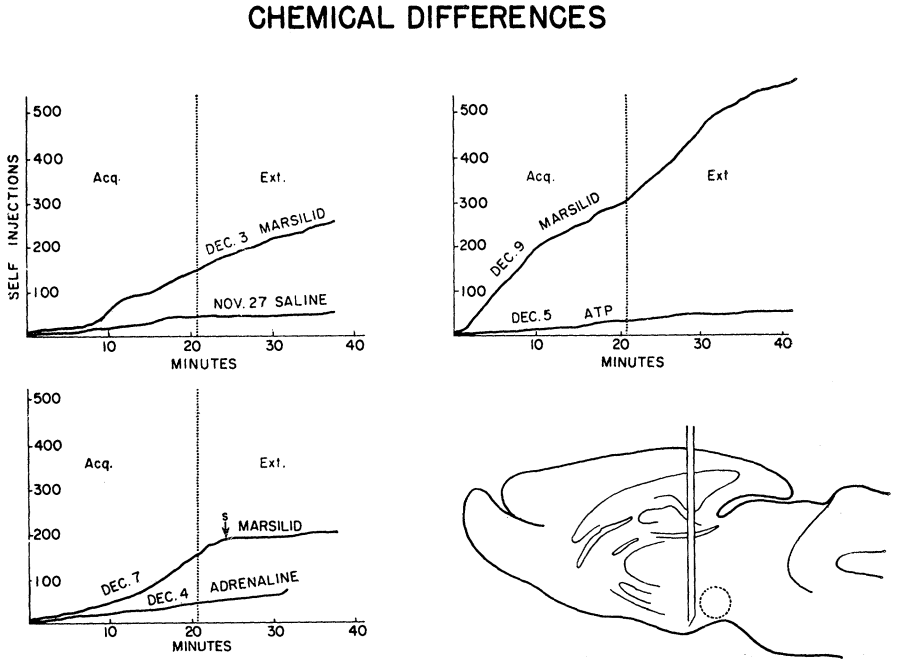

Fig. 1. Rate of response in tests with iproniazid (Marsilid) in relation to rate of response in tests with saline, adrenaline, and adenosine triphosphate (ATP). The total number of self-injections, for Marsilid, always rises to about 200 in the 20-minute acquisition period (area at left of dotted line). For saline and adenosine triphosphate, the rate does not rise above 50 (chance level). For adrenaline, the rise in rate of self-injection is slow but steady; this suggests the possibility that there are rewarding effects. Response is not rewarded by microinjections during the extinction period (area at right of dotted line). Responding tends to continue for long periods after termination of reward with Marsilid. At the point marked *S*, the animal had a seizure.