Pharmacological Effects of Anthranilic Hydroxamic Acid and Certain Other Hydroxamic Acids. (26169)

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The pharmacology of hydroxamic acids has not been extensively studied. Epstein and Freeman(1) have determined the approximate LD_{50} in mice for a number of aromatic and aliphatic hydroxamates and their effectiveness against sarin poisoning. Askew(2) tested the toxicity of a number of other aromatic hydroxamates and found that most of them produced no overt symptoms at a dose level of 150 mg/kg. We have prepared hydroxamic acids some of which were toxic, but others were well tolerated at relatively enormous doses despite the fact that these compounds are able to chelate cations such as iron, magnesium and calcium. One compound, anthranilic hydroxamic acid, showed interesting pharmacological effects and is the main subject of this communication.

Experimental. The hydroxamates were synthesized from hydroxylamine and the methyl esters of the acids and isolated as sodium salts. The citric, succinic and malonic hydroxamates were very hygroscopic. They were dissolved in water and injected intraperitoneally into mice. One mg/g of the citric hydroxamate killed all of 10 mice in 5 minutes; half the dose caused no deaths. One mg/g of the succinic hydroxamate caused no deaths, but the mice were very depressed for several hours. Four mg/g of the malonic hydroxamate was tolerated without symptoms.

The sodium salt of anthranilic hydroxamic acid was recrystallized several times. It was dissolved in water and HCl added until the acid just began to precipitate at approximately pH 8.3. When it was injected intraperitoneally into rats, 1.0 mg/g, the animals lost consciousness in 10 minutes and remained anesthetized for 10 hours or more. Upon recovery they appeared normal and remained so. One half this dose caused depression for 8-10 hours but no loss of consciousness nor analgesia. Some excess salivation was apparent. Rats were trained for conditioned avoidance in a box with an electric grid for shocking and a fence to climb to avoid the shock. The conditioning shock was large enough so that for several days following the rats gave at least 10 consecutive positive reactions. When 0.5 mg/g of anthranilic hydroxamate was injected intraperitoneally, the reaction became negative after 10 minutes and remained so for the next 10 consecutive tests. The animals each time responded to the shock.

One half mg/g was given to rats every day. On the third day, the animals became somewhat restless before they were depressed. At this time they had lost no weight. But regularly on the fourth day they went into clonic convulsions, accompanied by excessive salivation, at intervals during 90 minutes. Depression then set in for about 8 hours. Injection on the fifth day caused more severe convulsions, which ended fatally. If injections were discontinued after the fourth day and not resumed until the eighth day, the rats behaved as they had originally. The magnesium and calcium salts had the same effect as the sodium salt, so the convulsions were not the result of chelation or depletion of these ions. Probably the drug accumulated in the body.

To test this, the urine of a rat was collected for 48 hours after it had received 1.0 mg/g of the anthranilic hydroxamate. $FeCl_3$ was added and the color read against a standard. Less than 10% of the dose appeared in the urine during this period. As a control an equivalent amount of malonic hydroxamate was given to a rat, and 83% of the dose was recovered in the urine after 24 hours. The anthranilic compound must either be stored or metabolized. Homogenates of rat liver or kidney were unable to metabolize it.

Anthranilic hydroxamate protected mice against twice the lethal dose of strychnine. 0.025 mg strychnine killed all of 5 mice within 6 minutes. Forty mg anthranilic hydroxa-

mate given 10 minutes before the strychnine protected 7 out of 10 mice given 0.05 mg strychnine. Fifty mg and higher doses prolonged the life of mice given 0.1 mg strychnine, but they eventually died within a period of 90 minutes. The lives of mice given lethal doses of metrazol or picrotoxin were prolonged by anthranilic hydroxamate but there was no protection.

A dog was lightly anesthetized with nembutal. One g/kg of anthranilic hydroxamate was injected intravenously in a period of 30 minutes. During the next 2 hours, there were no changes in blood pressure, heart rate or EKG. The respiratory rate was slightly depressed at first, then became regular and shallow.

The hydroxamates of salicylic and benzoic acids in equivalent doses produced only transient depression, failed to abolish the conditioned avoidance response and could be given daily for 10 days without apparent ill effect as indicated by loss of body weight. They showed no protection against strychnine, metrazol or picrotoxin. The hydroxamate of phthallic acid is toxic; 0.25 mg/g is the lethal dose. When half this dose was given, tremors occurred at intervals for several hours, and the rat appeared very sensitive to touch. Respiration was of the Cheyne-Stokes type. Anthranilic hydroxamate antagonized these effects.

Discussion. Salicylic and anthranilic hydroxamates should be almost equally effective as chelating agents. Since their pharmacological actions are so different, chelation probably does not play an important part in the mechanism of action of anthranilic hydroxamate. This compound appears to penetrate the blood-brain barrier rapidly and the data suggest that its action is central. Despite the salivation, the absence of effect on blood pressure and heart rate indicate that the autonomic system is not affected centrally or peripherally. It will be of interest to study its effect on brain metabolism.

Summary. The hydroxamate of anthranilic acid injected intraperitoneally into rats in a dose of 1.0 mg/g causes anesthesia lasting 10 hours or more. Recovery is apparently complete. One half this dose causes tranquilization and suppression of the conditioned avoidance response. The compound protects mice against twice the lethal dose of strychnine. It merely prolongs the life of animals given metrazol or picrotoxin. A dose of 1.0 g/kg injected intravenously in a dog has no effect on heart rate, EKG or blood pressure and only minor effects on respiration. Equivalent amounts of the hydroxamates of salicylic and benzoic acids have none of the pharmacological effects.

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Effect of Arsenic on Growth of Mammalian Cells *in vitro*.* (26170)

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Arsenicals are useful in veterinary medicine (1,2), and in the past decade they have also acquired importance in animal nutrition as growth stimulants(3). Interest in the metabolic turnover of the growth promoting arsenicals and the question of tissue residues has spurred the need for additional basic information on the physiologic role of this class of compounds. The application of tissue culture methods to determine the direct response

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