USE OF THE ETHYL ETHER OF METAAMINOBENZOIC ACID (MS-222) FOR GENERAL ANESTHESIA OF COLD-BLOODED ANIMALS

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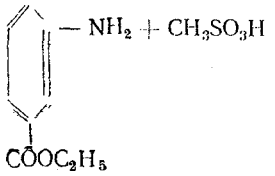
Because cold-blooded animals are widely used for various experimental studies [20], and particularly for a number of clinical endocrinological tests, the problem of general anesthesia for them has become of great methodological importance [10]. Ether, which has been most widely used for the elimination of general sensation from frogs and for preventing movement, causes partial paralysis of the respiratory center, it stimulates the skin, and increases the excretion of mucins and toxic substances from it [23], and causes clotting of proteins and dissolution of fat. Other anesthetics have many disadvantages and complications when applied to cold-blooded animals.

In the last decade, in physiological, experimental biological, ichthyological institutions, in scientific aquaria and animal houses, successful use has been made of the preparation MS-222 synthesized by the firm of Sandoz [17] in the course of their search for a substitute of cocaine.

MS-222 is the methylsulfonate of the ethyl ether of metaaminobenzoic acid, and is the isomer of anesthezin (benzokaine). The empirical formula is $C_9H_{11}O_2N \cdot CH_3SO_3H$, the molecular weight 261.30, and the structural formula:



It is a white crystalline powder having a melting point of 145-150°. It is soluble both in sea and in fresh water and forms a clear colorless solution having an acid reaction. It leaves scarcely a trace of ash. It should contain no chlorides, sulfates, alkaloids, or heavy metals. When dried at 105° it should not lose more than 0.5% weight. Quantitative estimation may be made by direct potentiometric titration with 0.1 M sodium nitrate, using a graphite rod and a calomel electrode. Titration is continued until a maintained deviation of the galvanometer spot occurs. A quantitative determination in biological fluids (blood, urine etc.) may be carried out calorimetrically by methods applying to substances containing a primary aromatic amino group. When a 10% solution is kept for 3 days at room temperature, there is no loss of effectiveness. After 10 days it turns brown, but its biological action is reduced only by 5%. For best results, the solution should be prepared fresh and kept in a cool dark place (2-8°) in tightly stoppered bottles.

Strzhizhovskii [22] was the first to test MS-222, and showed that the golden carp Carassius carassius var. auratus could be anesthetized by 1:10,000 of the solution acting for 5-10 minutes, or by a 1:1,000 solution acting for 60-90 seconds. Some of the fish died after 6-hour immersion in this concentration, while after 12-24 hours the death rate was 100%. When frogs (Rana temporaria) were immersed in 1:1,000 MS-222, anesthesia developed in 5-6 minutes, and lasted for 42 minutes. In frogs immersed for 20 minutes, the anesthesia lasted for $2\frac{1}{2}$ hours. Frogs immersed in the solution for 1, 2, or 4 hours remained anesthetized for $3\frac{1}{2}$, 6, and 10 hours. Immersion for 6-18 hours caused most of them to die, while those which survived remained anesthetized for up to 46 hours.

According to Boden [1], during anesthesia induced by MS-222, in the carp Carassius carassius var. auratus, the oxygen demand is reduced. The fish remained alive after remaining in a concentration of 1:20,000 at 16° for 30-60 minutes. They could remain in the solution for more than 24 hours without any harmful after effects.

Rotlin [14] points out from experiments on mice, guinea pigs, and rabbits that MS-222 is approximately 3 times less toxic than is procaine, and 10 times less toxic than cocaine. Cold-blooded animals tolerate MS-222 as an anesthetic much better than novocaine, alypin, stovaine, borocaine, tutocaine, cocaine, and pantezin. Frogs immersed in 1:2000 or 1:3000 MS-222 become deeply anesthetized in 5-7 minutes, and remain so for several hours. After they have been transferred to pure water, they recover in 30-60 minutes.

Buru [25] also obtained satisfactory results when operating on frogs (Rana temporaria) using 1:10,000 MS-222 as an anesthetic.

Sato [18] successfully used 1: 3,000 MS-222 to anesthetize Triturus vulgaris for removal of the crystalline lens, in order to subsequently observe its regeneration.

Karchmar and Kopani [10] used 1:7,500 MS-222 in experiments on larvae of the salamander Amblystoma punctatum, at various stages of development.

Rothman and Glyukson [6] used 1: 3,000 MS-222 to photograph and mark the salamanders Triturus vulgaris and Triturus cristatus.

Christianson [3] castrated male and female frogs (Rana pipiens) anesthetized with 2% MS-222.

Gadien and Duni [8] operated on the fish Carassius carassius var. auratus, which they anesthetized by immersion for 10-15 minutes in 1:10,000 MS-222 1:3000. McGovern and Pugh [12] showed that a solution of 1:3000 MS-222 has no effect on the motility or on the fertilizing power of frog sperm. Frog eggs fertilized in this solution by being left to stand in it for one hour developed normally. A longer exposure increased the percentage of abnormal developments.

Steinbrecht[21] used various fish from an aquarium, and 1:2000 MS-222. Anesthetized fish placed in Petri dishes containing filter paper soaked in MS-222 remained alive for more than 24 hours. After they had been transferred to the aquarium tanks they rapidly recovered from the anesthetics. In several experiments with anesthesia after fertilization, the aquarium fish Lebistes reticulatus produced normal offspring. Wilwock [24] showed that the use of MS-222 at a concentration of 1:1000 may be used with great advantage to take close-up photographs of aquarium fish, and that the effect of the anesthetic is to produce a maximal dilation of the chromatophores lasting for two minutes. Manner [11] used 1:1500 MS-222 to study regeneration in worms from which proteins had been cut off. Gilbert and Wood [5] have reported briefly that MS-222 may conveniently be used to anesthetize large sharks and skates. They investigated the effect of a hypophyseal injection on copulation and fertilization in elasmobranchs. They found MS-222 to be the most reliable anesthetic. Because it is not convenient to place large fish in a bath for anesthesia, they employed a technique of squirting the solution. The best results were obtained when MS-222 was diluted 1:1000 with sea water. The solution was introduced into the mouth of the shark or into the spiracle of the skate by water pumps, or with "pistols", or by a hand syringe (100 ml for the small and up to 1 liter for the large fish). Absorption by the gills was rapid and anesthesia occurred within 15 seconds. In sharks of 400 lb (180 kg), anesthesia is usually established within less than one minute, and lasts for 5-30 minutes. Gilbert and Wood [5] claim that this method can also be used for telosts of various sizes.

Hublou [9] used MS-222 when marking and measuring fish; by this means millions of fish were marked. He found that the method was convenient and that the fish were better preserved after the marking plates had been fitted. Butler [2] also reported favorably on the use of 1:75,000 MS-222 as an anesthetic when marking fish. At this concentration and at 10°, the fish were anesthetized in 10 seconds.

In aquarium management MS-222 is used chiefly in connection with the transportation of fish, for investigations, and for treatment of fungoid and parasitic infestations. Gossington [7] found that MS-222 greatly facilitated the transport of excitable fish, such as male Betta pugnax or Pirhanha (Serra salmo). They could be moved in their usual tanks and the concentration required was then lower, because the sedative action was maintained longer.

Recently Steganova, Pukhta, and Romanovsky [19] of the Prague Zoological Institute have investigated the anesthetic action of MS-222 on Rana temporaria tadpoles and adults. For both, a concentration of 1:2000-1:3000 was found to be optimal. The drug was entirely harmless, and induced the complete muscular relaxation required for surgical operations.

SUMMARY

The paper refers to the chemical and physiological properties of the preparation MS-222 used successfully for total anaesthesia of cold-blooded animals. According to published reports, MS-222 possesses many advantages involved in the management of and suitable not only for biological experiments and investigations but also for many procedures involved in the management of cold-blooded animals.

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