9294

Selenium Poisoning in Fishes.*

M. M. Ellis, H. L. Motley, M. D. Ellis and R. O. Jones.

From the Department of Physiology and Pharmacology, University of Missouri, and U. S. Bureau of Fisheries Laboratories, Columbia, Mo.

In studies on the toxicity of selenium we have found marked pulmonary edema in dogs following the intravenous injection of sodium selenite equivalent to 1.83 mg. of selenium per kg., and in rats following intraperitoneal injections equivalent to 3.7 mg. of selenium per kg. As these findings on dogs and rats, and the various published observations on the volatile odoriferous compounds in the expired air from man[1] and other mammals[2] which have received small quantities of selenium, direct attention to the lungs in selenium poisoning, a series of studies on selenium poisoning in gill-breathing fishes has been made.

One hundred fifty goldfish (average length *circa* 80 mm.) were carried in individual glass jars each containing 4 liters of well aerated tap water to which known quantities of sodium selenite were added. Analyses at frequent intervals showed that the quantities of sodium selenite used made no appreciable changes in the dissolved oxygen, pH, or conductivity of the water. Each fish was changed to fresh seleniferous water every 48 hours and fed 3 standard pellets of shrimp meal immediately after being transferred.

In waters containing 2 parts per million of selenium the fish showed no symptoms or distress for the first 8 days, taking their food promptly. On the 8th day of exposure to 2 ppm. of selenium the fish began to refuse food, or if food were taken to regurgitate it quickly. This reaction was followed by marked anorexia for a period of several days. The first fish in this series died on the 18th day of exposure to 2 ppm. of selenium and deaths became progressively more frequent between the 25th and 37th days of the experiments, the longest survival being 46 days. The onset of anorexia always marked the beginning of the fatal phase of selenium poisoning. During the next few days the fish frequently showed incoordination and a definite disturbance of equilibrium and as the poisoning progressed became more lethargic and feeble. These changes in behavior were not due to lack of food alone, for these

* Published by permission of the Commissioner of Fisheries.

[1] Dudley, H. C., *Am. J. Hygiene*, 1936, **23**, 181.

[2] Franke, K. W., and Moxon, A. L., *J. Pharm. Exp. Ther.*, 1936, **58**, 454.

symptoms did not develop in a comparable unfed control series of fish not exposed to selenium. In other tests in which the surrounding water carried 5 ppm. of selenium goldfish died in from 4 to 10 days, the sequence of poisoning being the same as in 2 ppm. series. The goldfish experiments showed that selenium could be taken up in cumulatively lethal quantities by fish from the surrounding water.

As the actual selenium intake was not determined in the goldfish series, known quantities of sodium selenite (in a 0.5% solution or less) were injected intraperitoneally into 550 catfish, *Ictalurus punctatus,* averaging 160 mm. in length and 54 gm. in weight. Control fish given comparable injections of physiological saline developed no symptoms and remained healthy throughout the tests. All fish were bathed in salt solution immediately after each handling to prevent fungus infections, and held out-of-doors (these experiments were conducted at Ft. Worth, Texas) in concrete hatchery raceways through which unpolluted water flowed continuously.

Two types of reactions were obtained from single injections of sodium selenite, namely, acute and delayed. Sodium selenite in excess of *circa* 3 mg. of selenium per kg., *i. e.,* 0.15 mg. of selenium per fish was fatal in less than 48 hours, usually in less than 7 hours at water temperature around 10°C. The toxicity of the selenite increased markedly with temperature as 0.35 mg. of selenium per kg., *i. e.,* 0.018 mg. per fish killed in 24 hours or less at 27°C. Fish dying from acute selenium poisoning consistently showed contraction of the dermal chromatophores, so that fish receiving selenium injections were definitely lighter in color often for several hours before death than control fish receiving comparable quantities of physiological saline. This reaction of the chromatophores was readily produced locally on any part of the body by subcutaneous injections of selenite. Shortly before death the poisoned fish developed incoördination and often made spasmodic movements.

At any given water temperature the break between the dose of selenite producing these acute effects and that producing delayed effects was quite abrupt. Fish receiving less than the immediately toxic dose of selenite showed no symptoms for 4 to 10 days after the injection. However, about the 7th day after injection in most cases the eyes began to protrude and the abdomen became more or less pendulous. These conditions grew progressively worse for the next several days at which time the eyes of many fish were almost extruded from the head. Autopsies of fish dying in this "pop-eye" condition of extreme exophthalmus showed the body cavities to be more or less distended with ascites often containing blood, and the

periocular spaces to be filled with highly edematous tissue, that is, the eyes had been extruded by pressure from behind.

Fish in this "pop-eye" condition usually died about 20 to 25 days after receiving the injection of selenium. At water temperatures of 12-13°C. a single injection of 0.9 mg. of selenium per kg., or about 0.05 mg. per fish, was sufficient to produce fatal cases of this "pop-eye" disease, and smaller amounts were effective at higher temperatures as has been noted in connection with the quantities producing acute lethal poisoning.

At water temperatures of 10-13°C. daily injections of 0.04 mg. per kg. of selenium that is, 0.002 mg. per fish produced the exophthalmus after 5 injections, the fish having received a total of 0.01 mg. of selenium.

Blood studies made on 19 catfish, 6 normal and 13 in various stages of selenium "pop-eye" disease showed that although the red blood cell count in the selenium poisoned fish averaged less than one-half million below the normals (2,053,000 normal; 1,847,000 selenized fish), the total hemoglobin of the poisoned fish (Sahli Method) was about 3 gm. per 100 cc. below normal, (normal 9.8 gm., selenized fish 6.9 gm. per 100 cc.). As this hemoglobin level is far out of proportion to the slight lowering of the red blood cells found, it suggests that the selenium was interfering with normal hemoglobin formation. In the selenium-treated fishes many immature red blood cells were present in the blood, indicating a disturbance of the hematopoietic functions. The white blood cell count averaged about 4,000 per cu. mm. higher in the selenium-poisoned fish than in the normal, (14,200 normal, 18,300 selenized fish), and differential counts showed a lymphocytosis from normal to 82% of all white cells.

The blood of the selenium poisoned fishes was also more watery than that of normal fishes, the average specific gravities (Barbour method) being 1.0247 and 1.0338 respectively.

The microscopic pathology of catfish receiving selenium showed extensive areas of degeneration in the liver about the central veins. For the most part nuclei were preserved, but seemingly were pushed apart by the excessive accumulation of fluid, which in some areas had disrupted about one-half of the liver pulp cells. Hyaline degenerative changes were present in the walls of the hepatic arteries.

The spleen was extensively infiltrated with fluid which spread the elements apart, with dilatation of the smaller blood vessels and capillaries. The fibrous tissue was increased, replacing the lymphoid elements, and the capsule was distended by fluid in the subcapsular

region, which in many areas separated it from the underlying tissue.

The mesonephros had wide separations of the glomerular and tubular elements, by the excessive interstitial accumulation of fluid. The cellular elements were definitely pale in appearance as compared to the normal due to intrastitial accumulation of fluid, although the tubules were not disrupted. Many glomeruli showed accumulation of fluid between the glomerular tuft and Bowman's capsule.

The edema of the stomach was the most extensive of any of the organs studied. The submucosa showed the greatest change, with wide dilatation of capillaries, arterioles and venuoles. The accumulation of fluid increased the width of the submucosa approximately 4 times, making it about twice as thick as the mucous membrane. The mucous membrane was detached from the underlying submucosa in some areas, apparently being pushed out by the fluid below. The muscle layers of the wall showed considerable edema, with separation of the muscle fibers, especially in the longitudinal group. Similar changes but less severe in degree were found in the small intestine. Some sections showed excessive accumulation of fluid beneath the serosa, tending to separate it from the muscular layer.

The ovaries showed extensive intercellular accumulation of fluid, the degree of edema being very pronounced.

The spaces along the cartilaginous supports of the gill filaments were dilated with fluid and some of the gill filament processes were swollen and distorted. The striated muscle bands at the base of the filaments were edematous with separation of the fiber bundles.

The pathology of the various organs revealed an upset in permeability with excessive accumulation of fluid giving rise to edema in all of the structures studied. Apparently the edema was more extensive in the stomach than in any of the other organs, with the greatest involvement in the submucosal layer.