EFFECTS OF COPPER ON THE CRAYFISH ORCONECTES RUSTICUS (GIRARD)

II. MODE OF TOXIC ACTION

BY

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INTRODUCTION

Waterways and reservoirs are often treated with toxic materials for herbicidal and pesticidal purposes. Copper is one of these materials. There are a number of papers documenting the acutely lethal nature of copper in aqueous solution but little is known of the toxic mechanisms in relation to aquatic life. Pollution abatement measures could be made more reasonable and intentional application of copper more controllable if more information were available on the mode of toxic action.

Previous work in this laboratory has shown that the toxic effect of copper to crayfish is dependent not only upon concentration and duration of exposure, but also on the age (or size) of the animal. Continuous flow experiments with newly hatched crayfish have demonstrated that copper interferes with growth and development at levels far below those that are acutely toxic. These experiments suggest that toxicity is not based upon the simple relationship of protoplasmic mass to ion concentration alone (Hubschman, 1966). Voegtlin, Johnson & Dyer (1925) discussed the relation of the size of the exposed organism to the lethal concentration of copper sulfate. They suggested that there are two modes of action: with high concentrations, toxicity may be due to the coagulating action on cellular proteins; at lower concentrations, it is due essentially to the disturbance of glutathione equilibrium. My preliminary experiments suggested that several toxic mechanisms are indeed operative. The mode of action at high concentrations apparently involves respiratory processes, whereas long exposure to low concentrations results in degenerative changes of certain tissues.

Jones (1942) studied the effects of copper on the amphipod Gammarus pulex (L.) and the flatworm Polycelis nigra Ehrenberg. While showing that exposure to copper did in fact result in a reduced respiration rate, he interpreted the change as no more than a “symptom of the toxic process”. He did not propose an alternative scheme for the mode of toxic action. The effects of mercury and copper

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on the marine amphipod *Marinogammarus marinus* (Leach) were studied by Hunter (1949). Although some of the results presented allow questionable interpretation (e.g., that copper is “virtually non-toxic” in concentrations below 50 mg per liter in sea water), he concludes that the toxic action of heavy metals may vary depending upon concentration. Hunter’s experiments indicate that the toxic action of copper on *M. marinus* results from interference with either the respiratory system or osmoregulatory system, or both. Corner & Sparrow (1956), working with the brine shrimp *Artemia salina* (L.), the copepod *Acartia clausi* Giesbrecht, and the barnacle *Elminius modestus* Darwin, concluded that the mode of action of copper is specific in its effect on respiratory mechanisms. Kerkut & Munday (1962) found that in *Carcinus maenas* (L.), not all tissues are equally affected by copper. Their in vitro studies indicated that in *Carcinus*, the heart and gills are most sensitive. In their experiments to determine the effects of high and low concentrations of copper in vivo, however, the copper concentrated in the hepatopancreas and “seriously impaired” the amylase activity of that organ.

The binding of copper in rat liver slices has been studied by Saltman, Alex & McCormack (1959). They suggest that the accumulation of copper is passive rather than dependent upon active transport. Their data indicate that, at low concentrations, copper is accumulated at primary sites (e.g. certain copper binding proteins, nucleic acids and their derivatives) whereas at higher concentrations, the secondary effect of copper itself (that of denaturation) leads to increased uptake at previously unavailable sites. Keilin & Hartree (1949) worked with preparations of horse heart muscle and pigeon breast muscle in vitro. They showed that copper (*2 × 10⁻³ M*) was completely inhibitory to succinate systems in those tissues.

Evidence of glycolytic and citric acid cycle pathways in crayfish tissue metabolism has been presented by McWhinnie & Kirchenberg (1962). Working with *Orconectes virilis* (Hagen), they showed that oxygen consumption of hepatopancreas homogenates is significantly increased upon the addition of sodium succinate. Similar systems are probably operative in other crayfish.

This report describes experiments conducted to determine the possible modes of toxic action of copper in the crayfish *Orconectes rusticus* (Girard). The objectives of these experiments were twofold. First, to determine the mode of action in cases of continuous exposure and thus contribute to the knowledge of the consequences of and the precautions demanded by the introduction of copper to the aquatic environment; secondly, to develop a method for early detection of heavy-metal poisoning in crayfish.

**METHODS**

**Experimental animals**

The collection, holding, acclimation, and exposure methods have been described previously in connection with the study of the acute toxicity of this metal to crayfish (Hubschman, 1966). According to Weins & Armitage (1961), oxygen consumption by crayfish varies according to the size of the experimental animal;