THE EFFECT OF IONIC COPPER ON THE OXYGEN CONSUMPTION OF GAMMARUS PULEX AND POLYCELIS NIGRA

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(With Five Text-figures)

INTRODUCTION

The oligodynamic action of copper and certain other heavy metals is a somewhat mysterious chapter in physiology. Many workers have found that solutions of copper, silver, mercury and gold are toxic to bacteria and fresh-water animals and plants at dilutions that are almost fantastic; to cite some of the writer’s results, a $4 \times 10^{-6}$ N solution of copper nitrate is fatal to Gammarus pulex in less than 4 hr., a $4 \times 10^{-5}$ N solution fatal to Polycelis nigra in 6½ hr., and a $3 \times 10^{-5}$ N solution fatal to toad tadpoles in 2 hr. The toxicity of silver and mercury is even more remarkable; $10^{-5}$ N AgNO₃ kills Polycelis in 30–40 min., and HgCl₂ is fatal to this animal at $10^{-6}$ N, 1 g. Hg in 10,000,000 c.c. water. No satisfactory explanation has been advanced for the high toxicity of these metals at great dilutions, and there has been some discussion as to whether these metals exert their poisonous effect in the colloidal or the ionic state. Earlier workers seem to have favoured the former alternative, and in support of this is the fact that colloidal gold and silver have been employed as germicides with some success. On the other hand, Freundlich & Sollner (1928) have shown that when silver foil is kept in distilled water for 3 days $2 \times 10^{-6}$ g./l. of silver goes into ionic solution, and that the poisonous effect of this solution is imitated by a silver nitrate solution of equivalent concentration. Leitner (1930) also concluded that the oligodynamic metals acted in ionic form. According to one theory, discussed by Seifriz (1936, p. 119), bacteria are killed because they become coated with a layer of metal; while Heilbrunn (1928, p. 144) states that solutions of CuCl₂ in sea water effect a coagulation of the protoplasm of sea-urchin eggs, clearly shown by centrifuge tests, even at a dilution of $10^{-5} M$.

Voegtlin et al. (1925), observing that copper sulphate and gold chloride reacted with reduced glutathione, suggested that the oligodynamic action of these metals might be due to disturbance of the glutathione equilibrium, and that the death of the organism ‘might be conceived as a special type of asphyxia’. It is now believed that copper is one of the metals whose catalytic action is an essential feature of the glutathione system, though whether this is so in the case of the organisms to which the metal is so highly toxic is uncertain. Meldrum (1934, p. 40), remarking that
nothing is known of the mechanism of oligodynamic action, also puts forward the suggestion that it is exerted by the inactivation of catalytic mechanisms, and cites the work of Wieland & Mitchell (1932) who have shown that Cu, Ag, Au and Hg retard the enzymic dehydrogenation of xanthine. Cook (1926) states that copper chloride solutions cause a rapid decline in the respiration rate of *Aspergillus* and *Nitella*. The list of substances whose toxic action is due to their power of reducing or inhibiting cellular respiration is a growing one; HCN, H$_2$S, CO and iodoacetic acid have been recognized as respiratory inhibitors (the action of the first three is fully discussed by Meldrum, 1934), and Keilin (1933, 1936) has shown that sodium azide has a physiological action very similar to that of cyanide. The possibility that the oligodynamic action of the heavy metals may be due to the inactivation or destruction of substances essential for the maintenance of cellular respiration thus appeared worthy of investigation, and prompted the present study.

**EXPERIMENTS WITH GAMMARUS PULEX**

**Method**

The technique employed for observing the changes in respiration rate occurring during the survival time will be understood from the following description of a typical experiment with $4 \times 10^{-5} N$ CuSO$_4$. Fifteen animals were placed in a 70 c.c. bottle and after two preliminary rinsings this was filled with tap water of measured oxygen content and closed with a perforated stopper. After 15 min. a sample of water was drawn off, its oxygen content measured, and the normal oxygen consumption of the animals thus determined. Two or three successive experiments usually gave results which did not differ by more than 2–3%. The bottle was then filled with the copper solution, 10 min. later a sample was withdrawn and after two rinsings the bottle was refilled with fresh solution. In this way the oxygen consumption of the animals was measured every 12 min., 2 min. of each interval being occupied with drawing off a sample, rinsing and refilling the bottle.

The animals remained in the bottle for the whole of the experiment. Filling the bottle, and drawing off a sample, took about 15 sec. To ensure consistency the filling of the bottle was started at the beginning of the 10 min. interval, and the withdrawal of the sample completed at the end of the 10 min. Water, solutions, and the bottle containing the animals were maintained at 18° C. in a thermostatically controlled electrically heated oven. In water, and during the early part of the survival time in copper solutions, the animals swam continuously and no agitation was necessary; towards the end of the survival time swimming power was lost and the bottle was gently agitated every 2 min. to avoid stratification. The time when the animals began to die (indicated by the cessation of pleopod movement) was observed, and the experiment was discontinued when half were dead.

Oxygen concentrations were measured by the Winkler method, using $N/56$ thiosulphate measured with a 5 c.c. micro-burette graduated in 1/100ths of a c.c. The presence of copper salts introduces a slight error into the Winkler method. At 0.01 $N$ CuSO$_4$ this error is about 2%, and with decrease in concentration it
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rapidly becomes inappreciable. The water used for the determination of the normal respiration rate, and for the solutions, was Aberystwyth tap-water slightly undersaturated with air at 18°C. In no experiment was the oxygen content of the water or solution permitted to fall to less than 75% saturation.

Fig. 1. The effect of 0.0002 and 0.00004 N CuSO₄ and 0.00012 N NaCN on the respiration rate of Gammarus pulex. Each plotted point records the respiration rate for the preceding time interval. Where the graphs become dotted the animals have begun to die. pH of copper solutions 6.2 and 6.4 respectively. pH of cyanide solution 6.8. Temp. 18°C.

Results

Representative results obtained with Gammarus are set out in Fig. 1. It will be noticed that the first response to the copper sulphate solutions is a slight rise in the respiration rate; this probably results from increased activity, for the animals swim furiously during the early part of the survival time. After about 35 min. in the
4×10⁻⁵ solution, or 25 min. in the 2×10⁻⁴ solution, the respiration rate falls rapidly, the animals become unable to swim, fall to the bottom of the bottle and continue feeble but regular movement of the pleopods. When the respiration rate is still 25% or more of the normal value it is noticed that some of the animals are quite inert.

A 0.00012 N solution of sodium cyanide, fatal in about the same time, rapidly depresses the respiration rate to less than 10% normal and inhibits swimming almost immediately. The respiration rate curve obtained is of quite a different type. Thus unless we are to believe that the copper ions take some time to enter the body, and then effect a sudden and very drastic reduction of the respiration rate, we must conclude that the death of the animal is not brought about by the inhibition of cellular respiration.

No further experiments were carried out with *Gammarus* and work was continued with *Polycelis nigra*.

**EXPERIMENTS WITH POLYCE LIS NIGRA**

**Method**

The technique adopted in experiments with *Polycelis* closely resembled that adopted with *Gammarus*. The number of animals used depended on the time intervals over which the respiration rate was measured. For 12 min. intervals 120-200 animals were necessary to give a reduction of oxygen concentration measurable with sufficient accuracy; for 30 min. intervals seventy animals were used, and for 2 hr. thirty sufficed. When the solutions rendered the animals incapable of ciliary locomotion the bottle was agitated gently at frequent intervals. The experiment was discontinued when the animals began to disintegrate; their disintegration appears to liberate substances into the solution which introduce a large error into the Winkler method, and an apparent rise in the respiration rate.

**Results**

The effect of CuSO₄ solutions over the concentration range 0.01-0.0004 N was studied, and a representative series of results is given in Fig. 2. It will be noticed that the 0.004 and 0.001 Cu solutions produce a considerable rise in the respiration rate, which is followed by a rapid decline. The preliminary rise appears to be due to increased activity; when the bottle is filled with tap-water the animals are mostly inactive, the majority crawl some distance up the side of the bottle and then remain still. When the water is replaced by the copper solution ciliary locomotion is instantly and completely inhibited, and the animals are thrown into a state of violent and continuous muscular movement which gradually becomes less energetic as the decline in respiration rate sets in. It appears that the cilia are at first paralysed and later destroyed, for if the animals are removed from a 0.004 N solution in 10 min. after immersion, washed, and placed in a Petri dish of tap-water, normal ciliary locomotion is almost immediately resumed; but removal in 25-30 min. results in
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failure to resume gliding and eventual death. A similar rise in respiration rate is induced by barium chloride, for this salt acts as a powerful stimulant to muscular movement. A 40% rise in respiration rate can be produced with the animals in tap-water if the bottle is agitated sufficiently frequently to keep them in a state of continuous activity, but the movement so produced mainly takes the form of active ciliary locomotion, not muscular movement.

A 0·0004 N copper solution does not inhibit ciliary locomotion; the animals glide slowly or remain quiescent during the early part of the survival time and no increase in respiration rate is observed. Later, as the respiration rate falls steadily, they become more and more inactive and eventually begin to disintegrate.

To see whether the depression of respiration rate caused by the copper solution is sufficient to account for the death of the animals we may compare the effect of

Fig. 2. The effect of 0·004, 0·001 and 0·0004 N CuSO₄, and 0·0002 N NaCN on the respiration rate of Polycelis nigra. pH of solutions 5·6, 5·8, 6·0 and 6·8 respectively. Other details as Fig. 1.
cyanide. The respiration rate curve for 0.0002 N NaCN drawn in Fig. 2 shows that in this solution the oxygen consumption rapidly falls to less than 20% of the normal value, whereas in the copper solutions the animals die when it is far above this level. And the cyanide solution is not fatal, the *Polycelis* were practically all alive in 4 days, when their respiration rate was about 16% normal, and were still capable of ciliary locomotion though their speed of gliding was reduced from the normal value of 1.7 mm./sec. to 0.5–0.8 mm./sec. The results thus appear to indicate that the death of the animals in the copper solutions does not result from the inhibition of cellular respiration, and that the depression of respiration rate observed is merely a symptom of the toxic process, not its essential feature. In a brief review of the physiological effect of toxic copper solutions Mitchell (1938, p. 296) concludes that the high
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toxicity of copper salts rests in the activity with which they combine with proteins, and that their penetration into living cells results in the precipitation of the cytoplasmic proteins as copper proteinates, and a gradual complete destruction of the entire protoplasmic structure. The writer's results appear to fit into this picture reasonably well.

Copper sulphate solutions are acid as a result of hydrolysis. The effect of hydrogen ions upon the respiration rate of *Polycelis* is shown in Figs. 3 and 4. It will be seen that hydrogen ion concentrations that are speedily fatal (2.6, 2.8) bring about a sharp decline in the respiration rate, that solutions of pH 3.0–4.0 have less effect, and that a pH of 5.0 has little action even in 24 hr. Very acid solutions are fatal too rapidly for the decline in respiration rate to be followed. This result is in agreement with the observations of Campbell (1923) who found that the injection of HCl reduced the metabolism of anaesthetized cats, and those of Haldane & Wigglesworth (1924) who state that the acidosis resulting from the ingestion, by man, of large doses of ammonium chloride seems to result in depression of the oxygen consumption. The pH of the most acid of the copper solutions used in the experiments with *Polycelis* was 5.6, and thus the hydrogen ions set free by hydrolysis do not appear to be responsible for the changes in respiration rate observed, nor is the acidity of the solution responsible for the inhibition of ciliary locomotion for at a pH of 4.4 H2SO4 has no apparent effect on the ciliary locomotion of *Polycelis*. At pH 4.0 gliding becomes erratic and intermittent, and at pH 3.4 the cilia are paralysed.

This, however, is not the only way in which hydrogen ions may enter into the picture. When heavy metal salts come into contact with living protoplasm the
precipitation of proteins by the cation results in the formation of free acid, and part of the salt's poisoning effect (at high concentrations, at least) takes the form of corrosion by the acid so formed. The nitrates of the heavy metals have a marked corrosive as well as astringent action, as their reaction with proteins liberates the highly ionized nitric acid; the acetates, citrates and tartrates, on the other hand, liberate weak acids and their corrosive is much less pronounced. (For a full discussion see Edmunds & Gunn, 1936, pp. 111-14.) Exactly how far the toxic action of heavy metals at great dilution is due to the formation of free acid within the living cells of the organism is another question which cannot be answered without further experimental evidence.

![Graph](image)

**Fig. 5.** The effect of 0.00001 AgNO₃ and 0.00015 HgCl₂ on the respiration rate of *Polycelis nigra*. pH of both solutions 6.6. Other details as Fig. 1.

**The effect of other oligodynamic metals**

Experiments with gold chloride were not successful, as the presence of this salt appears to introduce a very large error into the Winkler determination. Experiments with silver nitrate (in which glass-distilled water was used) and mercuric chloride yielded results essentially similar to those obtained with copper sulphate. Two representative results are given in Fig. 4; in each case the salt immediately inhibits ciliary locomotion and induces a preliminary phase of active muscular movement, disintegration of the animals beginning when the oxygen consumption falls to about half the normal value.
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SUMMARY

The suggestion has been put forward that the oligodynamic action of certain heavy metals is the result of the destruction or inactivation of substances essential for cellular respiration. In a study of the effect of copper sulphate solutions on the oxygen consumption of Polycelis nigra it is found that solutions of concentration 0.001-0.01 N, fatal in 2 hr. or less, induce a marked preliminary rise in the respiration rate; this appears to be due to the inhibition of ciliary locomotion and increased muscular activity. A similar increase is produced by increasing the activity of the animals by mechanical means, or by a muscle stimulant (barium chloride). Over the latter part of the survival time the respiration rate drops rapidly and disintegration of the animals begins when it falls to about 60% of the normal value. A 0.0004 N copper sulphate solution does not inhibit ciliary locomotion, does not stimulate muscular activity, and the oxygen consumption undergoes a steady decline. A 0.0002 N NaCN solution rapidly depresses the respiration rate to less than 20% of the normal value, but is not fatal, the animals surviving over 4 days. Hydrogen ions, at the concentrations resulting from the hydrolysis of the salt, have no appreciable effect on the oxygen consumption, but at lethal concentrations (pH 2.6, 2.8) effect a speedy depression. The results suggest that the depression of respiration rate observed is insufficient to account for the death of the animals, and is no more than a symptom of the toxic process.

A similar general result was obtained in experiments with silver nitrate and mercuric chloride, and also in experiments on the comparative effect of copper sulphate and sodium cyanide solutions on the oxygen consumption of Gammarus pulex.

REFERENCES