THE EFFECT OF COPPER ON THE TISSUE RESPIRATION OF THE CRAB *CARCINUS MAENAS*

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Résumé

I. — Les taux de respiration *in vitro* de quatre sortes de tissus du crabe *Carcinus maenas* ont été déterminés.

Les quantités d'O_2 consommé à 28° C sont : branchies 5,31 ; cœur 2,57 ; hépato-pancréas 0,82 et ganglion thoracique 2,77 ; les valeurs sont exprimées en mm^3 O_2/mg de poids sec/heure.

II. — Divers sels de cuivre tels que sulfate, chlorure cuivreux, chlorure cuivrique, citrate, amino-acétate, tartrate double de potassium et de sodium sont ajoutés aux tissus isolés pendant la respiration ; ils augmentent l'intensité respiratoire. Les sels les plus efficaces sont le sulfate de cuivre et le tartrate double de potassium et de sodium et cuivre.

III. — L'addition de tartrate double de potassium et de sodium et cuivre en solution 0,1 milliéquivalente provoque une diminution de la respiration de 34 % pour le cœur, 28 % pour les branchies, 6 % pour l'hépato-pancréas et 3 % pour le ganglion thoracique.

IV. — L'addition d'une solution 100 milliéquivalente provoque pour tous les tissus testés une diminution de plus de 50 % du taux normal de respiration.

V. — Les résultats sont discutés du point de vue de la toxicité des solutions de cuivre pour l'animal.

INTRODUCTION

Locke in 1895 found that metal contaminants in "distilled water" seriously affected the use of such water in keeping animals alive. Thus *Tubifex* died within eighteen hours and tadpoles became motionless after six hours. Locke showed that similar results could be obtained by adding bright sheet copper to glass distilled water and therefore suggested that the toxic agent might be copper ions.

Cahiers de Biologie Marine
Tome III - 1962 - pp. 27-35.

Since Locke's time many other research workers (Naegli 1903: Shaw-Mackenzie 1917; Voegtlin, Johnson and Dyer, 1925; Meldrum and Dixon 1930; Mitchell 1948; Saltman, Alex and Mc Cornack 1959) have confirmed the toxic action of very dilute solutions of copper salts. This has its practical application especially in the control of marine fouling (Ray 1959), but until recently there has been little understanding of the mechanisms by which copper exerts its toxic action.

The experiments reported in the present paper describe the effect of various copper salts at specified concentrations on the tissue respiration of the common shore crab *Carcinus maenas*, the concentration at which copper becomes effective, and the differential sensitivity of the various crab tissues.

Methods and Procedure.

Fresh adult *Carcinus maenas* were obtained daily from the Solent. On arrival in the laboratory, their tissues were dissected and the respiratory rate determined by Warburg manometer. The saline—2.5 ml in each manometer flask—had the composition given by Pantin (1946) and its pH was adjusted to 7.8 by addition of sodium bicarbonate. The pH of the freshly drawn blood under oil of *Carcinus maenas* was determined by glass electrode and shown to be 7.76 $\pm$ 0.02.

The manometers were set up and equilibrated for 15 mins. Respiration measurements were taken every ten mins and each experiment lasted for three hours at constant shaking rate of 130/min. The manometer bath temperature was set at 28°C. Each experiment consisted of a minimum of ten manometers.

In the experiments in which copper salts were added to the tissue saline, various copper solutions were used ; the main problem being the interaction of copper with the salts in the saline. This was particularly acute when high concentrations (10 m. eq.) were used, and here only organic complexes such as copper sodium potassium tartrate remained in a clear solution.

Since the volume of saline used in the main chamber of the flask was 2.5 ml, and the volume of saline plus copper in the side arm 0.5 ml, the copper saline on tipping would be diluted 6 times. The copper solution placed in the side arm to give 1 m. eq. on tipping was 74.913 mg Cu/100 ml. On tipping and dilution to 6 times, it was 3.177 mg Cu/100 ml. As the flask contained only 3 ml after tipping, this would contain 95.31 μG of Cu. As a rough approximation one could say that the flask containing 3 ml of 1 m. eq. Cu, contained 0.1 mg of copper.

RESULTS

Table I shows the effect of adding various copper salt solutions on the respiration of isolated gill tissue. The salts used were copper sulphate, cuprous chloride, cupric chloride, copper sodium potassium tartrate and copper glycine. The salt solutions were made up so

that when tipped the final volume in the respiration flask main chamber would contain 1 m. eq. of copper. Care was taken to ensure no change in the pH of the saline from 7.8.

Gill tissue is interesting amongst crustacean tissues in that it respires at a faster rate some time after isolation than it does immediately after isolation. Thus in the control example quoted in Table I, if the rate over the first hour after isolation is taken at 100%, the rate at the end of the second hour was 117% and the rate at the end of the third hour, 128%. Other tissues such as hepatopancreas, thoracic ganglion and heart did not show this effect; the respiration rate tending to fall off slowly with time. The average in QO_2 $\mu l/O_2$/mg dry weight/hr. at 28°C for the four different tissues recorded over the first hour in saline free from copper was as

TABLE I

Gill tissue

	QO_2 per hour			% QO_2 per hour		
	1st hr	2nd hr	3rd hr	1st hr	2nd hr	3rd hr
Normal rate - saline tipped	4.44	5.23	3.7	100	117	128
$CuSO_4$ $5H_2O$ 1 m. eq.	5.53	4.99	5.20	100	90	93
$CUCl_2$ $2H_2O$ 1 m. eq.	5.39	8.48	6.09	100	101	112
CUCl 1 m. eq.	2.99	3.19	3.55	100	106	118
$KNAC_4H_4O_6$ $4H_4$ + $CUSO_4$ $5H_2O$ 1 m. eq.	5.31	5.10	5.25	100	96	98
CU $(C_2H_4NO_2)_2$ H_2O 1 m. eq.	5.25	4.95	5.17	100	94	98

follows: gill 5.31; heart 2.57; hepatopancreas 0.82: thoracic ganglia 2.77.

The addition of copper to gill tissue decreased the rate of respiration. The effect was least in the case of cuprous chloride ; next in effect was cupric chloride, then copper-sodium-potassium tartrate and copper glycine, with copper sulphate the most effective in depressing respiratory rate.

Figure 1 shows the effect of adding different concentrations of copper-sodium-potassium tartrate on the respiration of isolated gill and heart. The manometers were set up and readings taken every ten minutes. After the first hour the manometers were tipped and the copper solutions or control saline added to the reaction vessel. The addition of copper caused a decrease in the respiration rate, the effect being more marked with the 10 m. eq. solution than with the 1 m. eq. solution. Experiments in which the 10 m. eq. of sodium potassium tartrate and sodium sulphate were added, showed no such marked effect on respiration ; the rate being similar to those of the saline controls. Thus we may conclude that the effect was due to

copper and not due to the sodium, potasium, tartrate complex or sulphate ions.

The effect of copper concentration is more clearly seen from Tables 2 A, B, C and D, where the respiration rate is given for four

TABLE 2 A

Gill

	QO_2 per hr			% QO_2 per hr		
	1st hr	2nd hr	3rd hr	1st hr	2nd hr	3rd hr
Normal rate - saline tipped	4.77	5.95	6.04	100	124	126
+ 0,1 m. eq. Cu Tartrate	5.31	5.10	5.25	100	96	98
+ 0.5 "	5.98	4.51	4.58	100	75	76
+ 1.0 "	6.25	4.16	3.94	100	66	63
+ 5.0 "	6.10	3.58	3.10	100	58	50
+ 10.0 "	6.73	3.95	2.9	100	52	43
+100.0 "	5.68	2.98	2.37	100	52	41
Control + 10 m. eq. NaK Tartrate + 10 m. eq Na_2SO_4	4.61	4.63	4.95	100	100	107

TABLE 2 B

Heart

	QO_2 per hr			% QO_2 per hr		
	1st hr	2nd hr	3rd hr	1st hr	2nd hr	3rd hr
Normal rate - saline tipped	2.06	1.83	1.54	100	89	74
+ 0,1 m. eq. Cu Tartrate	2.30	1.93	1.52	100	84	66
+ 0.5 "	2.80	2.09	1.53	100	74	54
+ 1.0 "	2.71	1.95	1.26	100	71	46
+ 5.0 "	2.39	1.62	0.65	100	67	27
+ 10.0 "	2.37	1.55	0.81	100	65	35
+100.0 "	3.36	1.21	0.64	100	36	19

crustacean tissues, both as QO_2, and also as a percentage change from QO_2 for the first hour.

Table 2 A indicates first of all the respiration rate of gill tissue in which saline was tipped as the control. The QO_2 in μl O_2/mg

dry weight/hr at 28°C was 4.77 for the first hour; 5.95 over the second hour and 6.04 over the third hour. This is then presented as a percentage change in QO_2 giving 100% over the first hour, 124% over the second hour, and 126% over the third hour.

TABLE 2 C

Thoracic ganglia

	QO_2 per hr			% QO_2 per hr		
	1st hr	2nd hr	3rd hr	1st hr	2nd hr	3rd hr
Normal rate - saline tipped	3.16	2.99	3.07	100	95	97
+ 0,1 m. eq. Cu Tartrate	2.48	2.60	2.42	100	105	97
+ 0.5 "	2.56	2.56	2.42	100	100	94
+ 1.0 "	2.80	2.63	2.77	100	94	82
+ 5.0 "	2.73	2.22	1.56	100	83	57
+ 10.0 "	2.89	2.27	1.71	100	78	59
+100.0 "	2.79	1.71	0.80	100	61	29

TABLE 2 D

Hepatopancreas

	QO_2 per hr			% QO_2 per hr		
	1st hr	2nd hr	3rd hr	1st hr	2nd hr	3rd hr
Normal rate - saline tipped	1.11	0.85	0.86	100	75	77
+ 0,1 m. eq. Cu Tartrate	0.98	0.75	0.92	100	76	94
+ 0.5 "	1.00	0.88	0.83	100	88	83
+ 1.0 "	1.07	0.97	0.84	100	91	78
+ 5.0 "	1.07	1.01	0.95	100	94	92
+ 10.0 "	0.83	0.84	0.65	100	100	78
+100.0 "	0.68	0.44	0.18	100	64	26

Copper-sodium-potassium tartrate solutions were added after one hour so that the tissues were exposed to copper concentrations ranging from 0.1 m. eq. to 100 m. eq. Care was taken to keep the mass of tissue comparable and constant throughout the experiments.

The higher concentrations of copper all caused a greater decrease in respiration rate, though it is worth noticing that the decrease was not directly proportional. Thus a ten times increase from 0.1 to

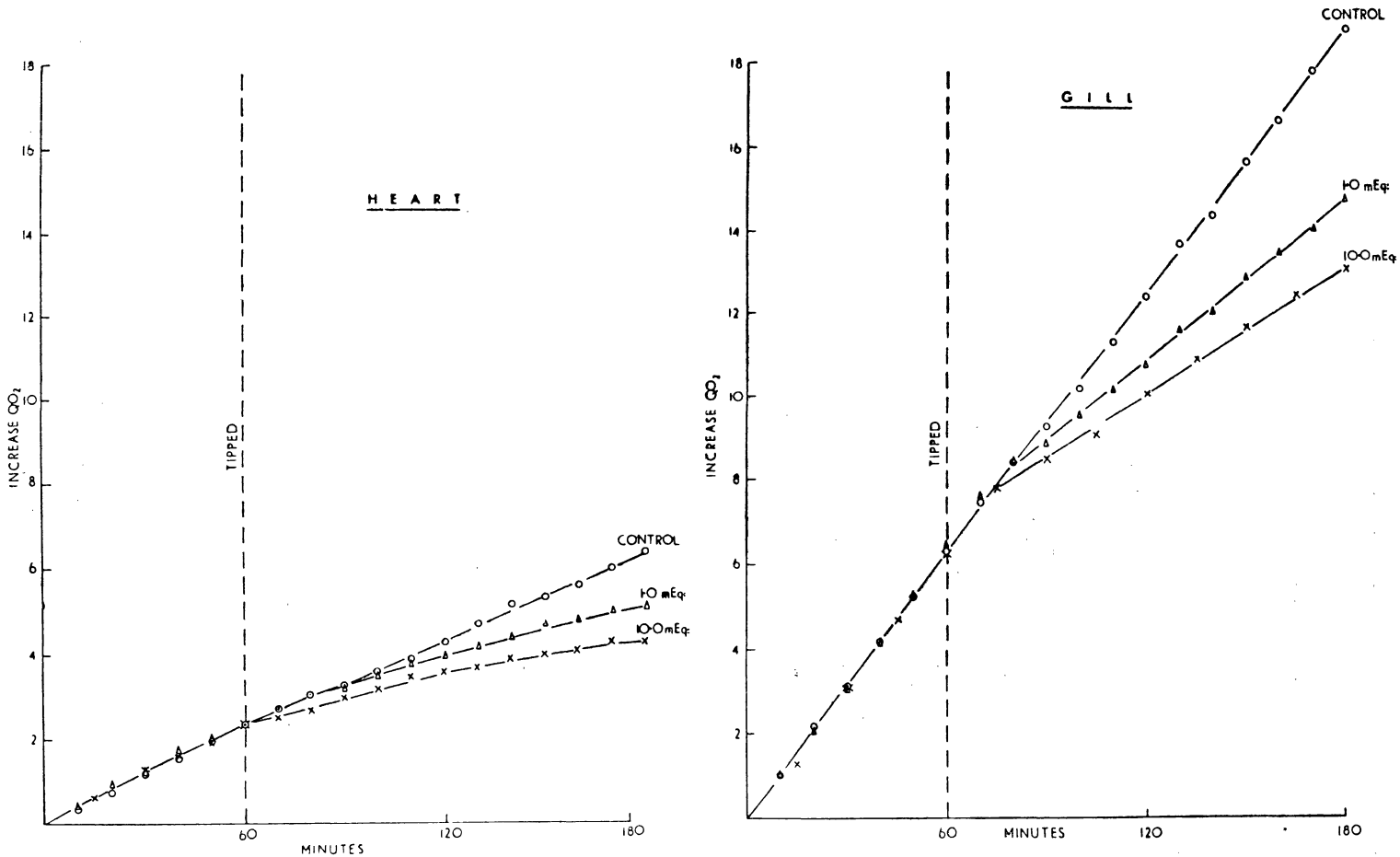

FIG. 1. — The effect of adding copper salts on the respiration of isolated gill and hearts. The amounts of copper added are indicated in terms of m. eq. of Cu/ml in the final flask solution.

1 m. eq. caused a drop from 98% to 63% (35%). An increase from 1.0 m. eq. to 10 m. eq. caused a change from 63% to 43% (20%) and an increase from 10 m. eq. to 100 m. eq. only a 2% fall from 43% to 41%.

A similar table is presented in 2 B for isolated hearts, 2 C for isolated thoracic ganglia, and 2 D for hepatopancreas. In general the higher concentrations of copper had a greater inhibitory effect on tissue respiration. But it will be noted that the hepatopancreas gave less consistent results than the other three tissues. Thus though the 100 m. eq. copper solution had a greater effect on the hepatopancreas tissue respiration than did the 10 m. eq. solution ; the 10 m. eq. solution had less effect than the 5 m. eq. solution and had a similar effect to the 1 m. eq. solution. This variation in the property of the isolated hepatopancreas might be explicable in terms of heterogeneity of the tissue.

It appears that the various crab tissues have differing susceptibility to the addition of the same strength and type of copper solution. Thus the heart is most sensitive; addition of a 0.1 m. eq. solution of copper-sodium-potassium tartrate caused a drop of 34% in the respiration rate measured as QO_2 whereas the same solution caused a drop of 28% in the gills, 6% in the hepatopancreas and only 3% for the thoracic ganglia.

DISCUSSION

The first problem in studying the effect of copper on tissue respiration, was the choice of a suitable copper salt.

Of the various 0.1 m. eq. copper solutions tested, copper sulphate was the most effective, causing a 35% decrease in the respiration of gill tissue. Copper sodium potassium tartrate and copper glycine came next, with a 31% decrease, cuprous chloride a 16% decrease, whilst cupric chloride caused only a 10% decrease. The objection to using copper sulphate throughout all the experiments was that in stronger solutions it reacted with the experimental saline in which the tissues were maintained and caused a precipitale. The only copper salt that gave a clear solution in quite high concentrations (100 m. eq.) in saline was copper-sodium-potassium tartrate and it was practically as effective as copper sulphate in affecting gill respiration.

The results described in the previous part of the paper indicate that copper salts at quite low concentrations can reduce the tissue respiration of the isolated crab tissues. Not all the tissues are equally affected; the heart and the gills being the most sensitive. If these results can be extrapolated to the tissues in the intact animal, then this differential sensitivity may be of considerable importance, since the gills would be one of the first tissues to come into contact with any copper salts placed in the environment. Once the copper salts had entered into the haemal system through the gills, they would be bound to reach the heart.

It is of considerable interest that the effects of copper on the respiration of the isolated heart agree very closely with its effect on the beat of the isolated heart. Experiments that have been carried out in our laboratory show that the spontaneous contractions of the isolated heart are regularly inhibited by concentrations of copper of 25 μg/ml. A similar concentration of 1 m. eq. (31 μg/ml) resulted in a metabolic depression of 34% of the QO_2 of the heart. This would suggest that the copper does not just affect the oxidative metabolism of the heart but also interferes with the mechanism of rhythmic contraction.

These concentrations of copper though low, are still higher than those required to kill intact crustaceans after prolonged immersion. Clarke (1947) showed that *Balanus balanoides* and *Balanus eburneus* could be killed in two days by a solution containing .0006 mg. Cu/ml, and Hunter (1950) found that it required 0.01 mg. Cu/ml to kill *Marinogammarus marinus*. On the other hand we have found from mortality/concentration curves of *Carcinus maenas* that the concentration of copper-sodium-potassium tartrate required to bring about 100% mortality within 12 hours is 130 mg%, though solutions of 20 mg% would cause death in 50 hours. In such animals the copper tended to become concentrated in the hepatopancreas, and furthermore, even in crabs containing sublethal concentrations of Cu the amylase activity of the hepatopancreas was seriously impaired. (Kerkut, Moritz and Munday 1962.)

It is not possible to state the precise cause of death when a crustacean is placed in a solution containing a lethal amount of copper. However, the experiments described in the present paper indicate that the gills and heart respiration are particularly affected by copper. We know that the heart beat is affected and also that the hepatopancreas will accumulate copper, which in turn affects the efficiency of the digestive amylase.

Crabs placed in sea water containing copper accumulate the copper on the slime on the gills. Furthermore, if copper salts are injected into the blood system of *Carcinus* and the animal quickly dissected, the heart will be found to have accumulated much of the injected copper.

All these effects will seriously impair the chances of the animals surviving and indicate possible causes of the toxic action of copper solutions on crustaceans.

We are indebted to The Director of Navy Contracts of H.M. Admiralty and Dr. C.D. Lawrence, Superintending Scientist of the Central Dockyard Laboratory for financial support on this project.

Summary

1. The *in vitro* respiration rates of four tissues from the crab *Carcinus maenas* were determined. The QO_2 at 28°C are gill 5.31; heart 2.57; hepatopancreas 0.82; and thoracic ganglia 2.77; the values being in μl O_2mg dry weight-hr.

2. Various copper salts such as copper sulphate, cuprous chloride, cupric chloride, copper citrate, copper glycine, and copper-sodium-potassium tartrate were added to the isolated respiring tissues and found to increase the rate of respiration. The most effective were copper sulphate and copper-sodium-potassium tartrate.

3. Addition of 0.1 m. eq. of copper-sodium-potassium tartrate caused a 34% decrease in respiration of the heart, 28% in the gills, 6% in the hepatopancreas and 3% for the thoracic ganglia.

4. Addition of 100 m. eq. caused tissue respiration for all tissues tested to be less than 50% of the normal rate.

5. The results are discussed in terms of the toxicity of copper solutions on the whole animal.

REFERENCES

CLARK, G.L., 1947. — Poisoning and recovery in barnacles and mussels. *Biol. Bull. Woods Hole.* 92, *pp.* 73-91.

HUNTER, W.R., 1950. — The poisoning of *Marinogammarus marinus* by cupric sulphate and mercuric chloride. *J. Exp. Biol.* 26, *pp.* 113-24.

KERKUT, G.A., MUNDAY, K.A., and MORITZ, P., 1962. — The effect of copper ions on the action of *Carcinus* amylase (In preparation).

LOCKE, T.S., 1895. — On a supposed action of distilled water as such, on certain animal organisms. *J. Physiol.* 18, *pp.* 319-331.

MELDRUM, N.U., and DIXON, M., 1930. — The properties of pure glutathione. *Biochem. J.* 24, *pp.* 472-96.

MITCHELL, P.H., 1948. — Textbook of General Physiology. *McGraw Hill. London and New York* (Chapter 4; Inorganic constituents of living matter).

NAEGELI, C., 1903. — Uber oligomynamische Erscheinungen in lebenden Zellen. *Neue. Denk. Schweiz. Gesell.* 34, *pp.* 1-15.

PANTIN, C.F.A., 1946. — Notes on microscopical technique for zoologists. *Cambridge University Press.*

RAY, D.L., (ed) (1959). — Symposium on Marine Boring and Fouling organisms. *Washington University Press.*

SALTMAN, P., ALEX, T., and MC CORNAK, B., 1959. — The accumulation of copper by rat liver slices. *Arch. Biochem. Biophys.* 83, *pp.* 538-47.

SHAW-MACKENZIE, J.A., 1917. — Toxic action of copper compounds of amino acids on protozoa. *J. Physiol.* 51, *pp.* iii-iv P.

VOEGTLIN, C.H., JOHNSON, J.M., and DYER, H.A., 1925. — Protoplasmic action of copper and gold. *Proc. Nat. Acad. Sci.* 11, *pp.* 344-345.