

Summary. Leghorn cockerels were fed for 20 months on diets containing either no supplement, whole egg powder, egg oil equivalent to that contained by egg powder or these diets in combination with corn sterols, the latter added in amounts equivalent to that supplied by 5% corn oil. An additional group was given egg powder for 12 weeks and then placed on the control ration for the remainder of the experimental period. The inclusion of corn oil sterol retarded the atherogenesis that was accentuated by the addition of egg powder or egg oil. Total fat deposition in the abdominal section of the aorta was reduced in the groups fed the sterols. The brief exposure to egg powder did not leave lasting effects on those animals that were changed over to the control diet. Egg oil appeared to be similar in its atherogenicity to egg powder. Sterol excretion patterns suggested that the beneficial effect of the corn sterols was not due to interference with cholesterol absorption alone.

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Effects of Copper Ion on Oxygen Uptake by Planktonic Cyprids of the Barnacle, *Balanus amphitrite niveus*.* (28383)

FRANCIS J. BERNARD AND CHARLES E. LANE

Institute of Marine Science, University of Miami, Miami, Fla.

Copper ion is absorbed by planktonic cyprids of *Balanus amphitrite niveus* mainly through the surface epithelium of the thorax (1), and is excreted into the lumen of the gut in certain regions by the lining epithelium. Concentration and storage of copper in specialized intracellular loci, in the cypris, suggest a role for this ion in normal development. Because it appeared to be involved in metabolism and because of its widespread use in antifouling coatings, the effect of various concentrations of copper ion on oxygen uptake by barnacle cyprids was evaluated.

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Materials and methods. Isotonic solutions containing 0.5, 1, 5, 10, 20, and 50 mg of copper ion per liter were made by adding appropriate volumes of a standard 500 mg Cu⁺⁺/l solution to Millipore-filtered Gulf Stream sea water. Solutions of 100, 200, 500, and 1,000 mg/l were made by dissolving cupric acetate directly in Gulf Stream water. At higher copper concentrations it was necessary to lower the pH slightly to dissolve sufficient copper acetate. The higher concentrations are well above those ever present in nature. Individual animals were studied in Kirk differential capillary respirometers(2). Oxygen consumption of individual animals was determined at 23°-25°C during a one-hour period. Values for 10 animals which survived this treatment and did not attach to the vessel wall during the period

TABLE I. Effects of Copper on Oxygen Uptake by Barnacle Cyprids.

Cu ⁺⁺ mg/l	μ l O ₂ /hr	S.D.*
.005	4.23	.49
.5	7.13	2.73
1	5.83	1.15
5	4.20	1.50
10	3.10	1.31
20	2.39	.83
50	2.10	1.05
100	2.61	.46
200	1.55	.33
500	.84	.25
1000		

* Standard deviation.

of observation were averaged for each concentration of copper (Table I).

Results. Copper concentration in normal sea water is about 0.005 mg/l(4). When this concentration is increased to 0.5 mg/l, oxygen uptake of barnacle larvae is elevated. Further increases in the amount of copper ion, however, up to 500 p.p.m., resulted in a steady decline in the quantity of oxygen used by the respiring cyprids (Fig. 1). For copper concentrations between 0.5 and 10 mg/l, oxygen uptake was an inverse exponential function of concentration. Between 20 and 500 mg/l the relationship is also linear, but shows a more abrupt rate decline with increased concentration than did the lower levels (Table I).

If continuing oxygen consumption is the criterion for life, there is no great variation in survival of cyprids between 0.5 and 500 mg/l copper ion. At 1000 mg/l survival rate is drastically reduced. Survival time (Table II) is markedly reduced between 100 and

200 mg Cu⁺⁺ per liter. Clarke(3) and others have equated motility with survival, suggesting a lower lethal concentration than our oxygen uptake measurements support. We have noted that activity is reduced above 50 mg/l and that no swimming or antennal movements occur above 100 p.p.m. It seems apparent, therefore, that attachment is prevented by a lower copper concentration than that required to kill cyprids.

The variability in oxygen uptake which is especially high in animals exposed to copper concentrations between 0.5 and 10 mg Cu⁺⁺/liter may reflect the presence of more than a single barnacle species in our cypris collections. The rearing experiments essential for resolution of this possibility are being conducted.

TABLE II. Effects of Copper on Survival of Barnacle Cyprids.

Cu ⁺⁺ in mg/l	% survival	Avg time of death (min)
.005	100	—
.5	80	51
1	77	63
5	91	80
10	91	30
20	91	80
50	83	73
100	77	95
200	71	18
500	83	22
1000	30	24

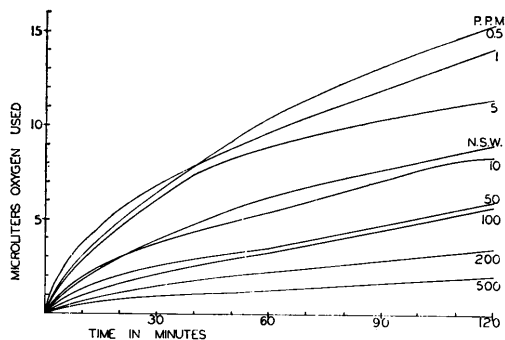


FIG. 1. Oxygen uptake by *B. amphitrite niveus* cyprids in the presence of varying concentrations of copper. "N.S.W." is normal sea water. Curves show avg uptake by 10 animals.

Discussion. These results suggest that the toxicity of the cupric ion in barnacle cyprids is the resultant of several interrelated metabolic responses, some of which are stimulated by copper and others depressed. Generally, cyprids appear to adapt to each copper concentration by adjusting metabolism to such a level that no more copper is absorbed through the respiratory surfaces than can be excreted. The enhanced oxygen uptake induced by copper ion occurred chiefly at low copper concentrations. The maximum response of cyprids occurred at 0.5 mg/l, when oxygen uptake exceeded the normal by more than 150%. Direct examination of specimens in appropriate containers suggests that the augmented rate of oxygen uptake in sublethal concentrations of copper proba-

bly reflects increased motor activity by the cyprids.

Changes in rate of oxygen uptake by barnacle cyprids reflect the energy cost of active transport of copper ions across excretory membranes of the cells of the intestinal epithelium. Earlier histochemical studies (1) suggested that copper ion is normally excreted by this route.

The progressive decrease of oxygen uptake as larger amounts of copper are added to the environment correlates as well with reduction of motor activity. Examination of a few individuals at each concentration revealed that duration of periods of swimming activity decreased progressively as more copper was added, except at very low concentrations. By 100 p.p.m. observable activity stopped immediately after immersion and the cyprid

shell remained tightly closed.

Summary. Oxygen uptake by planktonic cyprids of *Balanus amphitrite niveus* was elevated by addition of 0.5, 1, and 5 mg of copper ion per liter of sea water. Concentrations from 10 to 500 mg/l progressively depress oxygen absorption. Analysis of individual patterns relates increased oxygen consumption to increased motor activity and energy cost of active transport.

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Effect of Urease Immunity upon Rats with Controlled Vitamin A Intakes* (28384)

L. H. HARBERS, R. F. HENDRICKSON, H. N. BASS AND W. J. VISEK

Department of Pharmacology, University of Chicago

It has been reported that ammonia production due to bacteria in the gastrointestinal tract is depressed in animals fed levels of antibacterial agents known to stimulate growth(1,2). Of the various ammonia producing systems present in the gastrointestinal tract, urea hydrolysis is one which has been inhibited by antibiotics(2,3,4,5), sulfonamides(6), certain organic arsenicals(2), cyclic urea derivatives(7,8) and immunity to crystalline jackbean urease(9). Stimulation of growth concurrently with suppressed gastrointestinal ureolytic activity has been observed in both avian and mammalian species(2,8).

Present evidence indicates that animals immunized with urease excrete antiurease in the feces and urine(10). Presumably the

antibody enters the gastrointestinal lumen where it cross-reacts with urease(s) which is predominately of bacterial origin. Such immunity has suppressed *in vivo* C¹⁴ urea breakdown and has yielded antisera which depressed urease activity in homogenates of gastrointestinal contents from non-immunized animals(2). Unpublished data from this laboratory show that ammonia concentration is lower in the gastrointestinal tracts of immunized animals than in non-immunized controls.

Recently Guarrant(11) demonstrated increased growth in rats fed a diet containing limited amounts of Vit. A and supplemented with antibiotics. A similar approach has been used in these studies to investigate this response of rats to immunization with jackbean urease under conditions where dietary antibiotics have produced an increase in growth. Reported herein are 2 experiments with rats immunized with crystalline jackbean urease and fed the USP Vit. A Assay

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