Injurious Effect of Air-saturated Water on Certain Invertebrates

It is generally accepted by zoologists as a law of Nature that aquatic animals benefit by as much dissolved air as they can get. Consequently, after a catch is brought home from pond or shore the water is if possible aerated. Yet it is known, especially from the classical work of Juday¹, that whereas the oxygen content of the deep water of certain lakes falls very low and remains so for several months in summer, there is an active invertebrate fauna in and above the mud. It has never been suspected, however, that dissolved oxygen at atmospheric partial pressure, that is, in fully aerated water, might actually be harmful to certain mud dwellers in pond and sea; yet we have found this to be so.

Experiments have now been made in which the survival-time was compared, in water at two different degrees of aeration, of aquatic invertebrates that live in or near the surface of mud. In each experiment, through one sample of water, containing animals, air was bubbled continuously for several weeks, and through another a mixture of one part of oxygen to twenty-four parts of nitrogen, giving water approximately one-fifth aerated. The following animals survived longer in the less well-aerated water: the larvæ of *Chironomus* and of *Anatopynia* (another chironomid midge), *Heterocypris* (an ostracod), young *Tubifex* and young *Scoloplos* (a polychæte worm). The following animals lived equally well in one-fifth aerated and in fully aerated water: *Herpetocypris* (another ostracod), adult *Tubifex*, young *Arenicola*, adult *Planorbis*, *Physa* (another pond snail), *Pisidium* and *Phaenocora* (a rhabdocæle worm). The only animals which survived longer in fully aerated than in poorly aerated water were *Sabella*, young *Planorbis* and young *Limnaea*.

Not only did a number of species survive longer in poorly than in well-aerated water, but *Chironomus* larvæ, young *Tubifex* and young *Scoloplos* grew bigger in the poorly aerated water. In addition, although fewer young *Planorbis* survived in poorly aerated water, nevertheless they grew bigger in it, and *Phaenocora*, which survived equally well in well-aerated and poorly aerated water, grew bigger in the latter.

A full account of this work, in which specific names will be given, is to be published in the *Proceedings of the Royal Society*.

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July 9.

¹ Juday, C., *Trans. Wis. Acad. Sci. Arts Lett.*, 16, 10 (1908).

Chlorophyllide, the Probable Precursor of a Growth Inhibitor

WHEN investigating the production of growth substances by *Chlorella vulgaris* var. *viridis*, difficulties were met which were ascribed to a growth-inhibiting substance, possibly Pratt's 'chlorelline'. *Staphylococcus aureus* proved to be a suitable test object for the growth inhibition. Small pieces of filter paper or agar slices containing concentrated and dried crude ether extracts of *Chlorella* were placed on agar plates seeded with bacteria. After 2–5 hr. of diffusion of the extract, and incubation of the

bacteria at 37° C., clear inhibition zones were visible around the filter paper or agar slices.

When trying to separate the supposed chlorelline from the growth-promoting substances by shaking ether extracts with dilute hydrochloric acid or sodium bicarbonate, the chlorelline always disappeared from the alkaline as well as from the acid solutions, and also from any remaining ether extract. It was found that light—daylight and red light—inactivated the chlorelline during the working up of the extracts. Green light was inactive, so the manipulations were carried on in dim green light.

Bacillus subtilis proved to react differently from *S. aureus* with chlorelline. In the first place, the inhibition zones were larger than those of *S. aureus*. Secondly, light did not always inactivate the antibiotic action, and thirdly, after the plates had been developed, the inhibition zones expanded during several days in diffuse daylight.

This suggested that chlorelline consists of more than one component. The spectral activity indicating some relation with the chlorophylls, a mixture of benzene, petroleum ether and acetone (10:2.5:2), was used as a solvent for the separation of the components by paper chromatography. The ether extract was spotted on a paper strip (Whatman No. 4, buffered at pH 6, stored above sulphuric acid) and the ascending method was used. After drying, the paper strips were tested on agar plates seeded with bacteria. The plates received dim light in the incubator. *S. aureus* showed one zone of inhibition ($R_F = 0$) around the spot of the chlorophyllides on the strip; *B. subtilis* showed three zones of inhibition: (a) $R_F = 0$, expanding after development; (b) $R_F = 0.60$, just below the xanthophyll; (c) $R_F = 0.95$ at the same spot as the carotene.

Being mainly interested in the zone with $R_F = 0$, because of its expansion, we stopped the investigation of the two other inhibitors, which are only active on *B. subtilis*, for the time being.

We first examined the later expansion of the inhibition zone with *B. subtilis*, and found that this is due to the fact that *B. subtilis* can contract in spore form; *S. aureus* cannot do this.

When testing whether light is responsible for the expansion on the *B. subtilis* plates, we obtained varied results. Plates of *B. subtilis* and of *S. aureus* supplied with dried ether extracts on filter paper were incubated immediately in the light (daylight and red light) and in the dark (green light) respectively. In the dark no inhibition was found whatsoever and in the light scarcely any.

After testing several possible explanations with negative results, we came to the next hypothesis, namely, that there is a precursor of the inhibitor proper which is soluble in ether and in water. This precursor would diffuse into the agar plates and be transformed by radiation into an inhibitor which cannot diffuse. Experiment proved that this explanation is the correct one.

The extract was allowed to diffuse from the filter paper into water. The solution formed was tested by means of the cup test on plates with *B. subtilis*. All manipulations were carried out in green light. After incubation in the dark, no zones were present; but after a few hours exposure to daylight clear zones appeared. Controls, remaining in the dark, continued to show no inhibition. Also with *S. aureus* the inhibitor could be demonstrated, provided that the plates were illuminated only after the diffusion of the precursor had taken place. The zones produced