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Studies on the resistance of marine bottom invertebrates to oxygen-deficiency and hydrogen sulphide

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Abstract

Oxygen-deficient and H_2S-containing marine areas are characterized by a decline in the number of species. In laboratory experiments with bottom invertebrates from various biotopes of the North Sea and the Baltic, comparative measurements of the resistance to oxygen-deficiency alone, and to the simultaneous presence of H_2S, were carried out. The resistance values obtained show relations to the substratum on which the species naturally occur. The resistance to H_2S is greater in those macrofauna species which show higher survival rates under oxygen-deficiency. Further experiments with isolated tissues demonstrate that the species specific differences in resistance occurring in whole animals are already based on the cell metabolism. In general, oxygen-deficiency and simultaneous presence of H_2S were endured better in cold than in warmth, and at somewhat reduced pH-values (around 7). The dependence of this resistance on the salinity was only minimal in euryhaline species.

Introduction

Marine habitats with oxygen-deficiency offer, as a rule, only few possibilities for existence to marine animals. Especially among the lower invertebrates, however, there are a few representatives of different systematic groups (protozoans, rotifers, gastrotrichs, turbellarians, nematodes, polychaetes, oligochaetes, lamellibranchs, gastropods, crustaceans, tardigrades) which show a relatively high resistance to oxygen-deficiency. This can be concluded from a large number of ecological and physiological observations (for pertinent literature consult von Brand, 1946 and "Literature cited" of the present paper).

In the sea, the disappearance of oxygen is often correlated with the formation of hydrogen sulphide: When oxygen is completely absent, a reduction of nitrates becomes initially connected with the bacterial break-down of organic materials. When nitrates and nitrites have been reduced, this is followed by a corresponding reduction of sulphates, also caused by bacteria; this in turn leads to the formation of sulphides and H_2S. In addition, smaller amounts of hydrogen sulphide are formed by the putrefaction of protein (ZoBell, 1946; Kusnezow, 1959; Wood, 1965; Richards, 1965). Especially soft sea-beds, which are often poorly aerated (silt, slime, mud), are rich in sulphides and H_2S.

The knowledge of the degree of resistance to oxygen-deficiency is, therefore, not sufficient for assessing the capacities of marine bottom invertebrates to survive in such habitats. One must also study their reaction to hydrogen sulphide. Hence, we examined, comparatively, not only, the resistance of different marine bottom invertebrates to oxygen-deficiency, but also to H_2S. For further analysis, the cellular resistance in marine lamellibranchs to both factors was studied. The first results are presented in this publication. The investigations will be continued.

Pertinent literature

Waters poor in oxygen occur in all oceans (Wattenberg, 1929, 1939; Thomsen, 1931; Sverdrup, 1938; Sverdrup et al., 1946; Richards, 1957). In oceans, intermediate water layers, poor in oxygen, can be produced if oxidation processes (respiration, destruction of organic matter) exceed the rate of the oxygen supply through lateral exchange. Also in many subsidiary seas, such as the Baltic Sea (Segerstrale, 1957; Fonselius, 1962), and the Black Sea (Nikitine and Malm, 1934; Caspers, 1957; Zenkevitch, 1963), oxygen-deficiency can occur especially in greater depths. In some Norwegian fjords, a complete lack of oxygen may be caused by an excessively small exchange of the waters (Brongersma-Sanders, 1957).

In sea-water which is poor in oxygen, hydrogen sulphide is formed via reduction of sulphates and putrefaction of proteins. Fonselius (1962) found as much as 25 μgA H_2S-S/l in the Gotland-Deep. Almost throughout the Black Sea, hydrogen sulphide occurred below depths of 150 to 200 m (Zenkevitch, 1963). With increasing depth the concentration of hydrogen sulphide increases; at about 2000 m it reaches about 7 mg/l. Small amounts of H_2S (0.04 to 0.10 ml/l) occur also in the N.E. part of the Arabian Sea and in the N.W. part of the Gulf of Bengal at 100 to 1000 m (Ivanenkov and Rozanov, 1961). Thamdrup (1935) found as much as 6.13 mg H_2S/l in the soft bottom of North Sea mud flats.

For many animals, lack of oxygen or presence of hydrogen sulphide proves fatal. Marine regions poor in oxygen are, therefore, characterised by the presence of strikingly few species (Segerstrale, 1957; Caspers, 1957). In the oxygen-deficient bottom of the Gotland-Deep, only *Scoloplos armiger* in the mud, and *Pontoporeia femorata* and *Terebellides strömii* on loamy bottoms, were found (Hessle, 1924).

Demel and Mulicki (1954) recorded in the stagnating, O_2-deficient deeper waters of the Bornholm Basin, (O_2-content around 2.1 to 2.8 ml/l) at a depth of around 70 to 90 m, the following species: *Lineus ruber, Astarte borealis, Macoma calcarea, Scoloplos armiger, Harmothoe sarsi, Diastylis rathkei* and *Priapulus caudatus.* In 1964, Tulkki (1965) found *Halicryptus* *spinulosus* in these O_2-deficient areas, where other macrofauna representatives did not occur; at a somewhat higher O_2-content, (at which other macroscopic animals were already scarce) specimens of *Astarte borealis* were still alive.

MOORE (1931) found living nematodes in oxygen-lacking bottom layers of the Clyde Sea; he presumes that these are able to live there continuously without oxygen. Further correlations between the distribution of interstitial meio- and microfauna and the oxygen tension of the substratum have been reported for ciliates (FENCHEL and JANSSON, 1966), harpacticoids (JANSSON, 1967), turbellarians and nematodes (JANSSON, 1968a), and other species (FENCHEL et al., 1967; JANSSON, 1968b).

In the Black Sea, some polychaete larvae and *Sagitta euxine* survive at depths with only 2 to 5% oxygen-saturation (NIKITIN, 1931). In the same area, the lower plankton limit concurs with the boundaries of the oxygen-containing water. In the Arabian Sea, VINOGRADOV and VORONINA (1961) observed in the upper part of the oxygen-deficient mediary layer a decline in the amount of plankton forms and, between 500 and 1000 m, again a plankton maximum (mainly copepods). It is interesting to note that the lower distribution limit of some plankton species concurs with lines representing the same oxygen content. According to KINZER (1967) in the upper water layer of the Arabian Sea, which is rich in oxygen, the amount of plankton is 3 to 4 times greater than that of the oxygen-deficient mediary layer which contains only 0.08 to 0.04 ml O_2/l. At almost every observation point, however, the number of copepods in the oxygen-deficient layer was greater than in the surface layer. In the hydrogen sulphide-containing layer of the Arabian Sea, the biomass declines sharply and reaches its minimum in the zone of the H_2S-maximum (IVANENKOV and ROZANOV, 1961). Near the coast of California, CHILDRESS (1968) caught specimens of the mysid *Gnathophausia ingens* at depths with average O_2-contents of 0.5 to 0.2 ml/l; in experiments, this species survived O_2-contents of only 0.14 to 0.26 ml/l for at least 3 months.

Sudden occurrence of oxygen-deficiency and hydrogen sulphide can cause mass mortalities in the local fauna. Following influx of cold, high-salinity sea-water, the H_2S-containing lower water layers of some Norwegian fjords were forced to the surface in 1905, and the whole fauna destroyed as a result. In 1940, many fish died in the Black Sea after a sudden occurrence of hydrogen sulphide in the surface water (BRONGERSMA-SANDERS, 1957). Along the South American coast, the frequently occurring mass productions of plankton and the following breakdown of organic matter result in the formation of large amounts of hydrogen sulphide which, in turn, can cause mortality in fishes (GUNTHER, 1936). Also, along the south-west coast of Africa, between Walfish-Bay and Lüderitz-Bay, large amounts of hydrogen sulphide are formed in the bottom sediments by putrefaction of diatoms and dinoflagellates, and transported to the surface in spring by rising bottom waters. This process too causes mass mortality of fishes, as well as of crustaceans, molluscs and other invertebrates (BRONGERSMA-SANDERS, 1948).

Distinct differences in survival time were observed by JACUBOWA and MALM (1931) in various polychaetes and bivalves of the Black Sea. Animals buried in the mud survived at 12 °C for up to 14 days without oxygen. Most polychaetes and bivalves from the community of the mussel-banks, however, were already dead after less than a 24 h exposure to oxygen-deficient water; only a few species from this biotope survived up to 5 days without oxygen. MOORE (1931) reported that copepods (Harpacticoidea) survived in oxygen-deficient water (0.2 mg/l) for several hours, bivalves (*Syndosmya alba* and *Nucula tenuis*) for 3 and 17 days, respectively, and nematodes living deep in the mud for longer than 1 month.

THAMDRUP (1935) kept various bivalves and gastropods from the North Sea 7 days in oxygen-deficient water at about 10 °C. After this period, 100% of the *Mya arenaria* and *Littorina littorea* specimens were still alive, 96% of *Macoma baltica*, 80% of *Mytilus edulis*, and 50% of *Cardium edule*. According to HECHT (1932), *Arenicola marina* can survive 6 days and *Nereis* 9 days at temperatures between 15° and 18 °C. WIESER and KANWISHER (1959) found that animals exhibiting low degrees of activity, such as nematodes and mites (*Enoplus communis*, *Rhombognathides seahami*, *Halacarus basteri*), can survive anaerobically for 16 h at 25 °C. More active amphipods (*Calliopius laeviusculus*, *Gammarus oceanicus*, *Hyale prevosti*) survived only 2 to 3 h. In addition, there exists a relationship between oxygen consumption and survival time. The species with the highest oxygen consumption (*Calliopius* sp.) shows the shortest survival periods under oxygen-deficiency. In long-term experiments at 7 °C, several nematode species from a soft bottom biotope near Woods Hole (USA) survived longer than 60 days (WIESER and KANWISHER, 1961).

A dependence of survival periods on temperature in oxygen-deficient water was found by COLLIP (1921) in *Mya arenaria*. At 31 °C the test individuals survived for 1 day, at 14 °C for 8 days, and at very low temperatures for some weeks. LUND (1957) made corresponding observations on oysters. In winter (14° to 23 °C) the oysters survived for 18 days without oxygen, in summer (26.8° to 31.4 °C) for only 7 days. In freshwater animals too, survival periods under conditions of oxygen-deficiency depend on temperature. Thus *Limnaea stagnalis* can survive anaerobically for more than 7 days at 0 °C and at 8° to 10 °C, but only for $2^1/_2$ days at 20 °C (ALSTERBERG, 1930). According to LINDEMAN (1942), survival of freshwater benthic animals under anaerobic conditions is better at 0 °C and 5 °C than at 10 °C. *Chironomus*- and *Chaoborus*-

Table 1. *H_2S-resistance of bottom-invertebrates from the Black Sea* (after JACUBOWA and MALM, 1931)

Habitat	Species	Survival time in H_2S-containing water (days)	H_2S-content of the external medium (cm^3/l)
Biocoenosis of the *Zostera* roots (sand-mud-bottom)	*Pectinaria neapolitana*	8	0—1.68
	Sthenelais boa	2	0—1.80
	Staurocephalus rudolphii	3	0—1.80
	Glycera convoluta	10	traces
	Syndosmya ovata	10	traces
	Tapes rugatus	10	traces
Biocoenosis of the black mud	*Nereis diversicolor*	3	0—7.5
	Harmothoe incerta	5	0—7.9
	Eteone picta	6	0—8.2
	Cardium edule	4	0—6.1
	Syndosmya ovata	6	0—8.2
Mytilus galloprovincialis biocoenosis	*Perinereis cultrifera*	6	0—6.4
	Capitomastus minimus	8	0—20.4
	Capitella capitata	8	0—20.4
Biocoenosis of the mussel-banks	*Lysidice ninetta*	5	0—12.4
	Cardium edule	5	0—5.6
	Tapes rugatus	5	0—5.6
	Mytilus galloprovincialis	5	0—5.6
	Ostrea taurica	5	0—5.6

larvae and *Tubifex* survived for more than 120 days at 0° and 5 °C.

The critical concentration limits of oxygen have been determined, experimentally, for various plankton organisms of the Black Sea (NIKITINE and MALM, 1934). *Calanus finmarchicus* died when the oxygen content of the water decreased below 0.2 cm^3/l, *Acartia clausii* at 0.17 to 0.12 cm^3/l which corresponds to an oxygen saturation of 2.5 to 2%.

The resistance of various communities of the Black Sea to hydrogen sulphide has been investigated by JACUBOWA and MALM (1931). Polychaetes and bivalves of a mud community were more resistant than species of a mussel-bank community. Active and mobile species were more sensitive to H_2S than less mobile ones (Table 1). The authors suggest that active species have a greater requirement for oxygen, but also the possibility of searching for, and moving to, water layers rich in oxygen. Species which dig into sediments, however, require mechanisms which allow survival in O_2-deficient and H_2S-containing water. Resistant species like *Capitomastus minimus* and *Capitella capitata* still survived at 20.4 cm^3 H_2S/l. According to HECHT (1932) *Arenicola marina* died at an H_2S-content of 8.45 mg/l, *Nereis* spec. rarely below an H_2S-content of 22.23 mg/l.

Material and methods

The animals were selected from different benthal biotopes of the North Sea and the Baltic Sea. *Mytilus edulis, Littorina littorea, Littorina saxatilis, Cardium edule* and *Mya arenaria* were collected in the mud flats of the North Sea near Büsum. *Spisula solida* was obtained from the Biologische Anstalt Helgoland. *Modiolus modiolus* was caught in a 30 to 50 m deep trench in the Kattegat. The animals from the Baltic Sea were collected with a bottom-grab, dredge, or pile scrape net, in the southern part of Kiel Bay, the "Kieler Förde". Until the beginning of the experiments they were kept for 1 to 3 weeks at 10 °C in sea-water of 30‰ or 15‰ salinity, without being fed.

The species examined

Lamellibranchs

In the North Sea, *Spisula solida* is restricted to depths of about 10 to 15 m. It requires relatively high salinities. Distributionally, the species extends into the Kattegat, but does not enter the Baltic Sea. In comparison with species of the tidal zone, it has narrow thermal and osmotic resistance ranges (RESHÖFT, 1961). The species prefers sandy beds with continually moving water, and avoids mud and stagnant water.

The relatively euryoecous *Mytilus edulis* can withstand, with closed valves, exposures to air during low tides, and endure relatively wide fluctuations of temperature (considerable intensities of sunshine in summer; partial temporary freezing in winter). The species also exhibits a great range of osmotic resistance and penetrates far into estuaries. In the Baltic Sea its distribution is limited by salinities of about 4 to 4.5‰ (Gulf of Finland and Gulf of Bothnia); banks of *Mytilus edulis* can still be found on sandy and soft bottoms at depths of 20 to 30 m. Sometimes specimens occur down to a depth of 250 m.

Mya arenaria lives buried some 20 to 30 cm deep in sandy and silty mud flats of the boreal region. During low tide it is not as much exposed to intensity changes of abiotic factors (e.g. temperature and salinity) as is *Mytilus edulis*. It penetrates far into the Baltic Sea and extends into the Gulf of Bothnia (about 4.5 to $5^0/_{00}$ salinity).

In the North Sea and in the Kattegat, the circumpolar *Modiolus modiolus*, in contrast to *Mytilus edulis*, prefers deeper water with higher salinities and lower temperatures, as well as smaller fluctuations of these factors. According to Jaeckel (1952), it occurs primarily in habitats with stronger water currents. Experimental values for osmotic and heat resistance of isolated gill tissues lie between those for *Mya arenaria* and *Mytilus edulis* on the one hand, and those for *Spisula solida* on the other (Schlieper et al., 1958; Reshöft, 1961).

Cyprina islandica occurs in the subarctic-boreal region and is restricted to the low water mark (Jaeckel, 1952). Its vertical distribution extends down to 160 m. The species penetrates into the Baltic Sea as far as Warnemünde; here it remains restricted to cold, salty, deep waters below some 17 m, and prefers silt and mud.

Astarte borealis is arctic-circumpolar. It does not occur on the German North Sea coast; probably the high summer temperatures act as a limiting factor. In the Baltic Sea it penetrates eastward to the coast of Mecklenburg and the east coast of Bornholm in waters between 37 and 85 m (Jaeckel, 1950). While found even on sandy bottoms in the Kiel Bay, eastwards it prefers increasingly soft bottoms.

Scrobicularia plana occurs along the German North Sea coast on soft mud to depths of about 15 m. It lives buried in the bottom and, with its long incurrent siphon, searches the bottom surface for food. In the Baltic Sea it is found eastward as far as the coast of Mecklenburg.

Cardium edule lives on sandy and soft bottoms in the tidal zone of the North Sea to depths of about 10 m, and buries itself about 3 to 4 cm deep in the substratum. It also penetrates into the Baltic Sea. Variations in shell structure amongst separate populations make the systematic separation from *Cardium lamarcki* (Reeve) difficult (see also Eisma, 1965).

Gastropods

Littorina littorea thrives on substrate types ranging from hard bottom to mud; it occurs to depths of about 15 m, but is primarily found in the tidal zone on seaweed, stones and pilework. In the Baltic Sea it penetrates eastward as far as Bornholm and Rügen.

Littorina saxatilis occupies largely stones in the tidal zone above the high water mark. In the Baltic Sea it occurs eastward as far as the west coast of Rügen.

Polychaetes

The boreal lusitanian polychaete *Nereis diversicolor* is found on mud flats of the North Sea, frequently in company with *Arenicola marina*. It lives in burrows up to 20 cm deep and searches the substrate surface for food. The species is marine-meiomesohaline, penetrates far into estuaries, and occurs in the Baltic Sea as far as the entrance of the Gulf of Finland and the east coast of the Gulf of Bothnia on sandy to soft bottoms.

Crustaceans

Carcinus maenas inhabits the North Sea coast, particularly the tidal region; at low tide it is most abundant on breakwaters and beneath stones on the beach, where it may be partially buried in the substratum. In the Baltic Sea *C. maenas* extends its distributional area eastward to the Darss threshold, preferring sandy bottoms, mussel-banks, and phytal areas.

Crangon crangon lives on sandy and silty sea bottoms and prefers the shallow water near the coast; it occurs, however, to depths of about 90 m. By day it buries itself superficially in the substratum. In summer this shrimp penetrates far into estuaries; in the Baltic Sea it extends eastward as far as the Gulf of Finland.

The isopod *Idotea baltica* occurs on sandy bottoms, especially in the phytal, and may be found to depths of about 350 m. In the Baltic Sea this species is distributed as far as the Gulfs of Finland and Bothnia.

The amphipod *Gammarus oceanicus* lives in shallow coastal waters at depths between 0 and 25 m; it is frequently found in the phytal as well as amongst populations of *Mytilus edulis*. The species is also represented in the Baltic Sea; it is, however, absent in the Gulfs of Finland and Bothnia in salinities below about $2.5^0/_{00}$.

Echinoderms

Asterias rubens occurs on hard bottoms and stony sand, predominantly on banks of *Mytilus edulis*. It is distributed throughout the North Sea and occurs in the Baltic Sea, sporadically, down to $8^0/_{00}$ salinity.

Ophiura albida lives primarily on sand containing silt, but also on solid substrates at depths of 0 to 500 m. Like *Asterias rubens*, it is euryhaline and penetrates into the Baltic somewhat beyond the Darss threshold.

Further information on the descriptive ecology of the species mentioned may be found in Grimpe and Wagler (1926 and later issues), Dahl (1928 and later issues), Jaeckel (1952), and Reshöft (1961).

The methods used

Resistance to oxygen-deficiency

Pure nitrogen was bubbled through filtered seawater (filter from Schleicher and Schüll, No. 602 h), contained in a 40 cm high glass cylinder, until the oxygen content had reduced to below 0.15 ml/l. This content was controlled using the Winkler method and the water transferred via an overflow tube into transparent glass bottles. For experiments with whole animals, bottles of 250, 100 or 50 ml were used, for those with isolated tissue pieces 50 ml-bottles. The bottles, sealed and containing the experimental material, were kept at 0°, 10° or about 20 °C. At suitable intervals they were opened and the animals or tissue pieces transferred into well-aerated sea-water. After a recovery period of $^1/_2$ h, the number of surviving animals or tissue pieces was recorded.

In species without shells the criterion for survival was their ability for active movement. Mussels were regarded alive, if they closed their shells upon a mechanical stimulus, snails, if they withdrew into their shells. The criterion for survival in sea-stars was their capability to move their ambulacral feet and to crawl about. The degree of resistance of whole animals was measured in terms of the time (in hours) which 50% of the animals survived. These LD_{50}-values were read from graphs showing the number of the surviving and dead animals on a semilogarithmic scale as a function of exposure time.

The degree of damage to isolated gill tissues was carefully assessed, paying particular attention to the terminal cilia (Fig. 1). The decline of the ciliary activity during exposure to oxygen-deficiency or increased amounts of H_2S, was carefully tabulated and assessed according to the scale given in the legend to Fig. 1.

Resistance to hydrogen sulphide

In water, hydrogen sulphide has the properties of a weak acid. It dissociates in 2 steps into hydrogen sulphide- (HS^-) and sulphide-ions (S^{2-}):

$$H_2S \rightleftarrows H^+ + HS^- \rightleftarrows S^{2-} + 2H^+.$$

Below pH 11, this balance tends strongly to the left, so that only very few S^{2-}-ions are present. According to SKOPINTSEV (1957) the following equation for the calculation of the amount of sulphide ions in sea-water (at 25 °C) in the presence of hydrogen sulphide, can be used:

$$C_{S_2-} = C_{H_2S} \frac{K'_2 \cdot K'_1}{C^2_H + K'_1 \cdot C_H}$$

where $pK'_1 = 7.04 — 0.4\ (\mu)^{\frac{1}{2}}$

$pK'_2 = 14.92 — 1.2\ (\mu)^{\frac{1}{2}}$

and the ionic strength $\mu = 0.020\ S^0/_{00}$.

If an alkali metal sulphide, e.g. sodium sulphide, is dissolved in water, it is — as salt of a strong base and a weak acid — for the most part subject to hydrolysis:

$$S^{2-} + HOH \rightleftarrows SH^- + OH^-$$

and reacts, as a result, as an alkali. However, since the same decisive ions (HS^-, S^{2-}) occur in this reaction, the resistance of a marine animal to H_2S can also be tested by adding Na_2S to sea-water, provided the pH-value is recorded. For examining the resistance to sulphide ions and/or hydrogen sulphide, each time 50 mg $Na_2S \cdot 9H_2O$/l were added to oxygen-deficient sea-water. The amount of sulphur added to each litre of water in the form of this sulphide amounts to 6.67 mg. The concentration of hydrogen sulphide achieved in this way is approximately comparable to that of certain areas in the North Sea (THAMDRUP, 1935), Baltic Sea (FONSELIUS, 1962) and Black Sea (CASPERS, 1957; ZENKEVITCH, 1963). The pH-values of sea-water deficient in oxygen lie, after addition of the above-mentioned amount of sodium sulphide, between 8.2 and 8.45. Unless otherwise stated all experiments on H_2S-resistance were carried out at this pH-value.

In the presence of oxygen, sulphide oxidizes to sulphate, as well as to the intermediary steps pure sulphur, sulphite, thiosulphate and tetrathionate. The oxidation is limited by the amount of oxygen available. According to SKOPINTSEV et al. (1961) thiosulphate and sulphite are formed, when the ratio O_2:H_2S in the water is initially very low. High O_2-concentrations result in the formation of sulphate. The reaction may last from hours to days (RICHARDS, 1965). In the experiments reported in this paper, oxygen dissolved in sea-water was almost completely eliminated by nitrogen each time, prior to the addition of sodium sulphide, in order to prevent the oxidation processes mentioned above.

Further methodical details were the same as in the oxygen-deficiency experiments.

Results of the experiments

Resistance of whole animals to O_2-deficiency and H_2S

The results of our experiments on the survival capacity of marine bottom invertebrates in oxygen-deficient and hydrogen sulphide-containing water are summarized in Table 2. It gives, for each species, the duration of exposure survived by 50% of the test individuals. Under conditions of O_2-deficiency (at the beginning of each experiment 0.15 to 0.1 ml O_2/l) the resistance is, in most cases, greater than in the presence of H_2S. This holds especially for representatives of species which can endure long exposures under such conditions. Amongst these species are predominantly lamellibranchs and gastropods, which can protect themselves against harmful influences in an unfavourable medium by closure of, or withdrawal into, shells.

According to Table 2, *Cyprina islandica* survives longest (50% mortality after 55 days); it is followed by *Mytilus edulis* from the North Sea (35 days), *Scrobicularia plana* (21 to 25 days), and *Mya arenaria* (21 days). The resistance decreases in the order *Littorina littorea*, *Littorina saxatilis*, *Nereis diversicolor*, *Cardium edule*. In the sea-stars, brittle-stars, and crabs examined, the resistance of the more active species declines sharply with their concomitantly increasing rate of metabolism.

Cellular resistance to O_2-deficiency and H_2S

As pointed out by SCHLIEPER and his associates in numerous publications, the cellular resistance of lower marine invertebrates to different abiotic environmental factors can be used in the ecological-physiological characterization of the species. Pertinent values have become available for resistance to high and low salinities (SCHLIEPER et al., 1960; RESHÖFT, 1961; VERNBERG et al., 1963; THEEDE, 1965; SCHLIEPER et al., 1967), temperatures (SCHLIEPER et al., 1960; RESHÖFT, 1961; VERNBERG et al., 1963; USHAKOV, 1964, 1968; THEEDE, 1965; SCHLIEPER, 1966; THEEDE and LASSIG, 1967; ZHIRMUNSKY, 1967), and high hydrostatic pressures (SCHLIEPER, 1963; PONAT, 1967; PONAT and THEEDE, 1967; SCHLIEPER et al., 1967).

Table 2. *Resistance of marine invertebrates from the North Sea and the Baltic Sea to O_2-deficiency and H_2S. Temperature 10 °C. The experiments were conducted during May, June, and July, 1968*

Species	50% mortality (LD_{50}) was observed after the exposure times (h) listed	
	O_2-deficient sea-water (0.15 ml O_2/l)	O_2-deficient sea-water + addition of 50 mg $Na_2S \cdot 9H_2O$/l
Baltic Sea individuals tested at 15°/ooS		
Cyprina islandica	1320	800—1000
Scrobicularia plana	500—600	400—450
Mya arenaria	504	408
Nereis diversicolor	120	96
Asterias rubens	84	67
Ophiura albida	32	30
Carcinus maenas	48	32
Gammarus oceanicus	15	8
Idotea baltica	6	6
Crangon crangon	2	2
North Sea individuals tested at 30°/ooS		
Mytilus edulis	840	600
Cardium edule	102	96
Littorina littorea	365	180
Littorina saxatilis	144	72

Since it was shown in preliminary experiments that the isolated gill tissues of many species can survive for several days under oxygen-deficiency, it was first tested how long the tissue can be maintained alive under simple laboratory conditions, without oxygen-deficiency and without adding H_2S. RESHÖFT (1961) had already kept isolated gill pieces of a few bivalve species from the Kiel Bay in petri dishes, in water of 15‰ salinity at 10 °C, and observed in *Mya arenaria*, *Mytilus edulis*, and *Astarte borealis* a mean survival time of 14 to 15 days, and in *Cyprina islandica* of about 16 days. Gill pieces of *Mytilus edulis* from the North Sea survived, according to the same author, in 30‰ S and at 10 °C for an average period of 20 days, *Modiolus modiolus* 11 days. These survival times of isolated gill epithelium demonstrate the capacity of the tissues to survive in resistance experiments conducted over several days. The survival capacity could be still improved by simple means, e.g. by using filtered sea-water, keeping only a few gill pieces in 1 container (1 to 2 pieces in 40 to 50 ml sea-water) and maintaining the tissues in darkness. Nutrients and antibiotics were not added to the sea-water.

The experiments revealed that the isolated tissues of all examined species from the Baltic Sea survived in good condition for almost 3 weeks; after this time, the estimated ciliary activity is reduced on average to around 50%. The mean survival times (Table 3) lie between 19 days *(Cardium edule)* and 30 days *(Mytilus edulis)*.

When an isolated gill piece from a bivalve is kept in a sealed condition (e.g. beneath a cover-glass with the rim smeared with vaseline) in a small amount of water, the ciliary movement is soon halted due to the increasing CO_2 level and decreasing amount of O_2. The tissue can, however, survive a certain time via anaerobic metabolism; if brought in contact with air again, the ciliary movement is restored.

This survival capacity was now tested on gill tissues from several North Sea and Baltic Sea bivalves which occur in different biotopes.

The following species from the North Sea were examined: *Spisula solida*, *Mytilus edulis*, *Mya arenaria*, and *Modiolus modiolus* (Kattegat). The reaction of isolated gill epithelium of these bivalves to oxygen-deficiency (Fig. 1) demonstrates that, under similar conditions, the ciliary movement of *Spisula solida* declines fastest and is soon halted completely. Then follows the tissue of *Mytilus* and *Mya* and, finally, that of *Modiolus*. After cessation of ciliary activity, the tissues of all 4 species still survive for several days under these conditions. *Spisula* gill tissue shows irreversible damage (no recovery) at the latest after another 3 days. Under oxygen-deficiency gill pieces from *Modiolus modiolus* survive 6 more days counting from the beginning of absolute ciliary standstill. Hence, under oxygen-deficiency the survival capacity of *Modiolus* tissue is greatest of all tested species. After 11 days none of the gill pieces is capable of recovery after re-transfer into aerated sea-water. With *Spisula* this effect is already apparent after 4 days of exposure.

Under conditions of O_2-deficiency and simultaneous presence of H_2S, the survival capacity of the tissues reveals the same order: Lowest resistance for *Spisula*, almost identical resistance for *Mytilus* and *Mya*, and a somewhat higher one for *Modiolus*. In general, the survival times are for all species distinctly shorter than in O_2-deficient water alone (Fig. 2).

Table 3. *Survival capacity of isolated gill pieces of different bivalve species under laboratory conditions. (The tissue pieces were kept in sea-water of 15‰ S, without addition of foodstuff, at 10 °C, and darkness)*

Species	50% reduction of the relative ciliary activity (mean values) were observed after the following times of storage (days)	Mean values of survival times (days)	Absolute standstill of the gill cilia was found after the following times of storage (days)
Mytilus edulis	24	30	41
Cyprina islandica	26	28	37
Mya arenaria	23	25	29
Astarte borealis	23	24	29
Scrobicularia plana	21	23	26
Cardium edule	18	19	22

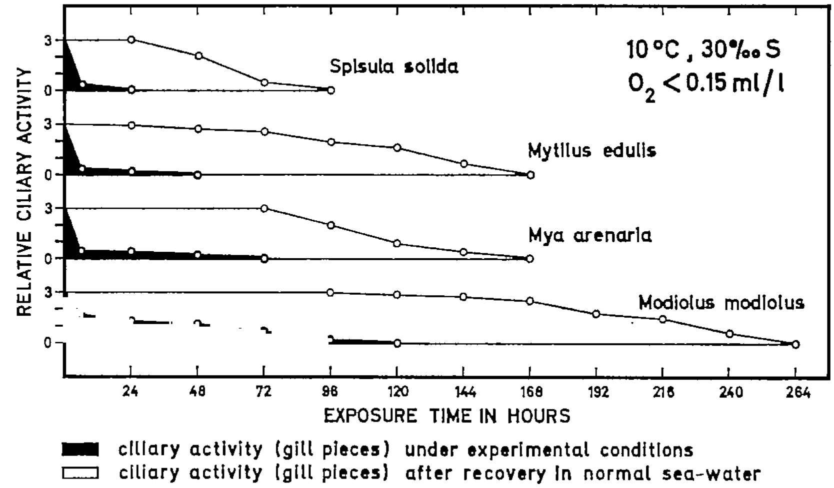

Fig. 1. Relative ciliary activity and survival capacity of isolated gill tissues of different bivalve species from the North Sea (~30°/oo S) in O_2-deficient sea-water. The activity of the terminal cilia of isolated gill pieces was observed during exposure in oxygen-deficient sea-water (< 0.15 ml O_2/l) and after subsequent recovery in well aerated sea-water. The activity was estimated and classified according to the following scale: 3 = normal activity; 2 = activity somewhat reduced; 1.5 = activity reduced to half of normal; 1 = activity reduced to less than 50%; 0.5 = 10—1% of cilia are still active; 0 = cessation of ciliary activity. The dots represent average values of 10 observations each

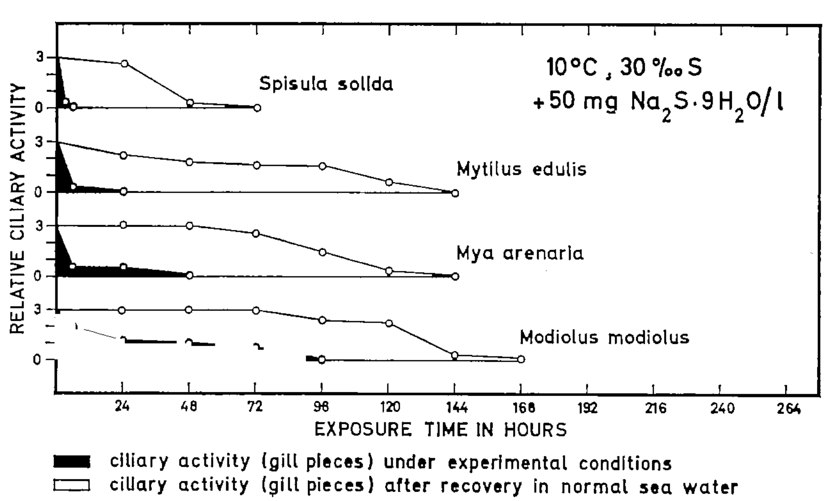

Fig. 2. Relative ciliary activity and survival capacity of isolated gill tissues of different bivalve species from the North Sea (~30°/oo S) in O_2-deficient water to which 50 mg $Na_2S \times 9H_2O$/l had been added. For further details see text to Fig. 1

From the Baltic Sea (Kiel Bay) the following species were tested: *Mytilus edulis*, *Mya arenaria*, *Cardium edule*, *Scrobicularia plana*, *Cyprina islandica*, and *Astarte borealis* (Figs. 3 and 4). The resistance of gill tissues from these species to O_2-deficiency increases in the following order: *Cardium edule* < *Mya arenaria* ≈ *Scrobicularia plana* < *Mytilus edulis* < *Cyprina islandica* ≈ *Astarte borealis* (Fig. 3). The tissue of *Cardium edule* was dead after 4 days oxygen-deficiency, that of *Astarte borealis* after 8 days.

The survival times of gill tissues in simultaneous presence of hydrogen sulphide (Fig. 4) are, on an

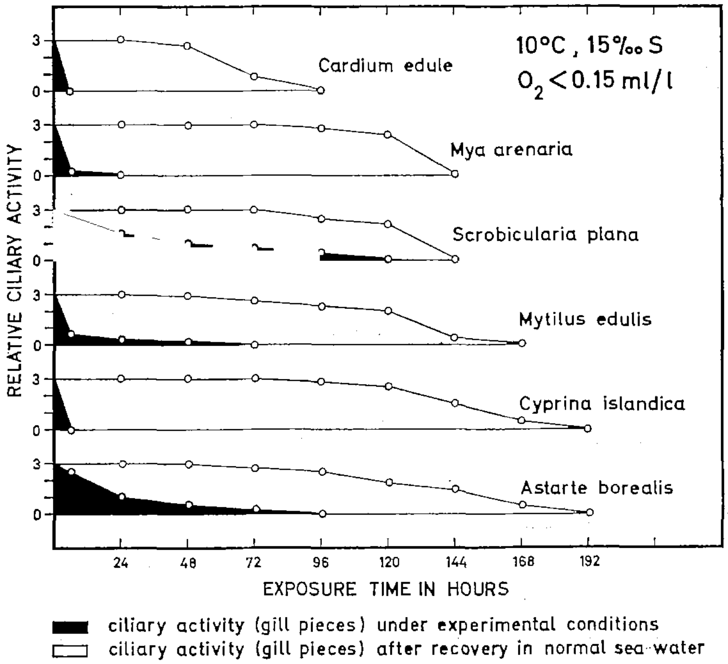

Fig. 3. Relative ciliary activity and survival capacity of isolated gill tissues of different bivalves from the Baltic Sea (~ 15°/oo S) in O_2-deficient water. For further details see text to Fig. 1

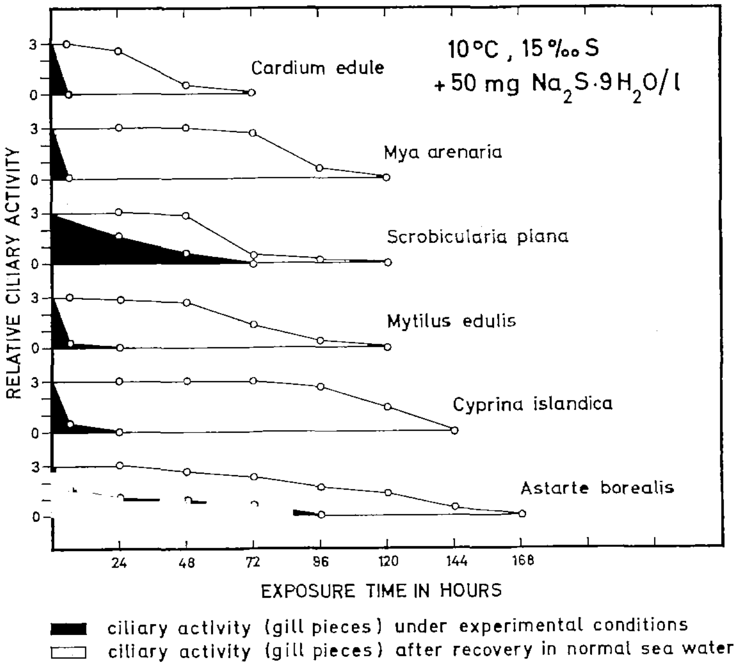

Fig. 4. Relative ciliary activity and survival capacity of isolated gill tissues of different bivalves from the Baltic Sea (~ 15°/ooS) in O_2-deficient water to which 50 mg $Na_2S \times 9H_2O$/l had been added

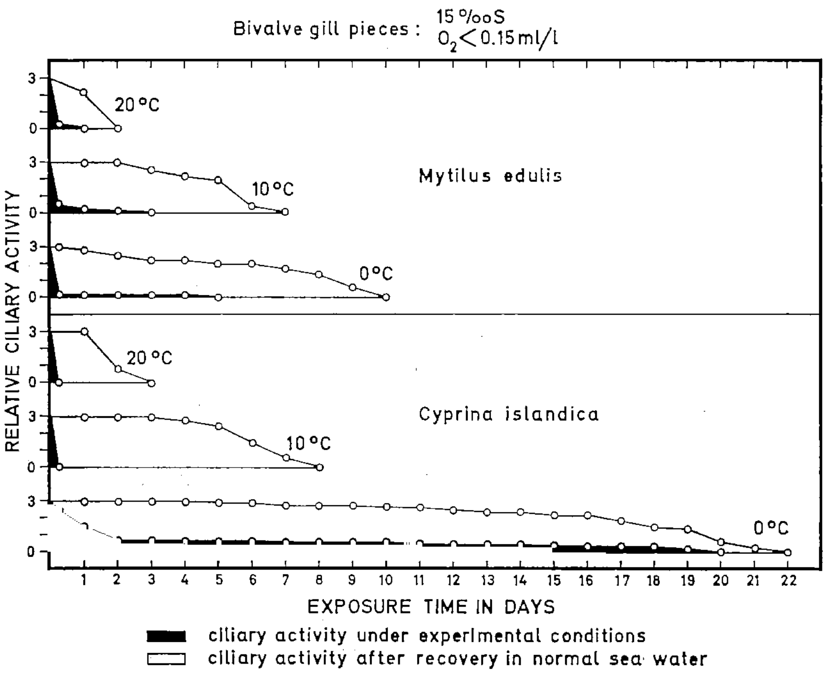

Fig. 5. Influence of temperature on ciliary activity and survival time of isolated gill tissues of two lamellibranch species from the Baltic Sea (~ 15°/ooS) in O_2-deficient sea-water. For further details see text to Fig. 1

average, 1 to 2 days shorter than under O_2-deficiency alone. Damage to tissues, however, definitely begins earlier, since the activity of gill cilia was no longer restored completely even if the gill tissue had been re-transferred into well-aerated sea-water (Fig. 4).

The tissue of all these species can, of course, survive under O_2-deficiency and simultaneous occurrence of H_2S, for several days. All bivalves examined, except *Cardium edule* and *Mytilus edulis*, can be found in the Kieler Förde in soft bottoms containing H_2S. *Mytilus edulis* occurs in localities containing H_2S, with hard substratum (stones, old bivalve shells) to which it can attach itself. *Cardium edule* is found in the Kieler Förde predominantly in well-aerated bottoms as well as above the bottom on mussel-banks.

In addition to resistance, the declining ciliary activity and the duration of the ciliary movement of gill pieces during O_2-deficiency and H_2S presence have been observed. In species from the North Sea, ciliary movement is maintained longer in forms whose cells generally survive longer (Figs. 1 and 2). The results given in Figs. 3 and 4 show, however, that this is not always the case. For example, the ciliary activity of gill pieces from *Cyprina* ceases rapidly, while that of *Astarte* is maintained much longer, although the survival capacity of both tissues is similar. Also, pieces of almost equally resistant gills from *Scrobicularia* and *Mya* react distinctly differently during O_2-deficiency and H_2S-influence. *Mya*-gills cease ciliary movement quickly, *Scrobicularia*-gills, on the other hand, slowly. These results suggest that the capacity for the maintenance of ciliary activity under almost anaerobic conditions does not correspond to survival capacity.

Temperature has an extremely strong effect on the cellular resistance to O_2-deficiency and the occurrence of H_2S (Figs. 5 and 6). At high temperatures, the survival capacity of tissues is notably less than at low

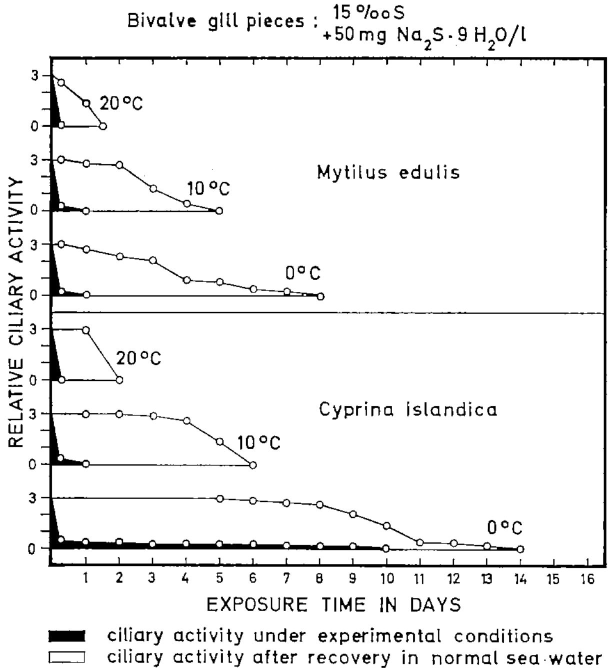

Fig. 6. Influence of temperature on ciliary activity and survival time of isolated gill tissues of two lamellibranch species of the Baltic Sea (~ 15°/oo S) in O_2-deficient sea-water to which sulphide (50 mg $Na_2S \times 9H_2O$/l) had been added. For further details see text to Fig. 1

Table 4. *Resistance of isolated gill tissues of different bivalve species from North Sea and Baltic Sea to oxygen-deficiency* (< *0.15 ml* O_2/l) *and hydrogen sulphide (addition of 50 mg* $Na_2S \cdot 9H_2O/l$). *Temperature: 10 °C*

Medium	Species	Salinity (‰)	Number of surviving gill pieces of 10 animals after exposure times (h) indicated						
			24	48	72	96	120	144	168
< 0.15 ml O_2/l	*Mytilus edulis*	30	10	10	10	10	10	4	0
		15	10	10	10	10	10	2	0
	Mya arenaria	30	10	10	10	10	6	4	0
		15	10	10	10	10	8	0	
	Cardium edule	30	10	10	7	5	0		
		15	10	9	4	0			
+ 50 mg $Na_2S \cdot 9H_2O$/l	*Mytilus edulis*	30	10	10	10	9	5	0	
		15	10	10	9	5	0		
	Mya arenaria	30	10	10	9	7	2	0	
		15	10	10	9	3	0		
	Cardium edule	30	10	6	0				
		15	9	2	0				

Table 5. *Influence of the pH on the resistance of isolated gill tissues of Mytilus edulis (Baltic Sea, ~ 15‰ S) to hydrogen sulphide (addition of 50 mg* $Na_2S \cdot 9H_2O/l$ O_2*-deficient sea-water); 10 °C*

pH	Number of surviving gill pieces of 10 specimens after exposure times (days) indicated											
	3	4	5	6	7	8	9	10	11	12	13	14
7	10	10	10	7	6	6	6	5	5		2	0
8	10	8	7	4	2	1	0	0	0	0	0	0
8.5	10	10	9	7	2	2	0	0	0	0	0	0

ones. Below 10 °C, the resistance of the cold-stenotherm *Cyprina islandica* increases more than that of the eurytherm *Mytilus edulis*. As a result, the degree of tissue resistance of each species differs considerably more at 0 °C than at 10° and 20 °C.

Salinity affects the cellular resistance to O_2-deficiency and H_2S relatively little. Euryhaline bivalve species, which penetrate far into the Baltic, are not significantly influenced as long as the lower critical salinity limit is not attained (Table 4). In all experiments, a tendency towards a reduced resistance in brackish water was noticeable.

The influence of pH on the survival capacity during exposure in sulphide-containing water is of particular interest, since the chemical balance between the components H_2S — HS' — S'' tends towards the right upon an increase, and to the left upon a decrease, in pH. In the experimental results summarized in Table 5, pH values, after addition of equal amounts of sulphide (50 mg $Na_2S \cdot 9H_2O$/l) to sea-water, were regulated by adding small amounts of weak soda lye or hydrochloric acid. The survival capacity of gill pieces from *Mytilus edulis* is similar at pH 8 and 8.5, but increases distinctly at pH 7. The damaging effect may have been reduced by an increase in undissociated H_2S.

Discussion

Our results reveal a high resistance of several lamellibranch species to oxygen-deficiency and hydrogen sulphide. Amongst these species are some which are able to live in mud flats *(Mya, Scrobicularia)* or other soft bottoms, *(Cyprina)*, i.e., in a substratum partly deficient in oxygen and often containing H_2S. Of course, the lamellibranchs mostly maintain contact with the medium above the substrate and extract from it oxygen for respiratory processes. Under unfavourable conditions they can close their shells temporarily. *Mytilus edulis* of the upper littoral keeps its shells closed when exposed during low tide. This reaction has been examined more closely (Schlieper, 1955; Schlieper and Kowalski, 1958). A short while after the closure of the shell, the heartbeat and the activity of the gill cilia slow down and finally cease. The mussels survive such periods almost anoxybiotically. *Littorina littorea* too, exhibits a considerable survival capacity under conditions of O_2-deficiency and H_2S presence. The resistances of *Cardium edule*, *Littorina saxatilis*, and the polychaete *Nereis diversicolor* are somewhat lower.

Asterias rubens, *Ophiura albida* and *Carcinus maenas* show a definite resistance; this fact allows us to establish correlations to their way of life. Sea-stars occur on mussel-banks often located in H_2S-containing areas. Brittle-stars move not only on top, but also in, the substrate. Shore crabs are occasionally found buried beneath stones or in soft ground. Under these circumstances respiratory conditions can be impaired. Inhabitants of sandy bottoms (e.g. *Crangon crangon*) and inhabitants of the phytal (e.g. *Idotea baltica*), on the other hand, quickly die under oxygen-deficiency and in the presence of H_2S. Thus, relationships between resistances and natural habitats can be noted.

Differences in resistance of whole animals suggest parallel differences at the cellular level. Ecologically, the resistance of whole animals is of primary interest. To the physiologist, however, resistance values obtained from homologous tissues of different species offer a valid basis for the comparative assessment of the cellular mechanisms involved. In whole animals, survival under extreme conditions may be modified through peculiarities of behaviour (protective reactions like shell closure, mucus secretion, retraction or reduction of exposed sensitive parts) and thus mask the critical limits for tissue responses.

Isolated gill tissues of bivalves may survive for days under oxygen-deficiency and simultaneous presence of H_2S. The externally visible ciliary activity soon ceases in *Mya arenaria* and *Cyprina islandica;* in other species, however, minimal ciliary activities are maintained much longer *(Scrobicularia; Astarte).* Therefore, no general conclusions can be drawn from the duration of the ciliary movements during oxygen-deficiency and H_2S influence, on tissue resistance to these factors, even if in several species (Figs. 1 and 2) such relationships do exist.

Comparative resistance experiments on isolated pieces of tissue reveal species specific differences, not only to oxygen-deficiency but also to the influence of H_2S. The reason for these differences can only be sought in different cellular properties. It should be noted that isolated tissues from bivalve species which endure oxygen-deficiency better, are also more resistant to H_2S. When the tested species are arranged according to their respective resistances to these 2 factors, almost the same order is obtained in both cases.

The long survival periods of isolated tissues from some lamellibranchs under O_2-deficiency may be due to their capacity to make use of the small amounts of oxygen available, to restrict drastically their energy requirements by reducing their cellular activities, and to meet the remaining requirements by anaerobic acquirement of biologically useful energy (glycolysis) (VON BRAND, 1946 and DALES, 1958). The toxic effect of H_2S on the cellular metabolism is primarily caused by the fact that it forms unsoluble sulphides with heavy metals. Its high affinity to iron causes a similar reaction to an elimination of the cytochrome-oxydases with hydrogen cyanide. This process interrupts the oxygen supply in the cells (KUSCHINSKY and LÜLLMANN, 1966). The decrease of the redox potential, which is connected with the presence of H_2S, may also inhibit oxidizing reactions in the cell. The effects of H_2S on single reactions in the cellular metabolism are not yet fully understood. Experiments on isolated enzymes must be performed.

The toxic effect of hydrogen sulphide on the tissues of marine bivalves is influenced by other external factors. A reduction of the pH in the external medium from 8 to 7 increases the survival capacity of the tissue in the presence of H_2S; this may be due to the fact that, with a decrease in pH, the balance between H_2S, $HS^{-\prime}$ and $S^{\prime\prime}$-ions is shifted towards undissociated hydrogen sulphide.

The survival capacity of tissues under O_2-deficiency and H_2S presence is greatly affected by temperature; at suboptimal temperatures it is greater than at supraoptimal temperatures. Whole animals survive in oxygen-free water better at lower temperatures (COLLIP, 1921: *Mya*; LUND, 1957: oysters) than at higher ones. These results suggest that the energy requirements for "maintenance metabolism" by predominantly anaerobic metabolic processes can more easily be met at lower temperatures.

Summary

1. Under laboratory conditions, marine bottom invertebrates show pronounced differences in resistance to O_2-deficiency and the presence of H_2S. These differences are parallel to the different substrates on or in which the species naturally occur. Inhabitants of soft substrates are more resistant than those of hard or sandy ones.

2. Corresponding differences in species specific resistances can be demonstrated at the cellular level. Resistances of whole animals are greater than those of isolated tissues.

3. The duration of continued activity (duration of ciliary movement) during exposure to O_2-deficiency and H_2S presence, likewise differs in different species of bivalves. It is longer at lower temperatures than at higher ones. The relation between active phase and duration of tissue survival shifts toward the active phase at lower temperatures.

4. Excised tissues of boreal marine species survive, under O_2-deficiency and in the presence of H_2S, better in cold than in warmth.

5. Isolated gill tissues of *Mytilus edulis* survive the influence of sulphide ions and H_2S better at pH 7 than at pH 8.

6. In euryhaline marine species, cellular resistance to O_2-deficiency und H_2S presence declines only very little upon a decrease in salinity.

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