Die Ergebnisse der oben skizzierten Versuche zeigen also, dass in Gegenwart von Liganden durch H_2O_2 ausgelöste Zerstörungsreaktionen von Cu-Ionen dorthin gelenkt werden, wo diese koordinativ gebunden sind. In weiteren Versuchen wurde noch für eine Reihe anderer biologisch bedeutsamer Metallionen untersucht, mit welcher Geschwindigkeit CTP in Form des entsprechenden Metallion-CTP-Komplexes durch H_2O_2 zerstört wird. Die ermittelten relativen Geschwindigkeitskonstanten $k_{M\text{-}CTP}$ bezogen auf $k_{Cu\text{-}CPT} = 1$ sind in der Tabelle aufgeführt. Wie zu erwarten, sind nur Redox-wirksame Metallionen katalytisch aktiv, da die Katalyse der H_2O_2-Zerlegung Redoxschritte des Metallions involviert[4]. Die relativen Wirksamkeiten der aktiven Metallionen Cu^{2+}, Fe^{3+}, Mn^{2+} und Co^{2+} sind im übrigen untereinander auffallend wenig verschieden.

Relative Geschwindigkeit der Zerstörung verschiedener Metallion-CTP-Komplexe durch 10^{-3} m H_2O_2

$k_{Cu\text{-}CTP}$	$k_{Mn\text{-}CTP}$	$k_{Co\text{-}CTP}$	$k_{Fe^{III}\text{-}CTP}$	$k_{Zn\text{-}CTP}$
1,00	0,54	0,63	1,22	0,03

Die strahlenbiologische Relevanz unserer Beobachtungen ist darin zu sehen, dass das Ausmass zumindest der auf das – durch die Strahlung gebildete – H_2O_2 zurückgehenden Strahlungsschäden zum Beispiel an Nukleinsäuren mit Sicherheit von Metallionen-Gleichgewichten abhängig sein muss. Da nun Komplexbildner in die Lage solcher Gleichgewichte im Sinne eines Metallionen-Entzugs eingreifen, ist zu verstehen, dass sie eine Prophylaxe des Strahlenschadens bewirken können.

Die Lage der für das Auftreten der Strahlungsschäden wichtigen Verteilungsgleichgewichte von Metallionen wie Cu^{2+} oder Fe^{3+} zwischen Nukleinsäuren, anderen im biologischen Medium vorhandenen Ligandsystemen und den Strahlenschutzsubstanzen ist naturgemäss in unserem Modellversuch nicht untersuchbar. Für die hochgeladenen Nukleinsäure-Anionen ist jedoch eine hohe Metallionen-Bindungstendenz zu erwarten[8].

Wir glauben, hiermit gezeigt zu haben, dass für eine genaue Kenntnis des Zustandekommens von Strahlungsschäden noch erhebliche Informationen über die Bindungsverhältnisse katalytisch aktiver Metallionen in den biologischen Systemen notwendig sind, und dass bei *in vitro*-Untersuchungen über die Strahlenempfindlichkeit biologischer Materialien, wie zum Beispiel Nukleinsäuren, auf definierte Verhältnisse hinsichtlich des Metallionen-Gehaltes geachtet werden muss.

Herrn Professor Dr. S. FALLAB danken wir für die Diskussion verschiedener Probleme, Fräulein HEDY KULL für die Ausführung experimenteller Untersuchungen und dem Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung, Kommission für Atomwissenschaften, für die gewährte Unterstützung.

H. BRINTZINGER, B. PRIJS und H. ERLENMEYER

Anstalt für Anorganische Chemie, Basel, 6. Juli 1960.

Summary

The catalysis of the reaction between H_2O_2 and nucleotides by biologically important metal ions has been studied spectrophotometrically, the importance of complex-formation equilibria for this catalysis was demonstrated. Radiobiological implications are discussed.

COGITATIONES

Temperature and Salinity Relationships in Marine Bottom Invertebrates[1]

By C. SCHLIEPER, H. FLÜGEL, and J. RUDOLF[2]

In recent times, the biological significance of temperature and salinity in sea water has been thoroughly discussed by GUNTER[3] and PEARSE and GUNTER[4]. A number of other excellent reviews touch on various aspects of the effect of temperature and salinity on marine organisms[5–8]. Here we should like to restrict ourselves to a few significant observations:

In order to study the influence of temperature and salinity on the viability and the metabolisms and thus on the distribution of marine species, it is not necessary to examine intact animals. In most cases, an analysis of surviving tissue pieces is sufficient, because thermal and osmotic resistance are largely based on local qualities in the cells. In order to prove this, we have compared the cellular thermal and osmotic resistance of some bivalves from the French Mediterranean coast taken out of different depths of water.

The temperature of sea water at 0–1 m depth on the French Mediterranean coast changes in the course of the year on an average of approximately from 13° to 23°C. The species which live in this area therefore are used to a yearly temperature variation of at least 10°C. The temperature changes rapidly with increasing depth. Already at 100 m depth, there occur only slight variations between 13° and 15°C. It is therefore to be expected that the species, living in these depths, are relatively stenothermic: that means that they can only sustain slight temperature changes.

The salinity of the sea water on the French Mediterranean coast changes very little, between 37 and 38‰. In the 'Etangs', however, it is different. In these lagoons, separated from the open sea, salinities from 37 to 15‰ and less do occur.

Seven species of bivalves were examined. The shore water forms, *Mytilus galloprovincialis*, *Tapes decussatus*, and *Cardium edule*, were easily obtained. *Mytilus galloprovincialis* and *Cardium edule* were obtained from the free sea shore as well as from the 'Étangs'. For purposes of comparison, *Mytilus edulis* and *Cardium edule* from the Baltic Sea were used later on. *Pinna nobilis* were obtained from medium depths by diving. *Pinna pectinata* and *Avicula hirundo* were finally dredged out of 100 m depth.

At first *the cellular thermal resistance* was measured on small surviving gill pieces in sea water at a temperature of 35°C and higher. The temperature was increased by 1°C every 15 min. At equal time intervals, the ciliary activity of the tissue was examined under the microscope. Typical and reproducible differences appeared. The gill cells of *Tapes decussatus* from the sand of the sea shore showed

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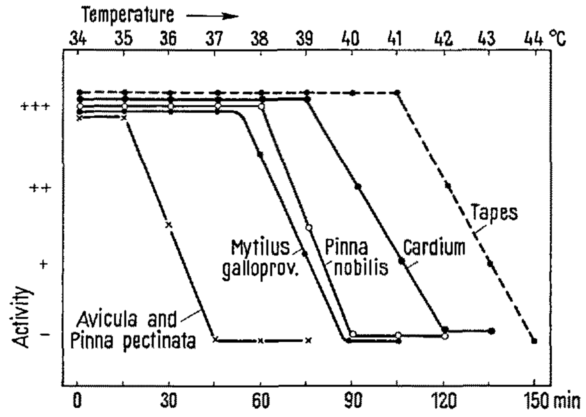

Fig. 1. Comparison of the cellular thermal resistance in the gill tissue of some bivalves from different depths.

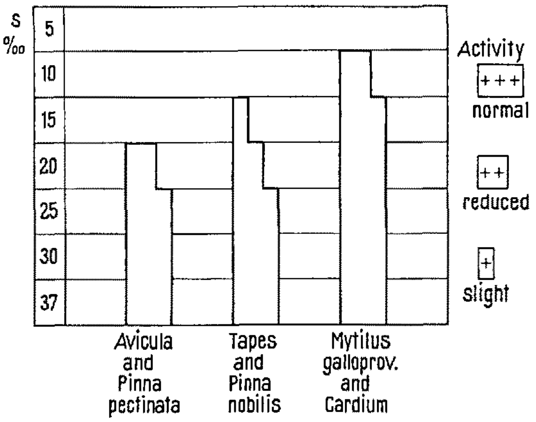

Fig. 2. Comparison of the cellular osmotic resistance in the gill tissue of some bivalves from different depths.

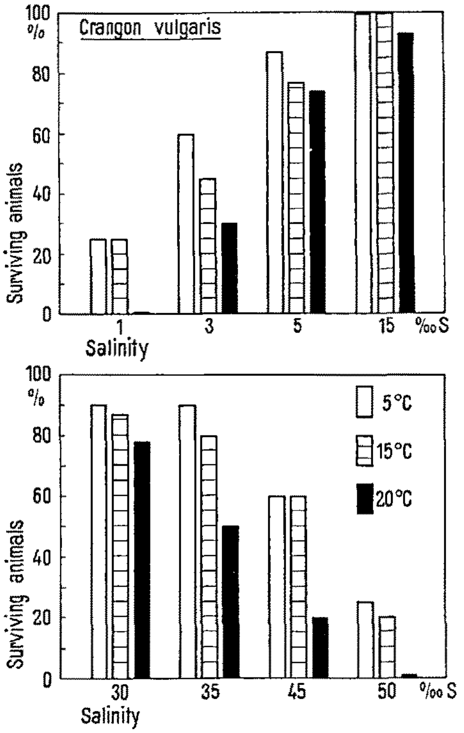

Fig. 3. Rates of survival for the shrimp *Crangon vulgaris* in sea and brackish water at different temperatures after 10 days acclimatisation.

the greatest resistance. *Cardium edule* also proved itself to be very resistant. *Mytilus galloprovincialis* and *Pinna nobilis* were a little less resistant. The bivalves *Avicula hirundo* and *Pinna pectinata* from the cool deep waters were rather sensitive to high temperatures (Fig. 1).

The same typical differences were found during other experiments in which the survival time was measured at various lethal constant temperatures.

Other experiments have shown that the salinity of the external medium influences the upper individual phenotypical temperature limit. Such observations can, of course, only be made with euryhalinic species. The tissues from *Cardium* were examined in this case. The bivalves were previously adapted to different salinities. Thereafter the individual thermal resistance had decreased with the lowering of the salinity. It is therefore necessary to work at the same salinity of sea water, if one wants to compare differences of genetic origin.

During the study of the cellular thermal resistance, the acclimation temperature must also be observed. Only when the objects have previously been acclimatised at the same temperature, can one measure comparable resistance differences. One must therefore consider the ability of individual phenotypical thermal acclimatisation. Such an ability does not exist in *Avicula hirundo* and *Pinna pectinata* living in cold deep water. But it is easy to demonstrate the individual thermal acclimatisation of the tissues in *Mytilus edulis* from the Baltic sea shore. Animals which have been acclimatised to 5°C, were, at the lethal temperature of 35°C, much less resistant than those individuals which were previously acclimatised to 20°C. If one changes the acclimatisation temperature from 5° to 20°C, or *vice versa*, then it takes approximately 4 days until the tissue has obtained the corresponding thermal resistance.

The cellular osmotic resistance can likewise be measured on small surviving gill pieces. The tissue pieces were immediately transferred to different sea water dilutions. The cell activity was controlled on the beating rate of the terminal cilias after 48 h. *Avicula hirundo* and *Pinna pectinata*, both from cold deep water, showed the least cellular osmotic resistance. The other species from higher water levels were, in comparison, much more resistant to the dilution of the external medium. But no common relationship between the thermal and osmotic resistance were found. *Tapes decussatus*, which showed by far the greatest thermal resistance, was osmotically more sensitive than *Cardium edule* and *Mytilus galloprovincialis* (Fig. 2).

Individual phenotypical differences may also appear during the examination of the osmotic resistance. The tissues of the *Mytilus galloprovincialis* from the brackish water of the 'Étangs' were plainly more resistant to the dilution of the external medium than the mussels from the more concentrated sea water of the free coast. The cellular resistance of the sea shore mussels, on the other hand, increased after being transferred into brackish water. The same observations, in the opposite sense, could be made on mussels from the 'Étangs' after being transferred into normal sea water. The individual acclimatisation of the cellular osmotic resistance to the changed salinity of the sea water, however, took up to 14 days' time. This process takes more time than the adjustment of the internal medium to the new concentration of the external medium. When the transferred mussels are already isotonic with the new external medium, after one day, the osmotic resistance bound to the colloidal structure of the protoplasma can still remain unchanged. The first signs of the resistance acclimatisation can be shown only after 2 or more days.

The process of the thermal and osmotic acclimatisation of the marine invertebrates, which posses osmoregulatory abilities, is more complicated. In this case, the osmotic resistance and *the osmoregulatory performance* depend largely upon the acclimatisation temperature. We have used the shrimp, *Crangon vulgaris*, in experiments with various temperatures and salinities[9]. In high and low salinities, the euryhaline shrimps are osmotically more resistant at low temperatures (Fig. 3). For a temperature range from 5° to 15°C, the optimal salinity of *Crangon* lies approximately between 15 and 30‰ salinity. At 20°C the resistance area or 'the zone of tolerance' is much smaller than at 15° and 5°C. These observations do not confirm earlier hypothetical conclusions[10] that for 2-year-old shrimps the optimum salinity decreases with a fall in temperature below 21°C. If, however, one reduces the temperature of the external medium below 3°C, the osmotic resistance of *Crangon* decreases more rapidly as the temperature approaches zero. The osmoregulation of *Crangon* in brackish water apparently functions sufficiently only within a middle temperature range, between 5° and 15–20°C.

In order to analyse the influence of the temperature upon the osmoregulation of *Crangon*, we determined the freezing point depression of the blood and the external medium at 5°, 10°, and 15°C. Figure 4 shows that sea water at 27‰ salinity is nearly isotonic with long-acclimatised individuals. The difference in the freezing points of the internal and external medium is very small in this case, and is possibly not caused by any activity of the animal. At all salinities of the external medium as applied by us, the osmoregulatory performance was observed to be strongest at 5° and not at 15°C.

At temperatures below 5°C, during which, as has already been pointed out, *Crangon* in brackish water survives only with difficulty, the osmoregulatory performance also decreases! It is not easy fully to demonstrate this decrease in the osmoregulatory performance at 2·5° or 1°C. During the first days, the osmoregulatory performance decreases only slowly, and then breaks down after a certain amount of time. When this happens, the animals will die quickly. If the animals in our experiments, for instance at 1°C, after a certain amount of time, were plainly weakened in their motions and reactions, then the concentration of the blood had always greatly decreased. These observations are contradictory to the previous data by BROEKEMA[10], according to which in all cases *Crangon* in warm brackish water is supposed to show a greater osmoregulatory performance than in colder water. But BROEKEMA has measured the electrical conductivity of the blood of *Crangon*. She therefore determined only salinity changes, not the osmotic changes due to organic molecules. We used the method of freezing point depression which gives more reliable figures for the osmotic concentration. We conclude that the shrimps are unable to live in brackish estuarine waters of low salinity in winter, because their osmoregulation breaks down at temperatures below 3°C. Furthermore, we believe that our observations show that one cannot explain the heavy immigration of marine invertebrates into tropical warm brackish waters simply by the hypothesis that osmoregulation is, on the whole, easier in a warmer medium[11,12].

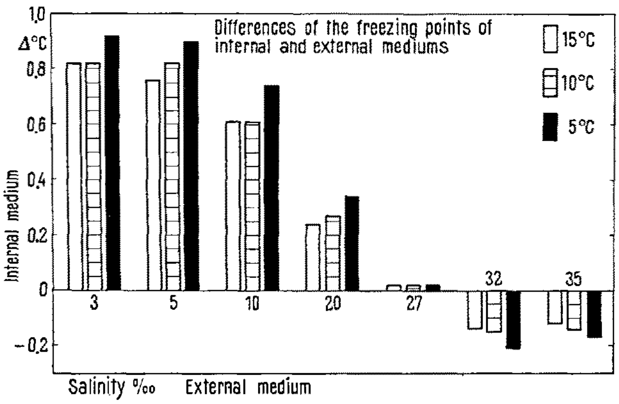

Fig. 4. The osmoregulatory performance of the shrimp *Crangon vulgaris* at various temperatures after acclimatisation in waters of different salinities.

Zusammenfassung

Das Ausmass des Temperaturbereiches mariner Arten ist in erster Linie durch erbliche zellphysiologische Eigenschaften bedingt. An isolierten Gewebestücken mariner Bodenevertebraten aus kaltem Tiefenwasser und aus wärmeren oberflächlichen Schichten lässt sich dementsprechend zeigen, dass ihr genotypischer thermischer Resistenz- und Leistungsbereich quantitativ verschieden ist und bei stenothermen Arten auch nicht individuell phänotypisch verändert werden kann.

Der Umfang des Salzgehaltsbereiches stenohaliner und euryhaliner Arten ist ebenfalls in erster Linie zellulär genetisch bedingt. Jedoch beruhen thermische und osmotische Resistenz auf verschiedenen zellphysiologischen Mechanismen. Nur bei euryhalinen Formen lösen Veränderungen der Salzkonzentration des Aussenmediums individuelle zelluläre Resistenz- und Leistungsverschiebungen aus.

Niedere Temperaturen begünstigen innerhalb des artspezifischen thermischen Resistenzbereiches Anpassung an extrem niedrige und hohe Salzkonzentrationen.

Die starke Einwanderung mariner Arten in tropische Brackwässer kann nicht durch die Annahme erklärt werden, dass die Osmoregulation in der Wärme leichter ist.

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[12] The authors wish to express their appreciation of the support of this work by the Universität Kiel, the Deutsche Forschungsgemeinschaft, and the Université de Paris, and in particular of the facilities and assistance so generously provided to the first author by Professor G. PETIT, Directeur, Laboratoire ARAGO, and his staff.

CORRIGENDUM

R. JAQUES and R. MEIER: *Pharmacological Characteristics of Bradykinin B.* Exper. vol. XVI, fasc. 8, p. 371 (1960).

The first sentence of the results (p. 371, left, 11th line from below) should read: A: 1. The isolated terminal guinea-pig ileum contracted upon addition of bradykinin B in final concentrations as low as 1 to 3 × 10^{-12} (g/l), i. e. 1 to 3 ng/ml.