The accumulation and utilization of algal metabolites dissolved in sea water by the medusa *Tiaropsis multicirrata*

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The accumulation and utilization of algal metabolites dissolved in sea water by the medusa Tiaropsis multicirrata

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It has been proved that the medusa Tiaropsis multicirrata is capable of accumulating algal metabolites dissolved in sea water.

C\textsuperscript{14}-labelled organic substances simulating dissolved algal metabolites, which are added to the solution, accumulate in the organs of the gastrovascular system, in the gonads and in the complex of marginal organs. It has been established that medusae are able to utilize the high-molecular metabolites of algae primarily by accumulating the products of hydrolysis of the initial substrate.

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We have obtained experimental data on the participation of certain marine invertebrates in the transformation of carbohydrate algal metabolites, including high-molecular ones. It has been established that C\textsuperscript{14}-labelled algal metabolites take part in plastic and energy metabolism.

* The numbers in the right-hand margin are the pages of the Russian text - translator.

**According to Ricker, metabolism associated with muscular activity - Translator.
The presence of the $^{14}$C label is registered in the basic biochemical components of the body - proteins, lipids, the calcified skeleton and the $^{14}$CO$_2$ discharged during respiration. The mechanisms of incorporation of the metabolites dissolved in sea water into the metabolism of organisms have not been clarified. It is therefore assumed that one of the initial stages of studying the mechanisms of utilization of dissolved organic substance by invertebrates should be the analysis of the incorporation of labelled compounds and their distribution in the morphological and biochemical structures of the organism.

We have attempted to determine the ability of the hydroid medusa Tiaropsis multicirrata to utilize the algal metabolites available in sea water.

The study is based on the principle of qualitative determination of $^{14}$C-labelled organic substances of autotrophic origin (found in the bodies of the medusae) which resemble the algal metabolites dissolved in sea water. The algal metabolites were taken in concentrations typical of the coastal zone of the Barents Sea where our investigations were conducted in the summer of 1968-1969.

The coastal zone in the study area is characterized by a significant (up to 40 mg/1) content of organic substance dissolved in the sea water /6/. According to our data, the partial concentration of dissolved carbohydrates in the surface layer of water reaches 3 mg/1. The basic source of organic substances in the coastal zone of the Barents Sea is macrophytes which discharge into the environment (during life and
after death) a considerable portion of the organic substance synthesized by them /4, 6/. Given this, the C\textsubscript{14}\)-labelled substrates used in our experiments were the following chemical compounds which represented the algal metabolites and were dissolved in the sea water: 1) a total hydrolyzate of \textit{Fucus vesiculosus} from C\textsubscript{14}-labelled thalli of macrophytes produced by acid-catalyzed hydrolysis (HCl) using standard methods; the molecular weight of the hydrolyzate did not exceed 600 according to K.M. Khailov; 2) high-molecular sugars (polysaccharide-C\textsubscript{14}) with a molecular weight of 50000 and greater, isolated using the usual method from C\textsubscript{14}-labelled cultures of the single-celled algae \textit{Platymonas viridis}; the polysaccharide-C\textsubscript{14} was purified using the method of gel filtration with neutral Sephadex G-75; 3) C\textsubscript{14}-glucose, a commercial product in the USSR, and C\textsubscript{14}-alginate isolated from labelled thalli of \textit{F. vesiculosus}. The Kilin method described by G.K. Barashkov /1/ served as the basis for the isolation and purification of C\textsubscript{14}-alginate.

The experiments were carried out with hydroid medusae \textit{Tiaropsis multicirrata} (M. Sars, 1835) from the Barents Sea. These medusae keep to small depths close to shore, often to the uppermost layers of water, preferring the places of strong ebb and flow. These medusae are a prolific species in the Barents Sea. They were caught in Yarnyshna Bay with a plankton net and hand nets, and were kept in aquariums without running water at a temperature of 9-10\textdegree C.

The method of autoradiography was used to determine the C\textsubscript{14}-organic substances which had accumulated in the bodies of the medusae. The
hydrolysis of high-molecular sugars and the incorporation of C$^{14}$ into the water-soluble chemical compounds of the animal body were judged on the basis of the radiometric processing of the molecular fractions isolated using the method of gel filtration.

In order to obtain the autoradiographs, the animals were incubated in a radioactive solution, usually 0.5 litres of sea water with a designated concentration of labelled compounds. The concentration of Fucus vesiculosus hydrolyzate added to the solution amounted to 1 mg/l. In the experiments with C$^{14}$-glucose, C$^{14}$-polysaccharide and C$^{14}$-alginate we used concentrations of the substrate which corresponded to the carbohydrate content in the coastal zone of the sea. The maximum density of the stocking of medusae did not exceed 120 specimens per litre, which approximated the density of the natural population in the places where T. multicirrata is concentrated. Most of the experiments were conducted with recently caught medusae.

The peculiarities of labelling through the bacterial link were determined by using sea water filtered through a No. 2 membrane filter (mesh size 0.5μ) prior to the experiment, sea water filtered through a No. 2 membrane filter with an addition of tetracycline (40 mg/l) and unfiltered sea water.

We had earlier shown /3/ that T. multicirrata with reduced mobility due to various physiological disorders (after two weeks in an aquarium without running water) are capable of accumulating high-molecular sugars
from the solutions more rapidly than recently caught medusae. Given this, we applied reversible anesthesia of the medusae using halopyridol. The controls were recently caught medusae and specimens which had lost their mobility after being kept in aquariums without running water for a week. The sea water in these experiments was also filtered through a membrane filter.

As a rule, incubation of the specimens in a radioactive solution lasted for 24 hours, after which the medusae were transferred to sea water and thoroughly washed. They were then placed on clock glasses, dried of excess water with the help of filter paper, fixed on No. 2 membrane filters and dried at a temperature of 30°C. The specimens prepared in this way were used for contact autoradiography on plates with MP-type emulsion. The exposure time was empirically selected, comprising six weeks for our preparations. An amidol developer was used to develop the autoradiographs /2/. The microphotographs were processed in the usual way.

The hydrolysis of high-molecular sugars in the presence of *T. multicirrata* was carried out as follows. Medusae were placed in sea water of a designated concentration of C\(^\text{14}\)-polysaccharide (0\(\mu\)g0000) which had been filtered through a No. 2 membrane filter and the content of C\(^\text{14}\)-polysaccharide in the solution and the products of its hydrolysis in the medium checked 3, 6, 12 and 24 hours later using the method of gel filtration with neutral Sephadex G-75. For this purpose, 15 ml aliquots of the solution were taken from the experimental vessels and
fractionated on a helium column into two components - high-molecular (M>50000), and average- and low-molecular (M<10000). After separation the fractions were concentrated by evaporation, distributed on plane tables, dried and counted with a T-25-6 bend-type counter, taking into account self-absorption in the salt layer.

In order to determine the labelling of water-soluble chemical compounds, samples of 60 medusae each from the experiments on the hydrolysis of C¹⁴-polysaccharide were dried down to a constant dry weight and then homogenized and extracted using distilled water for a period of 30 minutes at room temperature. The obtained water extract was centrifuged and the supernatant separated on a G-75 column. An ε²⁴A spectrophotometer was used to determine extinction (ε₂₃⁵), the proportional concentration of dissolved organic substance /7/ and the radioactivity of the different fractions.

Contact autoradiography has shown that T. multicirrata are capable of accumulating labelled low-molecular compounds from an aqueous environment. The medusae store up the algal metabolites added to the solution (the total hydrolyzate from the thalluses of F. vesiculosus) in the organs of the gastrovascular system, gonads and in the complex of marginal organs. The zones of maximum emulsion irradiation are observed in the region of the oral arms (lips) and the stomach (fig. 1).

There were no significant differences in any of the variants of the experiment as to the effect of sea water microflora on the labelling of morphological components in medusae. The T. multicirrata kept in filtered water incorporate labelled compounds in the same organs as
do the medusae incubated in unfiltered sea water rich in microorganisms. The use of antibiotics (tetracycline) does not alter the nature of the labelling either. Neither were there any differences revealed in the accumulation of dissolved organic substance by medusae which before the experiment had been kept in non-running water for quite some time and therefore had partially lost their capacity for movement, and by the medusae rendered motionless by halopyridol. The latter is of particular significance when determining how the labelled organic substance enters the organism and is distributed within it.

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**Fig. 1.** The distribution of C\(^{14}\)-labelled organic substances in the body of the medusa Tiaropsis multicirrata. The light areas on the autoradiograph signify the presence of labelled compounds in the bodies of the medusae. The zones of maximum emulsion irradiation are located in the region of the oral arms (lips) and stomach. The gonads and complex of marginal organs are less illuminated. Fixation occurs after 24 hours of incubation in a radioactive solution. The MP emulsion is used. Exposure time - 6 weeks. Enlarged 3.5 x.

The resulting autoradiographs do not indicate an accumulation of labelled compounds over the entire body surface of the animal, or an even distribution of them in the subumbrella. This fact either rules out the possibility of the predominance of an osmotic (diffuse)
mechanism in the process of label accumulation, or points to the presence of strictly defined zones in which the diffuse gradient manifests itself. We consider the most likely explanation to be an active permease transfer of substances through the cell membranes, though we do not exclude the existence of other mechanisms as well. The primary significance of permease transfer (or any other form of transfer effected by enzymes) is proved by the fact that the zones of emulsion irradiation on the autoradiograph correspond to the morphological structures which are assumed to provide the enzymes responsible for the transfer. As indicated above, these structures are primarily the gastrovascular system and the complex of marginal organs. The concentration of the label in the gonads points to the possibility of the dissolved organic substances being utilized by the growing planulae (either through the system of radial canals or direct).

It should be noted that the labelling of dissolved organic substances in the bodies of sea animals by means of autoradiography has not yet been investigated, with the exception of a series of studies by J.C. Ferguson /8, 9/ carried out with starfish. The experiments of Ferguson showed that amino acids can penetrate the cell membranes of the starfish and be evenly distributed in the cytoplasm of the epidermal cells in less than an hour. The author attributes the localization of the label in the epidermal tissue to the possible existence of several types of barriers which are capable of obstructing most of the internal migrations of amino acids, thus making the autonomous
existence of epidermal tissues possible. According to Ferguson, these barriers can be of great importance to the mechanism which prevents the loss of nutrients in the body fluids.

Fig. 2. Autoradiographs of T. multicirrata. Fixation after 6 hours of incubation of the medusae in a solution of C\textsuperscript{14}-glucose (A) and C\textsuperscript{14}-alginate (B). MP emulsion, exposure time 6 weeks, enlarged 3.5 x.

We also observed the concentration of the label in medusae in the experiments on the accumulation of C\textsuperscript{14}-glucose and C\textsuperscript{14}-alginate with a six-hour exposure of the animals to a radioactive solution (fig. 2). The latter is of particular interest in connection with the possibility of utilizing high-molecular compounds from sea water. The accumulation of high-molecular algal metabolites in the bodies of medusae is apparently due to both the physico-chemical sorption of alginate molecules on the body surface and the utilization of the transformed products of hydrolysis. It is a known fact that alginate is a compound which is poorly hydrolyzed by acids; however, this does not exclude the possibility of extracellular hydrolysis of this substance by the exoenzymes of alginolytic bacteria found in sea water /11/. Apart from that there are other pathways of alginate transformation in sea water, which can provide...
the organism with easily accessible and assimilable substances. The well-known fact that alginic acid dissociates in sea water is an indication of one of the pathways. The degree of dissociation depends largely on the chemical structure of the alginic acid, for example on the quantitative ratio of d-mannuronic and l-guluronic acid in the alginate /10/, as well as on the amount of admixtures of other polysaccharides containing fucose, xylose, uronic acid and sulphate groups.

Our experiments have confirmed the possibility of T. multicirrata utilising high-molecular sugars. Fig. 3 shows the decrease of $^{14}$C-polysaccharide in the solution (curve 1) and the appearance of low-molecular fragments (curve 2). Both values reflect the content of macromolecules ($M \geq 50000$) and the products of their hydrolysis ($M \leq 10000$) in percent of the initial amount of $^{14}$C-polysaccharide in the solution at different intervals. As can be seen, the amount of the products of
macromolecular hydrolysis does not correspond to the computed value.

Fig. 4. The absorption of the products of C\textsuperscript{14}-polysaccharide hydrolysis (mcg • 100 specimens\textsuperscript{-1}) by T. multicirrata medusae from the solution at different intervals from the beginning of incubation.

The difference in the content of high-molecular substrate and low-molecular fragments in the medium is due to the fact that the medusae (and partially the microorganisms) utilize the products of macromolecular hydrolysis found in the solution (fig. 4). In the initial stage, the utilization of low-molecular organic substances from the solution is uniform. However, six hours later, the specific rate of utilization diminishes, which is apparently due to the decrease in the amount of substrate in the medium. A certain effect may also be created by the accumulation of toxic metabolic products in the solution, the disturbance of the gas regime, etc.

In the conditions of our experiment, the specific rate of carbohydrate utilization by the medusae amounted to 0.22-0.27 mcg of the substrate per specimen over a one-hour period. Assuming that the density of the T. multicirrata population in natural conditions is equivalent to the experimental density, one can roughly estimate the degree
of transformation (ingestion) of dissolved carbohydrates in the biotope. With a 3 mg/l concentration of dissolved carbohydrates in the surface layer of water (the maximum observed in our experiments), the medusae transform up to 24% of the dissolved carbohydrates over a period of 24 hours. For the areas with a lower content of dissolved carbohydrates at a constant rate of utilization, this value will naturally be higher. It should again be noted that the given calculation is but an example which points to the necessity of taking into account the transformation of organic substances by invertebrates.

We already know of the possibility of sorptive accumulation of high-molecular compounds found in sea water by certain invertebrates /3/. The C\(^{14}\)-polysaccharide isolated from Platymonas viridis cultures can be sorbed on the body surface of the animals for subsequent utilization, whereas the polymers of humic nature are sorbed on surfaces without further assimilation.

The separation of the water extract from the bodies of T. multicirrata medusae using the method of gel filtration shows that the incorporation of compounds with a molecular weight not exceeding 10000 is prevalent.

The obtained data permit us to assume that the accumulation of macromolecules dissolved in sea water occurs mostly as a result of the utilization of low-molecular fragments—the products of hydrolysis of the initial substrate. According to the most recent conceptions /5, 6/, sea organisms interact via the water medium with the help of external
metabolites. The ability of *T. multicirrata* medusae to assimilate dissolved algal metabolites found in sea water is of great importance for the understanding of ecological relations in the coastal zone of the sea and for the evaluation of the flow of energy and matter from the autotrophic to the heterotrophic level.

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