ΑΝΑΚΟΙΝΩΣΕΙΣ ΜΗ ΜΕΛΩΝ

ΒΙΟΧΗΜΕΙΑ.— **Contribution to Plancton chemistry,** *by* ***A. Christomanos, A. Dimitriadi and V. Gardiki****. Ἀνεκοινώθη ὑπὸ τοῦ κ. Γεωργ. Ἰωακείμογλου**.

Plancton from the Ionian Sea was quantitatively analysed about his composition of amino acids, traces elements, fats and carotenoids.

During the oceanographic cruise[1] «Tithys» by H.M.S. «Leros» in September 1961, we collected[2] in the Ionian Sea, (between 38°.15′2 and 38°.13′2 North, and 21°.20′2 and 21°.19′6 East), samples of zooplancton containing mainly the species of Centropagus Hamatus, Oithona Similis, Temora Longicornis, and Acartia Bifilosa (pict. I). The zooplancton was collected from the sea surface during the night and was kept at 5°C in 5% Formalin until the end of the expedition.

EXPERIMENTAL METHODS

A. Amino acids.

The Formol fixed Plancton was washed with distilled water centrifugated and dried at 50° in vacuum.

The total N content was determined by the semi Mikrokjeldahl method.

A definite quantity (8-10 mg) was hydrolysed with 6 N HCl for 24 hours, then the HCl evaporated. An aliquot part of the aqueous solution of the hydrolysate, corresponding to about 0.144-0.155 mg. of total N, was chromatographied, after desalting, with aqueous Phenol in Ammonia and HCN atmosphere for the first run, and with Butanol-acetone water (I + I + I) for the second run.

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* Α. ΧΡΗΣΤΟΜΑΝΟΥ, Α. ΔΗΜΗΤΡΙΑΔΟΥ καὶ Β ΓΑΡΔΙΚΗ, Συμβολὴ εἰς τὴν χημικὴν ἰδιοσυστασίαν τοῦ πλαγκτοῦ

** Ἀνεκοινώθη εἰς τὴν συνεδρίαν τῆς 8 Φεβρουαρίου ἐ.ἔ (βλ. ἀνωτ., σ 63).

[1] The cruise was sponsored by the Department of Navy and the National Hellenic Oceanographic Society. Leader of the expedition was Prof. Dr. An A. Christomanos.

[2] The samples were collected by the expedition's assistant D. Giannitsis from the Department of Biochemistry of the University of Thessaloniki.

The quantitative estimation of the amino acids was carried out by measuring the colour intensity of their Ninhydrin spots by means of a densitometer and by multiplying the values of the densitometer by the square area of each spot. The resulting values were compared with spots of the same free pure amino acids of known quantities, and the absolute chromatographied quantity deduced in this way. The obtained results were in good agreement with blank determinations, where an extraction of the spots by acetone gave no satisfactory results.

Pict 1. Species of Plancton examined.

An exception of the values measured by the densitometer, was mainly observed for arginine, which was always giving very large spots with rapidly fainting coloration.

As location reagents, Ninhydrin and a by $Na_2CO_3$ alcaline solution of b-naphthoquinonsulfonic sodium, were used at first instance. For individual amino acids, the Sakaguchi reagent for arginine, and the Ehrlich p-Dimetylaminobenzaldehyd reagent for tryptophane, were used.

For the determination of the Sulphur containing amino acids the technique of Dent (1-2) was used by moistening the dry point of application of the sample to be chromatographied, first with few drops of an 1 % so-

lution of Ammonium molybdate, and after drying by applying 4-5 drops of 30% perhydrol, in order to oxidize the two Sulpho amino acids, methionine to methionine Sulfone and meth. Sulfoxide, which do not overlap with Valine like methionine, and respectively cystine to cysteic acid, or other positive Ninhydrin reaction giving products.

B. Fats and Lipids.

For the determination of the fats and the lipids, a quantity of zooplancton (416.2 mg.) was extracted in the Soxhlet apparatus with Acetone for 24 hours. The resulting Acetone extract was evaporated at room temperature, dried in vacuum and extracted repeatedly with Petrolether of low boiling point. The quantitatively filtered extract was evaporated, and the residue weighted after drying in vacuum.

A chromatographic separation of the intense orange-yellow coloured Petrolether extract was attempted using a chromatographic column (1 × 25 cm.) filled with an homogeneous mixture of $CaCO_3$ and Celite (3 : 1). The small available quantity of Plancton did not permit any further investigation and isolation of the resulting zones.

C. Trace elements.

For the qualitative and quantitative estimation of the trace elements contained, probably as complex with enzymes, or accumulated in Zooplancton (3), the sample was successively treated with conc. $HNO_3$ and HCl p. anal. until complete dissolution occured. The acid solution was evaporated and the residue dissolved in redistilled water, filtered and diluted with water to 10 ml. Aliquots of the solution were taken for one and two ways, chromatographic, detection of the kations Fe, Cu, Mn, Pb, Ca, Mo, Zn, Cr, and Al (4-6).

The chromatographic separation solvents and location reagents which were used for the different kations are listed in the table I.

Plancton hydrolysate (0,05 - 0.1 ml.) were applied on several Whatman No I paper strips 15 × 40 cm., and the strips placed in chromatographic tanks containing each a different separation solvent. After the solvent has ascended for the required time (in average 4 hours) the dry strips were sprayed each with a different location reagent for tracing the kations overmentioned. The colour intensity of the appearing spots was measured 6 hours after their location by means of the densitometer, in the same way, as previously by exposed for the quantitative determination of the amino acids.

TABLE I.

| Separation solvents | Kations | Location reagents to be sprayed | Colour |
|---|---|---|---|
| Methanol, 36 % HCl, water 8 : 1 : 1 | Fe | 0.5 % aqu. sol. $K_4Fe(CN)_6$ | blue |
| | Ca | conc. ethanol. sol. of Alizarine, afterwards spraying with 1N NaOH | violet |
| Acetone 36 % HCl, water 8 : 1 : 1 | Mn | 1 % aqu. sol. $AgNO_3$ and $NH_4OH$ | brown |
| | Cu | 0.5 % ethan. sol. of Diphenylcarbazid | red - brown |
| | Pb | » » » » » | white - green in U.V. |
| | Co | conc. ethanol. sol. of Diphenylthiocarbazon afterwards spraying with $NH_3$ | pink |
| | Mo | » » » | pink - violet |
| | Al | con. ethanol. sol. of Alizarine, spr. afterwards with $NH_4OH$ | pink - red - orange |
| | Cr. | conc. ethanol. sol. of Alizarine, spr. afterwards with 1N NaOH in $NH_3$ atmosp. | pink - violet |
| Ethanol, 5N HCl, 90 : 10 | Cr. | conc. ethanol sol. of Alizarine sp. 1N NaOH in $NH_3$ | violet |
| Butanol, 36 % HCl, 100 : 2 | Zn | 0.5 % ethanol. sol. of Diphenylcarbazid | dark red |
| Ethanol, 5N HCl, 90 : 10 | Zn | conc ethanol. sol. of Diphenylthiocarbazon sp. 1N NaOH in $NH_3$ | pink |

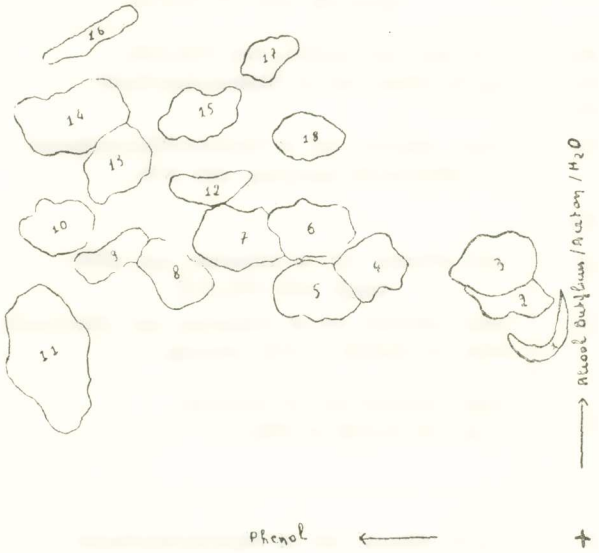
Blank determinations were carried out with different quantities of the chlorides of the kations mentioned. The colour intensity of the spots was found to be in good agreement with the values given by the densitometer and the different concentrations of the kations. Lead was detected by its fluorescence in U.V. light, after spraying with Diphenylcarbazid.

## RESULTS AND DISCUSSION

A. Proteins and amino acids.

The total N determination of the dry Plancton gave the values 8.1 - 8.4 mgs. %.

The chromatographic analysis of Plancton demonstrated the presence of 17 amino acids which are listed in the table II. Curiously we could not detect lysine which probably is present in very minute quantities. On the other hand glutamic acid is contained in remarkable quantity as well as arginine, leucine and isoleucine (pict. 2).




Pict. 2 Chromatogram of the hydrolysate of 1.72 mg. of Plancton. 1. Oxyd. product of Cystine, 2. Aspartic acid, 3 Glutamic acid, 4 Serine, 5 Glycine, 6. Threonine, 7. Alanine, 8 Histidine, 9. b-Aminobutyric acid, 10. Proline, 11. Arginine, 12. Methionine sulfone, 13. Valine, 14. Leucine - Isoleucine, 15. Tyrosine, 16. Phenylalanine, 17. Dijodotyrosine.

If we take in account that some amino acids, especially the aromatic amino acids are destroyed during the chromatographic run at a rate of 5 - 10 %, we must assume that the value found for the amino acid content of Plancton is relatively higher. The N content of Plancton proteins was calculated to 12.3 %, in average. Bearing in mind that the sum of the total amino acids determined in 100 g. Plancton represents the total amount of proteins, we can calculate the amount of amino acids per % of Plancton proteins. It is surprising that the per cent composition of the Plancton amino acids resembles very much to the composition of some prolamines

such as hordeine, with the high content of glutamic acid, leucine and isoleucine.

The nutritive value of Plancton proteins has a high biological value because they contain some essential amino acids, like threonine, phenylalanine, leucine and histidine.

We hope for a more detailed investigation of Plancton proteins, about their properties, as well as their physicochemical behaviour.

TABLE II.

Approximate N content and amino acid composition of Plancton and Pl. Proteins. The values are given in mgs °/₀ Plancton.

| Amino acids | Value of amino ac. N per °/₀ Planc. | Amino acids per °/₀ Planc. | Amin. acid N per °/₀ of the total amin. acid N. | Amino acid. per °/₀ of Planct. Prot. |
|---|---|---|---|---|
| Aspart. ac. | 0.23 | 2.90 | 3.90 | 4.50 |
| Glutam. ac. | 1.30 | 13.70 | 21.70 | 28.07 |
| Serine | 0.15 | 1.10 | 2.60 | 2.25 |
| Glycine | 0.38 | 2.08 | 6.40 | 4.26 |
| Threonine | 0.16 | 1.30 | 2.70 | 2.66 |
| Alanine | 0.32 | 2.06 | 5.40 | 4.22 |
| Histidine | 0.27 | 1.00 | 4.50 | 2.04 |
| b-aminobutyr. ac. | 0.05 | 0.42 | 0.96 | 0.86 |
| Proline | 0.06 | 0.52 | 1.06 | 1.06 |
| Meth. sulfone | 0.03 | 0.45 | 0.58 | 0.92 |
| Arginine | 0.74 | 2.29 | 12.30 | 4.69 |
| Tyrosine | 0.28 | 2.68 | 4.70 | 5.49 |
| Valine | 0.90 | 7.52 | 14.02 | 15.40 |
| Leuc. Isol. | 0.88 | 8.26 | 14.70 | 16.82 |
| Phenylal. | 0.09 | 1.16 | 1.60 | 2.37 |
| Dijodotyros. | 0.05 | 1.79 | 0.96 | 3.66 |
| Oxyd. prod. of Cystine | 0.01 | 0.16 | 0.29 | 0.32 |
| Amide $NH_2$ | 0.01 | 0.01 | 0.19 | 0.02 |
| Total | 5.91 | 48.80 | 98.56 | 99.51 |

* The values above are given for dry Plancton.

** The N content of Plancton proteins was calculated.

$$\frac{5.91 \cdot 100}{48.80} = 12.3\ \%$$

B. Fats and Lipids.

The Petrolether extract of Plancton which contained fats, sterols and lipids, were found to be 7.05 % of the weight of dry Plancton. The Petrolether extract showed, as previously mentioned, an intense orange-yellow colour, and exhibited after saponification, by column chromatographie, six distinct zones. The coloration of the zones, beginning from the top of the column was:

Pict. 3. Chromatogram of the Petrolether extract of Plancton

I. orange.
II. pink, very faint.
III. orange-red, very faint.
IV. grayish - pink.
V. orange, very intense.
VI. orange-red, very faint.

The fact that we could distinguish six zones, using $CaCO_3$ as absorbent indicates the presence of carotenoids, probably carotrenoid alkohols, which as known, are absorbed from $CaCO_3$, though the carotenes travel through the column.

Unfortunately the small quantity of lipids available did not permit any further investigation.

C. Trace Elements.

In dry Plancton* the following kations were detected and quantitatively determined:

Al = 2.6 mg. %
Fe = 0.56 mg. %
Co = 2.10 mg. %
Cu = 0.03 mg. %
Zn = 1.90 mg. %
Cr = 0.5 m. %
Pb = was detected but not quantitavely determined.
Mn = » » » » »
Mo = » » » » »
Ca = 1.1 mg. %.

* The $H_2O$ content of fresh Plancton represents, about 75 % of its weight.

The quite remarkable high values of Al and Co can be explained as a specific accumulation of these elements by planctonic organisms, in as much as we do know that several marine organisms accumulate different elements, especially Co, Mo, Pb (8 - 10) e.t.c Nevertheless it is unquestionable that extensive and minute new analyses about the presence of trace elements in Plancton should be undertaken.

*(From the Department of Biochemistry of the Aristotelian University of Thessaloniki and the Marine Biochemical Station of St. George, Limni Director: Prof. Dr. An. A Christomanos).*

ΠΕΡΙΛΗΨΙΣ

Εἰς τὴν παροῦσαν ἐργασίαν ἐξετάζονται ὡρισμένα εἴδη τοῦ ζωοπλαγκτοῦ τοῦ Ἰονίου πελάγους, συλλεγέντα κατὰ τὴν ὠκεανογραφικὴν ἐξερεύνησιν «Τηθύς», διὰ τοῦ πλοίου τοῦ Β. Ν «Λέρος», κατὰ τὸν Σεπτέμβριον 1961.

Τὰ ἀποτελέσματα τοῦ προσδιορισμοῦ τοῦ ὁλικοῦ N κατέδειξαν, ὅτι τοῦτο ἐνέχεται μεταξὺ 8.1 - 8.4 % τοῦ ξηροῦ πλαγκτοῦ. Ἡ χρωματογραφικὴ ποιοτικὴ καὶ ποσοτικὴ ἔρευνα τοῦ πλαγκτοῦ κατέδειξεν, ὅτι περίπου ποσοστὸν 48 % τοῦ βάρους τοῦ ξηροῦ πλαγκτοῦ ἀποτελεῖται κατὰ πᾶσαν πιθανότητα ἐκ πρωτεϊνῶν. Τὰ ἐκ τῆς ὑδρολύσεως τῶν πρωτεϊνῶν αὐτῶν προκύπτοντα 17 ἀμινοξέα, ὡς καὶ αἱ ποσοτικαὶ αὐτῶν ἀναλογίαι ἀναφέρονται λεπτομερῶς εἰς τὸν ἐπισυναπτόμενον πίνακα. Ἡ ἀνευρεθεῖσα μεγάλη σχετικῶς ποσότης γλουταμινικοῦ ὀξέος, λευκίνης καὶ ἰσολευκίνης προσεγγίζει τὰς πρωτεΐνας αὐτὰς πρὸς τὰς προλαμίνας. Λυσίνη καὶ τρυπτοφάνη δὲν ἀνευρέθη *. Εἶναι μᾶλλον πιθανόν, ὅτι ἀμφότερα τὰ ἀμινοξέα ταῦτα ἐνέχονται εἰς μικροτάτας ποσότητας μὴ δυναμένας νὰ πιστοποιηθοῦν, δεδομένου ὅτι ὡρισμένον ποσοστὸν τῶν ἀμινοξέων, ἰδίως ἡ τρυπτοφάνη, καταστρέφεται κατὰ τὴν χρωματογραφίαν. Ἐκ τῶν βιολογικῶς ἀναντικαταστάτων ἀμινοξέων ἀνευρέθησαν ἡ μεθειονίνη, ἡ φαινυλιοαλανίνη, ἡ θρεονίνη καὶ ἡ ἱστιδίνη. Συνεπῶς τὸ πλαγκτὸν περικλείει πρωτεΐνας μεγάλης βιολογικῆς ἀξίας.

Περαιτέρω διεπιστώθη, ὅτι τὸ πλαγκτὸν περιέχει μέχρις 7.05 % λιπαρὰς εἰς πετρελαϊκὸν αἰθέρα διαλυομένας ἑνώσεις. Ἡ χρωματογραφία τοῦ εἰς πετρελαϊκὸν αἰθέρα πορτοκαλεοχρόου διαλύματος τῶν ἑνώσεων τούτων διὰ στήλης ἐνεχούσης $CaCO_3$ κατέδειξε τὴν παρουσίαν καροτινοειδῶν ἀλκοολῶν.

Διὰ τῆς ἀνοργάνου χρωματογραφίας τῶν κατιόντων ἐδείχθη, ὅτι ἀνευρίσκονται ἐντὸς τοῦ πλαγκτοῦ τὰ κατωτέρω ἀναφερόμενα κατιόντα, εἴτε ὑπὸ μορφὴν τῶν προσθετικῶν αὐτῶν ὁμάδων εἴτε καὶ πιθανῶς ὡς εἰδικῶς συσσωρευόμενα ἰχνοστοιχεῖα Τὰ χρωματογραφικῶς μετὰ βεβαιότητος προσδιορισθέντα στοιχεῖα ἦσαν:

F, Cu, Mn, Pb, Ca, Cr, Zn, Al, Co καὶ Mo.

Ἐκ τούτων τὰ περισσότερα προσδιωρίσθησαν ποσοτικῶς μὲ πλάτος λάθους πιθανῶς ± 10 %.

Ἀξία μνείας τυγχάνει ἡ ἰδιαιτέρως μεγάλη ποσότης κοβαλτίου καὶ ἀργιλλίου.

* Τρυπτοφάνη δὲν ἀνευρέθη ἕνεκα τῆς καταστροφῆς αὐτῆς κατὰ τὴν ὀξίνην ὑδρόλυσιν.

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Ὁ Ἀκαδημαϊκὸς κ. **Γεώργ. Ἰωακείμογλου,** ἀνακοινῶν τὴν ἀνωτέρω μελέτην εἶπε τὰ ἑξῆς.

*Ἡ μελέτη τοῦ καθηγητοῦ κ. Α. Χρηστομάνου καὶ τῶν συνεργατῶν του Ἀ. Δημητριάδου καὶ Β. Γαρδίκη «Συμβολὴ εἰς τὴν Χημείαν τοῦ πλαγκτοῦ τοῦ θαλασσίου ὕδατος», προέρχεται ἀπὸ τὸ Βιοχημικὸν Ἐργαστήριον τοῦ Ἀριστοτελείου Πανεπιστημίου Θεσσαλονίκης καὶ τοῦ Ἐργαστηρίου Θαλασσίας Βιοχημείας Ἅγ. Γεώργιος, Λίμνη. Οἱ ἐρευνηταὶ ἐξήτασαν ἐνταῦθα ὡρισμένα εἴδη τοῦ ζωοπλαγκτοῦ τοῦ Ἰονίου Πελάγους, τὰ ὁποῖα συνελέγησαν κατὰ τὴν ὠκεανογραφικὴν ἐξερεύνησιν «Τηθὺς» διὰ τοῦ πλοίου τοῦ Β. Ν. «Λέρος» κατὰ τὸν Σεπτέμβριον 1961.*

*Ὁ προσδιορισμὸς τοῦ ὁλικοῦ ἀζώτου ἀπέδειξεν ὅτι τὸ στοιχεῖον τοῦτο ὑπάρχει μεταξὺ 8,1 - 8,4 % τοῦ ξηροῦ πλαγκτοῦ. Ποσοστὸν 48 % τοῦ ξηροῦ πλαγκτοῦ ἀποτελεῖται, κατὰ πᾶσαν πιθανότητα, ἐκ πρωτεϊνῶν. Εἰς ἐπισυναπτόμενον πίνακα ἀναφέρονται τὰ ἐκ τῆς ὑδρολύσεως ἐκ τῶν πρωτεϊνῶν προκύπτοντα ἀμινοξέα.*

*Ἐκ τῶν βιολογικῶς ἀναντικαταστάτων ἀμινοξέων ἀνευρέθησαν ἡ μεθειονίνη, ἡ φαιυνλιοαλανίνη, ἡ θρεονίνη καὶ ἡ ἰστιδίνη. Τὸ πλαγκτὸν περικλείει συνεπῶς πρωτεΐνας σημαντικῆς βιολογικῆς ἀξίας. Ἀνιχνεύθησαν ἐπίσης λιπαραὶ οὐσίαι διαλυόμεναι εἰς πετρελαϊκὸν αἰθέρα, καθὼς καὶ τὰ ἑξῆς στοιχεῖα: F, Cu, Mn, Pb, Ca, Cr, Zn, Al, Co, Mo.*

*Τονίζεται ἰδιαιτέρως ἡ σχετικῶς μεγάλη ποσότης κοβαλτίου καὶ ἀργιλλίου.*