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De novo biosynthesis and base composition of total and ribosomal ribonucleic acids during early development in *Artemia* sp.

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De novo synthesis of total and ribosomal ribonucleic acids has been studied during the early stages of *Artemia* sp. development. By in vivo incorporation studies of $[^{14}C]HCO_3^-$ an increase has been found in both total and ribosomal RNA synthesis post hatching, with a similar distribution of radioactivity and base composition.

Synthesis, de novo; Ribonucleic acid; Development, early; (*Artemia*)

1. INTRODUCTION

Nucleotide metabolism and its biosynthesis - as components of ribonucleic acids - have been extensively studied in *Artemia* sp. [1] because of the particular features connected with its larval development. Several authors have demonstrated changes in nucleic acid concentrations during larval development of this crustacean [2,3]. On the other hand, evidence of the capacity of *Artemia* nauplii to synthesize de novo purine nucleotides and of the increase of this synthesis during larval development has been reported previously by our laboratory [4].

In this paper, we have studied the changes in the de novo synthesis of total and ribosomal RNA and the base composition of the RNAs during the early stages of *Artemia* development by in vivo incorporation of $[^{14}C]HCO_3^-$ as a de novo precursor.

2. MATERIALS AND METHODS

2.1. *Materials*

Artemia cysts were obtained from San Francisco Bay Brand Co., Menlo Park, CA. $[^{14}C]HCO_3^-$ was purchased from The Radiochemical Centre (Amersham, UK). All nonradioactive purine bases and their ribonucleotide derivatives were obtained from Sigma Chemical Co., St. Louis, MO. Eagle's Minimal Essential Medium containing balanced salt solution was purchased from Grand Island Biological Co. (GIBCO), Grand Island, NY. Other fine chemicals were reagent grade from Sigma Chemical Co., or Carlo Erba (Milan, Italy).

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2.2. *Methods*

The dried embryos were treated and nauplii grown synchronously as described [5], except for the salt concentration of the growth medium which was diluted 5-fold. Nauplii at different times of development were incubated for in vivo incorporation studies under the same conditions as in [4], in the presence of $[^{14}C]HCO_3^-$ (spec. act. 60 Ci/mol), at a final concentration of 2 mM. After 3 h of incubation at 30°C, nauplii were collected by filtration and washed with 10 mM $NaHCO_3$. The different molecular fractions were then obtained as described [6] by standard methods involving homogeneization in 0.5 M perchloric acid. The nucleic acid fraction was obtained from the acid insoluble material by saline extraction as described [7]. Ribosomal RNA from nauplii of *Artemia* was prepared as described previously [8]. The amount of RNA was estimated by optical density at 260 nm and its de novo synthesis by in vivo incorporation of the de novo RNA precursor and subsequent high-performance liquid chromatographic (HPLC) analysis of nucleotides obtained by alkaline hydrolysis.

The RNAs were selectively hydrolyzed by 0.3 M KOH at 37°C for 20 h, and 2'- and 3'-RNA nucleotides were analyzed by HPLC using a Waters liquid chromatograph (Waters Assoc., Milford, MA, USA) 6000-A pump, a U6K injector, a 440 UV detector at 254 nm and a μBondapak C_{18} column (300 × 4 mm, 10 μm particles) under isocratic elution conditions.

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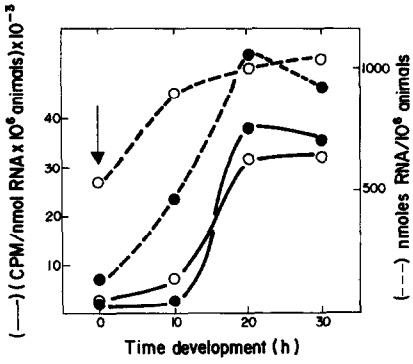

Fig.1. Content and de novo synthesis of total (○) and ribosomal (●) RNAs from *Artemia* nauplii at different development times. The arrow indicates the moment of hatching.

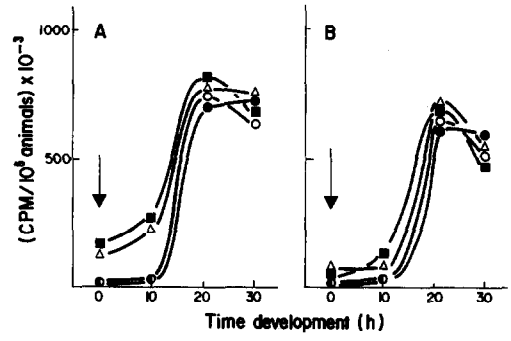

Fig.3. In vivo incorporation of $[^{14}C]HCO_3^-$ into total (A) and ribosomal (B) RNA bases: cytosine (■), uracil (△), guanine (○) and adenine (●), from *Artemia* nauplii at different stages of development. The arrows indicate the hatching of the nauplii.

3. RESULTS AND DISCUSSION

The content of total and ribosomal RNAs increases with the age of the nauplii (fig.1) in a qualitatively similar form to the increase obtained in the level of labelled RNAs in the presence of $[^{14}C]HCO_3^-$ as de novo precursor, with the highest incorporation of radioactivity taking place in 20–30-h-old *Artemia* nauplii under our experimental conditions.

The incorporation of $[^{14}C]HCO_3^-$ into alkali-derived RNA nucleotides is shown in fig.2, which represents the chromatographic profile of 2′- and 3′-RNA nucleotides and the radioactivity incor-

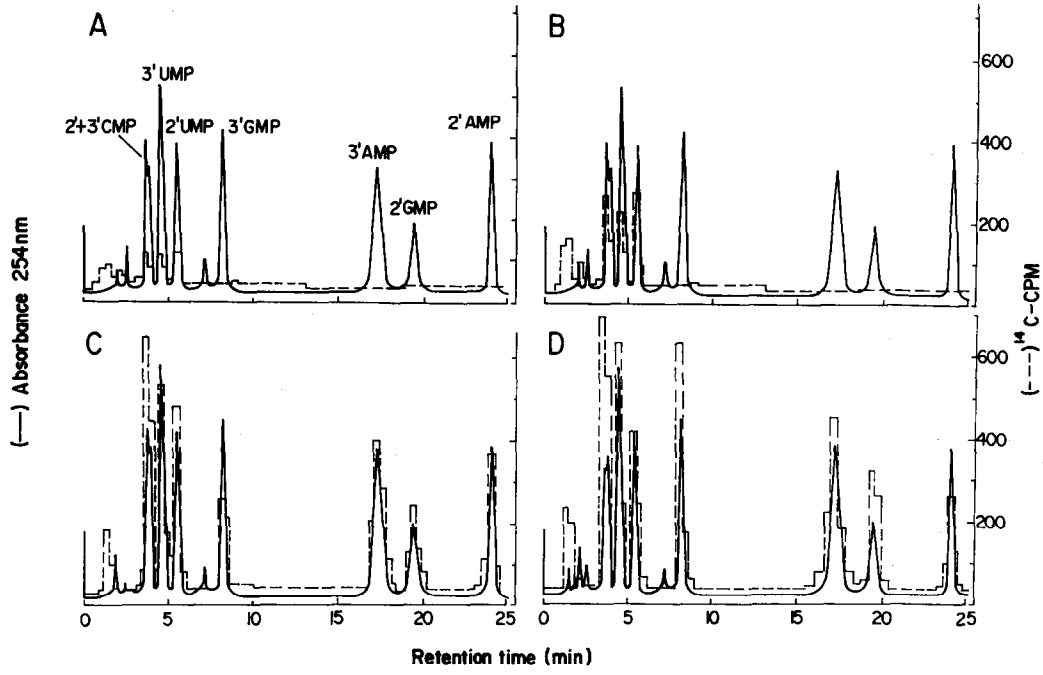

Fig.2. HPLC analysis of alkali-derived RNA nucleotides from newly hatched (A), 10 (B), 20 (C) and 30 (D) -h-old *Artemia* nauplii, incubated in the presence of $[^{14}C]HCO_3^-$. Conditions: μBondapak C_{18} column eluted with 0.1 M KH_2PO_4, 2% methanol, pH 4.0, flow rate 2 ml/min for 15 min, and then isocratic application of 0.1 M KH_2PO_4, 10% methanol, pH 4.0.

porated by newly hatched *Artemia* nauplii, as well as at different developmental times.

In recently hatched nauplii, the label is detected in pyrimidines, while the radioactivity in purines appears approximately 20 h later. Fig.3 shows data of incorporation of the labelled cytosine, uracil, guanine and adenine nucleotides into total and ribosomal RNAs. The incorporation increases during *Artemia* nauplii early development and a maximal value is obtained at 20 h post hatching. Under our experimental conditions of development a lower metabolism in 30-h-old nauplii is found in the absence of particulate food.

The distribution of the radioactivity incorporated into the 4 types of RNA nucleotides is similar for total and ribosomal RNAs during the early development of *Artemia* nauplii, as shown in table 1. In newly hatched nauplii and until 10–15 h post hatching incorporation is detected only into pyrimidine nucleotides, while from 20 h on, an incorporation into both purine and pyrimidine nucleotides is found and represents approximately 25% into the 4 base types.

In our study, the base composition of total and ribosomal RNAs from *Artemia* nauplii at different developmental times shows no differences and represents 21% for cytosine, 17% for uracil, 32% for guanine and 30% for adenine. On the other hand, the G+C content and the (G+C)/(A+U) ratio were 53% and 1.0, respectively, during larval development for both ribonucleic acids.

In conclusion, the results presented here show that de novo synthesis and the cellular content of total and ribosomal ribonucleic acids increase during *Artemia* nauplii development as a function of de novo pyrimidine synthesis early post hatching, and of de novo purine synthesis after 20 h, with unchanged base composition. This is in agreement with data from cryptobiotic embryos of *Artemia* sp. previously reported [9]. In connection with these results and with respect in particular to the value of G+C content obtained, we may consider the crustacean Artemia at a low level in the phylogenetic scale, because its G+C value is lower than that of other biological systems from mammalian tissues earlier described [10,11] and more related to that found in yeast or certain bacteria [11].

Table 1

Distribution of radioactivity from $[^{14}C]HCO_3^-$ into RNA nucleotides at different stages in the development of *Artemia* nauplii

Development[a]	Fraction	CMP	UMP	GMP	AMP
Hatching	RNA	63[b]	37	0	0
	rRNA	62	38	0	0
10	RNA	52	48	0	0
	rRNA	59	41	0	0
20	RNA	28	26	25	21
	rRNA	26	25	24	25
30	RNA	24	27	23	26
	rRNA	26	25	25	24

[a] Time of development as hours post hatching

[b] Data as percent of radioactivity from HPLC chromatograms with respect to total incorporation into 4 types of RNA nucleotides

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