CELL BIOCHEMISTRY AND FUNCTION VOL. 11: 193–200 (1993)

Effect of Diets Containing Adenosine, Guanosine, Inosine or Xanthosine on the Nucleotide Content of *Artemia*. Influence of Mycophenolic Acid

ANTONIO SILLERO[†], MARÍA A. GÜNTHER SILLERO[†] AND ARANTXA HERNANDORENA[‡]

[†]*Departmento de Bioquímica, Instituto de Investigaciones Biomédicas del Consejo Superior de Investigaciones Científicas, Facultad de Medicina, Universidad Autónoma de Madrid, 28029 Madrid, Spain;* [‡]*Laboratoire du Muséum National d'Histoire Naturelle, Centre d'Etudes et de Recherches de Biarritz, 64200 Biarritz, France*

Artemia uses the stored diguanosine tetraphosphate as a source of adenine and guanine nucleotides during development from the encysted gastrula to the free swimming larva. Further development of the larvae depends on a dietary source of purine rings. We have investigated the growth of *Artemia* in axenic cultures supplemented with 0·6 mg ml^{-1} of adenosine, guanosine, inosine or xanthosine. The total protein and soluble nucleotide content of *Artemia* grown in the presence of adenosine, guanosine or inosine was very similar, around (2 A_{260} units and 500 mg protein) and (4 A_{260} units and 1000 mg protein) after 4 and 6 days of postlarval development, respectively. The nucleotide pattern of those extracts subjected to HPLC were almost identical, the major peaks corresponding to ATP, ADP and AMP. Other nucleotides, not well characterized, were also present in those extracts. Mycophenolic acid (10 μg ml^{-1}) inhibited the growth of *Artemia* (as measured by their protein and soluble nucleotide content) in the presence of adenosine and inosine as the purine source, and had no appreciable effect in the presence of guanosine. A quantitative analysis of the chromatographic peaks obtained from *Artemia* grown in the presence of any of the three nucleosides ± mycophenolic acid showed that the effect of the antibiotic on each one of the chromatographic peaks was very similar, suggesting that *Artemia*, and probably other organisms as well, tend to maintain a balance between all nucleotides and to adjust the overall level to the limiting step(s) in their rates of synthesis/interconversion. Xanthosine was not able to support the development of *Artemia*.

KEY WORDS—*Artemia*; purine nucleotide interconversion; purine nucleosides; adenosine; guanosine; inosine; xanthosine; diet; mycophenolic acid.

INTRODUCTION

Artemia is a crustacean of the order Anostraca whose embryos become encapsulated at the gastrula stage. The cysts are viable for years in a dry environment. When placed in a suitable saline medium, the eggs resume their development and differentiate into free swimming larvae in about 24 h. During this time, *Artemia* uses the maternal stored diguanosine tetraphosphate (Gp_4G) as a source of guanine and adenine nucleotides during development from encysted gastrulae to free swimming larvae.[1–3] *Artemia* cysts contain the appropriate enzymatic machinery for the conversion of Gp_4G into AMP and GMP, i.e. *bis*(5′-guanosyl)tetraphosphatase (EC 3.6.1.17),[4–6] GMP reductase (EC 1.6.6.8),[7] adenylosuccinate synthetase (EC 6.3.4.4)[8] and adenylosuccinate lyase (EC 4.3.2.2).[9] The occurrence of IMP dehydrogenase (EC 1.1.1.205) in *Artemia* cysts has also been reported.[10]

Artemia is apparently unable to synthesize purines *de novo*[11] and the larvae depend on a balanced dietary source of purines (nucleotides, nucleosides or bases) for adequate growth and survival to adulthood. This has been well documented in axenic nutritive media whose composition can be controlled.[12] Larvae reared in purine-deficient media develop into abnormal adults.[12–14] Another potential way to change the inner balance in the nucleotide content of *Artemia* is to grow them in the presence of an antimetabolite which interferes with some steps of the interconversion of

Addressee for correspondence: Antonio Sillero, Departmento de Bioquímica, Facultad de Medicina, UAM, Arzobispo Morcillo 4, 28029 Madrid, Spain.

0263–6468/93/030193–08$09.00

purine nucleotides. For instance, we had observed that mycophenolic acid treatment during early postembryonic development disrupted pattern formation in postcephalic segments.[15] Mycophenolic acid is an antimetabolite known to inhibit IMP dehydrogenase,[16–18] a key enzyme for the synthesis of GMP from IMP[16–19] (Figure 1). This enzyme seems to be important for the supply of nucleotides during cell growth as its level increases during some proliferative processes.[19] The biochemical basis of the influence of dietary nucleotides on animal development is largely unknown and *Artemia* could be a good experimental model for the study of the influence of nutritional conditions on pattern formation.

We are starting a systematic exploration aiming at elucidating the molecular basis of the morphological changes in *Artemia* induced by dietary

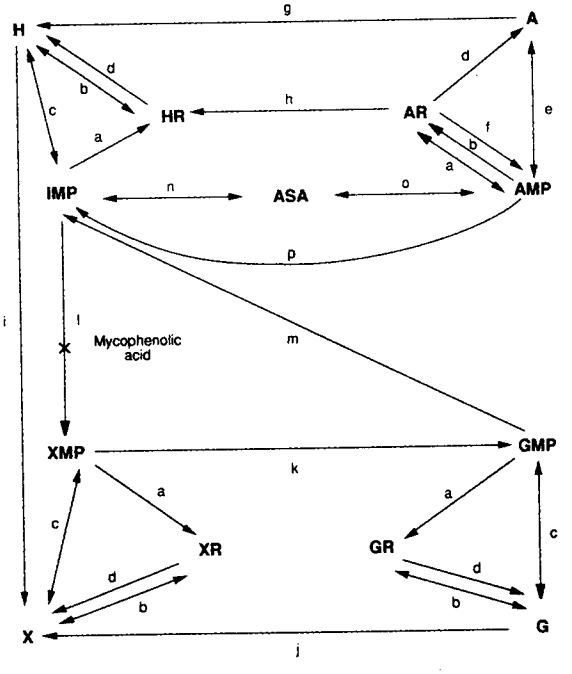

Figure 1. Interconversion of purine nucleotides. Effect of mycophenolic acid as inhibitor of IMP dehydrogenase. The letters in the figure refer to the following enzymes: (a) 5′-nucleotidase, EC 3.1.3.5; (b) purine-nucleoside phosphorylase, EC 2.4.2.1; (c) hypoxanthine phosphoribosyltransferase, EC 2.4.2.8; (d) nucleosidase, EC 3.2.2.1; (e) adenine phosphoribosyltransferase, EC 2.4.2.7; (f) adenosine kinase EC 2.7.1.20; (g) adenine deaminase, EC 3.5.4.2; (h) adenosine deaminase, EC 3.5.4.4; (i) xanthine oxidase, (EC 1.2.3.2); (j) guanine deaminase, EC 3.5.4.3; (k) GMP synthetase, EC 6.3.4.1; (l) IMP dehydrogenase, EC 1.1.1.205; (m) GMP reductase EC 1.6.6.8; (n) adenylosuccinate synthetase, EC 6.3.3.4; (o) adenylosuccinate lyase, EC 4.3.2.2; (p) AMP deaminase, EC 3.5.4.6.

(aminoacid and purine–pyrimidine) imbalances.[13,14] As part of this investigation we present here an analysis of the nucleotide composition of *Artemia* growing in the presence of dietary adenosine, guanosine, inosine or xanthosine as the only source of purine nucleotides. Cultures of *Artemia*, with the additional presence of mycophenolic acid were also done in parallel.

MATERIALS AND METHODS

Materials

Nucleotides and nucleosides used as standards, and mycophenolic acid were from Sigma. The HPLC equipment was composed of a Hewlett Packard Liquid Chromatograph (model 1090), with a diode array detector, commanded by an HPLC Chemstation.

Animals

The effect of one or two different concentrations of mycophenolic acid was tested on *Artemia* larvae from Utah grown during 4 or 6 days, under axenic conditions, in the presence of adenosine (Ado), or guanosine (Guo), or inosine (Ino), or xanthosine (Xao). Experimental details of the cultures were as follows. Larvae were reared in medium C at 0·7 per cent NaCl (20 ml sea water per 100 ml of medium).[20] Torula yeast RNA included in medium C was replaced by a chemically defined mixture of purine and pyrimidine compounds. The purine requirement was met by adenosine, guanosine, inosine, or xanthosine using a concentration of 0·6 mg ml^{-1}. The pyrimidine requirement was met by cytidine using a concentration of 1 mg ml^{-1} throughout experiments. Nucleosides were used to meet the purine requirement because differences between the dietary efficiencies of nucleosides (Ado, Guo or Ino) are less prominent than those observed between purine bases (A, G or Hyp).[12] Xanthine and Xao are apparently unable to satisfy the purine requirement of *Artemia*.[21] Medium C was supplemented with phosphate known to be required for an efficient utilization of purine nucleosides.[12] *Artemia* larvae were also treated with mycophenolic acid (MA) at concentrations of 10 $\mu g\,ml^{-1}$ (MA_1) or 20 $\mu g\,ml^{-1}$ (MA_2). *Artemia* larvae reared in MA-free or MA-containing media were collected after 4 and 6 days in culture.

Artemia *Extracts*

Artemia larvae were sucked from the culture, one by one, with the help of a Pasteur pipette and transferred into several baths of diluted sea water. In order to get them free from the culture medium, *Artemia* were kept swimming for 1 h, filtered gently under vacuum through filter paper and washed with 5 ml of deionized water. The small piece (2·5 cm diameter) of filter paper was then located on the wall of a small funnel, introduced into an empty tube immersed in boiling water. The *Artemia* on the filter were plunged to the bottom of the tube with the help of 2 ml of boiling water. The tube, with the *Artemia* suspension was kept at around 95°C for 3 min and then chilled. The suspension was homogenized (six treatments of 10 s each, with intervals of 4 s) with a Polytron. The resulting brei was centrifuged for 15 min at 15 000 *g*. The precipitate was used for the determination of protein (see below). The supernatant was lyophilized and resuspended in 0·2 ml of distilled water, heated for 1·5 min at 95°C, centrifuged (to remove some insoluble material), and the supernatant used to measure the absorbance at 260 nm (A_{260}) and for the nucleotide analysis by HPLC (see below).

Protein Determination

The precipitate obtained as above described was resuspended in 2 ml of 0·2 M perchloric acid. The extract was kept at 4°C for 20 min, centrifuged, and the precipitate washed once with 2 ml of glass-distilled water, resuspended in 0·2 ml of 1 M NaOH, 5 per cent SDS and proteins determined by the method of Lowry *et al.*[22]

Nucleotide Analysis by HPLC

The supernatants of the *Artemia* extracts obtained as described above were filtered through a nitrocellulose membrane (Millipore HA 0·45 μm) and a 0·05 ml aliquot was injected into a Hypersil ODS column (2·1 mm × 100 mm). Elution was performed at a constant flow rate of 0·5 ml min^{-1} with a 20-min linear gradient (5–30 mM) of sodium phosphate (pH 7·5) in 20 mM tetrabutylammonium, 20 per cent methanol, followed by a 10-min linear gradient (30–100 mM) of sodium phosphate (pH 7·5) in 20 mM tetrabutylammonium, 20 per cent methanol. Appropriate nucleotide standards were run under the same conditions.

RESULTS AND DISCUSSION

Hydrated *Artemia* cysts and newly hatched nauplii use most of the endogenous nutrients after 1–2 days of development. Beyond this period, unfed *Artemia* larvae die massively. Infections of the cultures and the need for a food supply are the main reasons for the mortality of the larvae. In order to avoid the cumbersome task of feeding and because of need to handle enough material, most of the biochemical investigations in *Artemia* have been made in encysted gastrulae and/or unfed larvae of 1–2 days. Hence classical biochemical analysis performed in growing *Artemia* reared under controlled axenic conditions, using chemically defined nutritive media, is rather scanty.

The development of more sensitive techniques of high pressure liquid chromatography (HPLC), particularly the possibility of measuring ultraviolet spectra of the eluting peaks, allowed the possibility of a more accurate study of the nucleotide composition of extracts obtained from a few number of *Artemia* larvae grown in axenic conditions. The extracts were obtained (see Materials and Methods) with a minimum of manipulations in order to standardize the experimental conditions better and to get the smallest loss of biological material.

Total Content of Proteins and Soluble Nucleotides

Artemia (200 animals) grown during 4 or 6 days were processed and the total protein content and UV absorbance was determined in the precipitates and supernatants. The total A_{260} units found in the supernatant was taken as a rough indication of the soluble nucleotide content. Taking the protein content of the precipitates as an indication of the degree of development, it can be concluded that *Artemia* can grow equally well in the presence of adenosine, guanosine or inosine but not in the presence of xanthosine (Table 1). In all cases, except for xanthosine, there was a net increase in protein and in the total A_{260} units from the 4th to the 6th day of development. Concerning the soluble nucleotide content, the total A_{260} units, after 4 days of development, were 2·5, 2·0 and 1·7, in the presence of adenosine, guanosine and inosine, respectively. These figures tended to duplicate from the 4th to 6th day in culture. In contrast, the A_{260} content of the soluble nucleotide pool decreased in the presence of xanthosine.

The development of *Artemia* was also followed

Table 1. Total protein and soluble nucleotide content of 200 *Artemia* larvae grown in the presence of different purine nucleosides ± mycophenolic acid.

Additions to cultures		Incubation time							
		4 days				6 days			
Nucleoside	Mycophenolic acid	Nucleotide Material		Protein		Nucleotide Material		Protein	
(0·6 mg ml^{-1})	(μg ml^{-1})	%	A_{260}*	%	μg	%	A_{260}*	%	μg
Ado	—	100	2·5	100	624	100	4·1	100	987
Ado	10	47		47		23		20	
Guo	—	100	2·0	100	430	100	4·0	100	900
Guo	10	62		110		83		83	
Guo	20	89		119		103		105	
Ino	—	100	1·7	100	525	100	4·5	100	1083
Ino	10	60		54		36		43	
Ino	20	36		41		28		29	
Xao	—	100	0·7	100	200	100	0·4	100	99
Xao	10	38		48		75		62	

* Units of absorbance at 260 nm.

in cultures containing mycophenolic acid in addition to each one of the four purine nucleosides. The effect of this antibiotic on the nucleotide content was almost undetectable in the case of *Artemia* reared in the presence guanosine. The amount of total protein and A_{260} units were very similar in the control and in *Artemia* grown in the presence of both concentrations of mycophenolic acid. This finding agrees with the known effect of this drug as an inhibitor of IMP dehydrogenase, i.e. it inhibits the synthesis of GMP from IMP[15–17] (Figure 1). The supply of guanosine in the diet, in the presence of the antibiotic, is sufficient to act as the source of both guanine and adenine nucleotides (Figure 1). The effect of mycophenolic acid was more evident in the case of *Artemia* grown in the presence of adenosine. In this case, the protein and A_{260} content decreased drastically relative to the control, particularly after 6 days of development. The synthesis of GMP from adenine nucleotides requires the action of IMP dehydrogenase,[10] a process inhibited by the antibiotic[18] (Figure 1). Mycophenolic acid also had an inhibitory effect on *Artemia* grown in the presence of inosine. This effect was less pronounced than in the case of adenosine probably because, when mycophenolic acid was present, the concentration of IMP reached *in vivo* could be higher in the presence of inosine than in the presence of adenosine (Table 1). As mycophenolic acid is a non-competitive inhibitor of IMP dehydrogenase (versus IMP),[18] less inhibition by mycophenolic acid will be reached at higher IMP concentrations. *Artemia* reared for 6 days in the presence of xanthosine were so damaged (even assessed visually and as deduced from their protein content) that further addition of mycophenolic acid to the medium would not have been meaningful.

Nucleotide Analysis

The soluble nucleotides present in the lyophilized supernatant from *Artemia* extracts were chromatographed on a Hypersil ODS column. As mentioned above only 200 animals were used and with so scarce material it is impossible, even with this advanced technology, to characterize each one of the peaks of the resulting chromatograms and only an overall picture of the nucleotide profile could be obtained.

Chromatograms of the total soluble nucleotide content corresponding to 50 *Artemia* larvae grown during 4 (not shown) and 6 days in the presence of the four nucleosides were obtained. To compare the chromatographic results; (i) we tried to perform the experiments under identical experimental conditions; nevertheless it is convenient to recall that the elution time of the same compound may change, with the repeated use of the same column, when

buffers are prepared and/or the temperature of the chromatographic runs changes slightly; (ii) the chromatographic profiles of the extracts obtained from *Artemia* grown in the presence of adenosine, guanosine, inosine and xanthosine were represented on the same scale and using two sets of time, from 0–3·2 min and from 3–20 min; (iii) the major chromatographic peaks were numbered from 1–18, (in Figures 2 and 3, and Table 2), in order to describe their changes with the different diets and during development more easily; nevertheless, as deduced from the spectra and from the purity of the peaks reported by the software of the chromatograph, there were several types of nucleotides under most of the peaks, whose nature it was not possible to know with the available amount of material; (iv) standards of several purine and pyrimidine nucleotides/nucleosides were run under the same experimental conditions (results not shown) to help in the partial characterization of some of the peaks that appeared in the chromatograms obtained from *Artemia* extracts.

The nucleotide profiles of *Artemia* grown during 4 (results not shown) or 6 days (Figure 2) in the presence of Ado, Guo or Ino (but not in the presence of Xao) were grossly similar, indicating that *Artemia* can salvage any of those three bases/nucleosides and synthesize, from any of them, the rest of nucleotides. No detectable peak of Gp_4G was seen after 4 or 6 days of post-larval development. Peaks 1, 5, and 12 correspond mainly to ATP, ADP and AMP, respectively. GTP, GDP, and GMP were under the peaks 2–3, 8, and 14, respectively, in almost the same chromatographic position as the corresponding uridine nucleotides. CTP, and CDP were under peaks 3–4 and 10, respectively (Figure 2). Peaks 15, 16 and 17 were of unknown nature and did not have spectra attri-

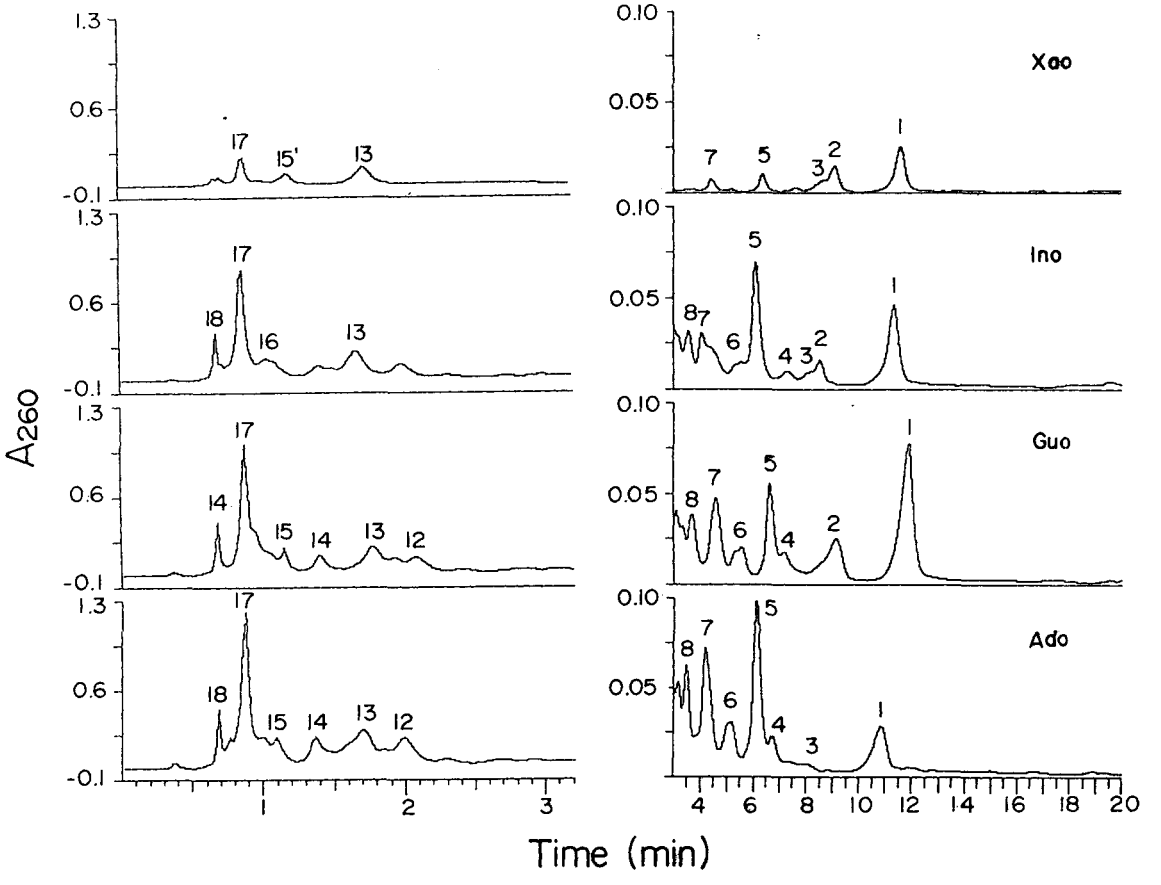

Figure 2. Profile of the soluble A_{260} content of *Artemia* grown for 6 days in the presence of adenosine, guanosine, inosine or xanthosine. Where known, the nature of the peaks is indicated in the text.

Table 2. Integrated values of the chromatographic peaks corresponding to nucleotides from 50 *Artemia* larvae grown during 6 days in the presence of purine nucleosides and in the absence and presence of mycophenolic acid (MA) ($10\,\mu g\,ml^{-1}$).

Peak*	Ado	Ado+MA	Ratio	Guo	Guo+MA	Ratio	Ino	Ino+MA	Ratio	Xao	Xao+MA	Ratio
1	4759	953	5·0	2674	3001	0·9	3215	2032	1·6	404	229	1·8
2	—	—	—	773	689	1·0	687	683	1·0	192	85	2·2
3	2076	529	3·9	396	—	—	—	—	—	96		
4	—		—	1012	1401	0·5	1558	469	3·3	—	—	—
5	2947	821	3·6	3146	2417	1·3	3782	1207	3·1	260	170	1·5
6	2116	365	5·8	1779	1443	1·3	2046	634	3·2	—	—	—
7	3365	745	4·5	3640	2916	1·2	3945	1221	3·2	178	107	1·7
8	2350	429	5·5	2349	1818	1·3	2654	735	3·6	—	—	—
9	2682	506	5·3	2548	2024	1·3	2909	835	3·5	—	—	—
10	1459	343	4·3	1330	1129	1·2	1827	570	3·2	—	—	—
11	1508	278	5·4	1595	1153	1·4	1517	465	3·3	—	—	—
12	5439	873	6·2	5079	3728	1·4	4998	1517	3·3	1174	767	1·5
13	2860	926	3·1	3198	2870	1·1	3708	1571	2·4	—	—	—
14	4705	827	5·7	4789	3417	1·4	4740	1360	3·5	—	—	—
15	3011	729	4·1	5074	3956	1·3	5323	—	2·8	1233	921	1·3
16	2673	800	3·3	—			—	1901	—	—	—	—
17	7572	1835	4·1	6994	7608	1·1	8825	3494	2·5	907	677	1·3
18	842	748	1·1	1029		—	2338	753	3·1	759	486	1·6
Mean			4·5			1·2			2·8			1·6
±S.D.			±1·3			±0·1			±0·9			±0·3
$\sum$	50365	10959	4·5	47405	39570	1·2	54072	19447	2·8	5203	3442	1·5

* The identity of each peak is, when known, as indicated in the text.

butable to common nucleotides/nucleosides. A new peak (peak 15′) corresponding to xanthosine was also clearly identified in *Artemia* larvae grown in the presence of Xao (Figure 2).

Effect of Mycophenolic Acid

Newly hatched *Artemia* nauplii were treated for 4 or 6 days with $10\,\mu g\,ml^{-1}$ mycophenolic acid (in the case of nauplii grown in the presence of adenosine or xanthosine) or with 10 or $20\,\mu g\,ml^{-1}$ mycophenolic acid (in the case of nauplii grown in the presence of guanosine or inosine). The results obtained with *Artemia* grown for 6 days in the presence of the indicated concentration of the antibiotic are presented in Figure 3. The effect of $10\,\mu g\,ml^{-1}$ of mycophenolic acid on the nucleotide profile of *Artemia* grown in the presence of guanosine was minimal (result not shown), whereas mycophenolic acid at $20\,\mu g\,ml^{-1}$ (Figure 3) caused the almost complete disappearance of peaks 2–3 (corresponding mainly to CTP, GTP, and UTP), an increase of peak 4 and the appearance of a clear peak at the left of peak 7 (Figure 2 and 3). The total nucleotide content remained practically unchanged (Tables 1, 2). However, mycophenolic acid markedly decreased the total nucleotide content of *Artemia* grown for 6 days in the presence of adenosine or inosine (Tables 1, 2). Quantitative rather than qualitative differences were noticed in absence (Figure 2) or in the presence of the antibiotic (Figure 3), except for the preferential loss of peak 15 with adenosine or inosine (Figure 2 and 3). The nucleotide content of *Artemia* grown during 4 or 6 days in the presence of xanthosine was so low (Table 1, 2) that only slight changes in the size of some peaks were observed with further treatment with mycophenolic acid (Figs. 2 and 3).

The area of each one of the chromatographic peaks obtained in the different nutritional conditions, in the absence or presence of mycophenolic acid is presented in Table 2. From these results and those presented in Figures 2 and 3, some additional comments on the effect of mycophenolic acid can be made: (i) The ability of each one of the nucleosides to serve as a source of purine rings can be deduced from the sum of the areas of the peaks in the corresponding chromatograms; clearly, adenosine, guanosine and inosine (but not xanthosine) can support the development of *Artemia* (Table 2; see also comparable results in Table 1); (ii) the overall effect of the antibiotic can be deduced from the ratio of the sum of the areas of all the peaks obtained in the presence of each one of the nucleosides ± mycophenolic acid; again, maximum effect was obtained in the presence of adeno-

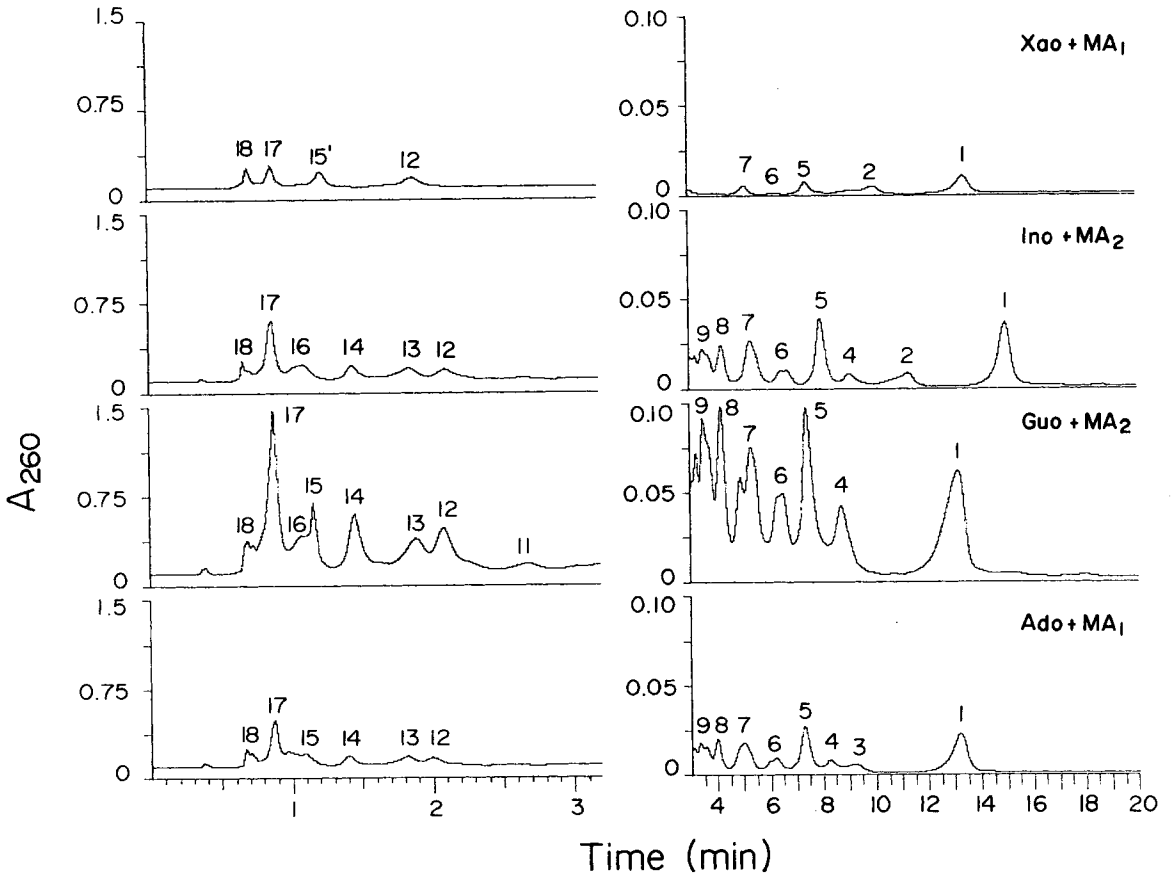

Figure 3. Profile of the soluble A_{260} content of *Artemia* grown for 6 days in the presence of 10 or 20 $\mu g\,ml^{-1}$ mycophenolic acid (MA_1 or MA_2, respectively) and in the presence of adenosine, guanosine, inosine or xanthosine. Where known, the nature of the peaks is indicated in the text.

sine (ratio=4·5) followed by inosine (ratio=2·8) and guanosine (ratio=1·2) (Table 2); (iii) roughly, those ratios are similar for each one of the corresponding peaks (1–18) (Table 2); (iv) as it is well-documented that mycophenolic acid inhibits IMP dehydrogenase, an increase in IMP could be expected in the presence of Ino and mycophenolic acid (Figure 1); no such increase was observed, suggesting that the excess of inosine/hypoxanthine was excreted (as such or as NH_4^+) into the culture medium; (v) neither was there observed an increase of IMP or AMP in the presence of adenosine and mycophenolic acid (Figure 1). In our view, these nutritional experiments indicate that *Artemia*, and probably other organisms, tend to keep a balance between the nucleotides and adjust the overall level to the limiting steps in the overall rates of synthesis/interconversion. This may explain why the ratio of the peaks obtained in the absence and presence of the antibiotic were kept rather constant in all the experimental conditions.

ACKNOWLEDGEMENTS

The investigation was supported by grants from Dirección General de Investigación Científica y Técnica (Grants PB87/0288 and PB91/107). The support of an Ayuda Integrada from both the Spanish Ministry of Education and Science and the French Ministry of Foreign Affairs is gratefully acknowledged. We thank Anabel de Diego, Elena Esguevillas and Javier Pérez for technical assistance.

REFERENCES

1 Finamore, F. J. and Clegg, J. S. (1969). Biochemical aspects of morphogenesis in the brine shrimp, *Artemia salina*. In: *The Cell Cycle. Gene–Enzyme Interactions*. (Padilla, G. M., Whitson, G. L. and Cameron, I. L., eds.) Academic Press: New York, pp. 249–278.

2 Warner, A. H. (1992). Diguanosine and related nonadenylated phosphates. In; *Ap_4A and Other Dinucleoside Polyphosphates*. (McLennan, A. G., ed.) CRC Press: Boca Raton, Florida, pp. 275–304.

3 Sillero, A. and Günther Sillero, M. A. (1987). Interconversion of purine nucleotides in *Artemia:* a review. In: *Artemia Research and its Applications*, Vol. 2. *Physiology, Biochemistry, Molecular Biology*. (Decleir, W., Moens, L., Slegers, H., Sorgeloos, P. and Jaspers, E., eds.) Universa Press: Wetteren, Belgium, pp. 289–307.

4 Warner, A. H. and Finamore, F. J. (1965). Isolation, purification and characterization of P^1, P^4-diguanosine 5′-tetraphosphate asymmetrical-pyrophosphohydrolase from brine shrimp eggs. *Biochemistry*, **4,** 1568–1575.

5 Vallejo, C. G., Sillero, M. A. G. and Sillero, A. (1974). Diguanosinetetraphosphate guanylohydrolase in *Artemia salina*. *Biochim. Biophys. Acta*, **358,** 117–125.

6 Prescott, M., Milne, A. D. and McLennan, A. G. (1989). Characterization of the bis(5′-nucleosidyl) tetraphosphate pyrophosphohydrolase from encysted embryos of the brine shrimp *Artemia*. *Biochem. J.* **259,** 831–838.

7 Renart, M. F., Renart, J., Sillero, M. A. G. and Sillero, A. (1976). Guanosine monophosphate reductase from *Artemia salina:* Inhibition by xanthosine monophosphate and activation by diguanosine tetraphosphate. *Biochemistry*,**15,** 4962–4966.

8 Faraldo, A., Pinto, R. M., Canales, J., Fernández, A., Sillero, A. and Günther Sillero, M. A. (1983). Presence of two isozymes of adenylosuccinate synthetase in *Artemia salina* embryos. Purification and properties. *Comp. Biochem. Physiol.*, **75B,** 221–226.

9 Pinto, R. M., Faraldo, A., Fernández, A., Canales, J., Sillero, A. and Günther Sillero, M. A. (1983). Adenylosuccinate lyase from *Artemia* embryos. *J. Biol. Chem.*, **258,** 12513–12519.

10 Canales, J., Fernández, A., Faraldo, A., Pinto, R. M., Sillero, A. and Günther Sillero, M. A. (1985). IMP dehydrogenase from *Artemia* embryos: molecular forms, purification and properties. *Comp. Biochem. Phys.* **81B,** 837–844.

11 Clegg, J. S., Warner, A. H. and Finamore, F. J. (1967). Evidence for the function of p^1, p^4–diguanosine 5′-tetraphosphate in the development of *Artemia salina*. *J. Biol. Chem.*, **242,** 1938–1943.

12 Hernandorena, A. (1985). Purine-mediated phosphate influence on *Artemia* morphogenesis. *Reprod. Nutr. Dev.*, **25,** 883–893.

13 Hernandorena, A. (1979). Relationships between purine and pyrimidine dietary requirements and *Artemia salina* morphogenesis. *Comp. Biochem. Physiol.*, **62B,** 7–12.

14 Hernandorena, A. (1990). Comparative study of nutritional requirements in two axenically reared *Artemia* strains. *Comp. Biochem. Physiol.*, **97A,** 153–158

15 Hernandorena, A. (1993). Guanylate requirement for patterning the postcephalic region of the brine shrimp *Artemia*. *Roux's Arch. Develop. Biol.*, in press.

16 Franklin, T. J. and Cook, J. M. (1969). The inhibition of nucleic acid synthesis by mycophenolic acid. *Biochem. J.*, **113,** 515–524.

17 Ullman, B. (1983). Characterization of mutant murine lymphoma cells with altered inosinate dehydrogenase activities. *J. Biol. Chem.*, **258,** 523–528.

18 Hupe, D. J., Azzolina, B. A. and Behrens, N. D. (1986). IMP dehydrogenase from the intracellular parasitic protozoan *Eimeria tenella* and its inhibition by mycophenolic acid. *J. Biol. Chem.*, **261,** 8363–8369.

19 Jackson, R. C. and Weber, G. (1975). IMP dehydrogenase, an enzyme linked with proliferation and malignancy. *Nature*, **256,** 331–333.

20 Hernandorena, A. (1991). Development of a new simplified medium for the axenic culture of *Artemia*. *Reprod. Nutr. Dev.*, **31,** 57–63.

21 Hernandorena, A. (1987). Circumstances affecting the efficiency of dietary purines in *Artemia*. *Comp. Biochem. Physiol.*, **80B,** 397–403.

22 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein Measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193,** 265–275.