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Study of the oxygen binding properties of *Artemia* hemoglobins

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Abstract

The physiological properties of total hemoglobin samples from *Artemia* populations of different geographical origin (Tientsin, Great Salt Lake, Chaplin Lake, Macau, Galera Zamba) have been studied *in vitro*. Measurements carried out with the diffusion chamber technique at different pH-values and temperatures, revealed significant differences in oxygen affinity (p50 values between 1.25 and 16.33 mm Hg), cooperativity (Hill coefficients between 1.07 and 2.18) and free oxygen-binding energy (between - 34.7 and - 56.2 Kj/mol). All the strains showed a decrease of oxygen affinity with increasing temperature, and a positive Bohr effect. Tientsin containing a considerable amount of Hbl (36.6 %), shows the lowest oxygen affinity, while Macau and Galera Zamba (except at pH 7.65) containing most of Hb3 show the highest affinity.

The highest cooperativity is found for Tientsin, and the lowest for Galera Zamba. Submission of the adult *Artemia* strains to cyclic hypoxic stress resulted in the production of hemoglobin. Hb2 was always the main pigment. Next to Hb2, Tientsin and Great Salt Lake produced preferentially Hb1, and Galera Zamba and Macau Hb3. Chaplin Lake produced the three types of hemoglobin.

Introduction

Earlier studies on the respiratory physiology of the adult brine shrimp *Artemia* have shown that this branchiopod adapts to low oxygen levels by hemoglobin synthesis in order to facilitate respiration (Decleir *et al.*, 1980). Bowen *et al.* (1969) have shown the existence of three types of hemoglobin in *Artemia*, which according to their electrophoretic mobility have been called Hb1, 2 and 3. „Total hemoglobin" extracts of a San Francisco Bay strain (Vos *et al.*, 1979) cultivated in laboratory circumstances (25 °C and 32 ‰ salinity) mainly contain Hb2 and small amounts of Hb1, while Hb3 is electrophoretically not detectable. This Hb composition can be influenced by submitting the animals to hypoxic stress. It has been demonstrated that responses to long-term hypoxic conditions include next to changes in reproductive behaviour (Dutrieu, 1960 ; Sorgeloos, 1975), a modified energy balance (Gilchrist, 1956) and an increase of the hemoglobin concentration, alterations in the procentual composition of the three hemoglobin types (Vos *et al.*, 1979). The observation, that the induced Hb3 shows oxygen-binding properties which are very suited for survival in a low oxygen environment (Van den Branden *et al.*, 1978), illustrates the existence of adaptional mechanisms to cope to a certain degree with changing environmental oxygen availability.

The work presented here is part of a program destined to examine and compare the oxygen-binding properties of the hemoglobins from a number of *Artemia* populations of different geographical origin. This paper describes the first results of the *in vitro* oxygen-binding properties of total Hb extracts of two North American, two South American, and one Asian population. In all the cases it was necessary to submit the *Artemia* cultures to cyclic hypoxic stress in order to obtain sufficient hemoglobin for extraction. The following functional properties of the Hb extracts were determined: the oxygen affinity (p50), the temperature and Bohr effect, the cooperativity (Hill coefficient n), the free oxygen-binding energy, and the Bohr factor.

Materials and methods

Cysts of *Artemia* populations originating from five geographical sites (Tientsin, China (T), Chaplin Lake, Canada (CH); Great Salt Lake, USA (GSL); Macau, Brazil (M); Galera Zamba, Columbia (GZ), were used. Mass cultures were run (at 25 °C and 35 ‰ salinity) as described by Sorgeloos (1979) and Versichele and Sorgeloos (1980).

Hemoglobin induction in adult *Artemia* was obtained by submitting them to cyclic hypoxic stress (Lavens and Sorgeloos, 1984). The hypoxia sensitivity of each particular population mostly showed considerable variability. After harvesting the animals were thoroughly washed with distilled water and frozen in flat layers of ± 1.5 cm thickness at - 70 °C until hemoglobin extraction. Total hemoglobin was obtained as described previously (Moens and Kondo, 1977).

The physiological properties of the hemoglobin samples were measured with the diffusion chamber technique (Sick and Gersonde, 1969 ; Van Pachtebeke *et al.*, 1982) at five temperatures and four pH values.

Electrophoresis was carried out on cellulose acetate strips or on 1 % agarose gels. The presence of hemoglobin was demonstrated using benzidine-H_2O_2 reagent, respectively 0.5 % Amido Black. Densitometric scanning was performed with a Kipp Microdensiscan (KS3).

Results

Table I presents the procentual hemoglobin composition in the five *Artemia* strains studied. The hemoglobin extracts of all the populations mainly contain Hb2 (Fig. 1). The induction of Hb1 is most pronounced in Tientsin and Great Salt Lake, and of Hb3 in Galera Zamba and Macau. Chaplin Lake contains the three types of hemoglobin. All the registrated oxygen-dissociation curves had a sigmoidal shape, which is conform with the findings of Van den Branden *et al.* (1978).

TABLE I

Procentual composition of *Artemia* hemoglobin extracts

	% Hb1	Hb2	Hb3	(n)
Tientsin	36.3 ± 3.3	63.7 ± 3.3	0	8
Great Salt Lake	22.2 ± 3.1	77.8 ± 3.1	0	24
Chaplin Lake	10.1 ± 1.8	82.6 ± 3.7	7.3 ± 2.1	12
Macau	0	91.4 ± 3.5	8.6 ± 3.5	10
Galera Zamba	0	73.4	26.6	3

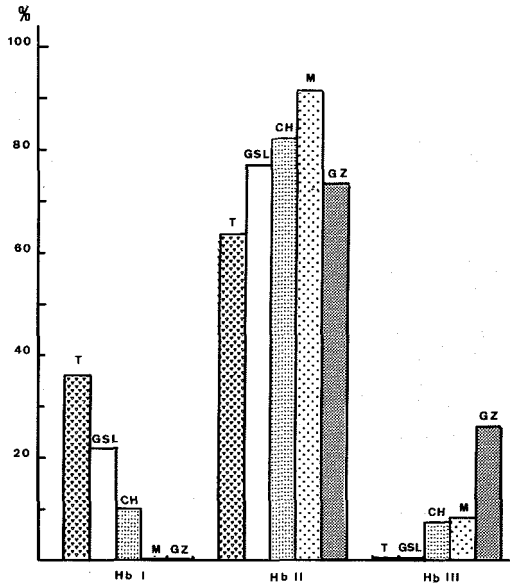

FIG. 1. Procentual distribution of the three types of *Artemia* hemoglobins. Tientsin (T) ; Great Salt Lake (GSL) ; Chaplin Lake (CH) ; Macau (M) ; Galera Zamba (GZ).

In Table II the data on oxygen affinity (p50 values) are presented. The five populations show a decrease in affinity with increasing temperature. In Fig. 2a, 3a, and 4a, oxygen affinity is plotted in relation to changing pH and temperature for respectively Tientsin, Great Salt Lake, and Chaplin Lake. These three populations show a positive Bohr effect which is more pronounced at higher temperatures, except for Chaplin Lake where at the highest temperature and the lowest pH values, a negative Bohr effect is observed. Tientsin hemoglobins show the highest pH sensitivity.

Table III contains the results on the cooperativity (Hill coefficients). The highest cooperativity is found for Tientsin (n = 2.14 at pH 8.25 and 8.60) and for Great Salt Lake (n = 2.18 at pH 8.60) and the lowest for Macau (n = 1.07 at pH 8.60) and Galera Zamba (n = 1.15 at pH 8.60).

In Fig. 2b, 3b, and 4b the evolution of cooperativity is plotted in function of pH and temperature for the same three populations mentioned before. Cooperativity in Great Salt Lake shows a maximum that shifts from 22 °C at lower pH values to 26 °C for increasing pH values. Tientsin shows no marked maximum for changing temperatures, and for Chaplin Lake a slight increase is noticed around 28 °C. Cooperativity of the last two strains seems to be more influenced by pH than by temperature. Insufficient measuring points made it impossible to realize similar graphics for Macau and Galera Zamba.

The mean values of free oxygen-binding energy (ΔH) and of the Bohr factors (Φ) are presented in Table IV. The ΔH ranges between - 34.7 and - 56.2 Kj/mole, differring as follows : Tientsin <Great Salt Lake <Chaplin Lake <Macau <Galera Zamba. The Bohr factors range between - 0.13 and - 0.45.

TABLE II

P-50 values of total hemoglobin extracts of *Artemia* populations from different geographical origin

		Tientsin		Great Salt Lake		Chaplin Lake		Macau		Galera Zamba	
	T°		n		n		n		n		n
pH 7.25	10	n.v.		2.65(± 0.11)	(8)	1.82(± 0.34)	(9)	1.58(± 0.07)	(11)	n.v.	
	18	n.v.		5.81(± 0.30)	(10)	4.11(± 0.33)	(13)	3.53(± 0.17)	(11)	n.v.	
	22	n.v.		6.92(± 0.33)	(8)	6.23(± 0.43)	(13)	4.90(± 0.38)	(13)	n.v.	
	26	n.v.		8.40(± 0.41)	(11)	7.25(± 0.54)	(10)	6.16(± 0.21)	(11)	n.v.	
	34	n.v.		n.v.		n.v.		n.v.		n.v.	
pH 7.65	10	2.80(± 0.21)	(8)	2.15(± 0.09)	(11)	1.55(± 0.09)	(9)	n.v.		1.44(± 0.11)	(12)
	18	6.61(± 0.25)	(8)	4.75(± 0.27)	(10)	4.27(± 0.20)	(10)	3.34(± 0.13)	(11)	4.97(± 0.51)	(10)
	22	9.01(± 0.22)	(11)	6.74(± 0.20)	(10)	5.79(± 0.18)	(12)	5.13(± 0.22)	(11)	5.23(± 0.26)	(7)
	26	11.17(± 0.42)	(15)	7.96(± 0.17)	(8)	7.08(± 0.41)	(9)	5.71(± 0.40)	(12)	8.48(± 0.64)	(11)
	34	n.v.		13.56(± 0.25)	(8)	11.43(± 0.91)	(9)	10.39(± 0.52)	(10)	n.v.	
pH 8.15	10	2.48(± 0.12)	(9)	2.16(± 0.13)	(8)	1.25(± 0.14)	(12)	n.v.		n.v.	
	18	6.23(± 0.28)	(14)	3.95(± 0.29)	(17)	3.26(± 0.11)	(8)	n.v.		n.v.	
	22	7.26(± 0.21)	(9)	5.99(± 0.27)	(8)	4.47(± 0.30)	(11)	n.v.		n.v.	
	26	9.22(± 0.26)	(10)	7.60(± 0.22)	(11)	5.80(± 0.29)	(13)	n.v.		n.v.	
	34	16.33(± 0.31)	(9)	12.32(± 0.37)	(11)	12.40(± 0.63)	(11)	n.v.		n.v.	
pH 8.60	10	n.v.		n.v.		n.v.		n.v.		n.v.	
	18	4.35(± 0.19)	(9)	3.88(± 0.16)	(10)	2.89(± 0.17)	(7)	n.v.		1.51(± 0.10)	(7)
	22	6.04(± 0.13)	(8)	5.08(± 0.27)	(10)	3.16(± 0.26)	(10)	n.v.		2.07(± 0.23)	(7)
	26	7.18(± 0.24)	(8)	6.83(± 0.24)	(10)	4.78(± 0.18)	(11)	n.v.		3.08(± 0.14)	(7)
	34	13.70(± 0.16)	(8)	11.66(± 0.47)	(10)	8.49(± 1.14)	(9)	n.v.		5.95(± 0.48)	(7)

n.v. = no values

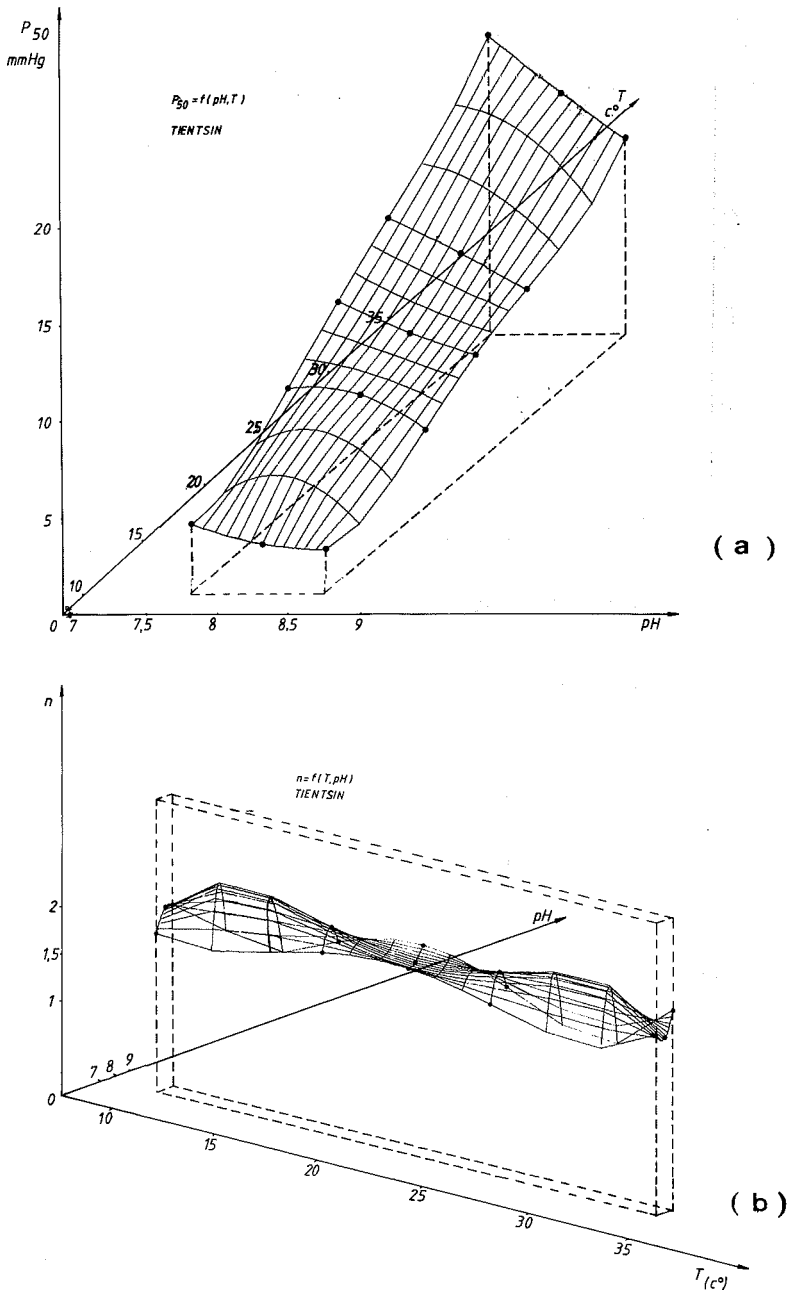

FIG. 2. Influence of pH and temperature on the oxygen affinity (a) and cooperativity (b) of hemoglobin extracts of Tientsin.

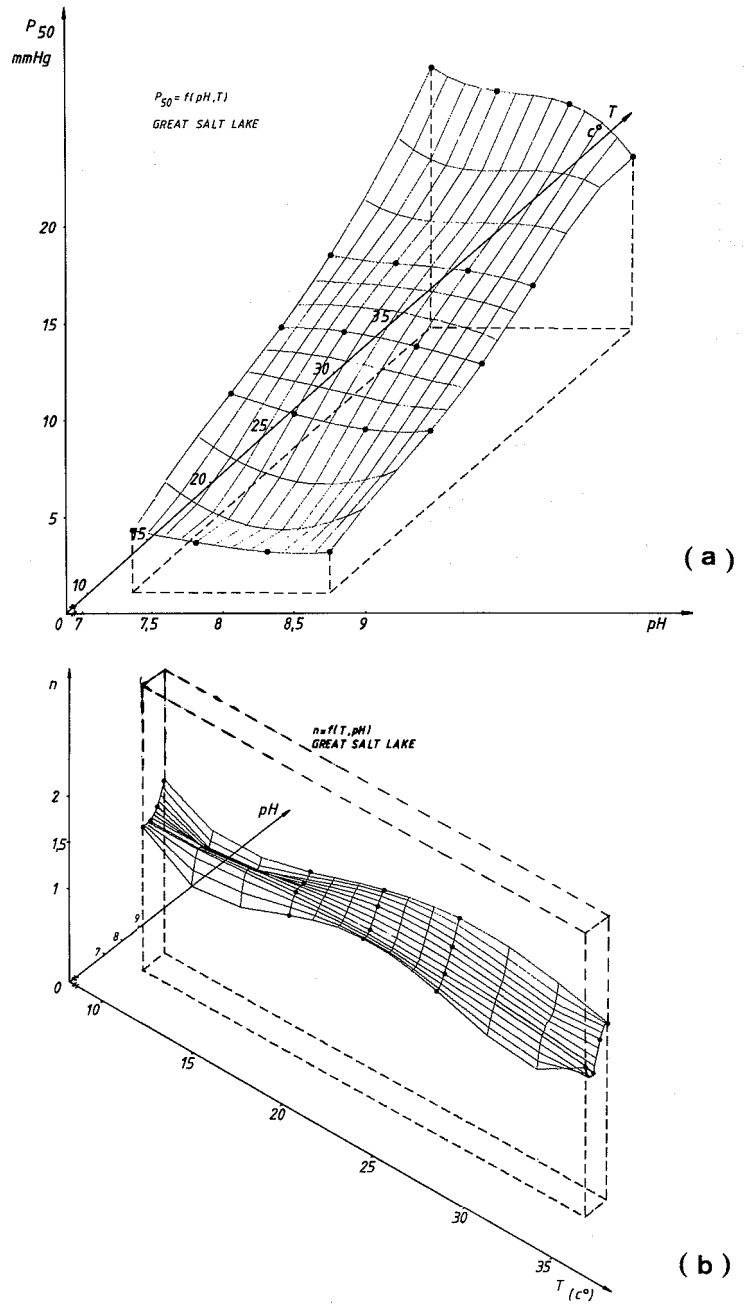

FIG. 3. Influence of pH and temperature on the oxygen affinity (a) and cooperativity (b) of hemoglobin extracts of Great Salt Lake.

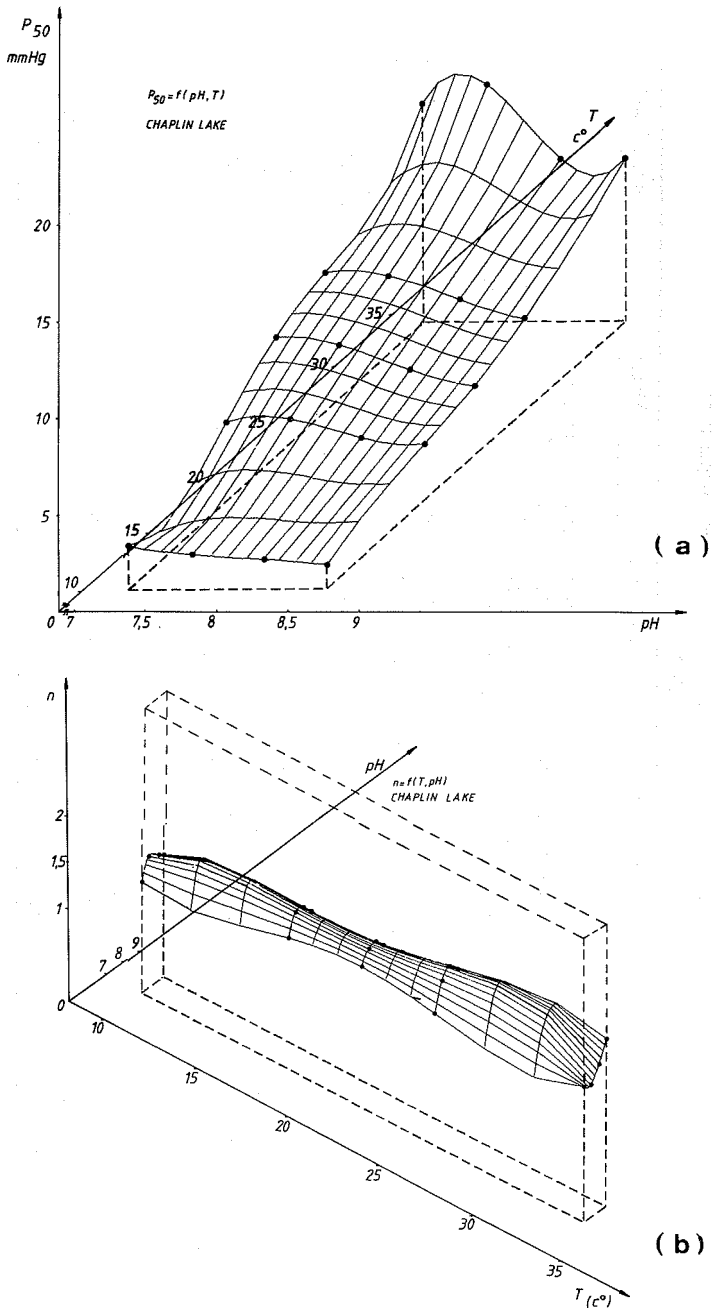

FIG. 4. Influence of pH and temperature on the oxygen affinity (a) and cooperativity (b) of hemoglobin extracts of Chaplin Lake.

TABLE III

Hill coefficients of total hemoglobin extracts of *Artemia* populations from different geographical origin

		Tientsin	n	Great Salt Lake	n	Chaplin Lake	n	Macau	n	Galera Zamba	n
	T°										
pH 7.25	10	n.v.		1.57(± 0.10)	(8)	1.56(± 0.09)	(9)	1.15(± 0.07)	(12)	n.v.	
	18	n.v.		1.50(± 0.08)	(10)	1.56(± 0.06)	(13)	1.08(± 0.07)	(9)	n.v.	
	22	n.v.		1.70(± 0.05)	(8)	1.67(± 0.08)	(13)	1.21(± 0.09)	(14)	n.v.	
	26	n.v.		1.57(± 0.06)	(11)	1.57(± 0.04)	(10)	1.18(± 0.11)	(10)	n.v.	
	34	n.v.		n.v.		n.v.		n.v.		n.v.	
pH 7.65	10	1.90(± 0.16)	(8)	1.66(± 0.14)	(11)	1.51(± 0.12)	(9)	n.v.		1.13(± 0.07)	(12)
	18	1.91(± 0.14)	(8)	1.69(± 0.07)	(10)	1.80(± 0.10)	(10)	1.26(± 0.08)	(10)	1.14(± 0.09)	(10)
	22	1.97(± 0.15)	(11)	1.73(± 0.04)	(10)	1.82(± 0.08)	(12)	1.33(± 0.10)	(7)	1.20(± 0.08)	(7)
	26	1.80(± 0.14)	(15)	1.70(± 0.09)	(8)	1.90(± 0.07)	(9)	1.32(± 0.11)	(15)	1.22(± 0.08)	(11)
	34	n.v.		1.64(± 0.13)	(8)	1.61(± 0.06)	(9)	1.07(± 0.07)	(10)	n.v.	
pH 8.15	10	1.90(± 0.17)	(9)	1.80(± 0.22)	(7)	1.45(± 0.15)	(12)	n.v.		n.v.	
	88	2.14(± 0.08)	(14)	1.72(± 0.13)	(17)	1.79(± 0.06)	(8)	n.v.		n.v.	
	22	1.98(± 0.05)	(9)	1.92(± 0.09)	(8)	1.83(± 0.07)	(11)	n.v.		n.v.	
	26	2.10(± 0.15)	(10)	1.93(± 0.13)	(9)	2.00(± 0.14)	(13)	n.v.		n.v.	
	34	1.92(± 0.06)	(9)	1.52(± 0.11)	(11)	1.57(± 0.11)	(11)	n.v.		n.v.	
pH 8.60	10	n.v.		n.v.		n.v.		n.v.		n.v.	
	18	1.95(± 0.08)	(9)	1.78(± 0.13)	(10)	1.69(± 0.08)	(7)	n.v.		1.26(± 0.25)	(7)
	22	2.14(± 0.04)	(8)	2.04(± 0.08)	(10)	1.73(± 0.11)	(10)	n.v.		1.24(± 0.24)	(7)
	26	1.91(± 0.12)	(8)	2.18(± 0.14)	(8)	1.90(± 0.13)	(11)	n.v.		1.17(± 0.06)	(7)
	34	1.85(± 0.11)	(9)	1.82(± 0.19)	(10)	1.75(± 0.20)	(11)	n.v.		1.15(± 0.17)	(7)

n.v. = no values

TABLE VI

Mean values of the free oxygen binding energy (ΔH) and of the Bohr factors (Φ) of different *Artemia* populations (all measurements between 18 and 26 °C, and pH between 7.3 and 8.5)

	ΔH (Kj/mole)	Φ (Δlog p50/ΔpH)
Tientsin	- 34.7	- 0.19
Great Salt Lake	- 41.0	- 0.13
Chaplin Lake	- 46.5	- 0.19
Macau	- 53.7	- 0.19
Galera Zamba	- 56.2	- 0.45

Discussion

All the experiments for determining the oxygen-binding properties of *Artemia* hemoglobins were carried out *in vitro*. This requires a careful interpretation in a *in vivo* context. Maybe the extracellular occurrence of these respiratory pigments make them probably less sensitive to specific local factors which are known to influence the intracellular hemoglobins to a larger extend (Moens, 1981).

The hemoglobin mixtures of the studied *Artemia* populations have different oxygen-binding properties. As we worked with total hemoglobin samples the influence of the procentual composition (Hbl + 2 + 3) on the oxygen-binding properties may be important since the physiological differences among Hbl, 2 and 3 were already established for San Francisco Bay (Van den Branden *et al.*, 1978). They demonstrated that the oxygen affinities of the three hemoglobins were moderately high and differred as follows Hb3 > Hb2 > Hb1. This was interpreted as a possible adaption to cope with different environmental pO_2, Hb3 being more efficient in a poorly aerated environment.

Although the cultivation conditions for all *Artemia* populations were standardized (temperature, salinity, food), the cyclic hypoxic stress applicated was different, which is due to the different response to hypoxic tolerance of each *Artemia* strain.

Artemia is a respiratory regulator and a critical oxygen tension exists, which decreases with acclimation to stronger hypoxic stress (Vos *et al.*, 1979 ; Decleir *et al.*, 1980). An interrelationship between this shift to lower critical oxygen tensions, and the occurrence of Hb3 is very probable. The degree of imposed hypoxia influences the procentual composition of the total hemoglobin, but this response is not identical for each of the *Artemia* strains used. The electrophoretic experiments show that Tientsin and Great Salt Lake produce next to Hb2, Hb1, while in Macau and Galera Zamba Hb3 was found. In Chaplin Lake the three types of hemoglobin were found. The Macau and Galera Zamba samples (except at pH 7.75) containing the highest Hb3 percentages show the highest affinity, while in Tientsin samples, containing a considerable amount of Hb1, the lowest oxygen affinity was observed. Our results suggest that hypoxic tolerance among these *Artemia* populations decreases as follows : Galera Zamba > Macau > Chaplin Lake > Great Salt Lake > Tientsin.

The relation between the procentual hemoglobin composition and the oxygen-binding properties seems to confirm that the conclusions of Van den Branden *et al.* (1978) can be extrapolated to other *Artemia* strains than San Francisco Bay, but the role of the genetical background of the hemoglobin synthesis of each population remains to be investigated.

Acknowledgements

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