REVISTA ESPAÑOLA DE FISIOLOGIA, 32, 307-312. 1976

Hemolymph Iron in Crustacea Decapoda During the Intermolt Cycle *

J. M. Recio ** y V. J. León

Laboratorio de Fisiología Animal
Facultad de Ciencias
Universidad de Salamanca
(España)

(Received on February 5, 1976)

J. M. RECIO and V. J. LEON. *Hemolymph Iron in Crustacea Decapoda During the Intermolt Cycle.* Rev. esp. Fisiol., 32, 307-312. 1976.

A study on the iron content in the hemolymph of *Austropotamobius pallipes* and *Carcinus maenas* has been made. The hemolymph iron content and the total iron binding capacity were investigated in males and females of both species. The values obtained for hemolymph iron content in the males were between 154 and 430 μg/100/ml and in the females between 134 and 431 μg/100/ml. The total iron binding capacity for the males was between 472 and 762 μg/100/ml and for the females between 390 and 698 μg/100/ml. The variations occurring in both parameters during different stages of the intermolt were also studied. The first increase in hemolymph iron coincides with the postmolt stage. During the intermolt, the need for iron decreases as the rate of tissue synthesis becomes lower. Finally an enormous increase in the hemolymph iron content was observed during the molt.

Extensive study has been done on the metabolism of iron in mammals and in birds (11). However, there is a lack of information concerning the content of iron in the hemolymph of crustacea decapoda. The key role played by copper in the metabolism of decapoda may explain this lack of interest shown in the other constituent metals.

However, iron has been studied as a component of erythrocruorin or hemoglobin in some crustaceans (8), in the phosphorylase system of lobster muscle extracts (1, 2), and the iron accumulated in the walls of cecal intestine, fat cells and maxillary glands has also been investigated (14).

The iron content of the exoskeleton, hepatopancreas, gills, hemolymph and muscle during the intermolt cycle has been studied recently by MARTIN (9) and a iron binding protein has been detected by GHIDALIA (6).

Bearing in mind the important metabolic changes which take place in crusta-

* With support from Ministerio de Educación y Ciencia. «Ayuda a la Investigación».

** Present addres: Cátedra de Fisiología general y especial, Facultad de Veterinaria. León (Spain).

ceans during the intermolt period it appears that a study of the variations in the iron levels of the hemolymph of both males and females may be of interest.

Materials and Methods

In order to carry out a comparative study, the river crab *(Austropotamobius pallipes lusitanicus)* and the sea crab *(Carcinus maenas)* were selected. 47 males and 36 females of the former and 41 male and 32 female marine crabs were used.

They were maintained in 300 l tanks with fresh water or marine solution respectively and filtration of fluids was carried through active coal in a closed circuit.

Care was taken to select specimens with a length of 5-7 cm in the case of the river crabs, while only those sea crabs with a cephalothorax breadth of 4 cm were selected.

Extraction of the hemolymph was effected by direct puncture of the ventral cavity. The material obteined was then centrifuged at 3,500 r.p.m. in order to separate the hemocytes in order to avoid coagulation.

Identification of the phases of development was done according to the criteria of Drach (4, 5). The phases A, B, C_1, C_2 and C_3 correspond to the postmolt; C_4 to the intermolt and D_1, D_2 and D_3 to the premolt.

The hemolymph iron (HI), the total iron binding capacity (TIBC) and the

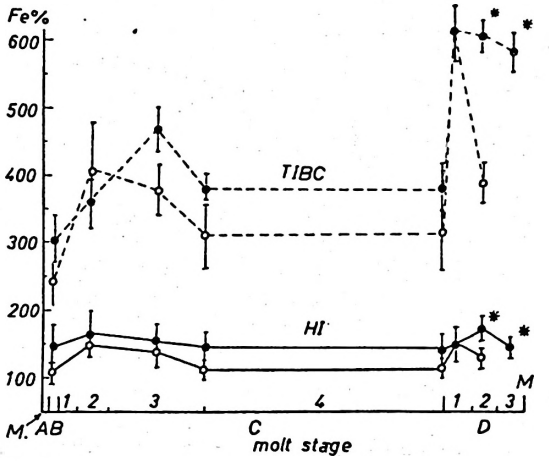

Fig. 1. *Variations of the hemolymph Iron (HI) and the total binding capacity (TIBC) during the different stages of Intermolt of* Austropotamobius pallipes. *Males* —●—●—; *females* —○—○—. *TIBC and HI μgFe/100 ml. M = time of molt.*

* Values obtained in the experimental molt produced by ablation of the ocular stems.

Table I. *Hemolymph Iron in* Austropotamobius pallipes *during the Intermolt cycle.* The hemolymph iron (HI), the total iron binding capacity (TIBC), the saturation coefficient (SC). Each value is the mean±SEM. The number of animals/group are show in parenthesis.
a. Values obtained in the experimental molt produced by ablation of the ocular stems.
B, C_2 and C_3: postmolt stage; C_4: intermolt stage; D_1, D_2 and D_3: premolt stage.

Males				Females			
Molt stage	HI μgFe/100 ml	TIBC μgFe/100 ml	SC %	Molt stage	HI μgFe/100 ml	TIBC μgFe/100 ml	SC %
B	(4) 144,2±37,8	(4) 305,8±36,6	47	B	(6) 113,2±19,1	(6) 245,1±38,9	46
C_2	(7) 164,7±34,7	(7) 360,4±41,4	46	C_2	(6) 147,8±12,1	(6) 410,1±67,5	36
C_3	(12) 153,1±24,1	(12) 465,4±34,2 *	33	C_3	(8) 140 ±30	(8) 380 ±40 *	37
C_4	(7) 146,9±20,6	(7) 378,4±25,1	39	C_4	(7) 116,3±10,3	(7) 315,3±53,4	37
D_{1a}	(8) 152,9±14,3	(8) 614,4±28,6 ***	20	D_1	(10) 156,7±32,8	(10) 605,1±31,3 ***	26
D_{2a}	(5) 174 ±21	(5) 605 ±26 ***	29	D_2	(7) 133,2±15,1	(7) 387,3±30,1 **	34
D_3	(4) 146,5±15	(4) 579 ±30 ***	26				

* $p < 0,05$. ** $p < 0,02$. *** $p < 0,001$.

saturation coefficient (SC) were determined in the hemolymph of each specimen.

Hemolymph iron was determined by the method proposed by the Expert Panel on Iron the International Commitee for Standardization in Haematology (ICSH) (7), thioglycolic acid being used as reducing agent and bathophenanthroline sulphonate as chromogen. In order to determine the TIBC, the hemolymph was satured with iron chloride and the excess was eliminated with magnesium carbonate, according to the directions of RAMSAY (12) but the concentration of binded iron was measured by the method mentioned above (7).

Determinations were made using 0.2 ml volumes of hemolymph and reading at 535 nm in a Pye Unicam 600 P spectrophotometer.

The complete available population of each species was arranged according to the sex and the state of intermolt of each individual specimen. No specimens were found in advanced stages of postmolt among the males of *A. pallipes*, thus experimental provocation of molting was made by means of ablation of the ocular stems, as described by DE LEERSNYDER (3).

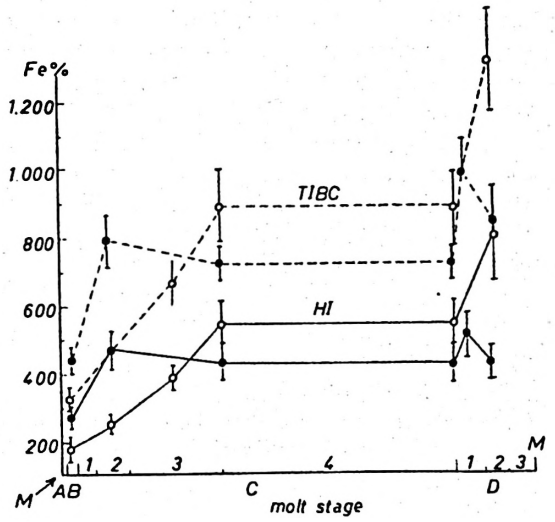

Fig. 2. *Variations of the hemolymph iron (HI) and the total binding capacity TIBC during the different stages of intermolt of* Carcinus maenas. *Males —●—●—; females —O—O—. TIBC μgFe/100 ml and HI μgFe/100 ml. M — time of molt.*

Results

Table I (fig. 1) shows the results obtained with specimens of *A. pallipes* for hemolymph iron, total iron binding capacity and saturation coefficient with respect to males and females in the different stages of intermolt.

In table II (fig. 2) the results are given for *C. maenas* of analogous parameters. These are also arranged according to sex and stages of intermolt.

Table II. *Hemolyhmp iron in* Carcinus meanas *during the intermolt cycle.*
The hemolymph iron (HI), the total iron binding capacity (TIBC), the saturation coefficient (SC). Each value is the mean±SEM. The number of animals/group are show in parenthesis.
B, C_2 and C_3: postmolt stage; C_4: intermolt stage; D_1, D_2 and D_3: premolt stage.

Males				Females			
Molt stage	HI gFe/100 ml	TIBC gFe/100 ml	SC %	Molt stage	HI gFe/100 ml	TIBC gFe/100 ml	SC %
B	(8) 282,9±28,9	(8) 437,9±37,7	65	B	(5) 172,1± 31,3	(5) 326,1± 16,1	53
C_2	(11) 483,4±60,5**	(11) 799,5±80,3**	60	C_2	(6) 233,5± 35	(6) 281,2± 32,1	83
C_4	(10) 428,5±56,5*	(10) 729,2±51,5***	59	C_3	(10) 388,1± 43,6**	(10) 673,1± 72,4**	58
D_1	(12) 519,3±69,9**	(12) 994,8±98,1***	52	C_4	(7) 543,1± 76,7**	(7) 887,1±114,5**	61
D_2	(7) 440 ±45**	(7) 850 ±70***	52	D_2	(4) 819,2±137,6**	(4) 1.323 ±152,7***	62

* $p < 0,05$. ** $p < 0,02$. *** $p < 0,001$.

Discussion

The high levels of hemolymph iron found in both species of crustaceans under consideration, and mainly in *C. maenas*, may account for the formation of myoglobins of the muscle tissue and cellular hemins, in both males and females but in females may be also due to a contribution of iron to the eggs.

The difference of iron content for males and females of *A. pallipes* is substantiated by this study. The average iron content for males during the intermolt cycle was 154,6 μg/100 ml and for females 134,5 μg/100 ml which is in agreement with the values obtained by MARTIN (9). In *C. maenas* the interferences due to the process of ovulation becomes more evident so that the difference has no significant value. With respect to TIBC both in *A. pallipes* and *C. maenas* a parallelism can ben observed in connection with the variations of the hemolymph iron content during each stages of the intermolt (figures 1 and 2). In both cases the levels of TIBC are higher in the males.

In figure 1 which refers to the intermolt period of *A. pallipes*, the first increase in hemolymph iron coincides with the postmolt stage (B, C_1, C_2 and C_3). At this stage a great amount of muscular tissue is synthesized in order to replace the water accumulated in the muscle during the molt (4) and leading to the increase in size. During the intermolt (C_4), the need for iron decreases as the rate of tissue synthesis is lower. Finally the enormous increase in the hemolymph iron content during the premolt (D_1, D_2 and D_3) may be due to the need to move iron towards the deposits, thus providing for a new cycle (13). The TIBC follows a pattern which is noticeably parallel.

Figure 2 shows the variations of hemolymph iron and TIBC with respect to the intermolt stages of *C. maenas*, with in implications similar to those of the river crab.

As molting may be impeded or prevented in other demands are made simultaneously on the organic reserves (10), it has been observed that during the intermolt and premolt the increase of Fe in females was higher than in males due to overlaping of the tissue formation processes and a hypothetical increase in the deposits occurring at the same time than the demand of iron for gonadal maturation. This increase is still more strinking in *C. maenas* than *A. pallipes* due to the higher proportion of eggs in relation to the body weight of former.

Work is in progress on the metabolism and the mechanisms of transference of iron in crustacea in oder to clarify the physiological role of iron in these species which have been so little studied.

Acknowledgement

The authors wish to thank the members of ICONA (Salamanca) for their help in capturing the specimens of *A. pallipes.*

Resumen

Se estudia el contenido de hierro hemolinfático y la capacidad total de fijación para machos y hembras de *Austropotamobius pallipes* y *Carcinus maenas*. En machos se obtienen valores de hierro hemolinfático de 154 a 430 μg/100 ml y en hembras de 134 a 431 μg/100 ml y una capacidad total de fijación de 472-762 y 390-698 μg/100 ml, para machos y hembras, respectivamente. También se han estudiado las variaciones de ambos parámetros en los diferentes estadios de la intermuda. El primer aumento en el hierro hemolinfático coincide con el estado de postmuda. Durante la intermuda, las necesidades de hierro decrecen al ser más baja la velocidad de síntesis de los tejidos. Finalmente, se observa un gran incremento en el hierro hemolinfático durante la premuda.

References

1. COWGILL, R. W.: *Biol. Bull.*, **111**, 300-301, 1956.

2. COWGILL, R. W. and CORI, C. F.: *J. Biol. Chem.*, **216**, 133-140, 1955.
3. DE LEERSYNDER, M. and GLACON, R.: *Cah. Biols. Mar.*, **15**, 505-512, 1974.
4. DRACH, P.: *Ann. Inst. Oceanog.*, **19**, 103-391, 1939.
5. DRACH, P. and TCHERNIGOVTZEFF, C.: *Vie et Milieu*, **18**, 3-A, 595-610, 1967.
6. GHIDALIA, W., FINE, J. M. and MARNEUX, F.: *Comp. Biochem. Physiol.*, **418**, 349-354, 1972.
7. IZAK, G. and LEWIS, S. M.: In «Modern concepts in Haematology». Academic Press, New York, 1972, pp. 69-160.
8. KEILIN, D. and HARTREE, E. F.: *Nature*, **168**, 266-269, 1951.
9. MARTIN, J. L. M.: *Comp. Biochem. Physiol.*, **46A**, 123-129, 1973.
10. PASSANO, L. M.: In «The Physiology of Crustacea», Vol. I (T. H. Waterman, ed.). Academic Press, New York, 1960, p. 483.
11. RECIO, J. M., LATORRE, J. L. and PLANAS, J.: *Rev. esp. Fisiol.*, **29**, 65-67, 1973.
12. RAMSAY, W. N. M.: *Clin. Chim. Acta*, **2**, 221-226, 1957.
13. RENAND, L.: *Ann. Inst. Oceanog.*, **24**, 259-357, 1949.
14. SMARIDGE, M. W.: *Quart. J. Microscop. Sci.*, **97**, 205-214, 1956.