The Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- Composition of Giant Fibers from *Balanus nubilus*

This is the first of a series of communications reporting the inorganic ion content of single muscle fibers from the barnacle *Balanus nubilus* Darwin. Until quite recently the favorite material for studies of this type has been frog or rat skeletal muscle. Both models are slowly being abandoned, since it is realized that an accurate quantitative analysis of the composition of a cell is feasible only when a single cell preparation is examined. It is also becoming increasingly clear that the properties of muscle fibers from the same muscle bundle may not be the same. The purpose of the experiments described in this paper was therefore to obtain information about the ionic composition of single barnacle fibers and to see whether there are any marked differences in ionic content between fibers from the same muscle bundle and between fibers from different barnacle specimens.

Single fibers from specimens of *Balanus nubilus* were isolated from one of the depressor muscle bundles under artificial sea water, using a binocular microscope, fine scissors and watchmaker's forceps. Isolation of these fibers from their basal attachment was done by first freeing the scutal-tergal end and then suspending the fiber in air before cutting at its origin. The fiber was next rinsed 3 times with distilled water (10 sec rinses), gently blotted, weighed on a microanalytic balance and put in a 'Vycor' vessel containing 1 ml of conc. HNO_3. The digest was ashed at 500 °C for 5 h and the ash was then dissolved in 1 ml of 1 *N* HCl and made up to 5 ml with cesium (4 *mM*) and lanthanum (15 *mM*). The concentration of Na, Ca and Mg was estimated by means of an atomic absorption spectrophotometer (Jarrell-Ash 82–500 model) using acetylene-air flame. The concentration of K was estimated by means of the Jarrell-Ash flame emission unit. For the estimation of Cl, separate batches of fibers were dissected. The fiber was placed in a 'Vycor' vessel and lysed with 1 ml of 0.5 *M* KOH. The digest was dried down at about 70 °C and 3 ml of nitric-acetic polyvinyl alcohol solution was added to the residue. The instrument used for chloride estimations was a chloride titrator manufactured by American Instrument Co., Inc.

The water content of single fibers was determined by oven drying at 120 °C for 15 h. The extrafiber water space was taken to be 6% of the total fiber volume, a value reported by GAYTON and HINKE[1] to largely represent the giant cleft and tubular system present in these fibers. The values for the extrafiber ion content were thus obtained from the measured concentration of ions in the artificial sea water. The intrafiber ion content was calculated by taking the difference between the total fiber and the extrafiber ion content, and dividing this value by the intrafiber water content.

Artificial sea water at 22–23 °C was used, the composition being (*mM*): Na^+ 465, K^+ 10, Ca^{2+} 10, Mg^{2+} 10 and HCO_3^- 10. The pH of the sea water was 7.8.

The water content of these fibers amounted to about 80% of their wet weight, a result which agrees with that of MCLAUGHLIN and HINKE[2] for fibers investigated in 1965 but not in 1964.

Table I gives the data on the internal Na, K, Ca, Mg and Cl concentrations of fibers freshly dissected from 4 specimens of *B. nubilus* (designated A, B, C and D). It will be seen that the mean Na content of fibers from specimen B of 14.7 (±12.6) *mM*/kg fiber water is lower than that found in fibers from specimen A. This value is of the same order as that reported by HAGIWARA, CHICHIBU and NAKA[3], BRINLEY[4] and BEAUGÉ and SJODIN[5], but not by MCLAUGHLIN and HINKE[2]. However, it is noteworthy that these workers carried out measurements on fibers that were immersed in artificial sea water containing 20 *mM* Ca^{2+} and varying concentrations of *Tris* as buffer. Table I also indicates that the K value of fibers from specimen B may be too low. This is interesting since GAYTON et al.[6] have pointed out that fibers having a low K content also have a low Na content. With one exception (fiber 9, specimen B) this appears to be the case here.

Table I. Intracellular ionic composition of *Balanus* muscle fibers

Specimen A	Na	K	Ca	Mg	Specimen A	Cl
Fiber 1	29.9	153	2.0	12.9	Fiber 1	24.8
2	33.8	149	2.0	12.5	2	16.2
3	61.1	139	2.6	12.9	3	22.5
4	26.7	162	3.9	12.7	4	22.6
5	30.5	167	3.4	13.0	5	24.3
6	22.1	170	2.8	12.7	6	55.7
Water content – 800 g/kg						
Mean	34.0	157	2.8	12.8	Mean	27.7
S.D.	±13.9	±11.8	±0.75	±0.18	S.D.	±14.0
Specimen B					Specimen D	
Fiber 1	5.1	112	1.9	9.0	Fiber 1	40.3
2	12.4	119	1.8	8.9	2	18.2
3	1.1	98	1.3	8.2	3	22.2
4	5.5	129	1.3	8.9	4	39.4
5	17.9	103	1.4	9.8	5	26.4
6	5.5	131	1.4	8.9	6	49.7
7	24.7	95	2.0	7.8	7	13.8
8	18.0	126	2.2	9.5	8	20.2
9	43.0	92	2.8	8.5		
10	7.1	105	1.6	7.9		
11	10.4	112	1.6	8.2		
12	26.1	129	1.4	10.9		
Water content – 800 g/kg						
Mean	14.7	112.6	1.7	8.9	Mean	28.8
S.E.	±12.6	±14	±0.45	±0.88	S.D.	±12.8
Specimen C	Na	K	Ca	Mg		
Fiber 1	14.6	133	1.2	10.1		
2	17.8	139	1.9	10.8		
3	42.8	130	2.5	10.9		
4	15.2	117	1.9	10.8		
5	19.1	122	0.68	10.4		
6	22.4	140	1.2	10.6		
7	25.9	138	1.2	10.6		
8	20.9	119	1.1	10.4		
9	18.5	120	1.2	9.6		
10	16.6	115	1.1	12.2		
11	20.0	127	1.1	10.7		
12	22.3	137	1.6	10.7		
Water content – 790 g/kg						
Mean	21.3	130	1.4	10.7		
S.D.	±7.5	±8.9	±0.49	±0.61		

[1] D. C. GAYTON and J. A. M. HINKE, Can. J. Physiol. Pharmac. *46*, 213 (1968).
[2] S. G. A. MCLAUGHLIN and J. A. M. HINKE, Can. J. Physiol. Pharmac. *44*, 837 (1966).
[3] S. HAGIWARA, S. CHICHIBU and K.-I. NAKA, J. gen. Physiol. *48*, 163 (1964).
[4] F. J. BRINLEY JR., J. gen. Physiol. *51*, 445 (1968).
[5] A. BEAUGÉ and R. A. SJODIN, Nature, Lond. *215*, 1307 (1967).
[6] D. C. GAYTON, R. D. ALLEN and J. A. M. HINKE, J. gen. Physiol. *54*, 433 (1969).

Measurements of the Ca^{2+} concentration in these fibers show not only significant differences between fibers from the 3 specimens studied but also between fibers from the same muscle bundle. Another clear-cut feature of these results is that the Na and Ca content of fibers from specimen A parallel each other. Both the Na and Ca values are high, and this is in striking contrast to the low Na and Ca values found in fibers from specimens B and C. The Mg^{2+} concentration seems to be quite steady and not very different from the findings of PAGE, MOBLEY and LEWIS[7].

The salient feature of the present results is that differences in ion concentration between fibers from the same muscle bundle show themselves when fibers are freshly dissected in artificial sea water containing HCO_3^- as buffer. So far as the mean values of the ionic content of these fibers are concerned, they are in fairly good agreement with those reported by other workers, as shown in Table II. MCLAUGHLIN and HINKE[2] found barnacle fibers (in 1964) to have a high Na content. The reason for this divergence could have been the use by them of *25 mM Tris* NO_3 as buffer, as well as allowing fibers to soak for varying periods in artificial sea water containing such a high concentration of *Tris*. This is thought to be so because there is evidence from experiments with barnacle fibers that the presence of *Tris* in the bathing medium leads to prolongation of the equilibration time of injected ^{22}Na (BITTAR et al. unpublished).

GAYTON et al.[6] have suggested that a low Na and K content in fibers is an indication of aging. However, the distribution of Na and K in relation to aging is supposedly reciprocal, i.e. there is a gain in Na and a loss in K. It would appear (to me) more likely that a low Na and K concentration is a characteristic of fibers from moulting barnacles. This possibility is now being investigated[9].

Table II. Various estimates of ionic concentrations in Balanus muscle fibres (m*M*/kg fiber water)

	Na	K	Ca	Mg	Cl
1. HAGIWARA, CHICHIBU and NAKA[3]	21	157			35
2. MCLAUGHLIN and HINKE[2a]	81 (in 1964)	168			10
	51 (in 1965)	143			
3. BRINLEY[4]	21	150			
4. BEAUGÉ and SJODIN[5]	21	167			
5. GAYTON and HINKE[1]					75.1 and 66.8
6. GAYTON, ALLEN and HINKE[6b]	21.8	160			36
7. ASHLEY[8]			0.78 m*M*/kg wet wt.		
8. PAGE, MOBLEY and LEWIS[7]				50 m*M*/kg dry wt.	
9. This paper	23.3	133	2.0	10.8	28.2

[a] Uncorrected for extrafiber space (6% of fiber water). [b] Corrected for extrafiber space (6% of fiber water).

Zusammenfassung. Es werden Analysenresultate über den Ionengehalt von einzelnen Muskelfasern der Entenmuschel, *Balanus nubilus*, mitgeteilt. Es wurden Unterschiede nachgewiesen in der Ionenkonzentration verschiedener Fasern, die von demselben Muskelbündel isoliert worden waren.

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[7] E. PAGE, B. A. MOBLEY and M. LEWIS, Proc. Am. Biophys. Society Meeting, Baltimore, February 1970, p. 224.
[8] C. C. ASHLEY, Am. Zoologist 7, 647 (1967).
[9] Acknowledgment. This research was supported in part by grants from the Wisconsin Heart Association, the Medical School Research Committee, the Graduate School Research Committee and the Office of Naval Research. E. E. B. wishes to thank Mr. GEOFFREY CHAMBERS for excellent technical assistance.

Penicillamine Induced Changes in Growing Rats. II. Liver Parenchymal Cell

D-Penicillamine is a chelating agent almost specific for copper, binding the metal present in the body[1] and inhibiting the intestinal uptake[2]. Therefore, penicillamine is generally applied for prophylaxis and therapy of Wilson's disease[3,4]. This autosomally inheritable copper storage disease is characterized by a deficiency of ceruloplasmin and deposits of copper in liver, brain, kidney and cornea[5]. The human body normally contains about 100 mg of copper, 98% of which is bound to ceruloplasmin, an α_2-globulin. This copper-protein complex is not toxic and acts as a ferrooxidase in synthesizing iron-containing enzymes in the respiratory chain[6,7]. Copper is a constituent of oxidases, e.g. xanthine oxidase, uricase, amine oxidase of microbodies[8] and cytochrome oxidase of mitochondrial cristae[9]. All these enzymes, except amine oxidase, function as so-called terminal oxidases in the last step of biological oxidation processes. Cytochrome oxidase transfers the hydrogen from substrate to oxygen at the end of the respiratory chain. Uricase and xanthine oxidase catalyse the terminal oxidation of purines, and are able to interact themselves with molecular oxygen[9].

The present paper deals with quantitative ultrastructural changes in rat liver cells after long-term application of Penicillamine, examined by means of stereological methods. Thereby, mitochondria and microbodies, the main sites of copper-containing enzymes, were especially considered.

[1] J. M. WALSHE, Am. J. Med. *21*, 487 (1956).
[2] P. M. WEBER, S. O'REILLY and M. POLLYCOVE, J. nucl. Med. *10*, 519 (1969).
[3] S. B. OSBORN and J. M. WALSHE, Lancet *1958*/I, 70.
[4] I. STERNLIEB and I. H. SCHEINBERG, New Engl. J. Med. *278*, 352 (1968).
[5] J. M. WALSHE and J. N. CUMINGS, *Wilson's Disease. Some Current Concepts* (Blackwell Science Publ., Oxford 1961).
[6] E. FRIEDEN, Scient. Am. *1968*, 103.
[7] S. SEN, J. Ind. med. Ass. *52*, 182 (1969).
[8] Z. HRUBAN and M. RECHČIGL, *Microbodies and Related Particles* (Academic Press, New York, London 1969), p. 149.
[9] A. WHITE, PH. HANDLER and E. L. SMITH, *Principles of Biochemistry* (McGraw-Hill, New York 1968).