lipid complexes, the tumour and liver were processed as described elsewhere[4]. After mineralization of the fractions obtained with $HClO_4$ plus HNO_3, phosphorus, calcium and magnesium were measured in aliquots of the mineralized material. Phosphorus was assayed colorimetrically using aminonaphtolsulfonic acid reagent[5]. Calcium and magnesium were determined with an Atomic Absorption Spectrometer. The results shown in the Table are the average and the range values corresponding to each type of tumour.

The observation that tumor tissue contains a much lower content of phospholipid than liver is in agreement with that of other authors[6,7]. The most striking difference between hepatoma and normal liver is a much lower amount of magnesium complexed by the phospholipids, while the complexed calcium does not show significant variations. Since it has been demonstrated that the binding of calcium and magnesium by the membranes exhibits a saturation type relationship characteristic of adsorption to binding sites and describable by the law of mass action[8], this difference may be due to a considerably higher calcium concentration at the cell membrane level. On the other hand, considering that tumor has shown a slightly higher proportion of cephalin than liver[9], and that in biphasic systems the acidic phospholipids have shown a far greater affinity for Ca^{2+} than for Mg^{2+}[10], it is possible that this small difference in the phospholipid composition may also contribute to the magnesium-binding behavior observed.

These findings raise the question whether this change in the divalent cation distribution may or may not be implicated in determining the tumor cell membrane characteristics. In relation to this, it is also very interesting to note that, with less than half of the complexed phospholipid amount, tumors bind almost as much calcium as liver does. This is an indication of a drastic change in the phospholipid-calcium relationship.

Zusammenfassung. Phospholipid-Kalzium- und Phospholipid-Magnesium-Komplexe wurden aus Hepatom und aus normaler Leber isoliert. Obwohl das Hepatom nur die Hälfte der in der Leber vorkommenden komplexen Phospholipide aufweist, ist die Kalziumbindung bei Hepatom und Leber gleich. Die Magnesiumbindung ist dagegen erheblich niedriger. Diese Unterschiede weisen auf eine mögliche Bedeutung der zweiwertigen Kationenbindung im Verhalten der Tumorzellmembran hin.

L. J. Anghileri[11]

Innere Klinik und Poliklinik (Tumorforschung), Klinikum der GHS Essen, Hufelandstrasse 55, D-4300 Essen (Federal Republic of Germany),
28 May 1974.

[5] G. F. Shinowara, L. M. Jones and H. L. Reinhart, J. biol. Chem. *142*, 921 (1942).
[6] J. H. Veerkamp, I. Mulder and I. van Deenen, Z. Krebsforsch. *64*, 137 (1961).
[7] P. H. Figard and D. M. Greenberg, Cancer Res. *22*, 361 (1962).
[8] A. P. Carvalho, H. Sanui and N. Pace, J. cell. comp. Physiol. *62*, 311 (1963).
[9] R. M. Johnson and P. H. Dutch, Archs Biochem. Biophys. *40*, 239 (1952).
[10] H. Hauser and R. M. C. Dawson, Europ. J. Biochem. *1*, 61 (1967).
[11] This work has been supported by the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen.

On the Quarternary Structure of *Carcinus moenas* (Arthropoda) Hemocyanin[1,2]

It is well enough established that the so-called minimum functional subunit of the hemocyanin (Hc) of several Arthropoda species (unlike Mollusca[3]) can be isolated without covalent bond cleavage[4,5]. This component includes one active site with 2 copper ions, weighs around 75,000 d and has been reported to be rather heterogeneous[6-9]. Three different hypotheses about its constitution have been proposed following the studies on minimum subunits regardless of their functionality: 1. Pickett, Riggs and Larimer[10] have reported that succinylated *Homarus americanus* Hc displays by sedimentation analysis a subunit of about 37,500 d, which probably consists of only one polypeptide chain; 2. according to another model[11], suggested in order to explain the low and 'continuously varying' sedimentation coefficient of *Cancer magister* Hc in 6 *M* guanidine hydrochloride solution, the minimum functional subunit is composed of 3 polypeptide chains of 25,000 d. Such a model was made probable by recent data obtianed in our laboratory on *Carcinus moenas* Hc by polyacrylamide gel electrophoresis and gel permeation chromatography in 0.1% SDS solutions at pH 7.0–9.3[12,13]; 3. the results of Loehr and Mason[8], confirmed by Carpenter and van Holde[9], seem to prove that the subunits around 80,000 d of *Cancer magister* Hc consist of single polypeptide chains. These results have been obtianed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) solutions at pH 8.0–9.0.

We have separated *Carcinus moenas* Hc polypeptide chains using gel permeation chromatography on Sephadex G-200 in 1% SDS solution[14,15]. Hc and apoHc (apohemocyanin) were prepared according to Ghiretti

[1] A preliminary report on this work was communicated at the 10th National Meeting of the Italian Society of Biophysics and Molecular Biology (Padua, 28/9/1973).
[2] This work was supported by CNR grants.
[3] R. Lontie, M. De Ley, H. Robberecht and R. Witters, Nature, Lond. *242*, 180 (1973).
[4] K. E. van Holde and E. F. J. van Bruggen, in *Biol. Macromolecules Series* (Eds. N. Timasheff and G. D. Fasman; Dekker, New York 1971), vol. 5, p. 1.
[5] F. Ghiretti, A. Ghiretti Magaldi and B. Salvato, in *Comparative Physiology* (Eds. L. Bolis, K. Schmidt-Nielsen and S. H. P. Maddrell; North-Hollans Publishing Co. Amsterdam 1973), p. 509.
[6] L. Di Giamberardino, Archs Biochem. Biophys. *118*, 273 (1967).
[7] P. Busselen, Archs Biochem. Biophys. *137*, 415 (1970).
[8] J. S. Loehr and H. S. Mason, Biochem. biophys. Res. Commun. *51*, 741 (1973).
[9] D. E. Carpenter and K. E. van Holde, Biochemistry *12*, 2231 (1973).
[10] S. M. Pickett, N. F. Riggs and J. L. Larimer, Science *151*, 1005 (1966).
[11] H. D. Ellerton, D. E. Carpenter and K. E. van Holde, Biochemistry *9*, 2225 (1970).
[12] B. Salvato, S. Sartore, G. Zaccaria and A. Ghiretti Magaldi, Boll. Soc. ital. Biol. sper. *47*, 777 (1971).
[13] B. Salvato, S. Sartore, M. Rizzotti and A. Ghiretti Magaldi, FEBS Lett. *22*, 5 (1972).
[14] W. W. Fish, J. A. Reynolds and C. Tanford, J. biol. Chem. *245*, 5166 (1970).
[15] K. Weber and D. J. Kuter, J. biol. Chem. *246*, 4504 (1971).

MAGALDI et al.[16]. The samples of apoHc and column calibration proteins, containing 2 mg/ml of protein in 1% SDS in sodium phosphate buffer (0.05 *M*, pH 7.2 at 25 °C), were deaerated with argon, with 1% (final concentration) 2-mercaptoethanol added, heated for 3 h at 40 °C, and dialyzed for several h against a frequently changed 1% SDS solution. Protein elution was carried out in ascending flow (5 ml/h) and followed at 280 nm.

Our results are reported in Figures 1 and 2. The apoHc shows a peak at 12,000 d. From the elution profile we always obtained two heavier components (50% of the total sample), the one weighting 23,500 d, the other with a MW close to BSA MW. The relative amount of these heavier components rises to 80% of the total if the sample is treated without any reducing agent (Figure 2).

The results previously obtained by SALVATO et al.[12,13], by LOEHR and MASON[8] and by CARPENTER and VAN HOLDE[9] may be due to incomplete dissociation and interaction with SDS of the Hc, owing to the short-time treatment and/or to SDS concentration lower than 1% during elution or electrophoretic run[15,17–19]. As a matter of fact, also some calibration proteins are reported to be completely or partially associated in oligomers. The result of PICKETT et al.[10] could be explained by a lowering of the sedimentation coefficient of the indissociated functional subunit following succinylation[20]; anyhow, although the dissociation observed by these authors had actually affected the functional subunit itself, the succinylation reaction does not necessarily cause a complete dissociation into single polypeptide chains[21]. The three hypotheses mentioned above could have been proposed, therefore, on the basis of an incomplete Hc dissociation. However one cannot yet exclude completely the possibility that the different data reported from this research and from the preceding ones are due to differences among zoological species and/or to the action of specific proteolytic enzymes during Hc samples preparation.

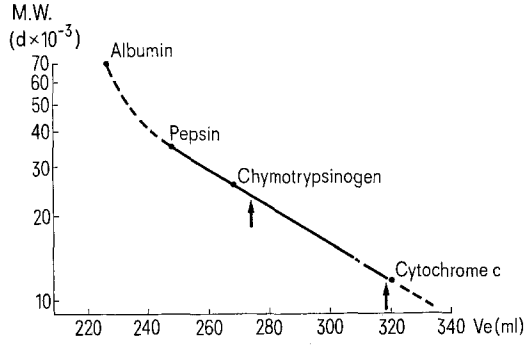

Fig. 1. Calibration curve of G-200 column with 1% SDS solution. The arrows show the position of the two lower peaks of *Carcinus moenas* Hc.

On the basis of the present results, the minimum functional subunit of *Carcinus moenas* Hc seems to be an aggregate of 6 polypeptide chains weighting about 12,000 d each. Such chains are not homogeneous as proved by the analysis of the N-terminal amino acids[22] and by the amino acid composition itself[23]. The amount of half cystines (3/75,000 d) excludes the possibility that all chains are held toghether by disulfide bonds, as shown also in the present work by the extensive apoHc dissociation without any reducing agent. Lastly, if we treat without any reducing agent the native Hc (which still contains copper) the dissociation is very small, and the low MW components represent only 5% of the total sample (Figure 2). This may be due to the stabilizing effect of copper and of the possible disulfide bond (which, on the other hand, could have been broken down by CN^- during the preparation of the apoHc [24]).

Riassunto. Utilizzando una metodica che offrisse la maggiore garanzia possibile di una completa dissociazione in catene polipeptidiche dell'emocianina di *Carcinus moenas* (Arthropoda), si è ottenuto un peso molecolare di 12000 d.

M. RIZZOTTI[25]

Institute of Animal Biology, University of Padua, Via Loredan 10, I-35100 Padova (Italy), 8 April 1974.

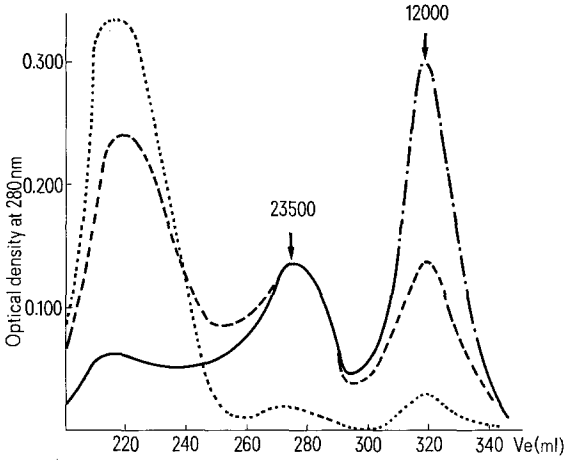

Fig. 2. Elution profiles of apoHc heated with a reducing agent (—), of apoHc heated without any reducing agent (– – –), of native Hc heated without any reducing agent (. . .).

[16] A. GHIRETTI MAGALDI, G. NARDI, F. GHIRETTI and R. ZITO, Boll. Soc. ital. Biol. sper. *38*, 1839 (1962).
[17] S. KATZ, M. E. SHAW, S. CHILLAG and J. E. MILLER, J. biol. Chem. *247*, 5228 (1972).
[18] J.-S. TUNG and C. A. KNIGHT, Analyt. Biochem. *48*, 153 (1972).
[19] C. A. NELSON, J. biol. Chem. *246*, 3895 (1971).
[20] W. N. KONINGS, J. DIJK, T. WICHERTJES, E. C. BEUVERY and M. GRUBER, Biochim. biophys. Acta *188*, 43 (1969).
[21] I. M. KLOTZ, in *Methods in Enzymology* (Ed. C. H. W. HIRS; Academic Press, New York 1967), vol. 11, p. 576.
[22] A. GHIRETTI MAGALDI, B. SALVATO and V. ALBERGONI, Boll. Soc. ital. Biol. sper. *46*, 943 (1970).
[23] L. TALLANDINI, personal communication.
[24] N. CATSIMPOOLAS and J. L. WOOD, J. biol. Chem. *239*, 4132 (1964).
[25] Acknowledgments. The author is grateful to Prof. A. M. TAMBURRO for profitable discussions and to Dr. R. MILANINO for reviewing the manuscript.