In conclusion, without considering their pathological or non-pathological nature, histiocytic cells have an in vitro collagenolytic activity in the EG as well as in other tumours.

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Ultrastructural localization of vanadium in the blood cells of Ascidiacea

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Summary. X-ray histospectrographic analysis at the scanning and transmission electron microscope (STEM) are made on the blood cells of *Phallusia mamillata* Cuvier and *Ciona intestinalis*, to study the 'direct' intracellular sites of accumulation of vanadium. The results show a clear accumulation of vanadium on the membrane and in the granules of vacuoles of amebocytes, signet ring cell, compartment cell and traces of metal in the 'vanadophores' of vanadocytes.

It has been reported that certain species of Tunicata manifest the faculty of absorbing great quantities of vanadium from the surrounding sea-water[2]. According to the majority of authors, this metal is selectively localized in a particular type of blood cell named 'vanadocyte'[3-5]. A vanadocyte measures 8-13 µm. Its cytoplasm contains voluminous acidophilic globules which, under the electron microscope, appear as more or less compact electron-dense masses, denominated vanadophores.

Vanadium, according to several authors, is found in the form of a tripositive valency and thus constitutes the native haemovanadin. This in turn has remarkable reducing influence on osmic acid[6]. This property has been utilized to asses indirectly the ultrastructural localization of vanadium and its compound in the blood cells during histogenesis[7,8]. Thus, in order to furnish information on sites of retainment of vanadium in the blood cells of the Ascidiacea, we have undertaken an ultrastructural histospectrographic microanalysis, by energy-dispersive X-ray analysis of different types of blood corpuscles of *Phallusia mamillata* Cuvier and *Ciona intestinalis*.

Materials and methods. Blood, collected through a puncture in the cardiac vessel, was centrifuged at 3000 rpm for 10 min. The pellet so obtained and preparation of the branchial epithelial cells were fixed in 10% neutral formalin in sea-water pH 6.9. The fixed material was dehydrated and embedded in epon[9]. 2-µm sections, obtained on a Ultrotome III, were examined by scanning transmission electron microscope Elmiscope ST 100 F Siemens (STEM) in combination with an energy dispersive X-ray detector. Attention was focused mainly on the microanalysis of the following categories of blood cells: amebocytes, signet ring cell, compartment cells and vanadocytes which are mainly involved in the accumulation and synthesis of vanadium complex.

Results and discussion. Data obtained from both species showed that: a) the vanadium is selectively concentrated in the vacuole membrane of amebocytes, signet ring cell and compartment cell; b) vanadium granules are present inside the vacuoles too; c) vanadium is very scanty in the vanadocytes. In fact the spectra, obtained by scanning of vacuole membrane and inside of the vacuole, showed peaks well corresponding to vanadium (figure A and B). On the other hand, spectrum of vanadophores showed a peak corresponding to the base line of the vanadium spectrum (figure C) which means just at the limit of the sensibility of the method employed (e.g. 1×10^{-19} g absolute weight of the element examined). Simultaneous control scanning of the blood cell cytoplasm without vacuoles and those of the resing used for embedding gave negative results. Besides

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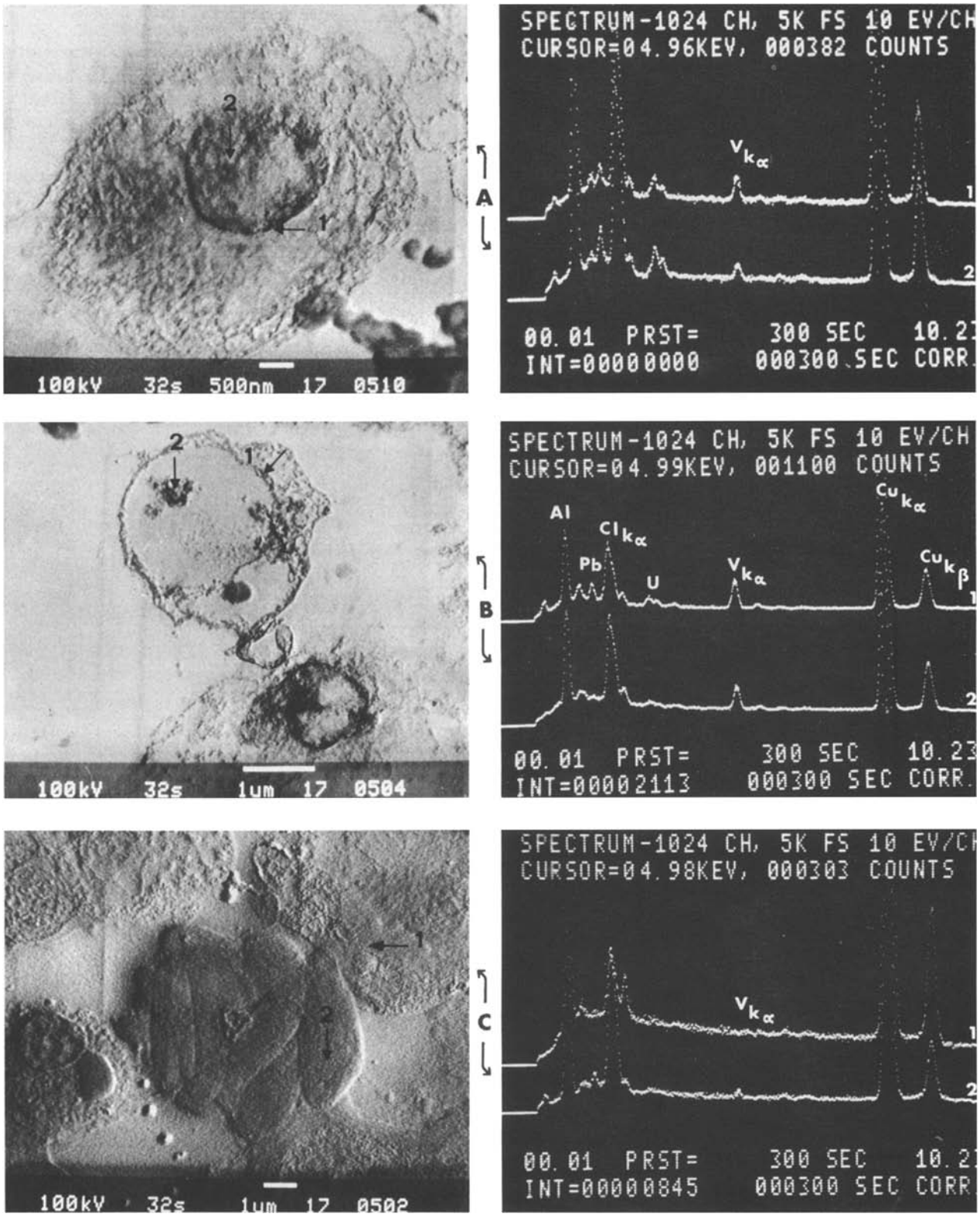
A Scanning transmission electron micrograph of a 'amebocyte' of the blood of *Phallusia mamillata* Cuvier. Presence of large vacuole with numerous clustered granules. The vacuolar membrane and the clustered granules inside of the vacuole are electron dense. - Energy dispersive X-ray spectra from the vacuolar membrane (1) and from the clustered granules inside the vacuole of the 'amebocyte'(2).
B Scanning transmission electron micrograph of a 'signet ring cell' of *Phallusia mamillata* Cuvier. The peculiarity of this cell is the presence of a large vacuole pressing upon the cytoplasm and nucleus in a polar cap. The vacuolar membrane and clustered granules are electron dense. - Energy dispersive X-ray spectra taken on the vacuolar membrane (1) and the clustered granules inside the vacuole of the 'signet ring cell' (2).
C Scanning transmission electron micrograph of a 'vanadocyte' of *Phallusia mamillata* Cuvier. Note the numerous electron dense globules 'vanadophore' around the nucleus. - Energy dispersive X-ray spectra of a tipical 'vanadophore' (2) and a control zone on the resin (1).



In the spectra there are, beside the peak corresponding to vanadium, other peaks that coincide with those of Cu(Kα) and Cu(Kβ) due to the grids; Pb are due to double staining of the section, and Al due to the 'specimen holder' of the STEM. Furthermore a peak corresponding to that of cloride is constantly present in all the spectra. - Spectrum legend: Spectrum 1024 CH = total spectrum consists of 1024 channels (horizontal scale). 5K FS = FS: full scale = 5000 counts. 10 EV/CH = the energy width of each channel = 10 eV. CURSOR 0.4.96-98-99 KEV = position of the bright spot (cursor) on the energy scale. COUNTS = number of added events. 0.0.01 PRST = Preset (of e.g. window region for integration of counts). 300 SEC CORR. = dead time connected time. 10.23 = 1023 KeV (energy value on horizontal scale). INT = Integral. STEM micrograph legend: KV = high tension of STEM. S = time of scanning. —— = magnification.

this, a microanalysis of the electron dense cytoplasmic granules, contained in the epithelial cell of the branchial chamber, and mucous present on the cell surface in direct contact with sea-water also showed total absence of vanadium. Quantitative analysis of the metal has not yet been done. Thus the present ultrastructural microanalysis of vanadium presents some new aspects concerning the distribution and metabolism of this metal in the ascidian blood cells. The present report demonstrates a direct localization of vanadium in the various types of blood cells. Earlier reports analized vandium with NMR[5] and with crude biochemical procedures in the blood cells in toto[10,11] and described a single value which according to several authors represented prevalently vanadium content of the vanadocytes. Furthermore it has also been shown here that the vacuole membrane of the blood cells examined possesses a high selectivity for the uptake of vanadium from sea-water. This is probably by simple diffusion through the branchial epithelium and against the concentration gradient in the vacuoles of blood cells. At last, these data showed scanty vanadium content in the vanadophores of vanadocytes. Contrary to claims of several earlier authors[3,7,8], the electron density of vanadium complex formed cannot be used as a marker in later stage of histogenesis of the blood cells, since the vanadophores of vanadocytes of *Phallusia mamillata* and *Ciona intestinalis* show a high electron density but very low vanadium content. This cellular type can no longer be considered as the main element for the selective uptake of vanadium. Vanadocytes could be destined to other metabolic functions[12] and probably also be the seat of catabolism of haemovanadin, as proposed earlier[13].

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Peritoneal macrophages from adjuvant arthritic rats enhance tumour cell growth in vitro

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Summary. Peritoneal macrophages from rats with adjuvant arthritis enhance the incorporation of ^{3}H-thymidine in 2 tumour cell lines in vitro. Maximal enhancement is found during the development of the secondary lesions, and it is suggested that the immunologic commitment of the macrophages could interfere with their regulation of tumour cell proliferation in vivo.

Macrophages appear to play an important role in the control of tumour cell growth[2]. Nonspecific activation of macrophages by i.p. administration of live organisms, such as *Corynebacterium parvum,* BCG or *Listeria monocytogenes,* elicits cytotoxic effects against a large variety of tumour cells, both in vitro and in vivo[3,4]. Recently Salmon and Hamburger[5] proposed that macrophages may also play a role as tumour promoters, and in the present report we show that, during the development of rat adjuvant arthritis, a chronic inflammatory disease triggered by immunologic mechanisms[6], peritoneal macrophages do enhance the growth of 2 rat compatible tumour cell lines in vitro, as compared to similar numbers of macrophages from non-arthritic rats.

Materials and methods. Adjuvant arthritis was induced in female, inbred Lewis rats (190–210 g) by injecting 0.3 mg of heat-killed *Mycrobacterium butyricum* (Difco) suspended in 0.1 ml of mineral oil, into the foot pad of the right hind paw. Saline-injected animals matched for sex and age served as controls. Peritoneal macrophages were collected from control rats and from arthritic rats 4, 7, 14, 21 and 28 days after the induction of adjuvant arthritis, by injection of 10 ml sterile saline into the peritoneal cavity. Cells from 5 control and 5 arthritic rats were poooled, respectively, and the cell suspensions were adjusted to 1×10^6, 5×10^5 and 1×10^5 cells/ml in culture medium (RPMI 1640, Gibco), supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. All cell suspensions were kept at 4 °C to avoid loss of adherent cells. Aliquots of 0.5 ml were pipetted into the flat-bottomed wells of tissue-culture treated multidishes (Flow Laboratories, cat. No. 75-033-05). The cultures were incubated at 37 °C in 5% CO_2 in air, nonadherent cells were removed 2 h later by washing. After overnight incubation, the medium was removed and 0.5 ml fresh medium containing 2×10^5 tumour cells/ml was added to each well. Cultures without macrophages were run in parallel. 24 h later 1 µCi/ml of ^{3}H-thymidine (^{3}H-TdR, 2500 mCi/mmole, Amersham, England) was added to each well, and the cultures were incubated for 4 h at 37 °C. At the end of incubation, the tumour cells were transferred into ice-cold trichloroacetic acid (12% TCA) and the precipitates were washed 3 times with 6% TCA, dissolved in 0.5 NaOH and counted in a liquid scintillation counter. The adherent cells left in the wells were dissolved in 0.5 N NaOH for determination of residual ^{3}H-TdR incorporation. All samples were performed in quadruplicate and the results were calculated as dpm/well ±SEM. Percent change in ^{3}H-TdR incorporation was calculated, compared to cultures with control macrophages. Tumour cell viability was assessed by the eosin exclusion method. The tumour cells used were the Yoshida Sarcoma cells, propagated in vivo in Lewis rats and the Ascites Hepatoma AH-13 cells, propagated in vitro for at least 20 years. These tumour cells were originally induced in Donryu rats by feeding with o-amino-azotoluene[7]. I.p. injection of $2\text{–}4\times10^7$ cells of each type in Lewis rats led to death in 8–10 days.

Results and discussion. Yoshida Sarcoma cells, propagated in vivo, were harvested from the peritoneal cavity of Lewis rats, inoculated 5 days previously with 2×10^7 living tumour